**Title**
Synthesis of analogs of saxitoxin



**Author**
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**Publication Date**
1977

Peer reviewed|Thesis/dissertation

# SYNTHESIS OF ANALOGS OF SAXITOXIN

by
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A.B., University of California Berkeley 1971

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

PHARMACEUTICAL CHEMISTRY

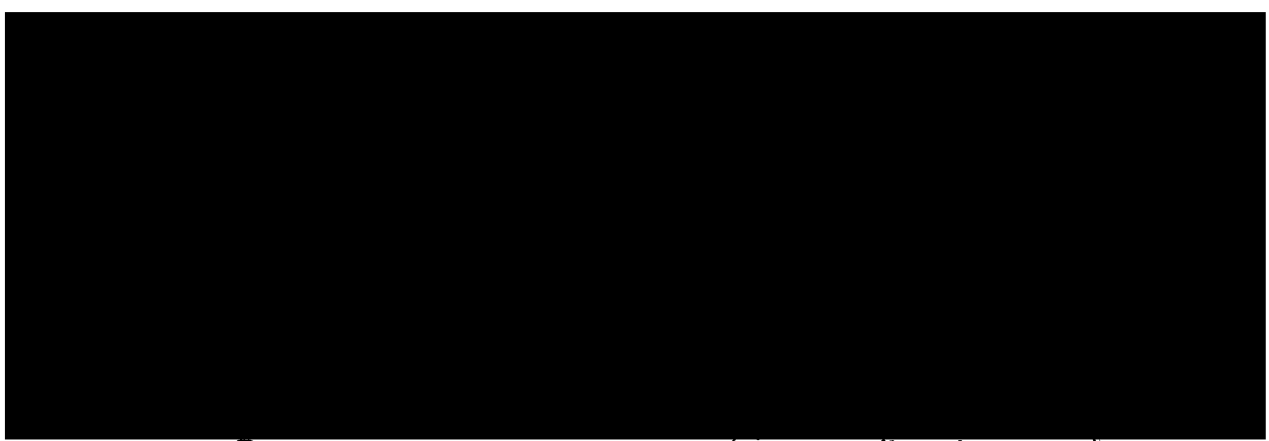
in the

GRADUATE DIVISION

(San Francisco)

of the

UNIVERSITY OF CALIFORNIA



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# SYNTHESIS OF ANALOGS OF SAXITOXIN: THESIS ABSTRACT

Jay F. Stearns

In the course of studies on the synthesis of saxitoxin, a new reaction was discovered involving the conversion of acylguanidines to alkylguanidines with lithium aluminum hydride. This reaction was studied in depth with seven different acylguanidines, and, with six of these, yields of alkylguanidines are 51% or better. One substrate for reduction, N,N,N',N'-tetramethyloctanoylguanidine, yields only non-guanidine products, four of which were characterized.

Substrate preparations include only known reactions with one exception, the synthesis of N-acetyl-N',N",N"-trimethylguanidine. This compound is obtained by diacetylation of N,N',N'-trimethylguanidine acetate with acetic anhydride followed by selective monodeacetylation with a quaternary ammonium resin in the hydroxide form. Three of the other substrates are prepared by direct monoacylation of the corresponding guanidine free base. In this manner, dodecanoylguanidine and N-dodecanoyl-N',N"-dimethylguanidine are obtained from methyl dodecanoate, and N,N,N',N'-tetramethyloctanoylguanidine is obtained from octanoylchloride. Creatinine is commercially available, and methylcreatinine and β-alacreatinine are synthesized by published methods.

In addition to developing practical syntheses of six specific alkylguanidines, several mechanistic studies are reported in order to make this new reduction as useful as possible. In general, the best conditions appear to be the use of excess lithium aluminum hydride in tetrahydrofuran at room temperature. The reaction times vary considerably with the substrate and must be carefully optimized

since the alkylguanidine products are themselves slowly reduced. Provided these conditions are met and the substrate has at least one hydrogen to nitrogen bond, this new reduction appears to be useful for the synthesis of alkylguanidines having a wide range of structural variation.

Roger Ketcham
Chairman, thesis committee

ACKNOWLEDGEMENTS

I would like to thank my thesis committee, Professors Roger Ketcham, Henry Rapoport, and Neal Castagnoli, for assistance and suggestions during the course of this work. Although the research reported here was done primarily in collaboration with Professor Rapoport, the assistance of my entire committee proved to be invaluable.

I would also like to thank my wife, Kathryn MacLeod, for her patience and support during the completion of this study.

Finally, I would like to express my gratitude to Sonja Scovel for her assistance in preparing this manuscript.

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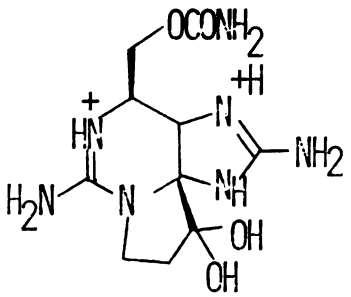
INTRODUCTION

This study was undertaken with two goals in mind, investigation of the reduction of acylguanidines with lithium aluminum hydride (LAH) and application of this reaction, if it worked, to the total synthesis of saxitoxin (1)[1].



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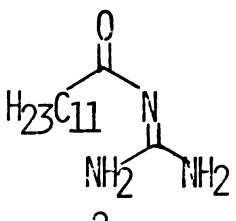
The reduction was tried on a total of seven acylguanidines, (2) through (8), and, in every case except compound (3), good yields of the corresponding alkylguanidines have been realized.



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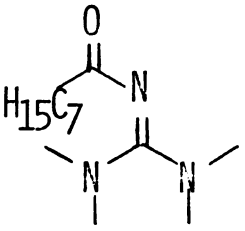

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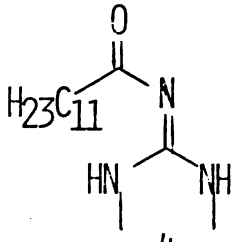

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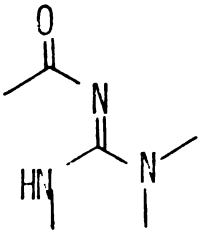

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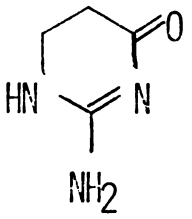

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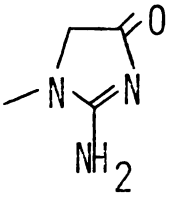

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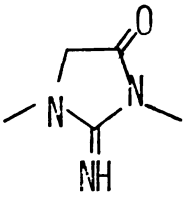

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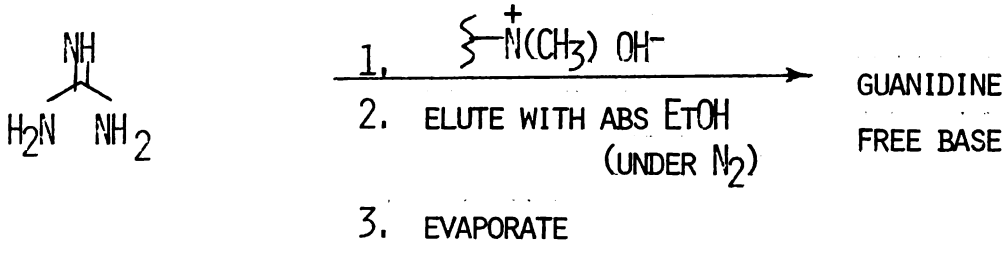
Although a literature search revealed no examples of the reduction of an acylguanidine with LAH, the clue that reduction of the carbonyl without reduction of the guanidine moeity might be possible came from a report that simple guanidines are not reduced by LAH[2]. This report turned out to be inaccurate since, as pointed out in the Discussion, LAH does indeed reduce alkylguanidines. However, in most cases, the carbonyl of an acylguanidine reduces (to a methylene) faster than the resulting alkylguanidine. To obtain a good yield of an alkylguanidine, then, the reaction conditions must be controlled very carefully to avoid subsequent reduction of the desired product.

This problem of over reduction was just one of the several obstacles which had to be overcome before this reaction could be considered synthetically useful. In several cases, the preparation of the substrates themselves was problematic; some were abondoned due to synthetic difficulties. In the following five sections, the problems encountered and the resolution of these difficulties are reported essentially as they occurred, each section covering about three to four months of research.

Although creatinine (7) is commercially available, each of the other substrates on the previous page had to be synthesized. Their preparation is outlined in the following Schemes.

Scheme A



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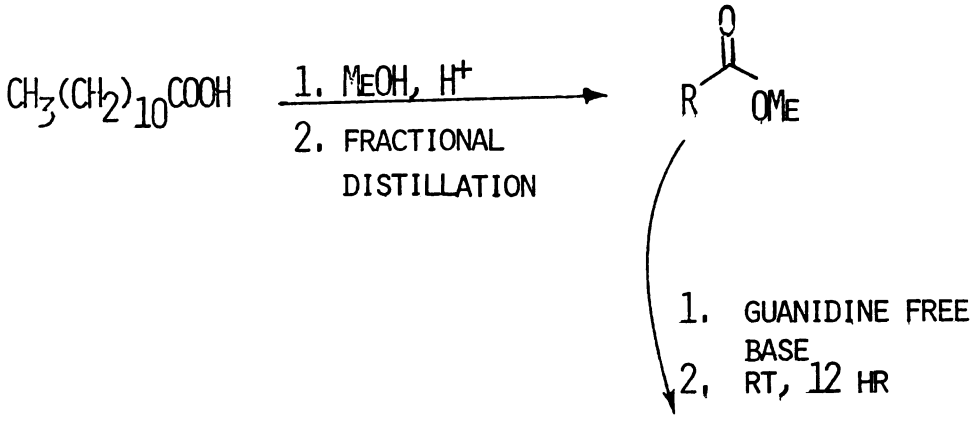


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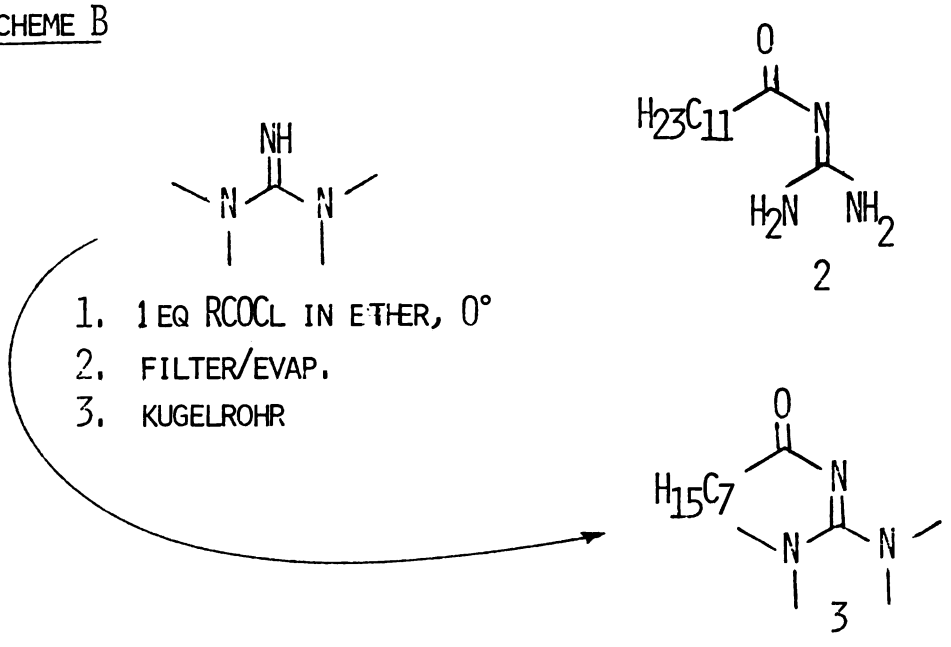


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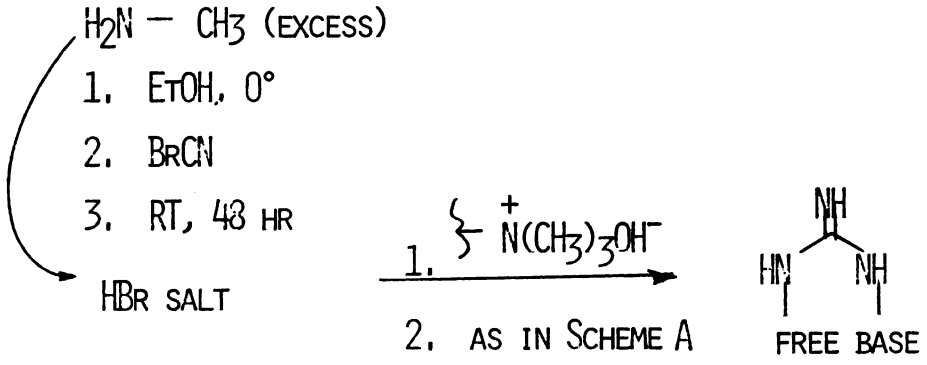

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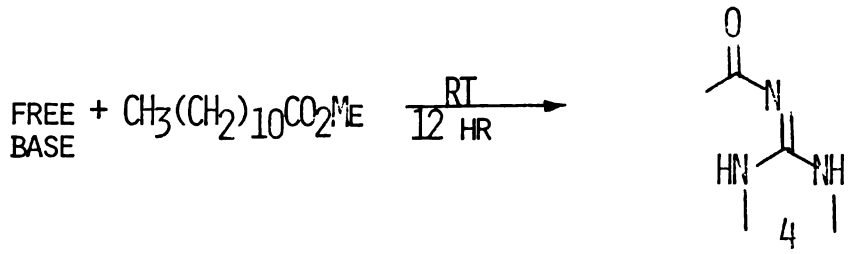


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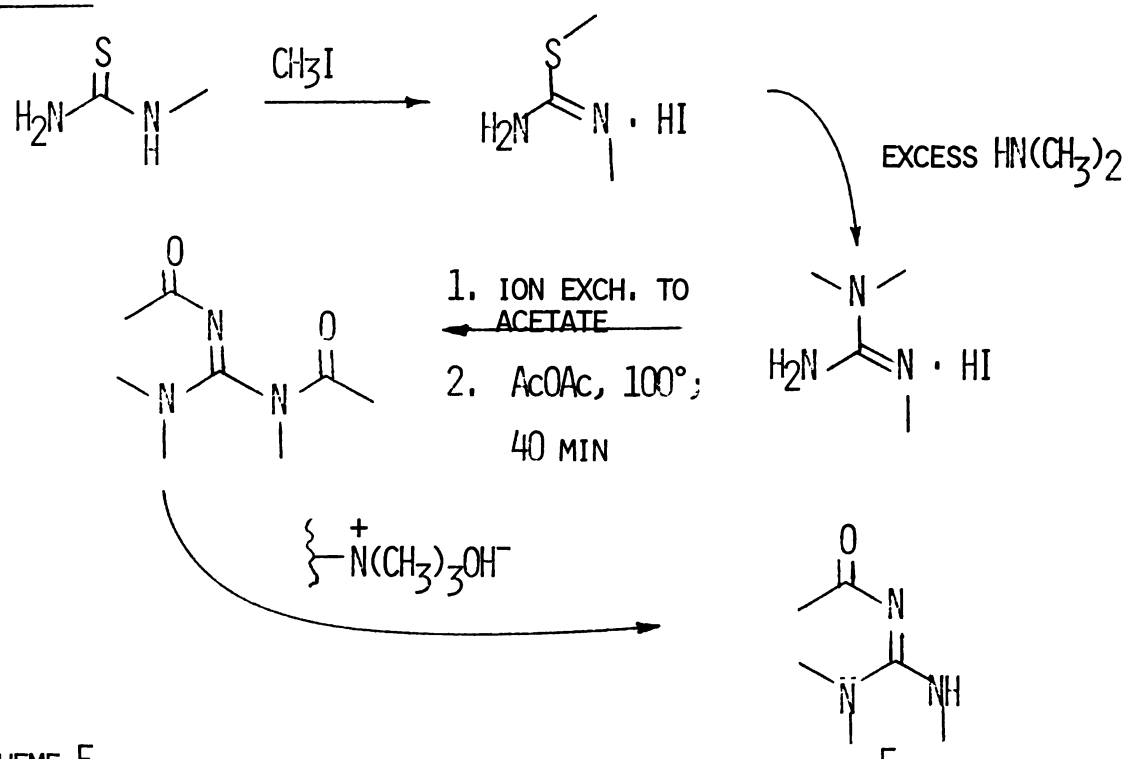


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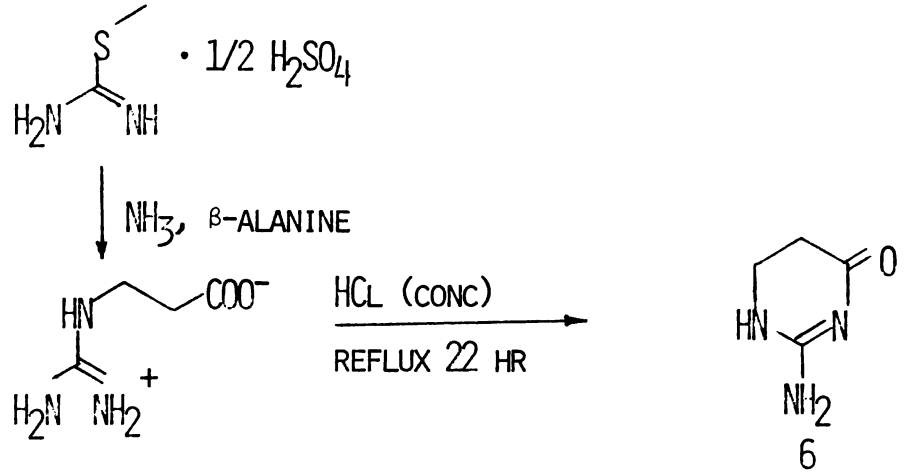


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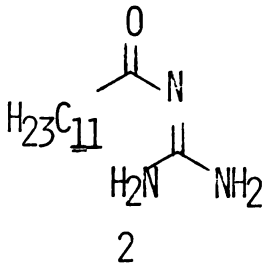
Creatinine (7) was purchased (Eastman) and methylcreatinine (8) was prepared from creatinine (7) and dimethylsulfate.

DISCUSSION

Section 1

All of the work described in this section centers on the preparation and reduction of compounds (2), (3), and (7).

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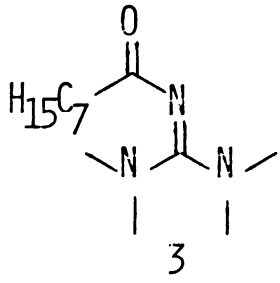


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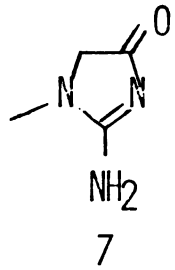


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Early work on the preparation of stearoyl guanidine was abandoned and replaced by the synthesis of lauroyl guanidine (2). Briefly, the work to date indicates that structures such as (2) may now be reduced smoothly to the corresponding alkyl guanidines, structures with no N-H bonds reduce easily to secondary N-methyl amines, and creatinine (7) must be reduced under more vigorous conditions (ether, LAH, and -30° for one hour was tried before the lauroyl guanidine reduction was accomplished).

Stearoyl guanidine was chosen first for preparation and reduction with the hope that the long hydrocarbon chain would produce favorable extraction properties (i.e., solubility in organic solvents). The procedure in the patent[3], essentially the same as that used by Matsumoto and Rapoport[4], consists of condensing ethyl stearate with free guanidine base. The product was contaminated with guanidinium stearate, and attempts to extract stearate into aqueous base or product into aqueous acid produced only stable emulsions. This preparation was abondoned and the shorter chain lauroyl derivative was considered for synthesis, since the shorter chain would, hopefully, be more managable and a literature melting point for lauroyl guanidine was available[5].

Unfortunately, the literature melting point for lauroyl guanidine (82°C) is wrong (should be m.p. 110-111°C, Experiment 1-3) and, thus, methyl laurate was condensed with guanidine by four different methods before it was discovered that the high melting material was indeed the desired product. Integration of the nmr implied less than three N-H protons, and initial melting points (106-107°C) suggested that the product was lauramide (m.p. 110°C)[6]. Suspicion was aroused when a very careful preparation of guanidine free base using Greenhalgh and Bannard's ion exchange technique[7] again gave the high melting material on condensation with methyl laurate. A uv was taken to test for the conjugation in lauroyl guanidine and the absorption found, $\lambda_{max}$ 232 (16,200) compared well with the uv of acetyl guanidine, $\lambda_{max}$ 231 (16,400)[4]. Thus, the melting point and the nmr suggested that the high melting material was lauramide, whereas, the uv implied that it was lauroyl guanidine. The confusion was cleared up when both a mass spectrum and an elemental analysis confirmed that the high melting material was lauroyl guanidine. Up to this point, three different methods of preparing guanidine free base were tried, and, as it turns out, all of them worked. The one attempted preparation of lauroyl guanidine which failed employed commercial guanidine (K & K Laboratories), and this run has been omitted from the Experimental Section. In the second and third preparations of lauroyl guanidine, the guanidine free base was used immediately without weighing (to minimize handling) since the quality of the guanidine was still in question at that time. This practice gave rise to the misleading low yields since the first preparation shows that considerable guanidine is lost on evaporation of the ethanol in which it is prepared.

The crude lauroyl guanidine thus prepared was purified by crystalization. The main impurity, which was probably guanidinium laurate, was

rather insoluble in most solvents and was best removed by hot filtration. Acetone appeared to be the best crystalizing solvent; most of the impurities were removed by hot filtration, and the solubility of the product in hot versus cold acetone was very favorable. The first reduction trial of the purified material with LAH was run at room temperature for one hour in ether. Workup of this reaction and treatment with HCl gave only the salt of starting material. At this point, a glc system was sought which would allow quick evaluation of subsequent reductions. Preliminary work with an Aerograph gc (TC detector) was disappointing, but subsequent studies with a Varian 2100 (FI detector) proved successful. Lauryl amine (Aldrich Chemical Co.), and lauryl guanidine[8] were obtained as standards, and retention times on OV-225 for the amine, the guanidine, and the acyl guanidine were determined. The glc data and yields for three reductions are summarized in Table 1.

Table 1. Reduction of Lauroyl Guanidine in THF with Excess LAH

| Reaction Number (Exp. No.) | Time/Temp | Yield of Lauryl Guanidine | GLC |
|---|---|---|---|
| 1 (1-4) | 21 hr/RT | 45% $CO_3^=$ salt | 1) mainly alk guanidine<br>2) sm amt acyl guanidine<br>3) trace lauryl amine |
| 2 (1-5) | 21 hr/RT<br>4 hr/reflux | 33% $CO_3^=$ salt | 1) mainly alk guanidine<br>2) trace acyl guanidine<br>3) large amt of $R\text{-}NH_2$ |
| 3 (1-6) | 33 hr/RT | 60% isolated as AcO salt | 15% $R\text{-}NH_2 \cdot AcOH$ isolated |

---

The structure of the product from reaction (1) was verified by uv, mass spectrum, and elemental analysis. Furthermore, the ir of the reduction product is identical with the ir of an authentic sample of lauryl guanidine. Of the four salts prepared (carbonate, sulfate, hydrochloride, and acetate), the most convenient one for isolation of product

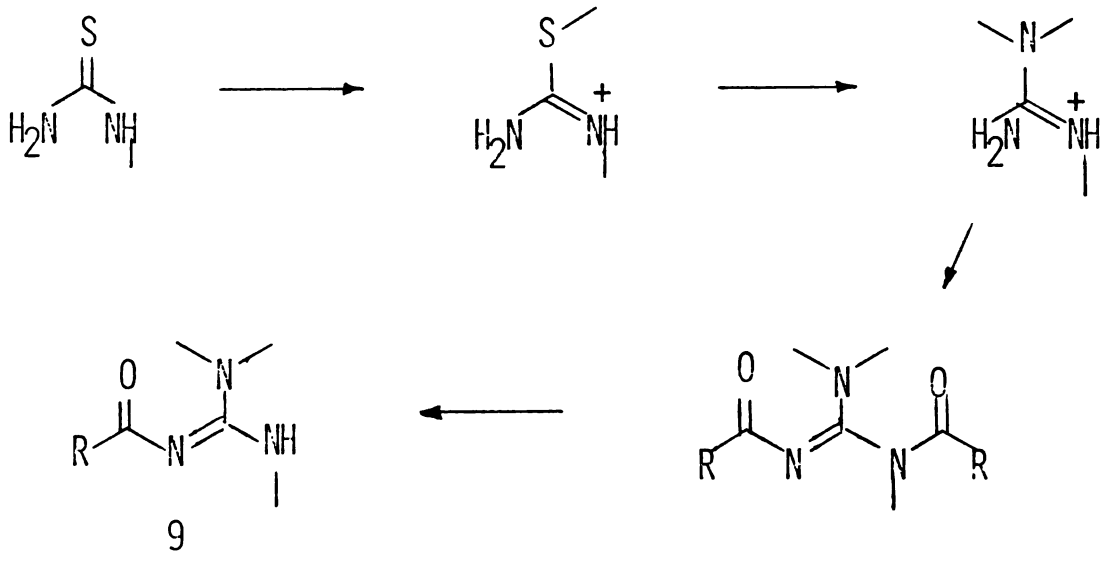
appears to be the acetate. N,N,N',N'-Tetramethyloctanoylguanidine (3) was prepared by condensing octanoyl chloride with commercial tetramethyl guanidine (Cyanamide Corp.). An attempt to prepare the lauroyl derivative from methyl laurate and tetramethyl guanidine failed, which is consistent with similar observations by Matsumoto[9]. The structure of the octanoyl guanidine obtained from the acid chloride was verified by uv and nmr, both of which suggested that the product was reasonably pure, and, consequently, the material was used without purification for two reduction experiments.

In sharp contrast to the results with lauroyl guanidine (2) the reduction of tetramethyl octanoyl guanidine (3) proceeds quickly and in high yield to N-methyl octylamine. The structure of this product was verified by nmr and by conversion of the product to its known picrate salt[10]. Sodium borohydride was then tried on the tetramethylacylguanidine with the hope that it might be mild enough to avoid total reduction. The nmr, however, showed only starting material and N-methyl octylamine. It was concluded from this contrast between the tetramethyl acylguanidine and the tetrahydro acyl guanidine that the presence of acidic N-H protons prevents over-reduction. Presumably, these acidic protons react with LAH to produce an N-Al bond which places high electron density on the nitrogen and thereby protects the guanidine moiety from hydride attack. Reduction of the tetramethyl compound by borohydride is probably facilitated by protonation of the starting material prior to hydride donation.

Section 2

In the previous section, a new reduction was described whereby an acyclic acyl guanidine having four N-H bonds could be converted to the

corresponding alkyl guanidine. Since it was also found that a tetra-alkylacyl guanidine reduces rapidly to an amine, the question arose as to how many N-H bonds are necessary for partial reduction to alkyl guanidines. To answer this question, compound (9) was prepared by the sequence shown;



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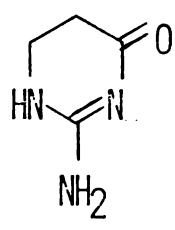
Since even non-alkyl acyl guanidines reduce partially to primary amines (Exp. 1-4, 1-5, and 1-6), the pathway for this over-reduction was examined. To look at the behavior of cyclic acyl guanidines with LAH, compounds (6) and (8) were prepared for reduction.



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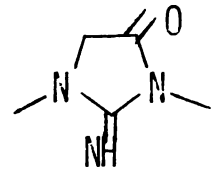

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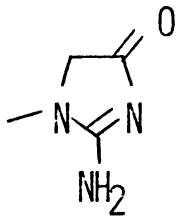
Compound (8) also raises the question of possible reactivity differences between acylamino (8) and acylimino (6) guanidines.

The actual reduction work described in this Section centers on the conversion of creatinine (7) to 1-methyl-2-amino-2-imidazoline (10) and 1-methyl-2-aminoimidazole (11).

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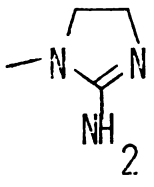


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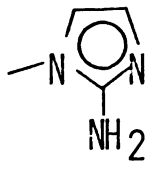


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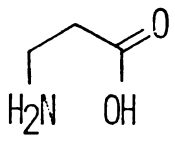
Several problems were encountered (especially in reaction monitoring and reaction work-up) which have yet to be solved.

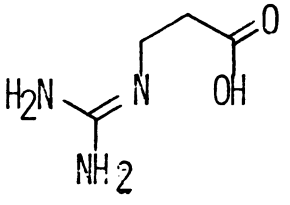
In order to maximize yields of alkyl guanidines, the mechanism by which amines are formed was investigated. If reduction of the guanidine moiety can occur before carbonyl reduction, then lowering the reaction temperature as much as is practical should favor the faster carbonyl reduction. If, however, reduction to an amine occurs by reduction of the newly formed alkyl guanidine, then the reaction time will have to be carefully optimized. To test whether an alkyl guanidine could be reduced to an amine, dodecyl guanidine sulfate was treated with LAH under the same conditions employed for reducing the corresponding acyl guanidine (Expts. 1-7). The production of amine under these conditions confirms the importance of finding an optimum reaction time. To do this efficiently, a method of monitoring reductions is necessary, but as yet no reliable method has been found; glc works only after the reaction is worked-up, and CIMS gives inconsistent results. A further complication is that even with homogeneous LAH solutions, all LAH reductions of

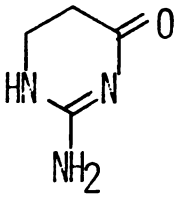
creatinine produced heterogeneous reactions making it difficult to obtain representative samples of the reaction in progress.

The possibility of reduction of the guanidine moiety prior to the carbonyl is much more difficult to establish. The problem is that the intermediate, an amide, is quite labile to LAH reduction and, therefore, difficult to isolate.

β-Alacreatinine (6), which was prepared by the sequence shown, suffered from a poor yield (38%) in the first step but this could probably be improved (the lit. procedure[11] was sketchy and no yield was given).







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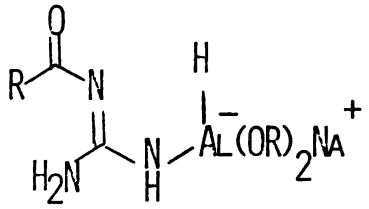
Using pure β-guanidinopropionic acid for this ring closure[12] is very important; an attempted ring closure with crude starting material (first β-alacreatinine prep; omitted from Experimental) afforded a very poor yield of material which was difficult to purify and therefore discarded.

The purification of methylated creatinine was unexpectedly difficult due to the tendency of the product to oil out rather than crystalize. Crystalization finally occurred on long standing, and subsequent crystalizations were initiated by seeding.

N-Methylthiourea was S-methylated three times with methyl iodide in both ethanol and acetone. Using acetone as a solvent gave good yields and relatively clean product, but this system has an irritating tendency to not react until all the methyl iodide has been added, at which point a sudden exothermic reaction occurs. Although the published procedure[13]

uses a stoichiometric amount of methyl iodide, an excess seems to improve the yield.

In the hope of finding a more convenient reducing agent than LAH, Redal, a dialkoxy sodium aluminum hydride, was tried on dodecanoylguanidine (Expts. 1-8). Using benzene as a solvent, a homogeneous reaction resulted which was worked-up after an arbitrary duration. All material was accounted for as the acetate salt of starting material, or the acetate of N-methyldodecylamine (only traces of dodecylguanidine were found in the CIMS and glc work; the mass spectrum implied a trace of primary amine, but the yield of N-methylamine was ∿20 times greater). The cause of this preferential production of the secondary amine with Redal (in contrast with excess LAH, Expts. 1-6) is difficult to explain at this time since the two reactions differred in solvent, reducing agent, and stoichiometry. Only two equivalents of hydride were used, and, since the initial acid-base reaction is very rapid (see Expt. 6-3 for example) a species such as (12) was probably produced quantitatively within a few seconds. This species remains dissolved in benzene and the first



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reduction step is probably intramolecular. With excess LAH in the THF, however, a second acid-base reaction probably occurs before any reduction (creatinine is insoluble in refluxing THF, but when it is treated with LAH at -40°, a clear solution results within 5 min. Upon warming to 0°, more $H_2$ is evolved and a fine white precipitate develops. Actual reduction would probably take several days at 0°). Thus, the guanidine moiety

Table 2. Creatinine Reductions

| Run (Expt. #) | Time (hours) | Yield of TsOH Salt & Characterization | Stoichiometry & Comments |
|---|---|---|---|
| 1 (6-1) | 29 | 36%, mp 170-1° CIMS (ppt $CO_3^=$ salt) 99 amu (10), trace of 97 amu (11), Anal. OK | 6X molar excess LAH (add at -30°) $CO_3$, HCl, $SO_4$ salts are all hygroscopic |
| 2 (6-2) | 30 | NMR of crude salt ppt from ether (29% total) 18% compound (11) CIMS: 97 sl. >99 amu | 1:1 mole ratio creat. to LAH (add at 0°) Rxn. sl. yl. (all other rxns. white) |
| 3 (6-3) | 28 | Reaction Discarded | 7X molar excess LAH CIMS: lg. incr. in amine from 10 to 23 hr. No (7). |
| 4 (6-4) | 2 | 21% mp 168-70° | 7X molar excess LAH CIMS: 1 hr aliquot shows no S.M. (7) (113 amu) CIMS: of Al salts; (7) present |
| 5 | 12 | 28% mp 168-70° | 7X molar excess LAH Rxn. not monitored. Time chosen from CIMS of run 3. |

Table 2 continued next page

Table 2. continued

| Run (Expt. #) | Time (hours) | Yield of TsOH Salt & Characterization | Stoichiometry & Comments |
|---|---|---|---|
| 6a | 22 | 37% mp 169-71° | 7X molar excess LAH |
| 6b | 26 | 39% mp 168-70° | This run was all one rxn. which was worked-up in 3 aliquots |
| 6c | 32 | 38% mp 168-70° | TsOH slat from i-PrOH; CIMS of filtrates shows 97 amu (11). |
| 7 | 52 | 23% mp 168-70° | 7X molar excess LAH |

Notes: Runs 5-7 were omitted from Experimental Section. In runs 3-7, powdered undesiccated creatinine was used. Homogeneous LAH solutions were used in all runs except run 1. LAH sol. was added to a -40° suspension of (7) except in runs 1 and 2.

in the LAH reduction probably has a formal double negative charge which may help explain the different product distribution from that obtained with Redal.

Despite the extensive reduction work with creatinine (7), no set of conditions have been found which are synthetically satisfactory. The maximum yield of guanidine (10) so far is 39% (see Table 2). The poor yields are partly due to partial reduction of the carbonyl of (7) followed by elimination to the imidazole (11). The only case where the extent of this side reaction is known, is for run 2 (see Table 2). In this experiment, p-toluenesulfonic acid (TsOH) was added immediately after filtration of the Al salts, so that no basic materials could have been lost during solvent evaporation. Although the nmr of the crude salts precipitated from ether integrated for 63% imidazole (see Expt. 6-2) versus 37% guanidine, two factors could be causing misleading results; 1) the two compounds may be trapped to different degrees by the Al salts, and, 2) this is the only run where a stoichiometric amount of LAH was employed which may favor a different reaction pathway (this may also be the case with Redal). In any case, the nmr and the CIMS (all runs except 1) verify the presence of large amounts of the imidazole (11). In run 1, $CO_2$ was added to form $CO_3^=$ salts some of which formed a sticky precipitate which was used for CIMS. If the guanidine $CO_3^=$ were preferencially precipitated, the lack of imidazole (11) in this spectrum would be explained.

Yields of guanidine are also diminished by entrapment of organic materials in the Al salts. This phenomenon conveniently removes left over starting material (7), but probably also removes substantial amounts of guanidine product (10) due to its high polarity and affinity for a protic phase, i.e., the hydrated Al salts. In run 1 where one of the

best yields was obtained (36%), most of the starting material had been consumed, yet the total yield of HCl salts (formed before evaporation) was only 50%. Thus, entrapment of material in the Al salts appears to be substantial.

The selection of an optimum reaction time has been very tedious since no effective method of monitoring the reaction has been found. At present, the optimum time for creatinine reduction appears to be ∿25-30 hr.

Since creatinine (7) never dissolved in THF, the solubility of (7) in various solvents was measured. No more than 1 mg of (7) could be dissolved in 100 ml each of refluxing THF, DME, triglyme, and toluene. Thus, creatinine (7) is essentially insoluble in any solvent suitable for LAH reduction. Once this was discovered, the starting material was powdered before use in subsequent reductions. Using powdered starting material and a homogeneous LAH solution, the first homogeneous reaction was observed after ∿5 min (-40°). Since (7) is insoluble, presumably this is an indication of the rate of the first acid base reaction at -40°. Within minutes, however, precipitation occurs which is probably due to a second acid base reaction (usually complete in ∿30 min at 0°). The continued use of homogeneous LAH solutions, however, will allow the use of trialkoxy derivatives, and may eventually provide a homogeneous reaction system.

Preparing a truly homogeneous LAH solution requires some special apparatus and careful technique, much of which is described by H.C. Brown[14]. Although partially hydrolysed LAH produces hydroxides which are insoluble in THF, completely clear solutions are obtained only if fresh LAH and carefully dried THF are used. Thus the apparatus is assembled hot

(dry 12 hr at 125°), and purged until cool (∿10 min) with dry $N_2$. The $N_2$ is introduced through a septum on the side arm of the receiving flask and vented through a #20 needle in the septum on the distilling flask. After purging, the vent needle is removed, and THF (∿400 ml reag.) and LAH (∿3-4 g) are added (THF first) through the top of the still (maintaining $N_2$ flow). After distilling most of the THF (∿300 ml), the condenser is removed from the receiving flask, fresh LAH (25 g, 95%+) is added to the receiving flask, and a rubber septum is wired snugly onto the neck ($N_2$ purge is maintained during these operations; lower the flow temporarily while adding the LAH to prevent blow-back). After magnetically stirring the suspension for 3 hr (∿23°), the $N_2$ inlet with a bubbler is replaced by a direct line to an $N_2$ tank regulator. Purge this line with dry $N_2$, insert the needle in the septum, and then close the needle valve on the tank regulator. The suspension is then transferred to the pre-dried Celite filled filter funnel via a double ended needle and moderate $N_2$ pressure. Constant monitoring of the needle valve on the $N_2$ regulator is necesaary to maintain a moderate flow (the only danger here is over-filling the filter funnel).

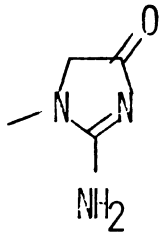
To achieve a clear solution, the filter cake must also be prepared carefully. A thick slurry is made from Celite (enough to fill about 2/3 of the funnel) and methanol. Enough of the slurry is then poured quickly into the filter funnel to give a 5-10 mm layer after the excess methanol is removed (moderate vacuum). The slurry is remixed and a second layer is similarly poured and sucked dry. Several flat layers are thus built up until the 100 ml filter funnel is about 2/3 full. After suction drying the filter cake for a few minutes, the funnel is gently laid down in a drying oven (filter cake will stay intact) and

dried along with a storage flask for 12 hr at 125°. The flask and funnel are assembled hot, and rubber septa are connected. Immediately, a dry $N_2$ purge is injected through the septum on the funnel and vented through the flask to an Hg bubbler. Purging the opposite direction will create channels in the filter cake. During the $N_2$ purge (∿10 min) the septum on the filter funnel is secured with wire (#16 copper was used) and plyers. The three septa must be wired securely to prevent them from popping off when under pressure. The filtrate thus obtained is a crystal clear solution of ∿1.5 M. The $N_2$ inlet needle is removed and inserted into the T-tube septum. The filter funnel may now be replaced with a greased glass stopper to seal the storage flask ($N_2$ flow in opposite direction from filtration). A spring clamp secures the glass stopper, and an unpunctured inverted septum of the proper size serves as a cap for the smaller punctured septum. Solutions may be stored in this manner at room temperature for several years.

The solution may be standardized by removing an aliquot (oven dry syringe and needle; flush and fill with dry $N_2$) and measuring $H_2$ evolution on hydrolysis[15]. A freshly dried syringe and needle should be used for every aliquot. Hand moisture is a nuisance when syringing LAH, and, therefore, disposable gloves are recommended. In an alternate standardization method, 2.0 ml LAH solution is added to ice water (10 ml). HCl (15 ml, 1N) is then added and the mixture is heated until homogeneous. Back titration is accomplished with NaOH (1N) in a 10 ml buret to a thymol blue end point (∿pH 3). If the LAH solution is clear, either standardization method gives good results (∿2% error).

Section 3

In the previous Section, two major problems were encountered in the development of the reduction of acyl guanidines. First, the yield of reduction product from creatinine (7) was greatly reduced by entrapment of the product in the aluminum salts. Second, attempts to monitor the reduction by CIMS proved futile.



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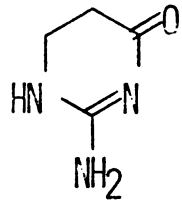

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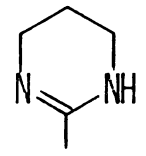
As suggested in the previous Section, attention was temporarily shifted from creatinine (7) to β-alacreatinine (6) since the former compound tends to aromatize in the presence of LAH (Expt. 6-2). β-Alacreatinine (6) was used both for a uv monitoring trial and for an attempted separation of the reduction product from the aluminum salts by physical methods. The general problem of separating guanidines from metal salts by chemical derivatization was also studied, and these studies consitute a major portion of the work in this section.

Since heterogeneous reactions are difficult to monitor, and since sodium bis(2-methoxyethoxy)-aluminum hydride (Redal) produces a homogeneous system with dodecanoyl guanidine (Expt. 1-8), an attempt was made to prevent over-reduction by use of this reagent. On the hypothesis that over-reduction occurred via intramolecular hydride donation, the dialkoxydihydrido aluminate species was converted to a trialkoxymonohydrido aluminate by addition of one equivalent of methanol to the commercial reagent, Redal (see Expt. 1-9). It was hoped that this trialkoxymonohydrido species could either give an acid-base reaction or hydride

donation but that any one given anion could not do both, thereby preventing intramolecular reduction. To further facilitate a bimolecular reduction mechanism, a large excess of reducing agent (∿12:1 mole ratio of hydride to substrate) was used. This reaction system did, indeed, take a different course from the first Redal reduction (Expt. 1-8); this time the predominate product was dodecylamine, whereas, in the first Redal reduction, the product was primarily N-methyldodecylamine. This difference is difficult to explain, but in any case, the over-reduction problem is still evident. Since substantial unreacted starting material was observed by glc, the over-reduction problem would not be solved by a shorter reaction time. These data suggest that further work with Redal is unwarranted.

Due to the low carbon content and highly polar nature of β-alacreatinine (6) and the reduction product, 2-amino-3,4,5,6-tetrahydropyrimidine (13), a high degree of trapping in the aluminum salts was both expected and observed (see Table 3 for yields). An attempt was made to




13

diminish this problem by using only a stoichiometric amount of $H_2O$ in the work-up (see Expt. 5-2), but, instead of improving the situation, this modification led to almost quantitative entrapment of all organic material in the aluminum salts.

Experiment 5-2 was monitored by uv (see Fig. 3). the absorbances were converted to percent starting material by assuming that the initial absorbance (t = 0 hr) reflected 100% starting material. The first two

points were verified by duplicate determinations. Values for t = 0 hr were within 3% of their average; values for t = 14 hr were within 1.4% of their average. Thus, determinations at a given time were quite

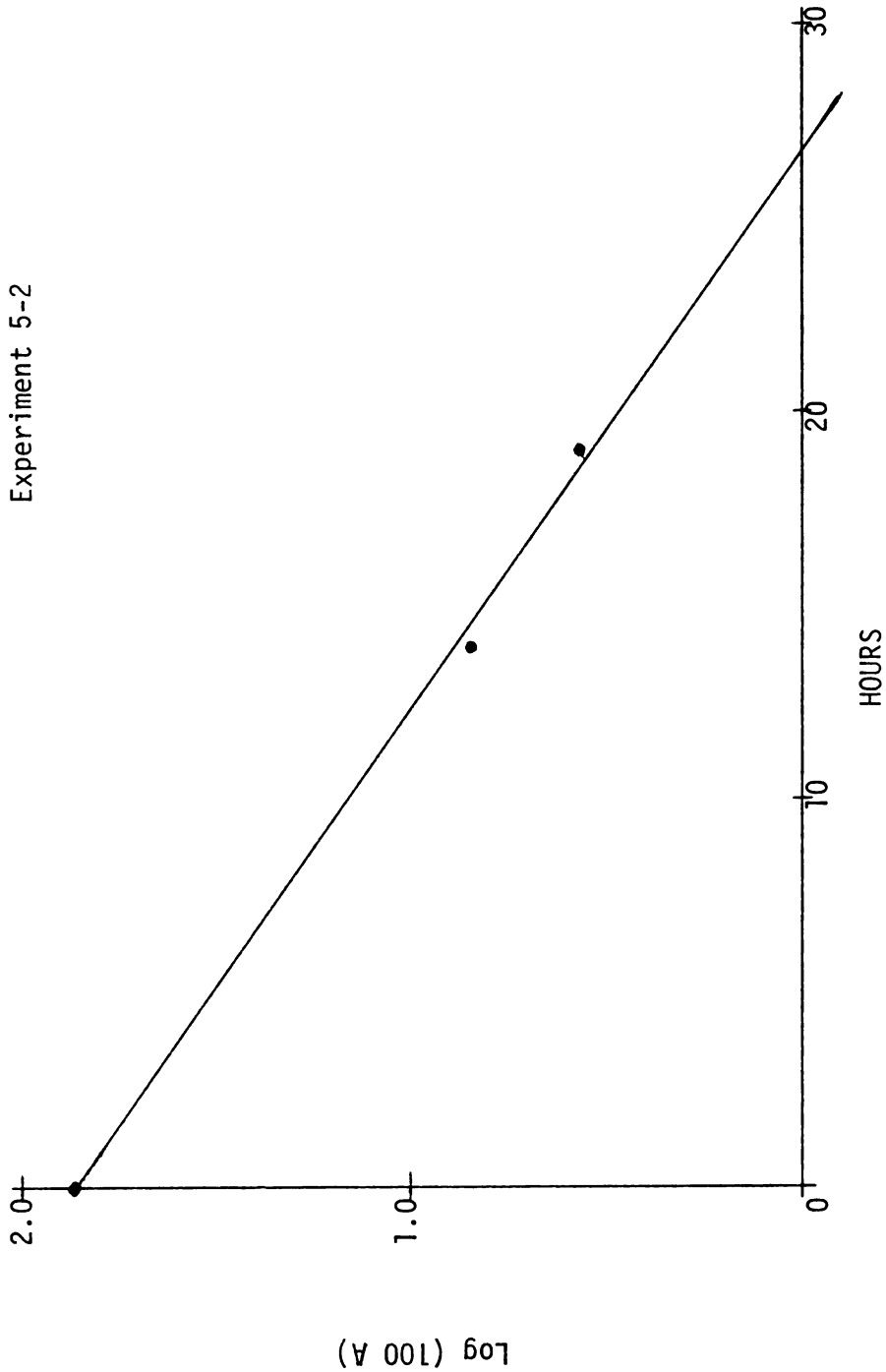
Table 3. β-Alacreatinine Reductions

| Run (Expt.#) | Time (hours) | Yield and Characterization | Stoichiometry and Comments |
|---|---|---|---|
| 1 Expt. 5-1 | 36.5 | 28% picrate; mp, NMR, CIMS | 8.5 molar excess LAH; Std. work-up |
| 2 Expt. 5-2 | 24 | ∿2% carbonate; discarded | 8.5 molar excess LAH; uv monitor; Stoic. $H_2O$ work-up |
| 3 Expt. 5-3 | 24 | 18.5% HCl; mp, NMR | 8.5 molar excess LAH; Std. work-up |

reproducible in spite of the heterogeneous nature of the reaction. Since a large excess of LAH (8.5 x molar excess) was used, one might expect pseudo first order kinetics. The semi-log plot shown in Figure 3 was generated by multiplying the absorbance by 100, taking the log, and plotting this log against time. This plot is indeed reasonably linear implying a first order reaction. By arbitrarily defining 98% loss of starting material as a complete reaction, Figure 3 predicts a reaction time of 24 hr. Therefore, the reaction was run a third time (Expt. 5-3) using a standard work-up after 24 hr. The resulting drop in yield compared to Experiment 5-1 (see Table 3) was both disappointing and unexpected. However, since changes in the work-up can have a profound effect on the amount of material trapped in the aluminum salts (compare runs 2 and 3 in Table 3), the isolated yield is not a reliable index of the completeness of a reaction. Thus, in order to properly evaluate any monitoring method, a procedure must first be developed for separating

Figure 3

Dissappearance of β-Alacreatinine (6)
UV Monitoring of a Reduction
Experiment 5-2



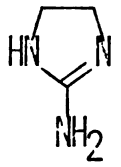
reduction products from the aluminum salts. Attention was, therefore, shifted to this purification problem.

Several preliminary experiments (Expt. 8-1) were tried on aluminum salts (which had been saved before a purification trial was attempted. Initially it was hoped that 8-hydroxyquinoline might form a complex with $Al^{+3}$ which could be extracted into an organic solvent, but, instead, the complex seemed to be insoluble in both $H_2O$ and toluene. This complex was easy to filter, however, unlike $Al(OH)_3$. An even better method of separating guanidine products from $Al^{+3}$ was found when it was discovered that $Al(OH)_3$ suspended in $H_2O$ forms a firm pellet on centrifugation. This information later was used for purification of (13) (Expt. 5-4). In the last part of Experiment 8-1, it was found that air drying of aluminum salts after an LAH reduction leads to the formation of base insoluble carbonates. This is no problem if the "trapped" products are simple guanidines since the carbonates dissolve in acid, and guanidines are quite stable to acid (see Expt. 8-16).

To test the viability of separating $Al^{+3}$ from a reduction product by centrifugation, the salts from the first β-alacreatinine reduction (Expt. 5-1) were digested in HCl (aq) and neutralized (with NaOH) to precipitate $Al(OH)_3$ (see Expt. 8-1). As anticipated from the previous experiment, this operation worked quite well. Lyophilization of the supernatant to near dryness followed by precipitation of metal salts with isopropanol left mainly a mixture of cyclic guanidine (as the HCl salt) and lithium chloride. These components could not be separated by a second isopropanol precipitation and the material was reserved for an ion exchange experiment.

An alternate approach to the purification problem consists of derivatizing a guanidine to make it soluble in an organic solvent. The

derivative is then extracted away from an aqueous phase containing the salts and then converted back to the free guanidine. To provide material to study this procedure, 2-aminoimidazoline (14) was prepared as the



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hydrobromide salt from ethylene diamine and cyanogen bromide essentially as described in the literature[16].

Since the carbobenzoxy protecting group is easily removed, carbobenzoxy chloride was used for all five derivatization trials (see Table 4, Expts. 8-3 through 8-7). The first acylation was run on 2-amino-3,4,5,6-tetrahydropyrimidine (13). For several practical reasons, subsequent acylations were performed on the five membered ring guanidine (14). Since this guanidine is cyclic and contains only three carbon atoms, it was felt that compound (14) would probably be the most difficult to desalt, and, therefore, an ideal compound for developing a general desalting method.

Conditions for the first Schotten-Baumann acylation (Expt. 8-3) were developed intuitively, and they seemed to work moderately well in terms of quantity of organic material extracted from the aqueous phase. The poor quality of the product was due to the use of HCl for benzyl cleavage; it was not yet known that HCl and HBr cannot be used for benzyl cleavage in this system. In subsequent acylations, a literature procedure for carbobenzoxylation of arginine[17] was used. This procedure appears to work quite well to produce an oily solid white derivative which precipitates from water and which may be conveniently extracted with methylene chloride. In runs 3 and 4 (Expts. 8-5 and 8-6; Table 4), glycine was

Table 4. Model Extractions

| Derivative Runs | Comments | Cleavage Method | Product |
|---|---|---|---|
| 1 (Expt. 8-3) | Acylation and extraction done with $Et_2O$. Substrate = (13) | Expt. 8-3 HCl (conc)/ AcOH, 23°, overnight, 95°, 30 min | <62%; white sticky material, discarded, |
| 2 (Expt. 8-4) | Acylation: used literature model. Extraction: 1 portion of $CH_2Cl_2$. Substrate = (14) | Expt. 8-4 HCl (conc)/AcOH, saturate with HCl (g). 95°, 3 hr (literature model used). | >100%; white sticky material. Several products (by NMR, CIMS). |
| 3 (Expt 8-5) | Acylation: as in run 2; added glycine treatment. Extraction: 1 x $CH_2Cl_2$. Substrate = (14). | Expt. 8-8 $H_2$ at 40 psig, 10% Pd/C, MeOH/EtOAc, shake 12 hr, ~20° | 73%; pure picrate (mp, Analysis, NMR and CIMS) |
| 4 (Expt. 8-6) | Acylation: as in runs 2 and 3. Glycine treated. Extraction: 4 x $CH_2Cl_2$, all material in first 2 portions. Substrate = (14) | Expt. 8-9 $H_2$ at 40 psig, 10% Pd/C, MeOH, shake 22 hr, ~20°. | ~40%; salmon colored HBr salt. NMR shows phenyl groups. |

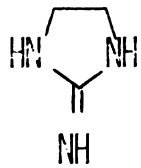
Table 4 continued next page

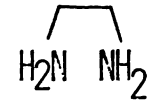
Table 4, continued

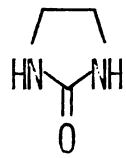
| Derivative Runs | Comments | Cleavage Methods | Product |
|---|---|---|---|
| 4 (8-6) cont'd | | Expt. 8-12 HBr/AcOH (anh, HBr sat'd). Stir 24 hr, 23° (literature model used). | 54%; white salt, mp >240°. NMR, CIMS imply diamine. |
| 5 (Expt. 8-7) | Acylation: as in runs 2, 3, and 4. Glycine omitted. Extraction: 3 x $CH_2Cl_2$. All work done in cold room. Substrate = (14) | Expt. 8-10 $H_2$ as in Expt. 8-9 for 14 hr. | 69%; purple HBr salt. NMR implies clean guanidine. |
| | | Expt. 8-11 $H_2$ as in Expts. 8-9 and 8-10 for 48 hr. | No yield; $H_2$ gives yellow solid; insol. in $H_2O$. NMR shows ∿1 phenyl per guanidine. |
| | | Expt. 8-14 HBr/AcOH as in Expt. 8-12 for 12 hr (done more carefully to verify results of Expt. 8-12. | 63%; white HBr salt mp > 300°/ Analysis, NMR, and CIMS all imply pure ethylene diamine dihydrobromide. |

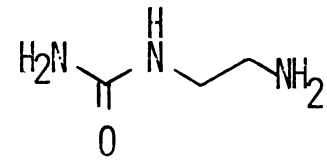
added to the basic acylation mixture to remove excess acid chloride by converting it to a water soluble anion. This glycine addition succeeded in removing most of the excess acid chloride, but the procedure was omitted from the fifth run because of mounting evidence that the carbobenzyoxyguanidine derivative is nucleophile sensitive.

Suspicion developed that the acyl derivatives of guanidines (13) and (14) were nucleophile sensitive when problems were encountered with acid cleavage of the carbobenzoxy groups. Cleavage with both HCl and HBr (Expts. 8-4, 8-12, 8-14) using a literature procedure for unblocking amino acids[18], led to anomalous results. In the case of HCl cleavage (Expt. 8-4), NMR and CIMS suggest that the isolated material contained hydrochloride salts of the compounds shown. When the cleavage




is done with HBr instead of HCl, the results are even more dramatic. Here, very good yields of ethylene diamine dihydrobromide may be isolated (Table 4, Expts. 8-12 and 8-14) with almost no evidence of other products (only a trace of guanidine (14) in the CIMS; NMR implied clean diamine).

To check whether the destruction of the guanidine moiety was occurring before or after acyl cleavage, three separate control experiments

were performed. First, the stability of 2-iminoimidazolidine (14) to acid was verified by refluxing some of this material (126°) in aqueous HBr (48% distilled) for 3 hr followed by recovery of the unchanged guanidine (Expt. 8-16). In addition, this guanidine (14) was treated twice with anhydrous HBr/AcOH in exactly the same manner as the acyl derivative (Expts. 8-13 and 8-15). The results of these control experiments were the same as with aqueous acid; no destruction of the guanidine was observed. These data confirm that acidic destruction of the guanidine moiety in the acyl derivative must occur before acyl cleavage. Since a conjugated monoacylguanidine is relatively stable to HCl (see, for example, the preparation of β-alacreatinine (6), ref. 12), it is probably the di and triacyl derivatives which are susceptible to nucleophilic attack. As the nucleophile gets weaker ($Cl^-$ vs. $Br^-$) one would expect more competition from the solvent (i.e., $H_2O$ or AcOH) and this explains the difference in products form HCl cleavage (Expt. 8-4) and HBr cleavage (Expts. 8-12 and 8-14).

This nucleophile sensitivity probably applies in basic media as well as in acid, because a diacylguanidine must have an acylamino moiety (carbonyl not conjugated with the guanidine) and non-conjugated acylguanidines typically have half times of minutes in base[4]. The diacyl compounds should, in fact, be more susceptible to nucleophiles than monoacylguanidines, since the diacyl derivatives are more electron deficient.

In general, then, it seems probable that any di or triacylguanidine is sensitive to nucleophilic attack in either acid or base. Furthermore, even a non-nucleophilic acid (e.g., $H_2SO_4$) would probably allow substantial solvent attack on the guanidine moiety, and, therefore, $H_2$ appears

to be the method of choice for cleaving carbobenzoxy groups from guanidines, especially if more than one acyl group is present.

Although $H_2$ cleavage seemed to work rather well the first time (Table 4, Expt. 8-8), three subsequent runs (Expts. 8-9, 8-10 and 8-11) gave poor results. The yields were erratic, the products were colored, and phenyl groups were clearly present in the products of Experiments 8-10 and 8-11. The simplest explanation for these results is catalyst poisoning. Greenstein and Winitz[19] claim that palladium on charcoal (used in these experiments) is less reliable than palladium black or palladium oxide for this debenzylation reaction, and, therefore, palladium oxide was used in later experiments.

Section 4

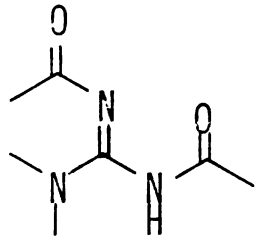
Work in this section centers mostly on new substrate preparation. Some LAH work was done, but due to difficulties encountered in substrate preparation, no new work was done on the carbobenzoxylation/extraction procedure.

The first experiments during this period, alkyl guanidine preparations, were merely repetitions of literature preparations, and the results were comparable to those in the publications. It should be noted that, while guanidine salts are generally very stable, N,N,N'-trimethylguanidine hydroiodide yellowed quickly if not stored in the refrigerator.

Since the Schotten-Baumann type acylation of a guanidine with carbobenzoxychloride worked well in the derivatization experiments (Expts. 8-4, 8-5, 8-6, and 8-7), two attempts were made to use this reaction to prepare substituted diacyl guanidines. Specifically, N,N'dimethylguanidine was acylated with propionyl chloride. Although the reactions appeared to "work", the yields were so low (1-2%) that this approach was

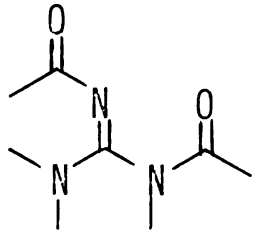
abandoned. Strongly basic conditions (pH 14) are required to deprotonate the guanidines for acylation, and it may be that the anhydride and the acid chloride are more sensitive to $OH^-$ attack than the alkyl chloroformate moiety.

Switching to a literature preparation[7] for compound (15), it was found that the preparation worked, but the yields ($\leq$ 14%) were far below those reported (31% and 46%) especially when a large scale preparation was attempted. Since isolation of pure product is both difficult and tedious, and, since the yields were so poor, work with this compound was abandoned.



15

In contrast to the acylation of the dimethyl guanidine, acylation of N,N,N'-trimethylguanidine by the same method to give compound (16) worked quite well (92% and 94%; this reaction has not been published). Although structure (17) is at least possible in theory, rotation about



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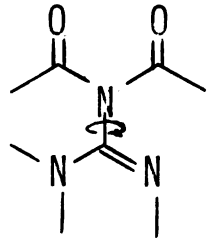
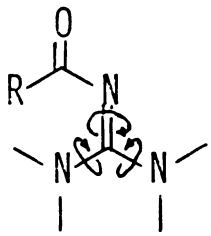


17

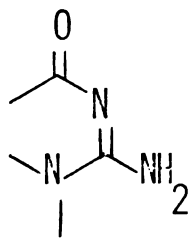
the bond shown would cause the two acetyl methyl groups to have the same nmr shift which they do not (see Expt. 4-2). Such a rotation should be possible since the nmr of octanoyl tetramethylguanidine (3) shows four equivalent methyl groups (Expt. 2-1), a situation which requires



3

free rotation about all of the bonds shown. Furthermore, the uv of the product obtained from diacetylation is characteristic of structures like (16)[7], and no structures such as (17) have yet been documented.

The objective in preparing the diacylguanidines (15) and (16) was to use these compounds for monodeacylation to structures (18) and (19), respectively, by selective alcoholysis. There is some precedent for this reaction[4,20], and, indeed, monodeacylation of the dimethyl compound (15)



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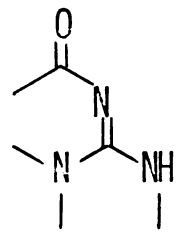
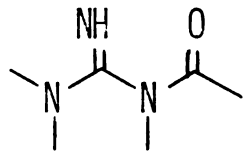


19

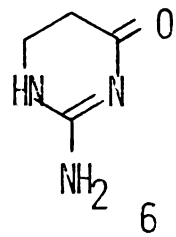
to the conjugated structure (18) seems to occur (omitted from Experimental Section). In the case of the trimethyldiacyl compound (16), monodeacylation does occur, but the uv spectra for products from both refluxing ethanol (Expt. 4-3) and base catalysis at 23° (Expt. 4-4) suggest that in each case, the product is the nonconjugated compound (20), a



20

result which is very difficult to explain mechanistically. Although this compound has yet to be fully characterized (it is a hygroscopic solid, and all attempts at salt preparation have failed), the mass spectrum clearly indicates the monoacyl compound, and the nmr, showing only three singlets (6H, 3H, and 3H), argues strongly against a mixture of conjugated (19) and non-conjugated (20) species. It is possible, however, that the product is indeed the conjugated compound, but that the expected uv absorbance is being masked by a trace side product which is a powerful chromophore. Indeed, a conclusive structure assignment may require reduction with LAH, and characterization of the resulting product.

Since material for reduction experiments on β-Alacreatinine (6) was running low, and, since material was also needed for the attempted preparation of N-methyl-β-alacreatinine (21), a large batch of β-alacreatinine (6) free base was prepared by treating the hydrochloride salt

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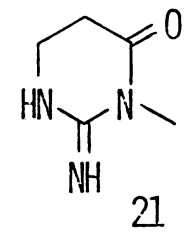
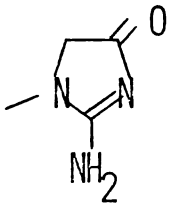



21

with an anion exchange resin in the hydroxide form (omitted from Experimental Section).

The conditions chosen for methylation of β-alacreatinine (6) were exactly the same as those used for methylation of creatinine (7)[21]. Monomethylation of glycocyamidine (22) on the acyl nitrogen is also known[22] and it was hoped that this reaction could be generalized to the 6-membered ring (6). Although the one experiment run on the 6-membered



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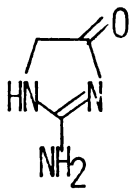
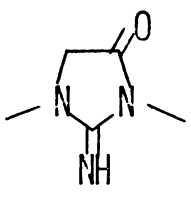


22

ring suggests that the reaction could be worked out, the total yield of monomethyl compound after one series of fractional cyrstalizations was only ∿15%, and this material contained over 40% starting material (by nmr and CIMS). In view of the poor yield, and the purification problems (dimethylation and hydrolisys also occurred, and the whole crude reaction mixture had to be converted from sulfate salts to hydrochlorides by ion exchange before any crystals could be obtained), further work on this reaction was set aside in favor of more pressing problems.

Only three reductions are included in this section, one of methylcreatinine free base (preparation and reduction, Expt. 7-1), and the other two relating to octanoyl-N,N,N',N'-tetramethylguanidine (3). the work with methylcreatinine indicates that an acylamino guanidine



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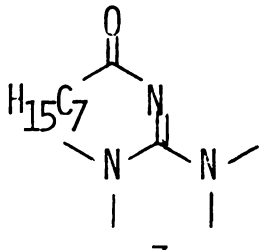
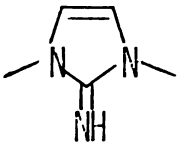


3

with only one N-H proton can, indeed, be reduced to a guanidine. The same two problems encountered with creatinine (7) reduction are also found with methylcreatinine (8), namely, aromatization to (23), and isolation difficulties. Aromatization was unexpected with methylcreatinine



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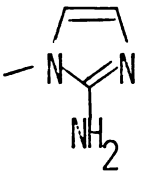
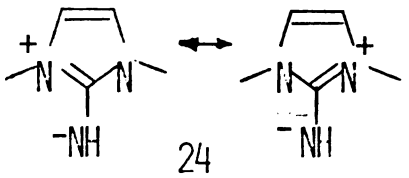


11

(8) since one does not normally think of a structure such as (23) as being aromatic. Although the aromaticity is probably lower than that of the creatinine reduction product (11), it can be argued that (23) is indeed aromatic (see resonance structures (24)) just as 2-pyridone (25) is considered to be aromatic.



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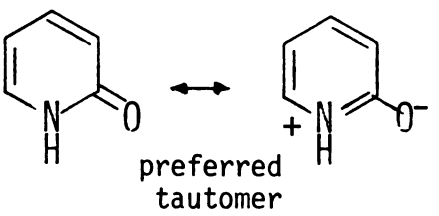
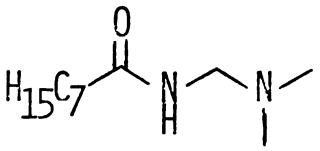


preferred tautomer

25

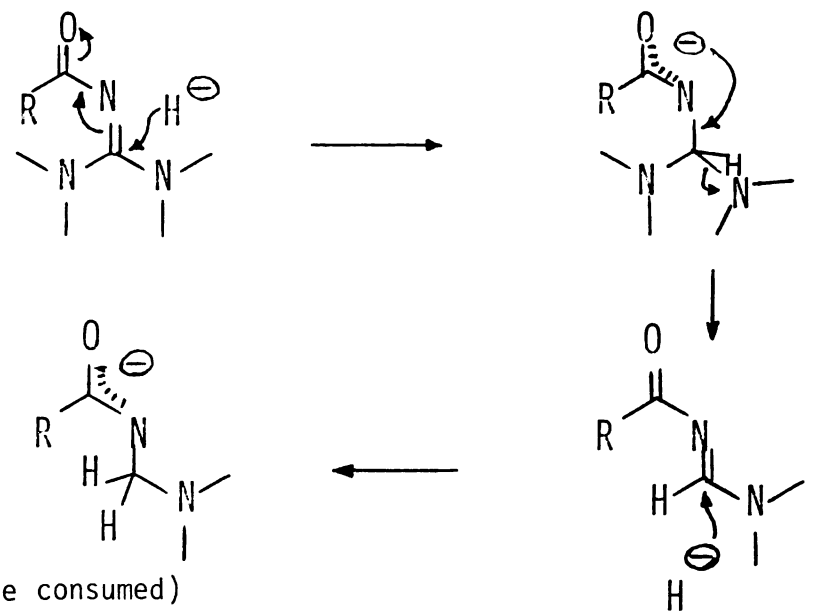
The only other acylguanidine reduction discussed in this section is that of octanoyl tetramethylguanidine (3) (Expt. 2-4). Although this compound was reduced once before with LAH (Expt. 2-2), an excess of solid hydride was used, with the result that a good yield (59%) of the picrate salt of N-methyloctylamine was obtained. Subsequently, the use of LAH powder was replaced by the use of homogenous solutions of LAH (in THF) of known concentration. Using such a solution, the reduction of octanoyltetramethylguanidine was repeated with precisely two hydride equivalents in the hope of stopping the reduction at the guanidine stage. The result appeared to be a 71% distilled yield of N-(dimethylaminomethyl)octanamide (26) (the product was later found to be a mixture

of (26) and two other components). A literature search revealed that



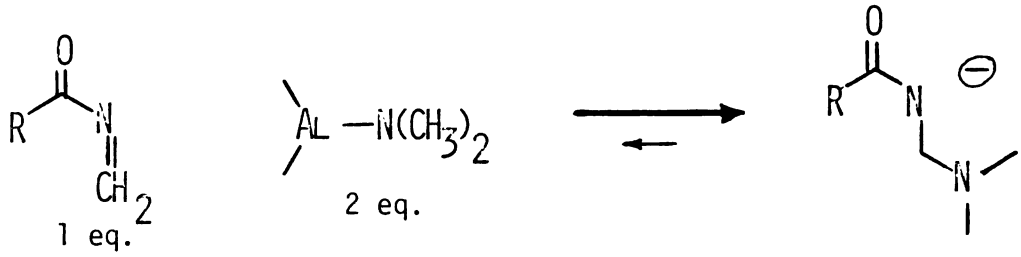
26

precedent for such structures does exist[23,24], and one can imagine a reasonable mechanism for the occurance of such a reduction:



(hydride consumed)

The second anion then gives the product (26) on work-up. The only discrepancy here is that the first anion generated eliminated dimethylamine whereas the second anion does not. One explanation is that the first anion can relieve more steric crowding by elimination than the second. The second anion must also be able to eliminate, however, if excess hydride is to be able to produce a high yield of N-methyloctylamine. This elimination probably does occur, but, since one equivalent of $N(CH_3)_2$ anion has already been elinimated, the equilibrium apparently favors the the condensed product as shown:

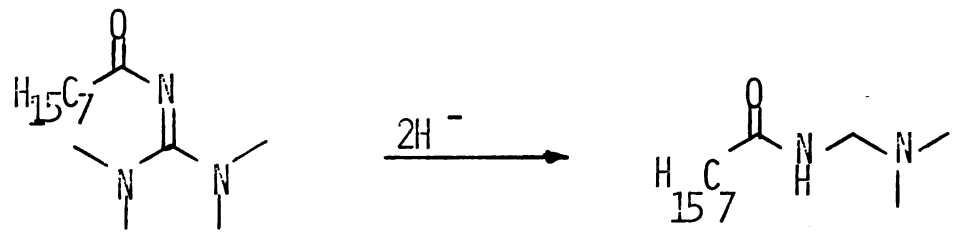



When excess hydride is present, the elimination product (an acylimine) could be trapped by reduction to the N-methylamide which in turn would reduce easily to the N-methylamine. To test whether structure (26) could be an intermediate in the reduction of the acylguanidine to the secondary amine, some of the N-(dimethylaminomethyl)-amide (26) was treated very cautiously with excess hydride (Expt. 2-6). The result, ultimately, was the isolation of a good yield (64%) of the expected product, N-methyloctylamine picrate.

Section 5

To complete the reduction study, further work was necessary on each of the seven substrates, (2) through (8), mentioned in the Introduction. The two most elusive problems encountered were identification of the reduction products from compound (3), and the unequivocal elucidation of the structure of compound (5).

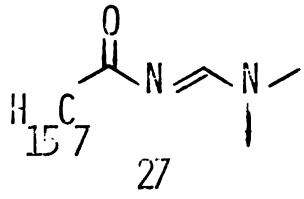
In order to verify the structure assigned to the major product (26) (Expt. 2-4) in the reduction of the tetramethylacyl compound (3), the




3 26

acylaminal compound (26) was prepared by an alternate method. This method employs a Mannich type condensation of a primary amide, formaldehyde, and a secondary amine. The only well described example of this

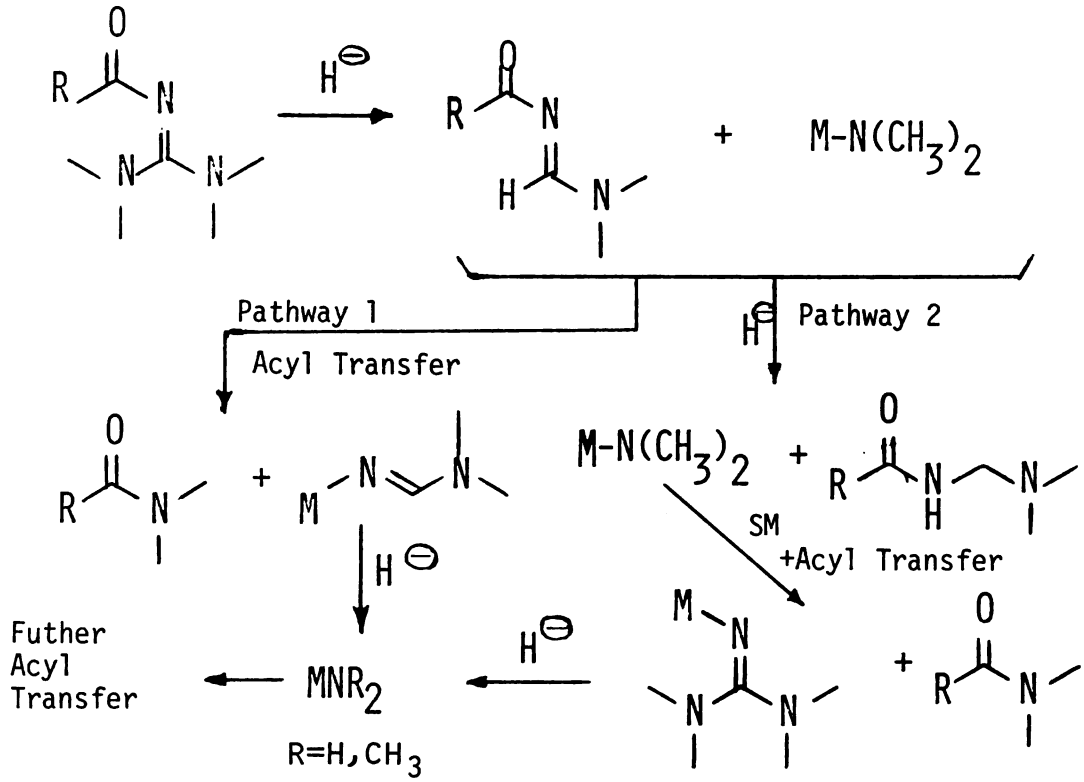
reaction in the literature[24], uses morpholine as the secondary amine, and their conditions proved to be too mild for preparation of compound (26). By employing excess dimethylamine, excess formaldehyde, and a longer heating period (Expt. 2-7), however, a substantial conversion (53%) of octanamide to the acylaminal (26) was obtained. The nmr of this material was not completely consistent with the nmr of (26) obtained by reduction (Expt. 2-4), the major difference being the absence of any absorbance around 2.75 to 3.07 $\delta$ with the Mannich product. The previous assignment of this absorbance to the $-COCH_2-$ methylene was obviously incorrect, and, therefore, an alternate explanation was sought. On the hypothesis that the product from the reduction may be a mixture of acylaminal (26) and acylamidine (27), the reduction of the tetramethylacyl compound (3) was run again (Expt. 2-5), this time with only one



27

equivalent of hydride in an attempt to increase the proportion of (27). The nmr of the material from this reduction indeed showed a much stronger absorption in the disputed region, specifically, two strong equivalent singlets at 2.88 and 3.00 $\delta$. These singlets could be explained by an energy barrier to rotation of the $-N(CH_3)_2$ group in (27) much the same as that seen in N,N-dimethylamides. The formamidine proton, however, could not be located in the nmr. A mass spectrum at this point (CIMS) revealed that the major component of the mixture of products had the mass of N,N-dimethyloctanamide (171 amu). This data was consistent with the nmr, but the dimethylamide is a more difficult product to rationalize than the acylamidine. The only reasonable explanation

requires some kind of acyl transfer to dimethylamide anion which has been generated from a reduction and an elimination. Either of the following two Pathways is plausible (M = Li or Al ion):




In addition to a mass of 171 amu, the CIMS also showed a mass of 143 amu which corresponds to octanamide. This mass could not be explained as a fragment of the acylaminal since the relative abundance varied drastically with time and temperature. Octanamide could arise, however, from acyl transfer during the reduction (amide ion is postulated by both Pathways) or a reverse Mannich during the work-up.

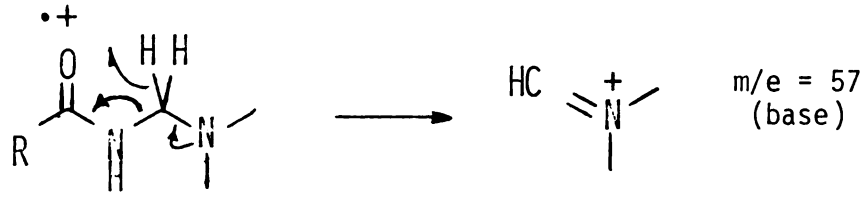
With a mechanistic rational in hand, further evidence for the presence of octanamide and the dimethylamide was sought. GC on OV-225 and OV-25 showed three major products for the stoichiometric reductions (Expts. 2-4 and 2-5), which corresponded in retention time to octanamide, N,N dimethyloctanamide, and acylaminal (26) standards. The relative gc yields (without correction for non-equivalent FI detector response)

are presented in Table 5.

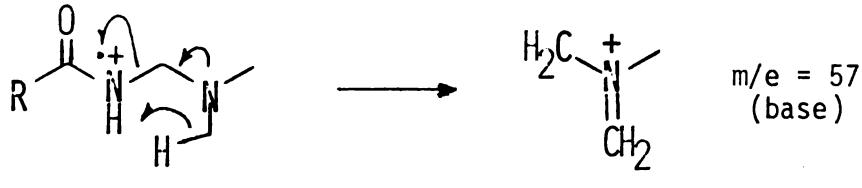
Table 5. Relative GC Yields

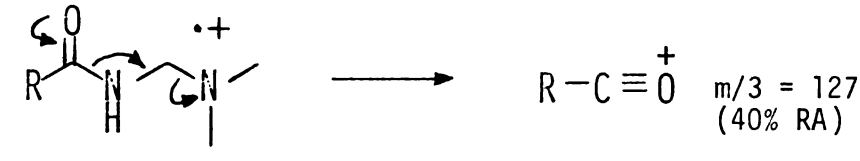
| | 3° amide | 1° amide | acylaminal |
|---|---|---|---|
| Third Reduction of (3) (Expt. 2-4) | 28% | 16% | 56% |
| Fourth Reduction of (3) (Expt. 2-5) | 78% | 12% | 10% |

To verify the identity of each of these three gc peaks, the mixture from the fourth reduction was analysed by gc (OV-25) interfaced with a low resolution ms (electron impact). As shown in Experiment 2-5, the molecular ions and predicted fragmentation patterns for both amides are observed. Furthermore, the empirical formulas for all of the parent peaks and their most important fragments were obtained by high resolution mass spectrometry on the mixture, thus confirming the identity of three major products. The major fragments observed for the acylaminal (26) may be explained as follows:




and/or



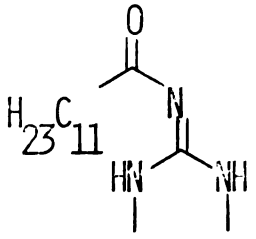




Considering the above data, a complicated but reasonably clear picture of the stoichiometric reduction of a tetramethylacyliminoguanidine

emerges. With two hydride equivalents, the predominant product is the acylaminal (26), with some acyl transfer to the tertiary amide (the primary amide is probably due to both acyl transfer and a reverse Mannich during the work-up). With one hydride equivalent, the acylaminal is a minor product, and formation of the tertiary amide (which requires less hydride) predominates. Although neither Pathway for the formation of tertiary amide can be discounted, extensive mass spectrometry verified formation of free tetramethylguanidine, but no evidence of the N,N-dimethylamidine was ever found. This observation seems to support Pathway 2, but the absence of free amidine may simply be due to its rapid reduction.

N,N'-Dimethylguanidine hydrobromide was prepared in good yield by a literature method[25], converted to the free base by ion exchange, and condensed with methyl laurate in a manner similar to the preparation of dodecanoylguanidine (Expt. 1-3). The acyldimethylguanidine (28) did not crystallize from the reaction mixture, however, making product isolation somewhat more difficult than with dodecanoylguanidine. A column of neutral alumina was sufficient to obtain pure material which was deliquescent and melted at 38° thus explaining its failure to crystallize from the reaction mixture.

The reduction of the dimethylacyl compound (28) to the corresponding guanidine (29) was considerably faster than expected, the first two runs

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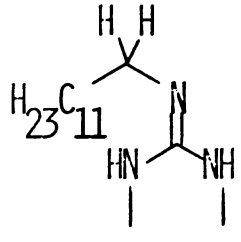
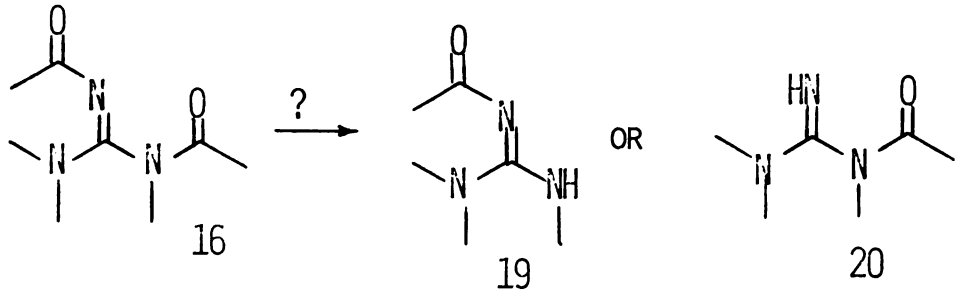



29

showing formation of extensive amounts of dodecylamine. The third run (Expt. 3-4) provided a good yield (62%) of the guanidine (29), thereby concluding work on this substrate.

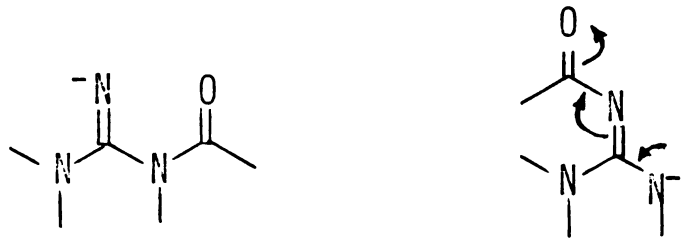
The previous Section describes the cleavage of diacetyltrimethylguanidine (16) to one monoacyl product (Expt. 4-4), the uv of which implied structure (20). The nmr, however, implied (19), and, so, several experiments were performed to confirm which monoacyl product was being formed. Since the conjugated carbonyl can get electron donation from



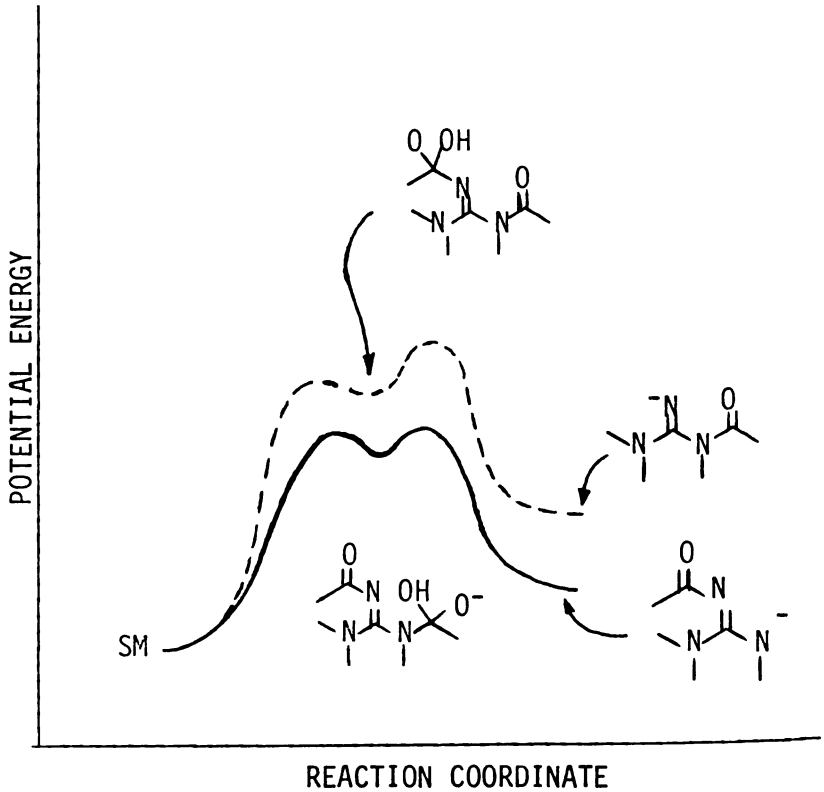

two nitrogens, whereas the non-conjugated carbonyl only gets donation from a nitrogen which can also donate to the conjugated carbonyl, one

would predict hydroxide attack to be on the non-conjugated carbonyl. In considering the transition state (or intermediate as the case may be), the two possibilities shown below also predict loss of the non-conjugated

carbonyl since the structure on the right has a lower energy $\pi$ electron system. Finally, the two possible leaving groups are quite different,



one being resonance stabilized and the other not. Once again the loss of the non-conjugated carbonyl appears to be favored. These arguments are summarized by the following energy diagram:

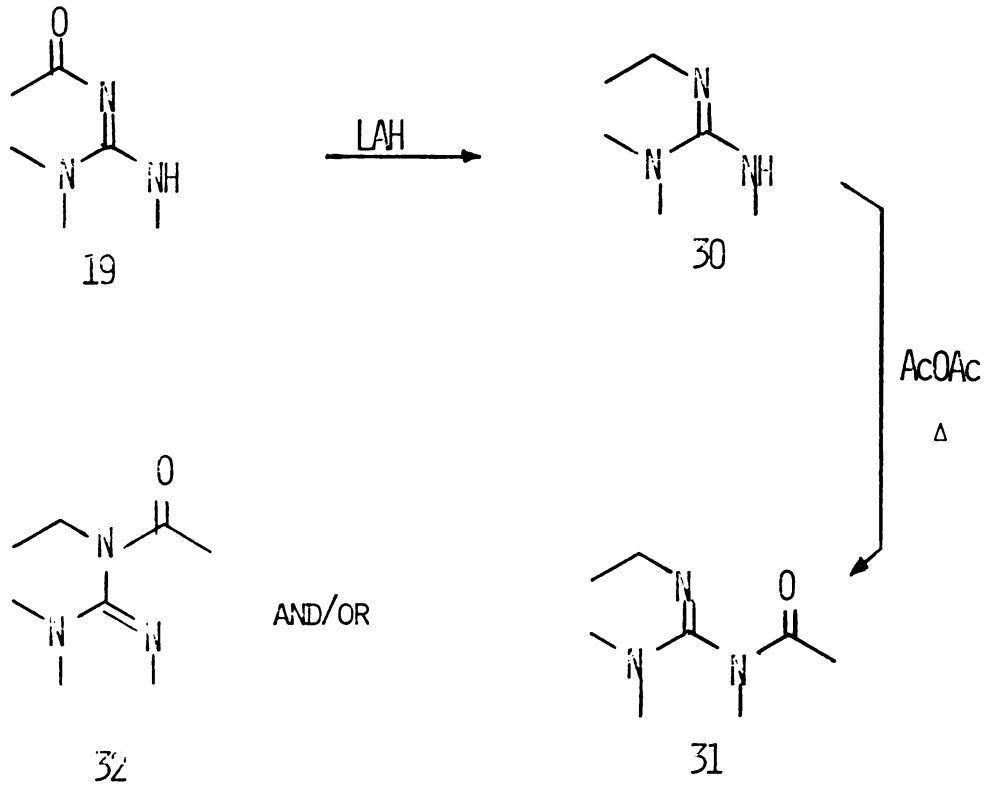



These arguments hold only in the total absence of acid catalysis; if protonation or complexing with a Lewis acid is allowed prior to hydroxide attack, prediction becomes very difficult. To verify that the cleavage product from the use of basic ion exchange resin (Expts. 4-4 and 4-5) would still be obtained in the absence of any acid stronger than

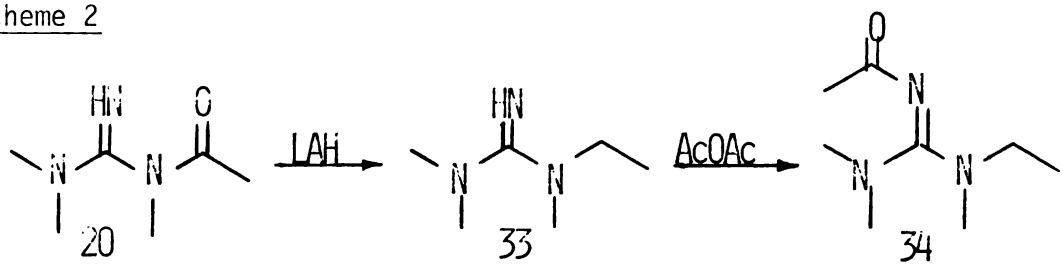
ethanol, a cleavage of the diacyl material was run under dry $N_2$ with potassium ethoxide in ethanol (Expt. 4-6). Here, in the absence of water, methanol, and Lewis acids, a product with a uv implying the acylamino compound (20) was once again obtained. Thus, the uv of the cleavage product remains inconsistent with that predicted by theory.

Some of the pure monacyl material (19) was then reduced with LAH to obtain the corresponding trimethylethylguanidine (Expt. 4-7). Some of this material was acetylated to see whether a conjugated or non-conjugated product resulted (Expt. 4-7). The possibilities in this experiment are summarized by Schemes 1 and 2; the trimethylacylimino compound

Scheme 1

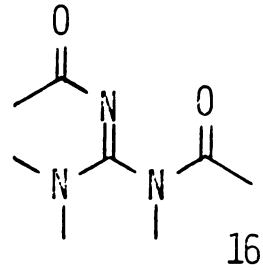


Scheme 2




20 33 34

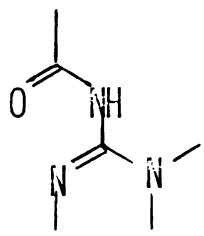
(19) can lead only to unconjugated products (31) and/or (32); the trimethylacylamino compound (20) can lead only to the conjugated (34). The corresponding tetramethylacetyl analogs of (31) and (34) are both known[4] and the nmr and uv data from the peracetylated product (Expt. 4-7) correlate well with the nmr and uv of the tetramethyl analog of (31). Surprisingly, no evidence was found for compound (32), but a large amount of the trimethyldiacetyl compound (16) was observed in the nmr. This material arose from the acetylation of monacetyl material (19) which had survived the LAH.



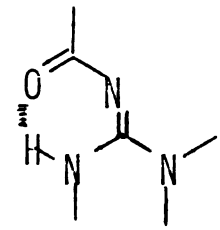

16

Given the uv and nmr evidence for the production of (31), the only data inconsistent with Scheme 1 is the uv of the monoacylimino compound (19). To check for solvent effects, the uv of (19) was retaken in dioxane $\lambda_{max}$ = 247 nm. With dodecanoylguanidine (2) and N-dodecanoyl-N',N"-dimethylguanidine (4) a bathochromic shift of only 5 nm was observed in changing from basic ethanol to dioxane. Clearly, the triaklyacyliminoguanidine (19) exhibits anomalous uv properties. There is considerable steric crowding in this molecule assuming a flat geometry,

and it may be that in a protic solvent, the non-conjugated tautomer (35) is preferred, whereas in aprotic solvents, the intramolecularly hydrogen



35

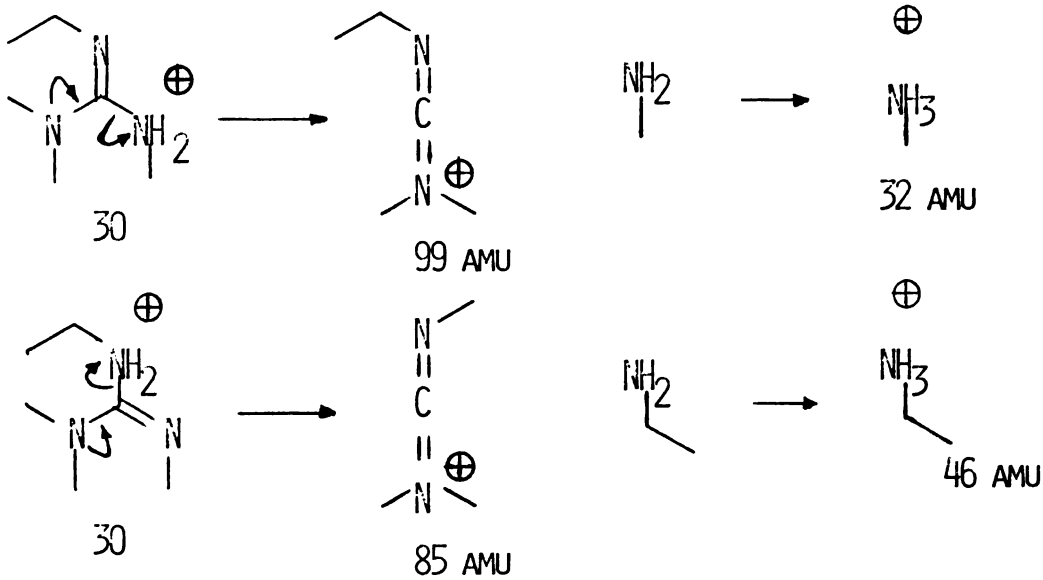


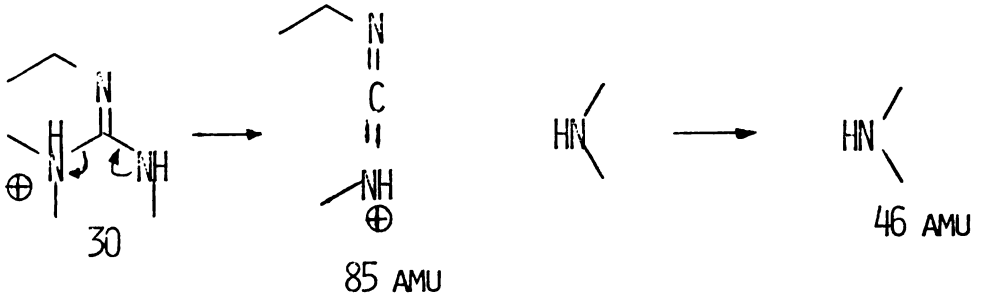
19

bonded tautomer is preferred.

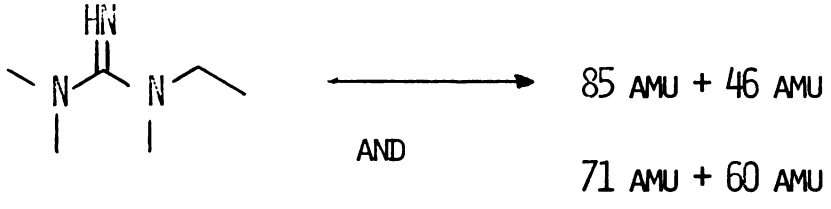
The data for the trimethylacylimino compound (19) and its reduction product (30) was entirely consistent with Scheme 1 at this point, but one more experiment was tried to leave no doubt about the structure of these compounds. If sufficient fragmentation in the CIMS were possible, one of the two following Schemes should be observed:

Scheme 1a (reduction product from Scheme 1)






Scheme 2a (reduction product from Scheme 2) using the same mechanisms as for Scheme 1a:

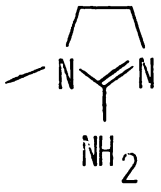



The loss of $NH_3$ in Scheme 2a would be very difficult since the volatile form of this compound has only one tautomer. As seen in Experiment 4-8, methane reactant gas did indeed give enough fragmentation, and all the fragments predicted by Scheme 1a were observed. Thus, the structure of

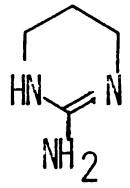
the trimethylacyl compound (19) is no longer in doubt.

The reduction of the trimethylacylguanidine (19) proved to be more difficult than the other substrates in several ways. First, the reaction was homogeneous with only one formal negative charge on the substrate; this resulted in short reaction times which varied considerably with changes in substrate or hydride concentration. Thus, it was difficult to pin down optimal conditions. Just reproducing hydride concentrations is in itself difficult; LAH must be syringed as a solution in THF, and the THF solvent must be distilled from LAH, stored, measured and transferred to a reaction vessel, all of these operations being done under dry $N_2$. The problem of storing and measuring dry THF was solved by closing a 1 liter graduate cylinder onto an outer 19/22 standard taper joint. A 10 mm O.D. side arm was then blown on for flushing with $N_2$. This cylinder serves simultaneously as a distillation receiver, storage vessel, and measuring device. It was oven dryed and flushed with dry $N_2$ (via a rubber septum in the side arm) just prior to filling with THF directly from a still (which was also flushed with $N_2$). The ground joint was then sealed with a septum, the cylinder was pressurized slightly with $N_2$, and the punctured rubber septa were both sealed with slightly larger inverted rubber septa. Transfers of known volumes were then made with a double ended needle and positive $N_2$ pressure via the side arm.

Another problem encountered in the reduction of the trimethylacyl substrate (19) was the handling of the substrate itself; it was difficult to purify, and once pure, it was difficult to handle, being deliquescent and sensitive to hydrolysis. The desiccated solid formed a hard cake which was difficult to break-up and had to be handled in a glove bag. A methylene chloride solution of the quarter hydrate proved




10

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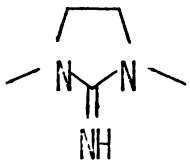



36

to be the most convenient means of handling this material (see Expt. 4-7).

Finally, once the reduction was accomplished, considerable difficulty was encountered in finding a salt suitable for analysis. After all the usual acids failed, p-bromobenzenesulfonic acid (which was prepared from its sodium salt by ion exchange) succeeded in producing a salt after some careful manipulation (see Expt. 4-8).

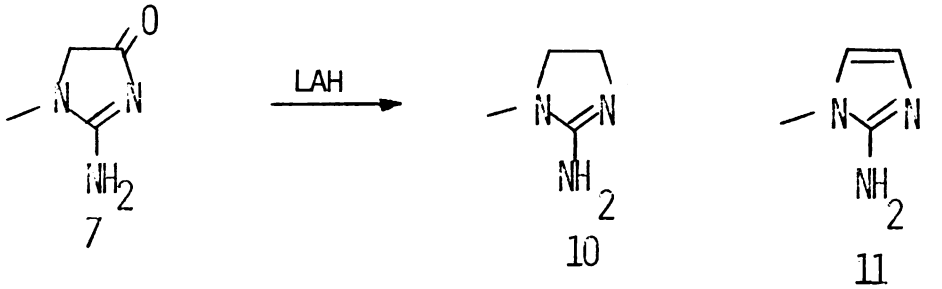
In the third Section, repeated efforts to purify compounds (10) and (13) are described. As it turns out, both of these compounds and compound (36) suffer from a common problem; they are all difficult to isolate in high yield from an LAH reduction mixture due to occlusion of organic material in the aluminum salts formed during work-up. This problem was severe only with the cyclic products, possibly because they are more polar than the acyclic guanidine products. Two approaches to this problem were partially developed in the third Section; one was to physically separate the guanidine from the metal salts; the other was to carbobenzoxylate the guanidine, extract this derivative into an organic phase, and then regenerate the guanidine. Each of these approaches has now been developed into a practical procedure.

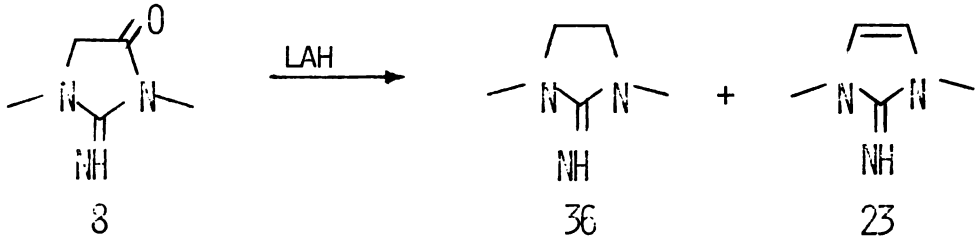
The method of physical separation employs a digestion of the mixed salts in HCl, followed by precipitation of $Al(OH)_3$ and NaCl (Exp. 5-4). The remaining mixture of guanidinium chloride and LiCl may now be separated on an anion exchange column. The ion exchange procedure was first perfected in a model system (Expt. 8-2) using N,N'-dimethylguanidine, and it was then applied to the purification of compound (13) in Experiment 5-4. It is a fortunate coincidence that Li ion, the most difficult to remove by selective precipitation, is the easiest to remove

by ion exchange chromatography (Li ions have the lowest known affinity for aromatic sulfonate resins - even lower than protons; guanidinium ions, conversely, have one of the highest affinities for these resins).

The other approach to this separation problem, the derivatization-extraction approach, was refined in a model system (Expt. 8-17) using N,N'-dimethylguanidine again and a new catalyst, palladium oxide, for removal of the carbobenzoxy groups. The catalytic hydrogenolysis of these groups was the one remaining obstacle in previous experiments on this procedure (Expts. 8-8 through 8-11). The palladium oxide (Englehard Industries) proved to be a much more reliable catalyst than the palladium on charcoal used previously.

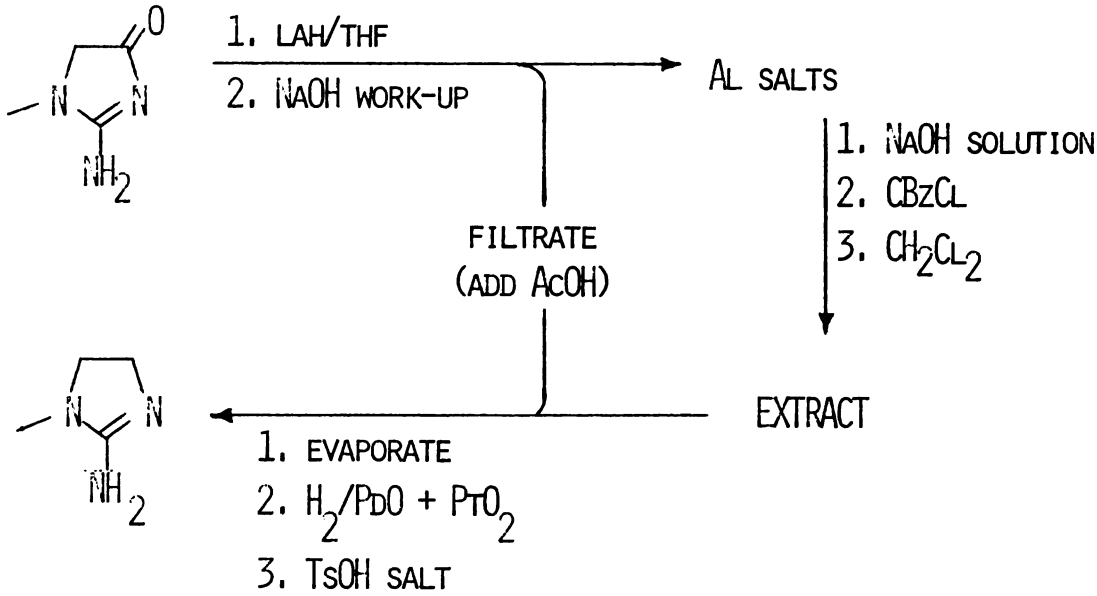
With two practical separation procedures available, only one problem remained in the reduction of creatinine (7) and methylcreatinine (8). The difficulty with these compounds is their tendency to form the



corresponding aromatic compounds, (11) and (23), in addition to the desired guanidines, (10) and (36). These aromatic compounds are apparently inert to further reduction by LAH. It was found, however, that these aromatic compounds may be catalytically hydrogenated to the corresponding guanidines (Expt. 6-6) using a mixture of palladium and platinum oxides. The palladium is probably unnecessary for this reaction, but it was added to simulate debenzylation conditions. Thus, by using the derivatization-extraction method to isolate the LAH reduction products, removal of the carbobenzoxy groups and conversion of the aromatic compounds to the corresponding guanidines can be accomplished in one step. The procedure, then, for reduction of either creatinine (7) (Expt. 6-5) or methylcreatinine (8) (Expt. 7-2) is outlined in Scheme 3.

Scheme 3




Of the seven substrates reduced by LAH, only one, the tetramethylacyl compound (3), failed to give a good yield of guanidine product. The substrates, their reduction products, and the yields are summarized

in Table 6.

Table 6. Summary of LAH Reductions

| Substrate Number | Yield (salt form) | Experiment Number |
|---|---|---|
| 2 | 60% (AcOH salt) | 1-6 |
| 3 | No guanidine products | 2-1<br>2-3<br>2-4 |
| 4 | 62% (TsOH salt) | 3-4 |
| 5 | 59% (pBromobenzene-sulfonate salt) | 4-9 |
| 6 | 53% (HCl salt) | 5-1<br>5-4 |
| 7 | 54% (TsOH salt) | 6-5 |
| 8 | 51% (TsOH salt) | 7-2 |

---

The data in Table 6 show that a wide variety of acylguanidines may be reduced to the corresponding alkyl guanidines in good yield providing one structural requirement is met; the substrate must contain at least one N-H proton in the guanidine moiety.

Having outlined the structural requirements for reduction to a guanidine, a few comments should be made about the relative rates of reduction. Since precise amounts of solvent were used only in the last few reactions, and since no standard concentrations were adopted for either substrates or reducing agent, only a rough comparison is possible. However, inspection of the reaction times and approximate concentrations leads to the following generalizations; the reduction of (3) (Table 6)

is very fast, the reductions of (4), (5), and (8) (Table 6) are moderately fast and the reductions of (2), (6) and (7) are slow. The sluggishness of (6) and (7) may be explained by the fact that these are the only two substrates which did not give a homogeneous reaction with LAH. Presumably, these two compounds precipitate as polyaluminates which then reduce rather slowly. If all the N-H protons on substrate (2) are replaced by aluminum, the slow reduction rate with this compound would be explained by the substrate having a formal -4 charge which would electrostatically repel an approaching hydride. To test whether all the N-H protons on dodecanoylguanidine (2) are removed by LAH, several $H_2$ evolution measurements were made with both this compound and creatinine (7) (Expt. 9). With both compounds, an initial surge of $H_2$ evolution was followed by a period of gradually decreasing gas evolution. With creatinine (7), slightly more than 1 eq of $H_2$ evolved in 10 min (0°), and, with dodecanoylguanidine (2), slightly less than 4 eq of $H_2$ evolved under the same conditions (longer reaction times gave more erratic results). Presumably, at room temperature, both compounds would eventually loose all their N-H protons in the presence of excess LAH. In view of this data, the electrostatic repulsion explanation for the slow reduction rate with dodecanoylguanidine (2) seems quite reasonable.

EXPERIMENTAL SECTION

Preparation of Dodecanoylguanidine (2): Experiment 1-1

Methyl laurate (26.0 g, 122 mmol, distilled[29]) and ethanol (50 ml, abs) were mixed with guanidine free base (7.3 g, 124 mmol, freshly prepared) and allowed to stand (23°, air atmosphere) for 12.5 hr. The reaction was protected from moisture (drying tube) and carbon dioxide (ascarite tube). Evaporation of the ethanol, addition of ether (30 ml, anh), and chilling gave an unfilterable cake of crystals. Therefore, hexane (150 ml, reagent) was added; the flask was stoppered and shaken vigorously. The mixture was then chilled (ice bath), vacuum filtered, and air dried to give 20.0 g crude product (68%) melting at 101-104° (lit. mp; 80-82°[5]). See structure proof after next two runs.

Second Preparation of Dodecanoylguanidine (2): Experiment 1-2

Guanidine hydrochloride (2.9 g, 27 mmols, Sigma, undried) was dissolved in ethanol (150 ml, abs) and the solution was chilled in an ice bath. Sodium (0.53 g, 22 mmol, MCB, 2 x hexane washed) was dissolved in ethanol (100 ml, abs) and also chilled before being poured slowly into the guanidine hydrochloride solution (magnetic stirring, air atmosphere). Within seconds, sodium chloride began to precipitate; the mixture was stoppered and refrigerated overnight to insure maximum precipitation. The guanidine solution was then decanted from the sodium chloride and evaporated (bleed with dry nitrogen). Methyl laurate (4.30 g, 20.1 mmol, fract. distilled) and ethanol (10 ml, abs) were immediately added to the evaporator flask which was sealed with a greased glass stopper. The reaction was allowed to stand and worked up essentially the same as the first run. Yield = 2.47 g (51% based on the ester), mp 104-105°.

Third Preparation of Dodecanoylguanidine (2): Experiment 1-3

Using the work by Bannard[7] as a guideline, free guanidine base was prepared by ion exchange and then used for condensation with methyl laurate. Anion exchange resin (BioRad AG 1-X8, hydroxide form, 20-50 mesh) was added to ethanol (50 ml, abs) until a wet resin volume of 50 ml (70 meq ) was obtained, and the resulting slurry was used to pour a column (probably two thirds this amount of resin would have been plenty). A one liter separatory funnel was then sealed to the top of the column (with a short piece of vacuum hose) and filled with ethanol. This reservoir was protected from moisture (drying tube) and carbon dioxide (ascarite) and the column was washed to remove all water and trimethylamine (total wash: 4.5 liters abs ethanol over a 40 hr period). After washing, the resin turned from a light reddish tan to a very dark reddish brown. Guanidine hydrochloride (2.0 g, mmol, Sigma) was vacuum desiccated for 2 days over calcium chloride. The dried salt was then dissolved in ethanol (30 ml, abs) without re-weighing. The resulting solution was cloudy even after another 10 ml of ethanol was added (some carbonate salt ?). The ethanol solution was applied to the column, and both the reservoir and the bottom of the column were protected from moisture and carbon dioxide. The guanidine was then eluted with ethanol (160 ml, abs) at a rate of 25 drops per minute. The elutant was evaporated (bled evaporator with dry nitrogen) and treated immediately with methyl laurate (5.0 g, 23.4 mmol, fract. dist.) and ethanol (10 ml, abs). The reaction was sealed with a greased glass stopper, allowed to stand for 12 hr, and worked up as before. Yield = 2.7 g (56% assuming 20 mmol of guanidine). Of the several crystalizing solvents tried (THF, ethanol/ether, ether, acetone, and chloroform), ethanol/ether (30 ml each, abs per 2.0 g crude product) gave the best melting point (110-111°) but the

lowest yield (0.25 g, 12.5%). Both ether (100 ml, anh per 2.0 g product) and acetone (150 ml, reagent per 11.0 g product) after hot filtration and chilling gave lower melting points (100-109° and 105-109°, respectively) but much higher yields (1.20 g, 60% and 6.95 g, 63% respectively).

UV 232 nm (16,000)

Chemical Ionization Mass Spectrum Parent Peak: 241 amu

Analyzed for $C_{13}H_{27}N_3O$:

| Calculated: | Found: |
|---|---|
| C = 64.69% | C = 64.66% |
| H = 11.17% | H = 10.80% |
| N = 17.41% | N = 17.34% |

Reduction of Dodecanoylguanidine (2) with LAH: Experiment 1-4

Lithium aluminum hydride (1.0 g, 26 mmol, Ventron, stored in desiccator) was added in a dry nitrogen stream to tetrahydrofuran (50 ml, reagent, fresh bottle) contained in a three neck flask. The mixture was stirred magnetically, kept under nitrogen, and the internal temperature was monitored with an alcohol thermometer. The suspension was then chilled to -30°, and dodecanoylguanidine (2) (500 mg, 2.1 mmol, recrystalized, undesiccated) was added in one portion (internal temp. rose immediately to -27°). The bath was changed to water alone (0°, no ice) and was allowed to come to room temperature. The reaction was stirred for 21 hr and worked-up by a modified Fieser and Fieser[26] method. Aqueous sodium hydroxide with a small amount of ethanol (1.0 ml, 5.0 M NaOH, 1 M EtOH) was added dropwise to the stirred chilled reaction (salt ice bath, -10°) over approximately 20 min through a number 25 stainless steel syringe needle. Some caking occurred under the stir bar and this

was broken up by a spatula. Stirring and cooling were continued until no cakes were visible. Water (3.0 ml, distilled) was then added in a similar manner until the mixture turned white. The reaction was vacuum filtered; the filtrate was placed in a stoppered flask and refrigerated. The crystals which formed were filtered after two days. Yield = 243 mg (45.4% assuming the product is the carbonate salt) mp 91-95°.

Second Reduction of Dodecanoylguanidine (2) with LAH: Experiment 1-5

A second reaction was run in parallel with the one above and under the exact same conditions with one alteration; after stirring at room temperature for 21 hr, the reaction was refluxed for 4 hr and then worked up as before. Yield = 176 mg (32.8%) mp 91-95°. GLC analysis of the filtrates (OV-225) shows substantial formation of dodecylamine in this run, but only a trace in the previous run.

UV: end absorption, no maximum

Chemical Ionization Mass Spectrum: 227 amu; major fragment, 211 (MH-ammonia)

Analyzed for $C_{13}H_{29}N_3 \cdot 1/2\ H_2CO_3$:

| Calculated (for carbonate): | Found: |
|---|---|
| C = 62.9% | C = 64.57% |
| H = 11.6% | H = 11.69% |
| N = 16.3% | N = 17.82% |

(The high value for nitrogen is probably due to contamination by carbonate salts of cleavage products, e.g., ammonia. Since this analysis is unacceptable, a better salt was sought.)

Conversion to the Sulfate Salt

Sulfuric acid (conc.) in abs ethanol (0.70 ml, 0.49 N, 0.34 meq ) was added to the crude carbonate (68 mg, 0.26 mmol) in ethanol (5 ml, abs). Yield = 67 mg (92.1%) mp 250-260° (decomp.), mp of standard 230-260° (decomp.[8]).

IR: KBr pellets of the above sulfate and of the sulfate prepared by the patent method[8] gave identical ir spectra.

Third Reduction of Dodecanoylguanidine (2): Experiment 1-6

Lithium aluminum hydride (2.0 g, 53 mmol) was suspended in tetrahydrofuran (120 ml, reagent, fresh bottle) under nitrogen and chilled as before. Dodecanoylguanidine (2) (1.0 g, 4.15 mmol) was added in one portion, as before, and the reaction was stirred 33 hr at room temperature and worked up as before. After filtering the aluminum salts, the THF solution was split into 64 ml and 74 ml portions for the preparation of the acetate and hydrochloride salts respectively. The hydrochloride salt was slightly hygroscopic, tended to oil out, and appeared to be a poor means of isolating product. The acetate salt, however, crystalized within minutes directly from the reaction filtrate (1.0 ml acetic acid, 64 ml THF sol., ice bath) to give analytically pure product. Yield = 329.7 mg (59.6%) mp 133-134°. Evaporation of the filtrate left an oil (139 mg) which crystalized in the freezer. Filtration gave 69 mg (14.6%) of lauryl amine acetate (mp 65-67°, CIMS, parent = 185).

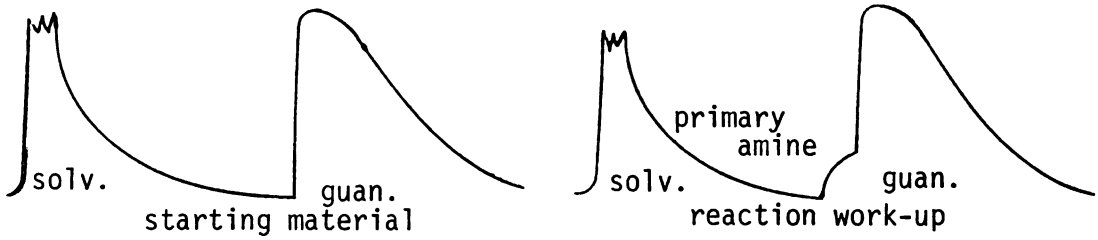
Analysis of First Crop (for $C_{13}H_{29}N_3 \cdot AcOH$):

| Calculated: | Found: |
|---|---|
| C = 62.67% | C = 62.86% |
| H = 11.57% | H = 11.30% |
| N = 14.62% | N = 14.81% |

Reduction of Dodecylguanidine Sulfate: Experiment 1-7

Dodecylguanidine sulfate[8] (500 mg, 1.81 mmol, crystals from EtOH) was treated with lithium aluminum hydride (1.00 g, 26.3 mmol, Alfa) in exactly the same manner as the original dodecanoylguanidine reduction (Experiment 1-4). Gas chromatographs (OV-225) of the starting material and the crude

reaction work-up show the development of a shoulder of primary amine just preceding the guanidine peak (see drawing). The glc conditions were the




same as those used in the previous work; a trace of sodium methoxide was added to the samples just prior to injection to neutralize carbonate salts.

Reduction of Dodecanoylgaunidine with Redal: Experiment 1-8

Dodecanoylguanidine (1.00 g, 4.15 mmol, Experiment 1-3) was suspended in benzene (100 ml, reagent) by magnetic stirring ($N_2$ atmosphere). A benzene solution of sodium bis(2-methoxyethoxy)-aluminum hydride (2.0 ml, 10.0 mmol, Aldrich's Redal) was pipetted into the flask (at ∿23°) instantly producing foam and a homogeneous solution (flask did not get warm to the touch). After stirring 24 hr, ($N_2$, ∿23°), $H_2O$ (1.3 ml in 4 ml THF) was dripped in with stirring at 23° and the reaction was then filtered. A 1 ml aliquot of the filtrate was removed and frozen for later glc work; the remainder of the filtrate was treated with acetic acid (1 ml, glacial) and the solution was refrigerated overnight. Filtration gave 802 mg (64%) of the acetate salt of the starting material, mp 120° sharp.

CIMS: Main peak: 241 amu (starting material)

Trace: 227 amu (dodecyl guanidine)

Evaporation of the filtrate produced 308 mg (29%) of crude N-methyl dodecylamine acetate.

Chemical Ionization Mass Spectrum: Main peak: 199 amu (N-methyldodecylamine or dodecanoamide)

Trace: 227 amu (dodecylguanidine)

IR (KBr): No carbonyl stretch at 1650 $cm^{-1}$ (excludes unsubstituted amide). C=O stretch at 1575 $cm^{-1}$ (ionized $AcO^-$) and at 1740 $cm^{-1}$ (unionized AcOH). N-H stretch at 3350 $cm^{-1}$.

GLC (crude reaction mixture): Under the same conditions used previously, two peaks were observed; 3.5 min (N-methyldodecylamine) and 11.3 min (starting material). Only a trace of dodecylguanidine was observed.

Second Reduction of Dodecanoylguanidine with Redal: Experiment 1-9

Dodecanoylguanidine (516 mg, 2.14 mmol, Experiment 1-3) was reduced with a benzene solution of sodium bis(2-methoxyethoxy)-aluminum hydride (5.00 ml, 25.05 mmol, Aldrich's Redal) in the same manner as before (Experiment 1-8) except for the following changes. The Redal was dissolved in benzene (50 ml, reagent) and treated carefully with methanol (1.00 ml, 25 mmol, reagent, $N_2$ atmosphere) to form the monohydridoaluminate before addition of the substrate. After 22 hr at ∿23°, $H_2O$ (3.25 ml dissolved 4 ml THF) was dripped in with continued stirring (at ∿23°) to give a milky suspension. After standing overnight (under $N_2$), the salts had settled and the benzene solution was decanted and evaporated. This crude reaction mixture was analyzed by glc and CIMS.

GLC: Under the same conditions used previously, only one peak was found; this corresponded to starting material.

Chemical Ionization Mass Spectrum: Strong peak: 241 amu (starting material)

Strong peak: 185 amu (primary amine)

Weak peak: 199 amu (2° methylamine)

Preparation of Octanoyl N,N,N',N'-tetramethylguanidine: Experiment 2-1

Prior to the reaction, crude octanoyl chloride (MCB) was fractionally distilled through a 1/2" x 6" vigreaux column (194-6°/760 mm Hg).

Octanoyl chloride (3.25 g, 20 mmol, freshly distilled) and tetramethylguanidine (5.00 g, 43.5 mmol, cyanamide corp.) were each dissolved in ethyl ether (50 ml each, Mallinckrodt anh, fresh can). The guanidine solution was chilled in an ice bath and the octanoyl chloride solution was poured slowly into the magnetically stirred guanidine solution (use hood, air atmosphere). A vigorous reaction occurred giving precipitation of the HCl salt of tetramethylguanidine (3.4 g, 22.4 mmol, wet ?) which was removed by filtration, and the filtrate was stripped on a rotary evaporator to give a slightly yellow oil. This oil was dissolved in $CCl_4$ (50 ml, Mallinckrodt reagent) and wahsed with aqueous base ( 2 x 20 ml, pH 13). The solution was then dried ($K_2CO_3$ anh), gravity filtered, and rotary evaporated. The resulting slightly yellow oil was twice dissolved in $CCl_4$ (2 x 30 ml, reagent) and evaporated to remove traces of ether before taking an nmr. Crude yield; 4.8 g (19.9 mmol, quantitative).

UV $\lambda_{max}$ 239 (13,000) (0.01 N NaOH/abs ethanol)

NMR: Integration implies ~5% excess octanoyl protons; probably due to octanoic acid.

This material was estimated (from the above spectra) to contain ~10% impurities, and was used for two reaction trials without further purification.

Reduction of Octanoyl N,N,N',N'-tetramethylguanidine: Experiment 2-2

A three neck flask (with magnetic stirrer and addition funnel) was purged with dry $N_2$ and charged with LAH (1.0 g, 26.3 mmol, Alfa) and ethyl ether (50 ml, Mallinckrodt, anh). Octanoyl tetramethylguanidine

(2.4 g, 10 mmol, ∿90% pure) dissolved in ether (50 ml, comm. anh) was then added to the dropping funnel. The starting material was dripped into the stirred chilled (ice bath) LAH suspension over a period of 15 min. The mixture was then stirred at room temperature for 1 hr under $N_2$. The reaction was worked up by hydrolysis of the aluminum salts followed by filtration essentially as described by Fieser and Fieser[26]. Evaporation of the filtrate gave an oil which was twice dissolved in $CCl_4$ (2 x 70 ml, reagent) and evaporated. Yield; 1.3 g oil.

NMR: suggests fairly pure N-methyl octylamine.

The oily product (1.0 g, 7.0 mmol) in ether, was added to picric acid (1.75 g, 7.65 mmol, sl. wet, dissolved in ether/benzene). Stripping the solvents yielded an oil which was azeotrope dried by dissolving in ethanol (anh)/benzene and evaporating. Shaking the oily residue with ether (anh) and filtration of the resulting yl. crystals produced 1.67 g (59%) of picrate salt, mp 93-95° (literature melting point[10], 98-98.5°).

Second Reduction of Octanoyl N,N,N',N'-tetramethylguanidine: Experiment 2-3

The reaction was run in aqueous phosphate buffer (pH 7.5) prepared as follows: sodium dihydrogen phosphate monohydrate (11.04 g, 80 mmol, reagent) and disodium monohydrogen phosphate heptahydrate (112.7 g, 420 mmol, reagent) were combined and diluted to one liter with distilled water. Octanoyl tetramethylguanidine (2.0 g, 8.3 mmol, crude) was dissolved in buffer (50 ml) and chilled briefly with an ice bath (prolonged chilling caused precipitation of sodium phosphate salts). Sodium borohydride (2.1 g, 12.4 mmol, MCB) was then added in portions over 0.5 hr with magnetic stirring and occasional chilling (air atmosphere). Considerable foaming was encountered apparently due to phosphate catalysis of borohydride decomposition (comparable foaming occurred when

sodium borohydride was later added to phosphate buffer alone). The reaction mixture was stirred an additional 0.5 hr and then the pH was shifted to 14 with sodium hydroxide solution (2.5 M, 100 ml). Water (100 ml) was added to prevent phosphate salt precipitation and the reaction was extracted with chloroform (1 x 300 ml, 1 x 100 ml, 2 x 50 ml). The combined extracts were dried with potassium carbonate (anh, reagent), gravity filtered, and rotary evaporated. The residue was dissolved in carbon tetrachloride (80 ml, reagent) which was evaporated to remove protic solvents. The nmr suggest mostly starting material, plus a small amount of N-methyloctylamine. Over-reduction was further corraborated by the presence of a strong amine odor (dimethylamine) which disappeared when the extraction solvent was evaporated.

Third Reduction of Tetramethyloctanolyguanidine: Experiment 2-4

Crude octanoyltetramethylguanidine (Experiment 2-1), which was cloudy and somewhat yellowed, was short path distilled (b.p. 111-134°/50μ) to give a clear colorless liquid ($\lambda_{max}$ = 239 nm (14,700), ∿98-100% pure). A portion of this material (1.479 g, 6.12 mmol) was treated with ∿2 eq of LAH (1.3 ml, 2.4 M, 3.12 mmol) in the usual manner (see previous experiment) except that ether (50 ml, abs, without distillation) was used instead of THF. Starting at -64°, the reaction was warmed gradually to 0° over 1 hr during which time the reaction remained homogeneous. The reaction was then maintained at 0° for 4 hr (turned cloudy after 1 hr at 0°) and 23° for 1 hr before the usual work-up[26]. The filtrate evaporated to 1.32 g of colorless oil. This material would not form a carbonate salt and it dissolved easily in $CCl_4$ for azeotrope drying and solvent removal (2 x 30 ml $CCl_4$). Evaporation of the second portion of $CCl_4$ left an oil (1.60 g) which gave the following data:

CIMS: 200 amu major peak

143 amu minor peak

NMR ($CCl_4$):

complex; 0.070 δ to 1.90 δ (>13H)(alkyl side chain)

complex; 2.75 δ to 3.07 δ (contamination, see Experiment 2-5)

triplet; 8.00δ (1H) (N-H proton) J = 6 cps

doublet; 3.92 δ(2H) ($N-CH_2-NH$) J = 6 cps

complex; 2.20 δ(8H) (N,N-dimethyl, $CH_2CO$)

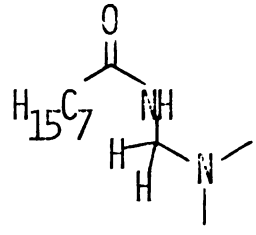
NMR (above sample $D_2O$ washed):

Exactly the same as the previous spectrum except:

(a) the triplet disappeared (N-H exchanged to N-D)

(b) the doublet collapsed to a singlet (3.92 δ, 2H); the methylene is no longer split by the N-H proton.

These data are consistent with the following structure:



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Of the 1.60 g of oil after $CCl_4$ evaporation, 604 mg were distilled (kügelrohr, 110-115°/400μ) to give 371 mg of hygroscopic solvent free oil. This constitutes a distilled yield of ∿71%. The nmr of this material was identical to the nmr before distillation.

IR (film):

1650 $cm^{-1}$ (strong) (C = O stretch of monosub. amide)

1540 $cm^{-1}$ (medium) (N-H bend of amide)

3300 $cm^{-1}$ (strong) (N-H stretch + $H_2O$)

Fourth Reduction of N,N,N',N'-Tetramethyloctanoylguanidine: Experiment 2-5

Tetramethyloctanoylguanidine (3.052 g, 12.6 mmol, distilled, Experiment 2-4) was reduced with ∿1.3 hydride equivalents of LAH (1.8 ml, 2.4 M, ∿17 meq, Section 2 of Discussion) in ether (100 ml, anh, reagent) under the same conditions as in Experiment 2-4, except that room temperature was maintained for 2 hr instead of 1 hr. The homogeneous reaction was worked up as before; evaporation of the crude reaction filtrate followed by evaporation of $CH_2Cl_2$ (5 x 30 ml) gave 2.9 g of oil containing three major components by glc (OV-225). Kügelrohr distillation (450 μ/108-114°) gave 1.90 g of oil, the three major components of which were identified by nmr and gcms (electron impact) with an OV-25 column. Integration of the glc peaks gave relative yields as follows: 78% of N,N-dimethyloctanamide, 12% of octanamide, and 10% of N-(dimethylaminomethyl)-octanamide. These values ignore differences in detector response.

NMR ($CCL_4$): Shifts are the same as product in Experiment 2-7. In addition, two singlets are observed at 2.88 δ and 3.00 δ due to N,N-dimethyloctanamide. Integration implies a ratio of 3.5:1 dimethylamide to acylaminal.

GCMS:

| Dimethyloctanamide: | | High Resolution Formula | Δ mmu |
|---|---|---|---|
| 87 amu | Base Peak (McLafferty) | $C_4H_9NO$ | -0.3 |
| 72 amu | 40% RA (α-cleavage) | $C_3H_6NO$ | -2.8 |
| 171 amu | 3% RA (parent) | $C_{10}H_{21}NO$ | -1.3 |

Also observed: loss of ethyl, propyl, butyl, and pentyl groups.

GCMS:

| Octanamide: | | High Resolution Formula | mmu |
|---|---|---|---|
| 59 amu | Base Peak (McLafferty) | $C_2H_5NO$ | 2.7 |
| 44 amu | 21% RA (α-cleavage) | $CH_2NO$ | -0.4 |
| 143 amu | ∿1% RA (parent) | $C_8H_{17}NO$ | 0.0 |

Also: ethyl, propyl, butyl, and pentyl fragments.

N-(Dimethylaminomethyl)-octanamide (26):

| | | | |
|---|---|---|---|
| 57 amu | Base Peak | $C_3H_7N$ | 3.6 |
| 127 amu | 40% RA | $C_8H_{15}O$ | 0.6 |
| 200 amu | (absent from GCMS) (parent) | $C_{11}H_{24}N_2O$ | -0.4 |

Reduction of N-(Dimethylaminomethyl)-octanamide (26): Experiment 2-6

N-(Dimethylaminomethyl)-octanamide (26) (207 mg, 1.04 mmol, distilled, Experiment 2-4) was reduced with excess LAH (1.25 ml, 2.4 M, 3.0 mmol) in ether (∿50 ml, fresh, abs) in a similar manner to the previous experiment. The temperature was maintained at -55° to 50° for 1 hr, then at 0° for 2 hr, then at 23° for 2 hr. After the usual work-up[26], a picrate salt of the product was prepared in the same manner as with the original tetramethyloctanoylguanidine reduction (Experiment 2-2) producing 248 mg (64%) of N-methyloctylamine picrate, mp 94-95° (literature mp, 98°[10]).

CIMS (crude material before salt prep.):

Major Peak 143 amu (N-methyl) Minor Peak 157 amu (N,N-dimethyl)

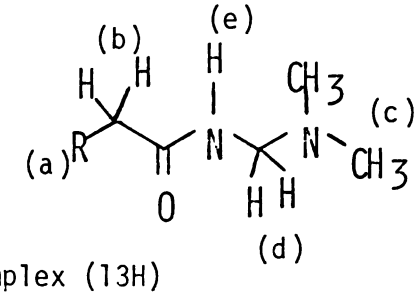
Trace Peak 129 amu (1° amine)

Preparation of N-(Dimethylaminomethyl)-octanamide (26): Experiment 2-7

Octanamide (300 mg, 2.10 mmol[27]), formalin (2.5 ml, 33.3 mmol, 13.3 M HCHO in $H_2O$, Mallinckrodt), dimethylamine (1.45 g, 32.2 mmol, Matheson

Gas), and t-butanol (20 ml, reagent) were added to a glass pressure bottle (120 psi test, Parr Inst. Co.) and a neoprene stopper was wired in. The bottle was placed on a steam bath behind an explosion shield, and the solution was heated 2 hr. Analysis by glc (OV-225) indicated ∿60-40 mixture of product to starting material. The reaction was evaporated to a dark yellow oil; $CCl_4$ (3 x 40 ml) was evaporated from the oil to remove $H_2O$ and other volatile materials. Petroleum ether (5.0 ml, reagent, was added, the solution was seeded with a crystal of octanamide, and the sealed flask was stored 3 days in the freezer. Filtration, evaporation, and kügelrohr distillation (400 μ/115-120°) gave 222 mg (53%) of colorless hygroscopic oil.

NMR ($CCl_4$):




(a) 0.070 to 1.90 δ complex (13H)

(b) 2.20 δ complex
(c) 2.20 δ singlet
} (8H)

(d) 3.92 δ doublet (2H, J = 6 Hz)

(e) 8.00 δ broad triplet (1H, J = 6 Hz)

CIMS: Base = 200 amu (parent)

Some contamination by 171 amu; either $R\text{-}CH_2CON(CH_3)_2$ or $R\text{-}CH_2CONHCH_2OH$; neither showed in the nmr.

Preparation of N-Dodecanoyl-N',N"-dimethylguanidine (4): Experiment 3-1

N,N'-Dimethylguanidine hydrobromide (6.45 g, 38.4 mmol[28]) was converted to the free base by ion exchange[7] (under $N_2$) before adding methyl laurate (10.5 ml, 42.7 mmol[29]). The flask was sealed with a glass stopper,

and the reaction was left at room temperature for 26 hr. Analysis by uv and tlc implied that the reaction mixture (which remained an oil) was predominantly acylguanidine. A column of neutral alumina (300 g, act. IV, 100-200 mesh, BioRad) was poured in hexane, and the reaction mix was washed onto the column with five portions of hexane (10 ml each, reagent). Separation of the three components was monitored by tlc on silica gel (Eastman, uv ind.) eluting with acetone (ester $R_f$ = 0.7, $I_2$ positive, uv and Weber[30] spray negative; product $R_f$ = 0.5, all vis. positive, guanidine $R_f$ = 0.05, $I_2$ and Weber positive, uv negative). The first fraction (ester) eluted from the column with 500 ml of 3:1 hexane to benzene. Elution solvent was switched to acetone and an intermediate fraction of almost pure solvent (∿300 ml) was collected. At this point the product abruptly "broke through" as a 2-phase system (which later became 1-phase again) and after collection of 375 ml, the eluant was essentially pure solvent again. Evaporation of this last fraction gave 6.5 g of oil which was pure product by tlc. Azeotrope drying and vacuum desiccation (3 days, $P_2O_5$, some subliming) yielded 5.171 g (50%) of analytically pure slowly deliquescent crystals, mp 38°.

Analyzed for $C_{15}H_{31}N_3O$:

| Calculated: | Found: |
|---|---|
| C = 66.87% | 66.8% |
| H = 11.60% | 11.5% |
| N = 15.60% | 15.3% |

NMR ($CCl_4$):

0.7 to 1.8 δ (complex, 21 H, alkyl chain)

2.0 to 2.3 δ (complex, 2 H, $-COCH_2-$)

2.84 δ (s, 6 H 2 $N-CH_3$)

UV (0.01 N NaOH in abs Ethanol):

$\lambda_{max}$ = 237 nm (15,300)

Reduction of N-Dodecanoyl-N',N"-Dimethylguanidine (4): Experiment 3-2

N-Dodecanoyl-N',N"-dimethylguanidine (4) (1.000 g, 3.71 mmol, Experiment 3-1) was reduced with LAH/THF (10.0 ml, 2.4 M) in the usual manner ($N_2$, magnetic stirring, int. therm.; chill to -60°, add LAH by syringe, stir at -55° for 20 min, 0° for 30 min). Using ∿100 ml THF (distilled from LAH), the homogeneous reaction was maintained at room temperature for 25 hr before chilling to 0° followed by the usual work-up[26]. Addition of $H_2O$ (0.5 ml, 28 mmol) and $CO_2$ (s) (∿1 g) to the filtrate followed by refrigeration (0°) overnight gave 351 mg (∿30%) of crude carbonate salt, mp 96-110° (d). This material will be referred to as A. The THF filtrate was evaporated and azeotrope dried (ethanol/toluene) to give 440 mg of low melting waxy solid ($CO_2$ evolved with strong acid) which will be called B. The aluminum salts from the reaction work-up were triturated under ethanol (200 ml, abs) and $H_2O$ (25 ml) with a stirring rod, and the mixture was alternately refluxed and treated with $CO_2$ (s) several times. The mixture was then filtered and evaporated to give the crude extract C.

A (first carbonate): This material gave one spot by tlc on silica gel (Eastman, uv ind.) eluting with acetone ($R_f$ =0.09 with tailing). The spot was uv negative and $I_2$ and Weber spray[30] positive (indicating an alkyl guanidine). Some of this carbonate (63 mg) was treated with conc. HBr (0.5 ml, 8.8 M) in ethanol (50 ml, abs). The salt was azeotrope dried (evap. 2 x 50 ml abs ethanol, 3 X 50 $Ch_2Cl_2$) but it refused to crystallize.

NMR (HBr salt in $CDCl_3$):

0.7 to 1.8 $\delta$ (complex, 23 H, alkyl chain)

3.0 $\delta$ (s, 6 H, 2 $NCH_3$)

3.4 $\delta$ (complex, 2 H, $NCH_2$-)

CIMS: Parent (Base): 225 amu (guanidine)

Trace: 185 amu (1° amine)

B (second carbonate): This material gave 4 spots by tlc (silica/acetone):

Major spot: $R_f$ = 0.38 ($I_2$ brn., uv negative, Weber spray[30] negative). Same $R_f$ as dodecylamine standard.

Minor spot: $R_f$ = 0.03 ($I_2$ brn., uv positive, Weber spray negative). Methylamine ?

Trace: $R_f$ = 0.52 ($I_2$ brn. uv positive, Weber spray positive). Same $R_f$ as starting material standard.

Trace: $R_f \sim 0$ ($I_2$ orange, uv negative, Weber spray positive). Same $R_f$ as A used as a standard.

CIMS: Major Peak 185 amu (1° amine)

Trace Peak 255 amu (guanidine product)

C (Al salt extraction) This material gave essentially the same profile as material B.

Using small amounts of carbonate salt (material A), salts of the mineral acids HCl, HBr, $H_2SO_4$, and $HClO_4$ were prepared and azeotrope dried, but none formed crystals. Finally, the $HNO_3$ salt crystallized (mp 65-70°) and, on recrystallization, melted at 78-9°. Good recoveries of the nitrate salt required taking several crops, however, and so a more efficient salt for isolation was sought. The tosylate proved to be a more convenient salt for isolation, mp 85-7°.

Analyzed for $C_{15}H_{33}N_3 \cdot TsOH$:

Dry 3 hr at ∿80°/25 μ (material melted).

| Calculated: | Found: |
|---|---|
| C = 61.79% | 62.09% |
| H = 9.66% | 9.53% |
| N = 9.83% | 9.89% |

Second Reduction of N-Dodecanoyl-N',N"-Dimethylguanidine (4): Experiment 3-3

N-Dodecanoyl-N',N"-dimethylguanidine (1.820 g, 6.76 mmol, Experiment 3-1) was reduced with LAH/THF solution (20.0 ml, 2.4 M, 48 mmol) in THF (100 ml, distilled from LAH) in the same manner as before (Experiment 3-2) except that the time at room temperature was 8 hr. The carbonate salt (863 mg, 45%) was isolated and identified as before. The material remaining in the filtrate again turned out to be mostly dodecylamine (characterized as before).

Third Reduction of N-Dodecanoyl-N',N"-Dimethylguanidine (4): Experiment 3-4

This reduction was run with exactly the same conditions and quantities as Experiment 3-2, except that the time at room temperature was shortened to 8 hr. The resulting carbonate was converted to the tosylate (Experiment 3-2) to produce 985 mg (62%) of colorless crystals, mp 84-6°.

Preparation of N,N,N'-Trimethylguanidine Acetate: Experiment 4-1

A quaternary ammonium cation exchange resin (36 ml, 44 m eq, $OH^-$ form, 20-50 mesh, BioRad) was converted to the acetate form by soaking with two portions of aqueous acetic acid (60 ml, 1 N). The column was then poured and washed with 100 ml $H_2O$. N,N,N'-Trimethylguanidine hydroiodide (5.00 g, 21.8 mmol[13,31]) was dissolved in 10 ml $H_2O$ and applied

to the column. Elution with 150 ml $H_2O$, addition of 30 ml ethanol, and evaporation gave 4.635 g (132%) of hygroscopic oil. Drying at 50μ/23° for 12 hr gave 3.481 g (99%) of deliquescent crystals which were used without purification for the next step.

Preparation of N,N'-Diacetyl-N,N",N"-Trimethylguanidine (16): Experiment 4-2

N,N,N'-Trimethylguanidine acetate (1.98 g, 12.3 mmol, Experiment 4-1) was magnetically stirred with acetic anhydride (53 ml, reagent, Mallinckrodt) under dry $N_2$ at 100° for a total of 40 min. Aliquots were taken at 10 min intervals for tlc on neutral alumina with uv indicator (1:1 ether/acetone). Two spots were found (with uv vis.), $R_f$ = 0.38 (diacetyl product) and $R_f \sim 0$ (probably monoacetyl). The faster moving spot gradually increased in intensity and the spot at the origin was barely visible at 40 min when the reaction was worked up. Excess acetic anhydride was evaporated; the residual oil was evaporated at 1 mm/60° for 30 min to remove the last traces of anhydride, thus producing 2.261 g (101%) of crude oil. This material was chromatographed on neutral alumina (60 g, act. IV, BioRad, 100-200 mesh) by eluting with commercial chloroform. The separation was followed with tlc; the first 60 ml were discarded; the next 200 ml contained all of the product (one tlc spot). Evaporation gave 2.122 g (93%) of a slightly yellow oil.

NMR ($CCl_4$):

singlets: 3.01 δ (6 H), 2.91 δ (3 H) (N-methyls)

singlets: 2.04 δ (3 H), 1.99 δ (3 H) (acetate methyls)

Some of the oil from the column (1.686 g) was distilled (kügelrohr/165°/4mm) to give 1.635 g (98% recovery) of colorless oil.

NMR ($CCl_4$):

Identical to nmr before kügelrohr distillation.

Analyzed for $C_8H_{15}N_3O_2$:

| Calculated: | Found: |
|---|---|
| C = 51.88% | 51.65% |
| H = 8.16% | 8.03% |
| N = 22.69% | 22.47% |

UV (abs ethanol):

$\lambda_{max}$ = 257 (14,400)

$\lambda_{max}$ = 205 (5,750)

Note: On standing 24 hr at 23° in a screw cap vial, the diacetyl product turned slightly yellow.

Second Preparation of N,N'-Diacetyl-N,N",N"-Trimethylguanidine (16)

N,N,N'-trimethylguanidine hydroiodide (23.7 g, 103 meq[13,31], was converted to the acetate salt (Experiment 4-1) and dried in a 1 liter r.b. flask at 100μ/23° for 4 hr. The acetate salt was treated with acetic anhydride (454 g, reagent, Mallinckrodt) as before but the $N_2$ was replaced with a drying tube and the chromatography was omitted. Short path distillation gave 20.01 g (96%) of slightly yellow oil. This material was redistilled through a 4 inch vigreaux (bp 120-125°/25μ) to give 19.62 g (94%) of colorless analytically pure oil.

Monodeacylation of N,N'-Diacetyl'N,N",N"-Trimethylguanidine (16): Experiment 4-3)

N,N'-Diacetyl-N,N",N"-trimethylguanidine (16) (960 mg, 5.18 mmol, Experiment 4-2) was dissloved in ethanol (100 ml, comm. abs), magnetically stirred, and heated to reflux under dry $N_2$. The reaction was monitored by tlc, uv, and glc. As with the diacetyldimethyl compound, pyrolysis occurred in the glc greatly limiting its usefulness. The tlc and the uv appeared to be consistent with each other however. The starting material had an $R_f$ = 0.48 (Eastman Silica, eluted with

8:1:1 n-BuOH/AcOH/$H_2O$, visualized with uv), and a new spot (uv vis.) gradually appeared with an $R_f$ = 0.15. In the uv spectrum, the maximum at 257 nm (starting material) gradually disappeared and absorption in the 200 nm region gradually increased. At no time was any maximum seen in the 230-235 nm region (conjugated monoacyl region). After 57 hr in refluxing ethanol, the starting material was totally consumed (by uv and tlc). The glc (OV-225), which gives three peaks for pure starting material, now showed more than 9 peaks (additional peaks may have been noise). The reaction was discarded.

Second Monodeacylation of N,N'-Diacetyl-N,N",N",-Trimethylguanidine (16): Experiment 4-4

N,N'-Diacetyl-N,N",N",-trimethylguanidine (16) (613 mg, 3.31 mmol, Experiment 4-2) was washed (with MeOH) into a flask containing 5.0 ml of cation exchange resin (7.0 m eq, BioRad AB 1-X8, quaternary ammonium type, hydroxide form, 20-50 mesh) which had been washed with methanol (3 x 30 ml, reagent). The reaction was diluted to ∿50 ml with methanol and allowed to stand at 23°. The uv showed only a very slight decrease at 257 nm after 4 hr, so the reaction was evaporated down to a total volume of about 20 ml. After another 18 hr at 23°, the uv showed a $\lambda_{max}$ = 207 nm with a very small absorption at 257 nm.

CIMS:

| | |
|---|---|
| Strong Peak: | 143 amu (monoacyl) |
| Trace: | 185 amū (diacyl) |
| Trace: | 101 amu (non-acyl) |

The reaction was worked up by diluting with ethanol (200 ml, abs), magnetically stirring (vigorously) for 10 min, filtration, and evaporation. Alcohols were removed by dissolving the resulting oil in $CCl_4$ (40 ml) and evaporating; this process was done three times before drying at

50μ/23° for 2 hr to give 533 mg (113%) of a slightly yellow oil.

NMR ($CCl_4$):

singlet: 2.95 δ (6 H) $N(CH_3)_2$

singlet: 2.67 δ (3 H) $N-CH_3$

singlet: 1.87 δ (3 H) acetyl

The spectrum was fairly clean, except for 4 small singlets corresponding to starting material (see Experiment 4-2 for shifts). Integration implied 5 to 10% diacyl material.

On storage in the refrigerator (0°) the oil began to crystalize, but the crystals had a soapy character and were hygroscopic; mp, 93-6°. In the hope of finding a salt which could be more easily purified, the following acids were tried; HCl, HBr, oxalic acid, $HNO_3$, $H_2SO_4$, $HClO_4$, and picric acid. Despite painstaking efforts to remove all $H_2O$ from these preparations and the use of ∿stoichiometric acid, all of the salts oiled out except the picrate which was deliquescent.

The remainder of the oil (∿150 mg) was then chromatographed on neutral alumina (5 g, BioRad, act. IV, 100-200 mesh) and the purification was followed by tlc (Eastman neutral alumina with uv indicator, acetone developer, diacyl $R_f$ = 0.47, product $R_f$ = 0.15). Starting with 2:1 ether/pet. ether and gradually increasing the solvent polarity, the starting material (diacyl) finally eluted with 100% acetone. The product was then eluted with 1:1 methanol/acetone which partially crystallized on storage at 0°. Stripping 3 portions of $CH_2Cl_2$ (30 ml each) to remove other solvents gave an nmr ($CCl_4$) the same as before chromatography except that three new singlets had appeared; weak sing., 2.62δ; larger singl., 2.16δ; strong sing., 1.25δ. A $D_2O$ wash removed the monoacylgaunidine leaving the above three peaks behind in the $CCl_4$

(same ratios).

Third Monodeacylation of N,N'-Diacetyl-N,N",N"-Trimethylguanidine (16): Experiment 4-5

In a manner similar to the previous run (Experiment 4-5), N,N'-diacetyl-N,N",N",-trimethylguanidine (16) (6.000 g, 32.4 mmols, Experiment 4-2) was mixed with methanol (50.0 ml, reagent) and quaternary ammonium ion exchange resin (60 ml, 84 meq , BioRad AG1-X8 hydroxide form, 20-50 mesh) which had been washed with methanol ( 4 x 100 ml). The flask was sealed and allowed to stand at room temperature with monitoring by uv and tlc as before. After 20 hr, the reaction was diluted with methanol (500 ml, reagent) and stirred for 10 min. The methanol was decanted and the resin was washed again with methanol (150 ml, reagent) and the combined methanol extracts were evaporated. The resulting oil was azeotrope dried (1 x 100 ml $CCl_4$; 1 x 100 ml 1:1 benzene-$CH_2Cl_2$) and mixed with benzene (7.0 ml, reagent) and $CH_2Cl_2$ (1.0 ml, reagent; benzene alone gives two phases) for crystallization. After chilling (0°, 3 days), layering with petroleum ether (20 ml, reagent), and chilling again (0°, 4 days), the crystalline monoacetyl product was found adhered to the flask wall. The supernatant solvent was decanted, the cold crystal cake was rinsed with hexane (2 x 15 ml, reagent), and the flask was pumped dry to give 3.50 g (75%) of crude deliquescent solid. This material was azeotrope dried with $CH_2Cl_2$ (1 x 100 ml, reagent), redissolved in $CH_2Cl_2$ (dilute to 100 ml, reagent), sealed in a flask, and stored at 0°. Remaining impurities form a precipitate which floats on the $CH_2Cl_2$. All subsequent transfers of solid were made in a glove bag filled with dry $N_2$. This monoacetyl product should be used within a few days since both the desiccated solid and the material in solution slowly hydrolyse.

An aliquot of $CH_2Cl_2$ solution (50.0 ml) was evaporated, pumped dry (12 hr, 50μ), and the resulting solid was vacuum desiccated ($CaCl_2$, 50μ) for 3 days to produce 1.658 g (69%) of analytically pure quarter hydrate, mp 99-107°.

Analyzed for $C_6H_{13}N_3O \cdot 1/4\ H_2O$:

| Calculated: | Found: |
|---|---|
| C = 48.79% | 48.5% |
| H = 9.21% | 9.1% |
| N = 28.45% | 28.5% |

UV: 0.01 N NaOH in ethanol (abs): $\lambda_{max}$ = 209 nm (~14,000)

dioxane (reagent): $\lambda_{max}$ = 247 nm

Note: Sublimation in a kügelrohr (105°/ 35μ) of impure solid material produced some purification (by nmr) but spattering from the pot caused considerable contamination of the sublimate. This problem can be avoided by removing all volatile solvents on a good pump for at least 12 hr followed by desiccation for 1-2 days prior to sublimation.

Fourth Monodeacylation of N,N'-Diacetyl-N,N",N"-Trimethylguanidine (16): Experiment 4-6

A suspension of KH in mineral oil (1.00 ml, 24.8%, 6.2 mmol, Alfa) was pipetted into cold ethanol (25 ml, -67°, abs, dryed over 3Å mol sieves) under dry $N_2$ with magnetic stirring. The oil congealed, but, on slow warming to room temperature, a homogeneous solution of potassium ethoxide was obtained. The diacetyltrimethylguanidine (1.00 ml, 5.4 mmol, Experiment 4-2) was added by pipet, and the reaction was stirred under dry $N_2$ at room temperature for 2.5 hr.

UV: (0.01 N NaOH in ETOH): 5.00 μl of the crude reaction mixture was diluted to 10 ml with basic ethanol; $\lambda_{max}$ = 209 nm, apparent $\xi \geq$ 13,000.

No absorbance for the starting material (257 nm) remained.

Reduction of N-Acetyl-N',N',N"-Trimethylguanidine (5): Experiment 4-7

A methylene chloride solution of N-acetyl-N'-N'-N"-trimethylguanidine (25.0 ml, 0.2245 M, 5.61 mmol, Experiment 4-5) was pipetted into a three-neck vessel and the $CH_2Cl_2$ was removed (in the hood) by blowing dry $N_2$ through the flask (magnetic stir; warm with heat gun). This evaporation produced a glass which partially dissolved on addition of THF (25 ml, fresh Mallinckrodt reagent), but, on continued stirring, crystallization occurred on the flask wall. The system was chilled to -67° (internal) and LAH/THF (5.2 ml, 2.4 M, 12.5 mmol) was added slowly by syringe (produced considerable foaming due to $H_2O$). The reaction was homogeneous in ∿5 min (-60°). After warming gradually to room temperature (1.5 hr) and maintaining room temperature for 1 hr, a 5.00 ml aliquot was romoved by pipet and quenched in methanol (30.0 ml, -60°, reagent). The methanol solution was warmed to room temperature for 5 min before adding HCl (2.7 M, aqueous) until pH = 2. This acidified aliquot was then stored in the freezer. The remainder of the reaction was maintained at room temperature for 11 hr before the usual work-up[26]. The filtrate was saturated with $CO_2$ and evaporated; the resulting oil was azeotrope dried ($CH_2Cl_2$, 3 x 50 ml) to give 460 mg of mixed oil and crystals.

NMR ($D_2O$):

quartet: 3.28 δ (2 H, J = 7 Hz, $N-CH_2-CH_2$)

singlet: 2.96 δ (6 H, $N(CH_3)_2$)

singlet: 2.90 δ (3 H, $N-CH_3$)

triplet: 1.20 δ (3 H, J = 7 Hz, $N-CH_2-CH_3$)

The spectrum contains several small extraneous peaks.

An attempt was made to prepare a picrate salt, but this material melted at 118°-130°. Recrystallization made matters worse (mp, 100-125°), and the picrate was, therefore, abandoned. To check the extent of reduction after 1 hr reaction time, the aliquot which had been taken after 1 hr reaction time and acidified was removed from the freezer and evaporated to dryness. The residue was azeotrope dryed (abs ethanol, 2 x 50 ml) before mixing with acetic anhydride (50 ml, reagent, Allied Chemical). The flask was then protected from moisture ($CaCl_2$ drying tube) and heated to 100° for 6 hr. The residue obtained by evaporation of the excess acetic anhydride (55°/1 mm) was partitioned between $CH_2Cl_2$ (50 ml, reagent) and aqueous NaOH (50 ml, 1.25 M) to remove salts. The $CH_2Cl_2$ layer was washed once with $H_2O$ (25 ml, distilled), dryed with $K_2CO_3$ (~5 g, anh, reagent) and evaporated to yield 36 mg crude oil. Dilution of 75 µl of this oil to 50 ml with basic abs ethanol (0.01 N NaOH) provided a uv with broad absorption maxima in the 257 nm and 225 nm regions. The 230-240 nm region showed a relative minimum indicating the absence of any conjugated monoacylguanidines.

NMR ($CCl_4$):

1.93 δ (s, $CH_3CON{=}C$)

2.00 δ (s, $CH_3CON$)

2.88 δ (s, $CH_3N$)

3.00 δ (s, $(CH_3)_2N$)

N-Acetyl-N'-ethyl-N,N",N"-trimethylguanidine (31):

1.09 δ (t, $CH_3CH_2$-N, J = 7 Hz)

3.05 δ (q, $CH_3CH_2$-N, J = 7 Hz)

1.83 δ (s, $CH_3CO$)

2.82 δ (s, $(CH_3)_2N$)

2.86 δ (s, $CH_3N$)

The integration is consistent with a 2:1 mixture of the diacetyl compound (16) to the monoacetyl material (31).

Second Reduction of N-Acetyl-N',N',N"-Trimethylguanidine: Experiment 4-8

N-Acetyl-N',N',N"-trimethylguanidine · 1/4$H_2O$ (1.300 g, 8.80 mmol, Experiment 4-5) was reduced with LAH/THF (12.0 ml, 28.8 mmol, 2.4 M) in THF (50 ml, distilled from LAH) in exactly the same manner as before (Experiment 4-7) except that the reaction time (at room temperature) was shortened to 3 1/4 hr. Addition of $H_2O$ (1 ml) and several pieces of $CO_2$ (s) to the reaction filtrated produced an oil. A trace of ethanol was added causing the oil to redissolve in the THF: refrigeration for 24 hr produec 807 mg (∿57%) of carbonate, mp 73-5° (d). Attempted preparation of all the usual salts (HCl, HBr, $H_2SO_4$, $HClO_4$, $HNO_3$, and TsOH) gave only oils. In order to prepare the p-bromobenzenesulfonate salt, the free acid had to be prepared by elution of the Na salt (5.8 g, 22.4 mmol, Eastman) through an anion exchange column (50 ml, 85 meq, BioRad AG 50W-X8, 200-400 mesh, Hydrogen form) with $H_2O$ (150 ml, distilled). Evaporation gave the free acid as a deliquescent solid. An ethanol solution of free p-bromobenzenesulfonic acid (2.2 ml, 0.8 M, ∿1.76 mmol) was mixed with the carbonate salt (280 mg, ∿1.75 meq) and the solution was evaporated to an oil. This material was azeotrope dried (3 x 30 ml, abs ethanol) and crystallized from ethanol (2-3 ml, abs)/ether (7 ml, abs) to give 222 mg (20%) of crystals, mp 95-6°. Recrystallization from ethanol/ether and drying at 80°25 μ for 3 hr produced analytically pure crystals, mp, 97-8°.

Analyzed for $C_{12}H_{20}BrN_3O_3S$:

| Calculated: | Found: |
|---|---|
| C = 39.35% | 39.41% |
| H = 5.50% | 5.48% |

N = 11.47% 11.50%

Br = 21.82% 21.92%

NMR ($D_2O$):

3.28 δ (q, 2 H, $NCH_2CH_3$, J = 7 Hz)

2.96 δ (s, 6 H, $N(CH_3)_2$)

2.90 δ (s, 3 H, $NCH_3$)

1.20 δ (t, 3 H, $NCH_2CH_3$, J = 7 Hz)

7.73 δ (q, 4 H, aromatic protons)

A small sample (20 mg) of the p-bromobenzenesulfonate salt was converted to the free base by eluting with abs ethanol through an anion exchange resin in the hydroxide form[7]. The extensive column washing and protection from air used previously were omitted. The eluant was treated with $CO_2$ (s) and evaporated to produce a sample for mass spectrometry.

CIMS ($CH_4$ reactant gas):

Parent: $MH^+$ = 130 amu

Major fragment = 85 amu

Minor fragments = 99 amu and 46 amu

Trace fragment = 32 amu

Third Reduction of N-Acetyl-N',N', N"-Trimethylguanidine (5): Experiment 4-9

A solution of N-acetyl-N',N',N"-trimethylguanidine (5) in $CH_2Cl_2$ (Experiment 4-5) was vacuum filtered (in the cold room) to remove precipitated impurities. The solution was transferred to a 3-neck reaction vessel and the $CH_2Cl_2$ was removed (see Experiment 4-7) before pumping the residue dry (24 hr, 50 μ, flask in a desiccator). This produced 593 mg (4.02 mmol) of 1/4 hydrate which was reduced as before (Experiment 4-8) with LAH/THF (5.5 ml, 13.2 mmol) in THF (25 ml, Distilled from LAH) for 4 hr at room temperature. A slight excess of p-bromobenzenesulfonic acid

(1.00 g, ∿4-4.2 mmol, Experiment 4-8) was added to the filtrate before evaporating the THF. Crystallizing twice from ethanol-ether produced 870 mg (59%) of salt, mp 97-8°.

Reduction of β-Alacreatinine (6): Experiment 5-1

Tetrahydrofuran (∿120 ml, Aldrich) was distilled from LAH (under $N_2$) directly into a 3-neck reaction flask containing a magnetic stir bar. β-Alacreatinine hydrochloride (614 mg, 4.11 mmol[21]) was powdered and added without desiccation to the reaction flask (briefly stop $N_2$ flow). The suspension was then magnetic stirred and chilled to -40° before addition (by syringe) of 15 ml of LAH/THF solution (2.35 M, 35 mmol). The reaction became homogeneous in 2-3 min, and $H_2$ evolution could be observed. The bath was changed to 0° and stirring was continued for 30 min before allowing the reaction to warm to ∿23°. Visible $H_2$ evolution had ceased and the reaction still appeared homogeneous. A fine white precipitate did develop slowly, however, suggesting further acid base reaction. After 36 1/2 hr at ∿23°, the reaction was chilled to 0° and NaOH (2.5 ml aq., 4 M) was added dropwise (under $N_2$). $H_2O$ (1 ml) was then added in a similar fashion but at ∿23° to facilitate mixing. After 10 min stirring (still under $N_2$) the reaction was filtered, and the Al salts were saved for later extraction. $H_2O$ (0.2 ml) and dry ice (∿1-2 g) were added to the filtrate to insure protonation of the product prior to solvent evaporation. The resulting white carbonate was dried at 15μ/23° for 2 hr to give 140 mg of crude product. After removing a trace for CIMS, the product was dissolved in methanol (100 ml, reagent) and 20 ml were removed for nmr. The remaining 80 ml of this solution were combined with a solution of picric acid (0.5 g wet, ∿50% molar excess, in 10 ml reagent methanol). Evaporation gave

a yellow solid which was azeotrope dried with a 60:40 mixture of ethanol (anh) and toluene. Crystallization from ethanol (10 ml, anh) produced 301 mg (28%) of yellow needles, mp 183-4° (lit. mp 185-6°[32]).

CIMS: Only peak, 99 amu

NMR ($D_2O$):

triplet: 3.37 δ (4 H) J = 6 Hz

quintet: 1.95 δ (2 H) J = 6 Hz

Second Reduction of β-Alacreatinine (6): Experiment 5-2

This reduction was run exactly the same as the previous one using 1.958 g starting material and 250 ml THF (except for uv monitoring and a change in the work-up). The reaction was monitored by taking aliquots in duplicate with two freshly dried 1 ml volumetric pipets (120°, 12 hr). Each aliquot was quenched by pipetting into cold methanol (10 ml, reagent, chilled in dry ice bath, protected from $CO_2$) followed by dilution to 60.0 ml in a graduate cylinder. Absorbances for aliquots 1a and 1b (1 mm cell, t=0 hr) were 0.750 and 0.710. For aliquots 2a and 2b, A= 0.071 and 0.073. Aliquot 3 (2.00 ml rather than 1.00 ml; dil. 25 x, not done in duplicate) gave an absorbance of 0.178 which implies A=0.0365 at the same dilution as points 1 and 2. The reaction times for these points were 0 hr, 14 hr, and 19 hr. After 24 hr, the reaction was worked up with stoichiometric $H_2O$ and LiOH in an attempt to minimize trapping of the product in the Al salts. Dropwise addition of $H_2O$ (1.41 ml in 5 ml THF, 0 to -10°, $N_2$) was followed by LiOH (1.00 g, anh, powdered, reagent). The mixture was then heated to 30-35° for 1 hr with vigorous stirring before filtration. Excess $H_2O$ and $CO_2$ were added to the filtrate as before to produce the carbonate salt. Evaporation gave only 10 mg of white solid (max. of 0.6%), and therefore, this work-up was abandoned.

Third Reduction of β-Alacreatinine (6): Experiment 5-3

β-Alacreatinine hydrochloride (6) (1.639 g, 10.96 mmol[12]), was reduced with LAH/THF (20.0 ml, 47.0 mmol) in ∿250 ml of THF under the same conditions as the first two runs. No aliquots were taken, the reaction time was 24 hr (the same as Experiment 5-2), and the work-up was exactly analogous to Experiment 5-1. The product was isolated as the HCl salt and crystalized from isopropanol/ether to give 274 mg (18.5%, vacuum desiccated over $P_2O_5$) of slowly deliquescent product melting sharply at 153° (lit. mp 127-129°[33]).

NMR ($D_2O$): Identical to run 1; very clean.

Purification of 2-Amino-3,4,5,6-tetrahydropyrimidine (13): Experiment 5-4

Al salts (2.120 g, 39.6% of total salts, air dried, saved from first reduction of β-alacreatinine (6), Experiment 5-1) containing a theoretical maximum of 159 mg (1.173 mmol) of product (13) were dissolved in HCl (24 ml, 4 M aq). This solution was chilled before careful neutralization with NaOH (∿7 ml, ∿12 M aq) to a pH of 8.5; the pH was re-adjusted to 7.00 by dropwise addition of HCl (10%, 2.7 M) giving a total of ∿35 ml of solution plus $Al(OH)_3$ precipitate. This suspension was chilled and centrifuged at 7700 g (0°, 15 min). The supernatant was decanted, and the pellet was resuspended in $H_2O$ (30 ml). This suspension was centrifuged as above, and the supernatants were combined and lyophilized to a volume of 25-30 ml. The resulting solution (with some salt precipitated) was shaken with isopropanol (100 ml, reagent) which precipitated most of the metal chlorides. This mixture was boiled 10 min (steam bath) before vacuum filtration. Evaporation of the filtrate gave a white precipitate which was again boiled with isopropanol (100 ml, reagent) and vacuum filtered (hot). The filtrate was evaporated to dryness,

and the precipitate was dried at 15μ/23° for 4.5 hr yielding 370 mg of salt (233%). A trace of this material gave a red flame test (Li ion present).

CIMS: Main peak: 99 amu

Small trace: 100 amu (cyclic urea)

This mixture of compound (13) and LiCl (370 mg, ∿4:1 LiCl to guanidinium salt) was dissolved in $H_2O$ (5 ml, distilled, deionized) and washed onto a cation exchange column (12.0 ml, preparation: see Experiment 8-2) which had been previously washed with HCl (aq) (500 ml, 1 M) and $H_2O$ (1 liter, distilled, deionized, slow drip overnight). As before (Experiment 8-2), the HCl from salt splitting was eluted with $H_2O$, and the elution of lithium ion with HCl (∿100 ml, 0.3 M, aq) was followed with the flame test. The guanidinium ion was then eluted with HCl (200 ml, ∿10 M, conc. aq). Evaporation to an oil, azeotrope drying with ethanol (2 x 50 ml, abs) gave 65 mg of crude material which was crystallized (isopropanol/ether) and vacuum desiccated ($P_2O_5$) to produce 55 mg (34.6% recovery or 25% yield, based on Experiment 5-1) of crystals mp 153°. Since the original reduction (Experiment 5-1) produced 28%, the total yield of (13) is 53%.

Reduction of Creatinine (7) in Tetrahydrofuran: Experiment 6-1

LAH (2.0 g, 52.6 mmol, Alfa) was suspended (magnetic stirring) in THF (100 ml, fresh bottle) under $N_2$. The suspension was chilled to -25°, and creatinine (7) (1.0 g, 8.85 mmol, Eastman) was added in one portion (internal temperature rose to -24°). The bath was changed to 0° $H_2O$ (no ice) and allowed to come to ∿22°. Stirring was continued for 29 hr, and the reaction was then chilled to 0° (ice bath). Water (1.0 ml in 5 ml THF) was dripped in slowly enough to maintain the internal

temperature <12°. Similarly, NaOH (2.0 ml, 5 M, aq) was added causing the reaction to turn to sludge and stop stirring. The final dropwise addition of $H_2O$ (3.4 ml) was made at ∿23° and mixing was accomplished by alternately swirling the flask and magnetically stirring the contents. The aluminum salts were vacuum filtered and washed with THF (40 ml, anh). The carbonate salt of the product was formed by blowing through a tube into the filtrate. The pricipitate was very hygroscopic, however, and therefore unsuitable for isolation of product. After removing a trace of the carbonate salt for a mass spec., the filtrate was evaporated and the residue was dissolved and diluted to 100 ml with methanol (anh, reagent). Aliquots (25 ml) were then taken to form various salts. Dry HCl and evaporation gave 134 mg (max yield possible, 52%) of mixed crystals and oil. This material was hygroscopic and was therefore discarded. The sulfate salt was prepared using ∿stoichiometric concentration of $H_2SO_4$ (assumed 60% yield), but this material was also hygroscopic. A third aliquot was combined with p-toluenesulfonic acid (190 mg, assumes 50% yield), and, after evaporation, dry white cyrstals were obtained. This material was dried at high vacuum, dissolved in hot ethanol, cooled and layered with ether (∿5 ml, anh). After diffusion at ∿23° and chilling (0°) overnight, the crystals were vacuum filtered and washed with ether (2 x 15 ml, anh). Yield: 199 mg (36%); mp 166-8°. Recrystallization from ethanol/ether and again from isopropanol produced analytically pure colorless needles; mp 170-1° (dried at 100°/25μ for 3 hr).

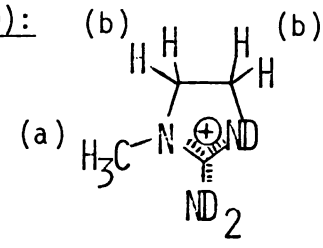
Analyzed for $C_4H_9N_3 \cdot TsOH$:

| Calculated: | Found: |
|---|---|
| C = 48.70% | 48.94% |
| H = 6.32% | 6.11% |
| N = 15.49% | 15.22% |

CIMS ($CO_3^=$ salt): Parent peak = 99 amu

Trace peak = 97 amu

NMR ($D_2O$):




a: 2.83 δ (singlet)

b: 3.53 δ (singlet)

Second Creatinine (7) Reduction in Tetrahydrofuran: Experiment 6-2

Creatinine (7) (496 mg, 4.38 mmol, desiccated 5 hr at 80°/15 μ) was suspended in THF (100 ml, freshly distilled from LAH, $N_2$ atmosphere, magnetic stirring) and the system was chilled to 0° (internal) witn an ice-salt bath. A homogeneous solution of LAH (3.3 ml, 1.66 M, 5.48 mmol, 25% molar excess, see Discussion Section 2 for prep) was syringed into the reaction; stirring was continued at 0° for 5 min, and then the bath was removed. Evolution of $H_2$ was observed upon addition of LAH, but at no time did the reaction become homogeneous. After stirring 30 hr at ∿23°, the reaction was chilled to 0° (internal). $H_2O$ (0.2 ml, in 5 ml THF) was added dropwise by syringe followed by NaOH (aq) (0.2 ml, 6.25 M) and finally, $H_2O$ (0.5 ml in 5 ml THF). The aluminum salts were vacuum filtered, and methanol (5 ml, anh, reagent) was added to quench peroxide formation. p-Toluenesulfonic acid (1.0 g, 5.8 mmol, slightly wet) was dissolved in methanol (∿10 ml, reagent) and added to the reaction filtrate. On chilling, no crystallization occurred, and evaporation produced an oil. After azeotrope drying (add ∿30 ml, anh ethanol and evap.), the oil was shaken with ether (∿30 ml, anh) to produce a crystaline solid which caked in the flask. The ether was decanted and discarded. The crude product was then scraped free from the wall of the flask, refluxed briefly with a second portion of ether (∿10 ml, anh),

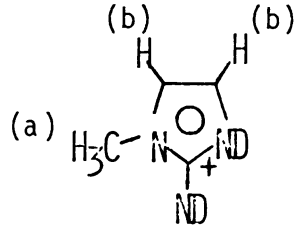
and vacuum filtered without cooling (this removes excess acid). Crude yield: 125 mg (29%) of mixed guanidine and imidazole.

CIMS (NaOMe added to TsOH salt):

Major peak = 97 amu; 1-methyl-2-aminoimidazole (11)

Minor peak = 99 amu; 1-methyl-2-amino-2-imidazoline (10).

NMR ($D_2O$):




a: 3.39δ (singlet)

b: 6.74δ (quartet)

63 mole %; imidazole (11)

37 mole %; guanidine (10)

Third Creatinine (7) Reduction: Experiment 6-3

Creatinine (7) (385 mg, 3.41 mmol, powdered) was suspended in THF (100 ml, distilled from LAH) by magnetic stirring ($N_2$ atmosphere) and then chilled to -40° (internal, dry ice-methanol-$H_2O$ bath). LAH solution (10.0 ml, 2.35 M; 6.9 x molar excess) was added quickly, by syringe, producing $H_2$ and a temperature rise to -35° (internal). After changing to a 0° bath and stirring 5 min, the entire reaction became homogeneous. Evolution of $H_2$ had slowed considerably but had not stopped, and, therefore, the reaction was stirred at 0° for 30 min (no $H_2$ bubbles). During this 30 min period, a fine white precipitate formed and persisted until work-up. After 30 min at 0°, the reaction was stirred 28 hr at ∿23°. During this 28 hr period, aliquots (0.4 ml) were taken at 10 hr and 23 hr. In each case, the aliquot was quenched in methanol (5 ml, abs) at -78° and then evaporated at 23°/∿1mm. A few drops of methanol and a few milligrams of $NaOCH_3$ were added just prior to mass spec. work.

Aliquot 1 (10 hr) CIMS:

1) Approximately equal amounts: guanidine (10) (99 amu) and imidazole (11) (97 amu).

2) Trace: N-methylethylenediamine (74 amu)

Aliquot 2 (23 hr) CIMS:

1) Major peak: N-methylethylenediamine

2) Approximately equal amounts: guanidine and imidazole

Since the mass spectra implied that the optimum reaction time had been passed, the reaction was discarded.

Fourth Creatinine (7) Reduction: Experiment 6-4

The reaction was set up exactly as in the third reduction except that an aliquot was taken after 1 hr at ∿23°.

Aliquot 1 (1 hr) CIMS:

1) Approximately equal amounts: guanidine and imidazole.

2) No starting material.

Since the starting material appeared to have been consumed, the reaction was worked up after 2 hr at ∿23° using the method described for the second creatinine reduction (1 ml $H_2O$ in 4 ml THF; 1 ml, 5 M NaOH, and 2.5 ml $H_2O$ in 2.5 ml THF). Crystalization from ethanol/ether gave 224 mg (21%) crystals, mp 168-70°.

CIMS: of material caked in the reaction flask shows mostly 113 amu (creatinine).

Fifth Reduction of Creatinine (7): Experiment 6-5

The conditions for the original creatinine (7) reduction (Experiment 6-1) were repeated with the following changes: the creatinine was suspended in THF (distilled from LAH) and chilled to -60° before adding the LAH in one portion (briefly stop $N_2$ flow). After 30 min at -56°

and 30 min at 0°, the reaction was stirred at room temperature for 30 hr. After the usual work-up[26], the filtrate was acidified (acetic acid, 1.0 ml, glacial) and stored at 0° during the extraction of the metal salts. The aluminum salts were suspended immediately in $H_2O$ (10 ml, dist.) and NaOH aq. (12.5 M, Mallinckrodt) was added slowly to pH = 14 (allowing the Al salts to air dry produced base insoluble carbonates). Dissolved products were then derivatized and extracted exactly the same as with the model system (Experiment 8-17). The dried, filtered $CH_2Cl_2$ extract was combined with the stored reaction filtrate and the solution was evaporated to an oil. Following the method in Experiment 6-6, the residue was shaken with methanol, $H_2$ (42 psig), PdO (600 mg), and $PtO_2$ (60 mg). After 12 hr, the reaction was filtered, tosic acid (1.683 g, ∿8.85 mmol, monohydrate, Eastman) was dissolved, and volatiles were evaporated. Traces of HCl, acetic acid, and $H_2O$ were removed by evaporating isopropanol (3 x 70 ml, reagent) before cyrstallizing from ethanol/ether to produce 1.299 g (54.1%) of tosylate salt, mp 169-70°.

NMR ($D_2$): Identical to NMR of the product (10) in Experiment 6-6.

Catalytic Reduction of 2-Amino-1-methylimidazole (11): Experiment 6-6

A mixture of 2-amino-1-methylimidazole (11) and 2-amino-1-methyl-2-imidazoline (10) as their tosylate salts (145 mg, 0.54 mmol, 2:3 imidazole to guanidine by nmr, combined material from LAH reductions ) was dissolved in methanol (50 ml, reagent) and added to a pressure bottle. Palladium oxide (100 mg, Englehard Ind.) was powdered, mixed with $PtO_2$ (10 mg, MCB), chilled (may not be necessary; reduced metals can ignite lower alcohols on initial contact), and transferred to the reaction bottle with methanol (50 ml, reagent). Acetic acid (10 ml, glacial, Du Pont) was added to maintain acidity and the mixture was shaken (Parr

low pressure shaker) with $H_2$ (40 psig, 20°) for 17 hr. The reaction was vacuum filtered (med. frit), evaporated, and protic solvents were purged by evaporating $CH_2Cl_2$ (3 x 30 ml, reagent) from the product.

NMR ($D_2O$):

2.82 δ (s, 3 H, guanidine methyl), 3.52 δ (s, 4 H, guanidine ring), 2.34 δ (s, 3 H, tosylate methyl), 7.56 δ (q, 4 H, tosylate Ar). Clean guanidine; no trace of imidazole (11) (shifts of analytically pure material; 2.83 δ and 3.53 δ, Experiment 6-1).

Reduction of Methylcreatinine (8): Experiment 7-1

Methylcreatinine free base (8) (257 mg, 2.02 mmol[34]) was placed in a 3-neck flask with a magnetic stirring bar and internal thermometer, the flask was flushed with dry $N_2$, and tetrahydrofuran (50 ml, reagent) was distilled from LAH directly into the reaction flask under $N_2$. The flask contents were stirred and cooled to -68° before addition of LAH/THF solution (1.50 ml, 2.4 M, 3.6 mmol). Some LAH appeared to precipitate temporarily at this temperature. On gradual warming, $H_2$ evolution occurred slowly, stopping again when the reaction reached -45° (over about 15 min); the reaction was very nearly homogeneous at this point. The bath was changed to ice/water and the reaction was allowed to stir and warm slowly overnight. After 10 hr the reaction was cloudy; a 2.00 ml aliquot was removed, quenched in methanol (20 ml, -65°), evaporated and a CI mass spectrum was taken.

CIMS:

clean 111 amu (aromatic compound)

small trace 113 amu (guanidine)

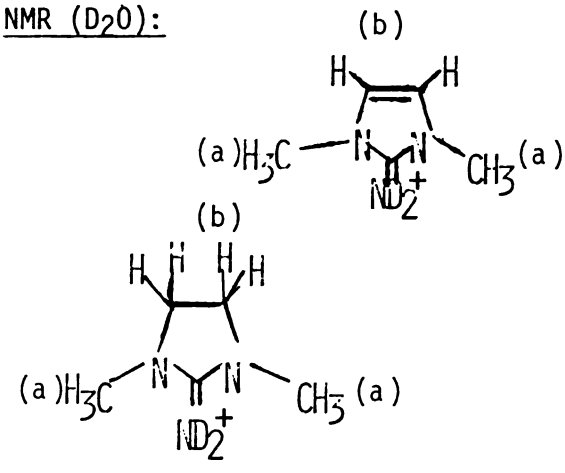
no trace 127 amu (SM)

In the hope that the 111 amu compound might be an intermediate which would reduce to the guanidine (113 amu), stirring (23°, $N_2$) was continued for an additional 8 hr before taking a second aliquot for mass spectrometry.

CIMS: Almost identical to the above spectrum.

The reaction was worked up by the usual method[26], and evaporation of the THF gave only 19 mg oil, the rest of the material being trapped in the aluminum salts. In order to get enough material for an nmr, the aluminum salts were dissolved in HCl aq, neutralized with NaOH aq and centrifuged to remove $(AlOH)_3$. The supernatant was evaporated and extracted with isopropanol to remove most of the alkali metal salts. This whole process has been described in detail (Experiment 5-4).

NMR ($D_2O$):




(a) 3.20 δ (s)
(b) 6.75 δ (q)

(a) 2.87 δ (s)
(b) 3.46 δ (s)

These tentative assignments were made by analogy with the results from the reduction of creatinine (Experiments 6-1 and 6-2), and integration suggests roughly equal amounts of each compound, plus the presence of other unidentified material. Estimated total material recovery was only about 35%.

Second Reduction of Methylcreatinine (8): Experiment 7-2

Methylcreatinine bisulfate (704 mg, 3.13 mmol, crystallized two times from isopropanol and vacuum desiccated over $P_2O_5$[34]) was reduced in THF (90 ml, distilled from LAH) with LAH/THF (9.0 ml, 2.4 M, 22 mmol) following the procedure in Experiment 3-2. The work-up, derivatization, and extraction was done exactly the same as in the creatinine reduction (Experiment 6-5). The hydrogenation was also the same as for creatinine, except that the catalyst loading was cut in half. Evaporation of ethanol (3 x 50 ml, abs), crystallization from isopropanol/ether, and vacuum desiccation ($P_2O_5$) gave 456 mg (51%) of tosylate salt, mp 180-1°.

NMR ($D_2O$):

2.87 δ (s, 6 H), 3.46 δ (s, 4 H), 2.34 δ (s, 3 H, tosylate methyl), 7.27-7.85 δ (q, 4 H, tosylate aromatic).

Analyzed for $C_5H_{11}N_3 \cdot TsOH$:

| Calculated: | Found: |
|---|---|
| C = 50.51% | 50.56% |
| H = 6.71% | 6.73% |
| N = 14.72% | 14.69% |

Aluminum Salt Experiments: Experiment 8-1 [Combined Material from Experiment 6-1 Through 6-4]

1. Al salts (suspended in $H_2O$, pH 14) were shaken with 8-hydroxyquinoline (dissolved in toluene). A yellow color appeared in the $H_2O$ layer, and substantial complexing appeared to occur but this complex formed a solid between the liquid phases rather than dissolving in the toluene as hoped.

2. Al salts were dissolved in concentrated HCl (aq) (gas evolved) and slightly more than a 3 x molar excess of 8-hydroxyquinoline was

added. The pH was raised to ∿7 with NaOH (10%, aq) producing an easily filterable solid.

3. Al salts without chelating agent were dissolved in concentrated HCl (aq) (gas again evolved) and the pH was raised to ∿7 with NaOH (10% aq) producing an unfilterable precipitate of $Al(OH)_3$. This suspension was centrifuged at ∿5000 g producing a firm pellet of $Al(OH)_3$ with a clear supernatant which could be easily decanted.

4. In experiment 1 of this series, the Al salts were not completely soluble in $H_2O$ (pH 14) as they should have been. The base solubility was rechecked by stirring Al salts (0.5 g) in $H_2O$ (400 ml, pH 14) for 30 min at ∿23°. A solid phase was still present. A second portion of Al salts (0.5 g) was dissolved in concentrated HCl (5 ml, aq) (gas evolved) and this solution was made basic with NaOH solution to give a pH of 13 (total volume of 20 ml) at which point all precipitated material redissolved. Apparently, air-drying of the Al salts after a reduction work-up causes formation of base insoluble carbonate salts.

Model Ion Exchange Removal of Lithium Ion: Experiment 8-2

A equimolar mixture of LiCl (360 mg, 8.5 m eq, Allied Chem.) and N, N'-dimethylguanidine hydrobromide (1.428 g, 8.5 meq[28]) was dissolved in $H_2O$ (20 ml), and the resulting solution was applied to a cation exchange column (BioRad AG 50W-X8, hydrogen form, 200-400 mesh, 20 ml bed volume, 34 meq , poured in 50 ml buret). Elution of HCl and HBr with $H_2O$ (salt splitting) was followed with pH paper and was complete after 50 ml of eluant. At this point no lithium ion or guanidinium ion had eluted, and, during the elution of acid, a band of slightly lighter colored resin developed at the top of the column. The front of this band continued to move down the column until ∿5 ml of the column bed

were lightened. The appearance of this band, later found to be associated with the guanidinium ion, proved to be helpful in monitoring the separation. After elution of the acids, the eluting solvent was changed from $H_2O$ to 1 M HBr (aq). After slow elution of 20 ml, the first positive lithium ion test (red flame) was observed (one drop of eluant was caught with a stainless steel spatula and heated with a Bunsen burner). Continued elution with 1 M HBr (aq) (total 68 ml) removed all the lithium ion at which point the front of the guanidinium band had traversed 95% of the column. Elution of the guanidinium hydrobromide occurred as follows: evaporation of 70 ml of 1 M HBr (aq) eluant gave 750 mg of salt; 110 ml yielded 350 mg; 120 ml produced 86 mg of salt (obviously considerable tailing occurs). Thus, a final combined fraction of 300 ml of 1 M HBr (aq) produced a total recovery of 1.186 g (83%) of salt.

Derivatization and Extraction of 2-Amino-3,4,5,6-tetrahydropyrimidine (13), First Model System: Experiment 8-3

2-Amino-3,4,5,6-tetrahydropyrimidine (13) picrate (140 mg, 0.427 mmol, Experiment 5-1) was suspended in $H_2O$ (35 ml, dist) and concentrated HCl (15 ml) was added slowly converting the yellow salt to near white needles of picric acid. The picric acid was extracted from the $H_2O$ with ethyl ether (1 x 50 ml, 2 x 30 ml, fresh anh reagent) and discarded. The $H_2O$ solution was placed in a round bottom flask and basified with NaOH (10 ml, 12 M) to pH 14 (with cooling in an ice bath). Ethyl ether (75 ml, anh reagent) and carbobenzoxychloride (1.0 ml, ~6 mmol[35]) were added to the flask, and the mixture was magnetically stirred vigorously for 1.5 hr at 23°. The $H_2O$ layer was separated and extracted with ethyl ether (2 x 30 ml, reagent). The pooled extract was washed with $H_2O$ (3 x 10 ml, distilled, final wash showed pH = 6). The ether was evaporated and 10 ml concentrated HCl was added for cleavage of the

carbobenzoxy groups; two liquid phases (one cloudy) resulted. Acetic acid (12 ml, glacial) was added to get a 1 phase system, and the reaction was allowed to stand overnight at 23° (air atmosphere). The reaction was then heated (steam bath) to check for $CO_2$, and gas evolution was indeed observed. Heating was therefore continued for 0.5 hr before evaporation of $H_2O$, HCl, and AcOH. The residue was dissolved in ethanol (4 ml, abs) and layered with ether (6 ml, anh reagent). Diffusion of the ether left a sticky white mass from which the solvent was decanted. A total yield of 36 mg (max of 62% as the HCl salt) was thus obtained. Both the procedure and the product were discarded as wholly unsatisfactory.

Derivatization; Second Model System: Experiment 8-4

Using a procedure for the carbobenzoxylation of arginine[17] as a guide, 2-aminoimidazoline hydrobromide (14) (3.00 g, 18.0 mmol[16]) was placed in a 250 ml round bottom flask and dissolved in cold aqueous NaOH (36 ml, 1 N). Aqueous NaOH (45 ml, 2 N, 0°) and carbobenzoxychloride (12.3 ml, 74 mmol, 0°[35]) were added alternately in five portions with continued cooling (ice bath) and shaking (glass stopper in flask). This addition process should be spread over 25 min (i.e., ∿5 min shaking and cooling time per addition). With the first addition, a firm white solid precipitated; this solid, by the end of the addition step, had become mushy due to the excess of acid chloride. Ethyl ether (100 ml, tech., 0°) was added to extract organic material, but most of the solid remained undissolved. The liquid phases were poured into a separatory funnel, and methylene chloride (200 ml, 0°, reagent) was added to the flask to dissolve solid material. The aqueous layer was separated from the combined organic material and discarded. The organic phase was washed

with $H_2O$ (1 x 100 ml, 3 x 50 ml, 0°, distilled, final pH = 5.5) and stored at 0° overnight. Solvent evaporation (∿30°) gave 13.2 g oil with a strong acid chloride smell. The oil was dissolved in acetic acid (20 ml, glacial), and concentrated HCl (5 ml, 37%) was added giving rise to a second liquid phase. The literature procedure for cleaving carbobenzoxy protected amines (using glacial acetic acid saturated with dry HCl[18]) calls for heating to 75° for 2 hr. The above two phase system, therefore, was saturated with HCl gas (giving a one phase system) and heated on a steam bath for 3 hr with continuous HCl (g) addition. The reaction was taken up in isopropanol (100 ml) and evaporated; this process was then repeated two more times. The product was then dissolved in isopropanol (10 ml) and precipitated with ethyl ether (50 ml, abs). Chilling and filtration produced 2.38 g (109%) of white sticky material (as in Experiment 8-3): Vacuum desiccation of the product over $P_2O_5$ gave 2.187 g (100.8%) of white caked material.

NMR (1:1, $D_2O/d_6$DMSO) very dirty:

Main peaks; 7.5 δ (phenyl)
3.65 δ (guanidine)
3.5 δ (diamine)

Minor peaks; 3.7 δ to 2.5 δ

CIMS, In order of estimated concentration:

85 amu: cyclic guanidine

86 amu: cyclic urea

60 amu: ethylene diamine

103 amu: $H_2NCH_2CH_2NHCONH_2$

Derivatization, Third Model System: Experiment 8-5

Using exactly the same derivatization procedure as in the second model system (Experiment 8-4) 2-aminoimidazoline hydrobromide (14) (1.000 g, 6.00 mmol[16]) was acylated with carbobenzoxychloride (4.0 ml, ∿24 mmol[35]). After acylation, glycine (2.70 g, 36 mmol, powdered, Aldrich) and methlyene chloride (80 ml, reagent) were added and the reaction was shaken and cooled another 5 min to remove excess acid chloride. The organic phase was separated and washed with $H_2O$ (1 x 20 ml, 3 x 10 ml; still basic, therefore 3 x 15 ml, final wash; pH = 6.0). The organic layer was dried with $Na_2SO_4$ (∿10 g, anh) and evaporated to 4.80 g of cloudy oil (faint acid chloride smell, some $H_2O$ probably present). Storage of this material in the freezer overnight produced white feather-like crystals which were used for the first $H_2$ cleavage (see Experiment 8-8).

Derivatization; Fourth Model System: Experiment 8-6

Conditions and quantities were the same in this run as in the previous one (Experiment 8-10), except that the glycine treatment and extraction were altered. In this case, glycine (5.4 g, Aldrich) was dissolved in $H_2O$ (30 ml) and the pH was raised to 14 with aqueous NaOH (5 ml, ∿12 M). This solution was chilled in an ice bath before the acylation reaction. Following the acylation, the glycine solution and methylene chloride (30 ml, 0°, reagent) were added to produce two liquid phases. The mixture was stirred vigorously (magnetic stirrer, ice bath, air atmosphere) for 30 min, whereupon methylene chloride (50 ml, 0°, reagent) was added before separation of the organic phase. The $H_2O$ layer was extracted with methylene chloride (1 x 40 ml, 0°, reagent) and the extracts combined to form extract A (total = 120 ml). The $H_2O$ layer was extracted

again with methylene chloride (2 x 40 ml) and this material was labelled B.

A. Extract A (120 ml, cloudy) was dried several hours with $K_2CO_3$ (6 g, anh) which removed most of the cloudiness. The extract was then filtered, the $K_2CO_3$ washed with methylene chloride (20 ml), and the solution washed with $H_2O$ (25 ml, 0°, pH = 6.5 after wash). The organic layer was dried again with $K_2CO_3$, filtered, and evaporated to give 4.05 g of oily acyl derivative which crystallized within seconds. Further drying (∿1 mm, 38°, 2 hr) gave 3.42 g (161% assuming pure diacyl derivative) of crystaline material which still smelled of acid chloride.

B. Extract B (80 ml) was treated exactly as extract A with proportionatly less materials to give 60 mg of oil with no acid chloride odor.

The material from extracts A and B was combined by dissolving in methanol (dilute to 200 ml, reagent, shake to dissolve). This methanol solution was split into two equal portions (100 ml each) and used for $H_2$ cleavage (Experiment 8-9) and HBr cleavage (Experiment 8-12).

Derivatization; Fifth Model System: Experiment 8-7

2-Aminoimidazoline (14) (2.922 g, 17.6 mmol[16]) was acylated in the same manner as the three previous runs (Experiments 8-4, 8-5, and 8-6) except that both the acylation and extraction were done in the cold room (0-4°) with ice baths (all equipment and materials were pre-chilled). No glycine was used in this run. Methylene chloride (70 ml, reagent) was added to the acylation product, and the mixture was shaken to dissolve the solids. The mixture was added to a separatory funnel and the flask was rinsed with methylene chloride (30 ml, reagent). After shaking and separating this mixture, the $H_2O$ layer was extracted again with methylene chloride (1 x 75 ml, 1 x 50 ml, reagent). All extracts were

combined and dried with $K_2CO_3$ (~5 g, anh, swirl until near clear). The mixture was allowed to settle and the solution decanted into a clean separatory funnel. The $K_2CO_3$ was rinsed with methylene chloride (30 ml) which was also slowly decanted into the funnel. The total extract (~260 ml) was then washed with $H_2O$ (50 ml, pH = 6.3 after wash) and redried with $K_2CO_3$ (5 g, anh). The extract was decanted into a graduate cylinder ($K_2CO_3$ washed with 30 ml $CH_2Cl_2$) and split into 2 equal portions, each of which were used for $H_2$ cleavage trials (Experiments 8-10 and 8-11). The extracts were stored over $K_2CO_3$ (2 g, anh) at 0° for 1-3 days pending hydrogenolysis. Total cold room time was about 2 hr.

First Hydrogenolysis: Experiment 8-8

Crude crystaline material from the third model derivatization (theoretically 6.0 mmol, Experiment 8-5) was shaken with aqueous methanol (100 ml, reagent, 10 ml $H_2O$ added) to dissolve it for hydrogenolysis. Since the material appeared to be dissolving very slowly, ethyl acetate (20 ml, reagent) was added, shaking was continued, and the mixture was also warmed gently until a solution was obtained. This solution was then added to a pressure bottle containing palladium catalyst (1.5 g, 10% Pd on charcoal, MCB, chilled and premoistened with methanol), and the mixture was shaken with $H_2$ for 12 hr at ~20° ($H_2$ at 40 psig or 3.72 atm. absolute). The catalyst was then removed by vacuum filtration through a fine fritted glass funnel and washed with ethanol (10 ml, abs). The filtrate was evaporated to 370 mg of yellow oil; this material was diluted to 100.0 ml and divided into two equal portions. Preliminary experiments with the first 50 ml portion suggested that the picrate salt would be a good method of product isolation. To get an accurate yield, picric acid (860 mg, 10-12% $H_2O$, ~3.3 mmol, J.T. Baker) was dissolved

in the second half of the cleavage product solution (in 50 ml methanol) and the methanol was evaporated. The resulting yellow solid was crystallized from $H_2O$ and air dired to give 740 mg (2.36 mmol, 78%) yellow needles, mp 215-7° (literature mp 223-4°[32]). A second crop (185 mg air dry, mp 119-120°) was a much lighter yellow, a different crystal form, and appeared to be nearly pure picric acid (literature mp 121-3°[36]). The first crop was recrystallized from $H_2O$ and vacuum desiccated (100°, 25 μ, 11 hr) to give 688 mg (73%) of analytically pure yellow needles, mp 222-3°.

Analysed for $C_3H_7N_3 \cdot$ Picrate:

| Calculated: | Found: |
|---|---|
| C = 34.40% | 34.59% |
| H = 3.21% | 3.30% |
| N = 26.75% | 26.83% |

NMR ($d_6$-DMSO):

Broad peak: 7.64 δ (3 H)

Sharp singlet: 3.55 δ (4 H)

The nmr of an authentic sample of 2-aminoimidazoline picrate in $d_6$-DMSO was identical to the nmr above in all respects.

CIMS: Only one peak: 85 amu

Second Hydrogenolysis: Experiment 8-9

A portion of the crude acyl derivative from the fourth model derivatization (theorectially 3.0 mmol, in 100 ml methanol, Experiment 8-6) was mixed in a 250 ml pressure bottle with premoistened catalyst (0.5 g, 10% Pd on charcoal, MCB). This mixture was shaken with $H_2$ (40 psig, 3.72 atm absolute) for 22 hr at ∿20° before filtration of the catalyst (fine fritted glass funnel) and evaporation of the methanol to give

0.42 g of mixed oil and crystals. This material was dissolved in methanol (100 ml, reagent), hydrobromic acid (3 ml, 5.9 M, slightly yellow commercial, 48%) was added, and the solution was evaporated to dryness. Attempts at crystallization were frustrated by formation of a red precipitate whenever the crude HBr salt was heated in an alcohol solution. Crystallization from methanol-ether gave 200 mg (40%) of salmon colored material which still contained benzyl groups by nmr.

Third Hydrogenolysis: Experiment 8-10

Half of the crude acyl material from the fifth model derivatization (theoretically 8.75 mmol, Experiment 8-7) which had been stored over $K_2CO_3$ (anh) in methylene chloride (at 0°) was filtered and evaporated (at 0°). The residue was taken up immediately in methanol (150 ml, 2.0 ml $H_2O$ added), and the solution was shaken with palladium (1.5 g, 10% Pd on charcoal, MCB) and $H_2$ (40 psig, 3.72 atm absolute) for 14 hr. Filtration of the catalyst and evaporation produced 1.1 g of yellow solid. This material was dissolved in methanol (dilute to 50 ml) and a 5.0 ml aliquot was removed for nmr. This aliquot was stripped dry, and the residue was dissolved in $D_2O$ (sample was slightly cloudly, filtered through cotton).

NMR ($D_2O$): Clean singlet: 3.7 δ

To the remaining 45 ml of solution, hydrobromic acid (5.0 ml, 5.9 M, distilled[37]) was added causing the solution to turn dark yellow. This material was evaporated, hydrobromic acid (5.0 ml) was added again, and the solution was again evaporated to give a mixture of crystals and viscous purple oil. The product was dissolved in $H_2O$ (100 ml, dist.) causing the color to change from purple to yellow, and this solution was extracted with ethyl ether (3 x 20 ml, reagent) which removed most

of the yellow color. On evaporating the $H_2O$ layer, however, a purple residue was again obtained. Crystallization from isopropanol/ethyl ether produced 900 mg (69%) of light purple solid which was not characterized.

Fourth Hydrogenolysis: Experiment 8-11

One third of the methylene chloride extract from the fifth model derivatization (theoretically 5.83 mmol, Experiment 8-7) was evaporated (at 0°) and taken up in methanol (100 ml, reagent, 1.3 ml $H_2O$ added). This solution was shaken with palladium (1.8 g, 10% Pd on charcoal, MCB) and $H_2$ (40 psig, 3.7 atm. absolute) for 48 hr at 17°. The catalyst was filtered, and a 10 ml aliquot of the filtrate was evaporated and pumped dry (25 μ, 23°, 3 hr) for an nmr. The resulting yellow solid was insoluble in $H_2O$.

NMR ($d_6$ DMSO): Singlet: 3.5δ
Singlet: 5.0δ(broad)
Singlet: 7.8δ(broad)

Hydrogen Bromide Cleavage of Carbobenzoxyderivative: Experiment 8-12

The methanol solution containing half the crude acyl material from the fourth model derivatization (Experiment 8-6) was evaporated and the residue dissolved in methylene chloride (dilute to 90.0 ml, reagent). Two thirds of this solution (theoretically 2.00 mmol) was evaporated to near dryness; the final solvent traces were removed with a stream of dry $N_2$ (atmospheric pressure, 23°) to avoid loss of material due to spattering (while on the evaporator). With the literature procedure for unblocking amines[18] as a guide, acetic acid saturated with HBr (10 ml solution; glacial AcOH was saturated with dry HBr by magnetic stirring at -10°; stored at 0°) was added to the acyl derivative (which was cloudy).

On addition of the acid, the cloudiness was replaced almost instantly by a white precipitate. Since a homogeneous reaction was both expected and desired, more acetic acid/HBr (60 ml) was added, but the white precipitate remained undissolved. Magnetic stirring overnight (drying tube, 23°) also failed to dissolve the suspended precipitate. After 24 hr, ethanol (100 ml, abs) was added and the mixture was evaporated to dryness to give 238 mg (54%) of crude salt which remained unmelted at 240°.

NMR ($D_2O$): One clean singlet: 3.5 δ

CIMS: Major peak: 60 amu (ethylene diamine)
Trace peak: 85 amu (guanidine)

First Hydrogen Bromide Control: Experiment 8-13

2-Aminoimidazoline hydrobromide (14) (339 mg, 2.04 mmol[16]) was treated with acetic acid saturated with HBr (70 ml, see Experiment 8-12) in the same manner as the derivative in the previous experiment. This time, however, the system became homogeneous. The slightly yellow reaction mixture was diluted with ethanol (100 ml, abs) and evaporated to dryness as in the previous experiment (8-12). The white solid thus obtained was crystallized from isopropanol/ether to give ∿100 mg (34%) air dried salt, mp 170-5° (literature mp 125-6°[16]).

NMR ($D_2O$): Singlet: 3.7 δ (4H)
Singlet: 2.3 δ (3H)

The isolated material was obviously not starting material. On the hypothesis that 2-aminoimidazoline acetate had been isolated, hydrobromic acid (5 ml, 5.9 M, distilled) was added to the product and the solution was evaporated. This addition and evaporation was repeated a second time and an nmr was taken.

NMR ($D_2O$): Singlet: 3.7 δ 2-aminoimidazoline (14)
Trace singlet: 2.3 δ trace of acetate

Second Hydrogen Bromide Cleavage: Experiment 8-14

To verify the results of Experiments 8-12 and 8-13, both the cleavage and the control experiments were repeated under exactly identical conditions. One sixth of the methylene chloride extract from the fifth model derivatization (theoretically 2.92 mmol, Experiment 8-7) was carefully evaporated and treated with acetic acid/HBr (60 ml, see Experiment 8-12). The resulting precipitate was magnetically stirred (drying tube) for 12 hr at 23°. The still homogeneous reaction was chilled briefly and ethanol (100 ml, abs) was added before evaporation to dryness. Four portions of hydrobrimic acid (5 ml each, 5.9 M) were added and evaporated to remove acetic acid. Drying under high vacuum (25 μ, 23°, 8 hr) produced 434 mg of white salt which was dissolved in $H_2O$ (dilute to 20.00 ml, distilled) and split into two equal portions. One portion was evaporated and crystallized from methanol (∿15 ml, reagent) to give 204 mg (63%) of analytically pure white plates, mp >300°.

CIMS: Major peak: 60 amu (ethylenediamine)

Small trace: 85 amu (cyclic guanidine, undetectable by analysis or nmr)

NMR ($D_2O$):

Clean singlet; 3.4 δ

This nmr sample was mixed with authentic 2-aminoimidazoline HBr[16] as a reference and a second nmr was taken.

NMR ($D_2O$):

Singlet: 3.4δ

Singlet; 3.7δ (difference = 18 Hz)

Analyzed for $C_2H_{10}Br_2N_2$:

| Calculated: | Found: |
|---|---|
| C = 10.82% | 11.03% |
| H = 4.54% | 4.56% |
| N = 12.62% | 12.68% |
| Br = 72.01% | 71.98% |

Second Hydrogen Bromide Control: Experiment 8-15

2-Aminoimidazoline hydrobromide (14) (483 mg, 2.91 mmol[16]) was dissolved in acetic acid saturated with HBr (60 ml, Experiment 8-12) and magnetically stirred (drying tube) for 12 hr at 23°. The homogeneous reaction was chilled briefly and ethanol (100 ml, abs) was added before evaporation to dryness. Four portions of hydrobromic acid (5 ml each, 5.9 M) were added and evaporated to remove acetic acid. Drying under high vacuum ($25_{\mu}$, 23°, 8 hr) gave 457 mg (94%) of crude slightly yellow solid which was dissolved in $H_2O$ (dilute to 20.00 ml, distilled) and split into two equal portions. One portion was evaporated and crystallized from isopropanol/ether. Vacuum desiccation ($50_{\mu}$, over $P_2O_5$, 2 days) gave 396 mg (82%) of white crystals, mp 123-5° (literature mp 125-6°[16]).

NMR ($D_2O$): Clean singlet: 3.7 δ

This nmr sample was mixed with authentic ethylene diamine dihydrobromide as a reference and a second nmr was taken.

NMR ($D_2O$):

Singlet: 3.7 δ (2-aminoimidazoline)

Singlet: 3.4 δ (difference = 18 Hz)

Acid Stability of 2-Aminoimidazoline: Experiment 8-16

2-Aminoimidazoline hydrobromide (200 mg[16]) was refluxed in hydrobromic acid (15 ml, 48%, 5.9 M) in an air atmosphere for 3 hr. Addition of ethanol (50 ml, anh) and evaporation to dryness produced a white crystaline solid.

NMR ($D_2O$): Clean singlet: 3.7 δ

Authentic ethylene diamine 2HBr (Aldrich) was added to the nmr above as a reference.

NMR ($D_2O$):

Singlet: 3.7 δ

Singlet: 3.4 δ

Model Derivatization and Extraction of N, N'-Dimethylguanidine Hydrobromide: Experiment 8-17

N,N'-Dimethylguanidine hydrobromide (1.000 g, 5.95 mmol[25]) was acylated with carbobenzoxychloride (4.0 ml, 24 mmol, Aldrich) in the cold room (0-4°) in the same manner as before (Experiment 8-6). The excess acid chloride was quenched with glycine (3.00 g, 40 mmol, Aldrich), and the acyl derivative was extracted (in the cold) with $CH_2Cl_2$ and dryed as before.

The evaporated extract was dissolved in methanol (80 ml, reagent), powdered premoistened PdO (1.00 g, Englhard Ind.) was washed into the hydrogenation bottle with more methanol (20 ml, reagent), and the mixture was shaken with $H_2$ (43.1 psig/20°) for 20 hr. The reaction mixture was vacuum filtered and HBr (5 ml, 5.9 M, concentrated aq) was added before evaporation. Traces of HCl were then purged by evaporating two portions of ethanol (50 ml, abs) containing HBr (2 ml, 5.9 M, concentrated). Evaporation of ethanol alone (50 ml, abs) and pump drying gave 802 mg of crude salt. Crystallization from ethanol/ether yielded 760 mg (76%) of colorless crystals, mp 142-144° (literature mp, 144[16]).

Hydrogen Evolution in LAH - Acylguanidine Reactions: Experiment 9

Using a gas buret similar to that described by Brown[38], the evolution of $H_2$ in the reactions of creatinine (7) (Eastman) and dodecanoylguanidine (2) (Experiment 1-3) with LAH was measured. Reaction flasks, syringes, and needles were oven dryed (24 hr, 120°) and purged with dry $N_2$ prior to use. The THF used was distilled from LAH and kept under $N_2$ (transfers made by syringe). Both acylguanidines were

used without desiccation (neither is measurably hygroscopic).

At the biginning of each determination, syringes and needles were removed from the oven, assembled hot, purged with dry $N_2$, and sealed until needed by pushing the needle into a rubber stopper. The reaction flask (100 ml rb with a small side-arm) was then removed from the oven, the side-arm was fitted with a rubber septum, and the flask was purged with dry $N_2$ (through the septum) while cooling. The preweighed acylguanidine and a magnetic stir bar were then added to the flask (briefly interrupt $N_2$ flow); the vessel was then attached to the gas buret (greased joint, spring clamp, resume $N_2$ purge through the cold trap). THF (20 ml) was then transferred to the flask and the contents were chilled to 0° (ice bath) with magnetic stirring (maintain slow $N_2$ purge). After cooling the reaction flask 10 min, the $N_2$ inlet was removed, the vent (between the cold trap and the buret) was closed, and excess LAH/THF (2.5 ml, 2.4 M, 6.0 mmol) was injected over 15 sec. A reaction time of 10 min at 0° was allowed ($H_2$ evolution very slow by this time). Once the volume was read, the entire process was repeated (fresh glassware) with NO acylguanidine present. The volume generated from the control was subtracted from the original reading to give the correct volumes. $H_2$ was assumed to be an ideal gas, the volumes being converted to mmol by the following equation:

$$n = \frac{273\ (P_{amb} - P_{H_2O})\ V_{H_2}}{760\ (22.4)\ T_{amb}}$$

$n$ = mmol of $H_2$

$P_{amb}$ = atmospheric pressure (mmHg)

$P_{H_2O}$ = vapor pressure of $H_2O$ (mmHg) at $T_{amb}$
(gas collector contains $H_2O$)

$V_{H_2}$ = measured $H_2$ volume ( in ml, minus blank volume)

$T_{amb}$ = ambient temperature (in °K)

Results:

| Creatinine (7) | $H_2$ (mmol) | Mole Ratios $H_2$/Creatinine |
|---|---|---|
| 226 mg (2.00 mmol) | 2.24<br>2.36<br>2.32 | 1.16 ± 0.06<br>(95% confidence) |
| Average $H_2$ mmols = 2.31 | | 1.16 ± 0.14 |
| Standard deviation ( δ ) = 0.0499 | | (99% confidence) |
| Dodecanoylguanidine (2) | | $H_2$/Dodecanoylguanidine |
| 169 mg (0.700 mmol) | 2.60 | 3.80 ± 0.77 |
| | 2.72 | (95% confidence) |
| Average $H_2$ mmol = 2.66 | | |
| Standard deviation ( δ ) = 0.06 | | |

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