I. BIOSYNTHESIS OF OPIUM ALKALOIDS - SUBSTRATE SPECIFICITY AND ABERRANT
BIOTRANSFORMATIONS

II. QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS OF AROMATIC ESTERS OF
1-METHYL-4-PIPERIDINOL AS ANALGESICS

by

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ABSTRACT

Part I:

Substrate specificity associated with the biotransformations of
($\pm$)-reticuline and thebaine in <u>Papaver</u> <u>somniferum</u> L. was investigated by
feeding experiments with labeled modified precursors. The 6-ethyl analog
of [2-^{3}H]thebaine was efficiently incorporated into codeine (8.84%) and
morphine (9.28%). Among the three ethyl analogs of [1-^{3}H, 3-^{14}C]-($\pm$)-
reticuline administered to the plant, the 6-ethyl analog gave good in-
corporation into morphine (3.28%); the 4'-ethyl analog was less efficiently
incorporated into morphine 3-ethyl ether (0.44%) and morphine (0.56%);
while the <u>N</u>-ethyl analog was apparently not metabolized by the plant enzymes
with practically all the radioactive precursor recovered unchanged. The
labels in the radioactive alkaloids isolated were shown by chemical degrada-
tion to be located in the expected positions, indicating that no randomization
of the labels had occured. The ^{3}H/^{14}C ratios in the isolated alkaloids and
in the recovered precursors were the same as that of the administered pre-
cursors within experimental error, indicating that racemization of any of
the three reticuline analogs did not take place during incubation. It
appears that when reticuline was modified by replacing the 6-methyl
group on the A-ring of the tetrahydroisoquinoline moiety with an ethyl
group, the transformation to morphine took place with about the same degree
of efficiency as with reticuline itself. However, when the ethyl group was
in the benzylic portion of the molecule, some interference with the conversion
was observed. Replacement of the <u>N</u>-methyl group of reticuline with an ethyl
group completely prevented further biotransformations. The racemization of
reticuline appears to be a process of strict substrate specificity.

The biosynthesis of hydrophenanthrene alkaloids in <u>Papaver orientale</u> L. was also investigated by precursor feeding experiments. $[1-^3H$, <u>N</u>-<u>methyl</u>-$^{14}C]$-($\pm$)-Reticuline was incorporated into thebaine (0.05%) and oripavine (0.31%) with all the ^{14}C activity located in the <u>N</u>-<u>methyl</u> group within experimental error. A decrease in the $^3H/^{14}C$ ratio with 51% loss of ^3H was observed, which indicated racemization of reticuline as occurs in <u>P</u>. <u>somniferum</u>. $[2-^3H]$Thebaine was incorporated into oripavine (0.17%) without randomization of the label. The results clearly demonstrated that oripavine is biosynthesized from reticuline <u>via</u> thebaine in <u>P</u>. <u>orientale</u>.

Part II:

Substituted benzoic acid esters of 1-methyl-4-piperidinol showed analgesic activity when assayed by the mouse hot-plate method, the more potent ones falling in the morphine-codeine range. To understand how substituents on the aromatic ring affect the analgesic potency, quantitative structure-activity correlations were carried out on a series of 44 derivatives. Among the various substituent parameters included in the study, $L_{\underline{ortho}}$ (length of ortho-substituents), and B_1 (minimal width of substituents) or E_s at <u>meta</u>- and <u>para</u>-positions gave negative correlation with the potency, while lipophilicity (esp. $\pi_{\underline{meta}}$) and the ability of being a hydrogen-bond acceptor enhanced the potency. Based on the QSAR results, a substitution pattern of the phenyl group was defined for optimal activity. Implications on drug-receptor interactions and the possible binding mode of these compounds were discussed.

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Chapter I. BIOSYNTHESIS OF OPIUM ALKALOIDS - GENERAL INTRODUCTION

Complex organic molecules are abundant in nature. How these so-called natural products are constructed in living organisms has been a subject of interest to many organic chemists. In the investigation of the biosynthesis of natural products, hypotheses based on relevant structural units and reaction mechanisms of similar compounds usually precede the exploration by experiments. After radioisotopes became available in the 1950's, radiotracer techniques[1] have been widely used to prove or disprove a biosynthetic hypothesis. The incorporation of a labeled compound, without scrambling of the labels, into a natural product often indicates that the compound is a natural precursor in the biosynthesis. The isolation from the organism of the presumptive precursor compound and key intermediates along the proposed biosynthetic pathway provides supporting evidence to the results of precursor feeding experiments. The confirming proof is often provided by the demonstration of the bioconversion of potential intermediates on the biosynthetic route to the natural end product.

The combination of mechanistic reasoning, feeding experiments and isolation experiments has been successfully employed in the biosynthetic studies of a large number of natural products existing in higher plants. Among the natural products investigated, opium alkaloids are of special interest because of their unusual abundance in the opium poppy, the large variety of structural types (more than 35 alkaloids of eight different groups[2]), and their long association with mankind.

Opium, the dried milky exudate of the incised unripe seed capsules of the poppy plant, has long been used as medicine (the first undisputed

reference to poppy juice is found in the writing of Theophrastus in the third century B.C.[3]) and simply for pleasure through the euphoric effect of its main component - morphine. Among the opium alkaloids, morphine (analgesic), codeine (analgesic and antitussive), noscapine (antitussive) and papaverine (smooth muscle relaxant) are still in clinical use. The abuse of and addiction to morphine and its synthetic derivative heroin remain major causes of crime and other social problems.

The account which follows on the biosynthesis of opium alkaloids in _Papaver_ _somniferum_ starts from the amino acid tyrosine (1), an aromatic $C_6 - C_2$ building block derived from shikimic acid _via_ prephenic acid.

$$CO_2 \rightarrow$$

shikimic acid   prephenic acid   4-hydroxy-phenylacetic acid   1
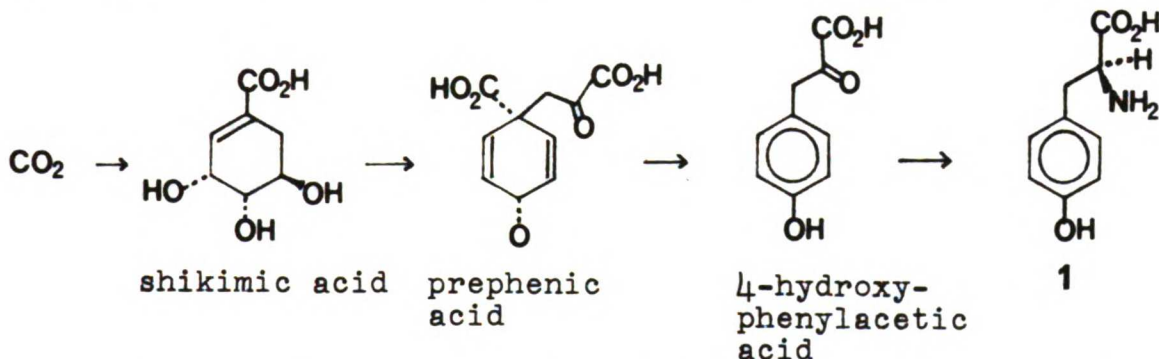
Before any experiments were performed, there had been important structural proposals which formed the basis of subsequent fruitful tracer experiments. In 1925, Gulland and Robinson[4] proposed that if bonds a and b in morphine (2) are broken and the molecule rotated, we obtain a structure whose carbon skeleton resembles that of 1-benzyltetrahydroiso-quinolines, e.g., norlaudanosoline (3).

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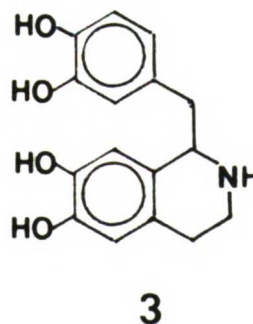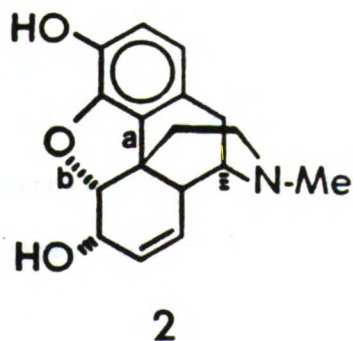
Derivatives of norlaudanosoline have been isolated from opium.

Another important proposal was made as early as in 1910 by Winterstein and Trier.[5] It was proposed that 1-benzyltetrahydroisoquinolines might be derived in the plant from two eight-carbon units, originating from the essential amino acid tyrosine (1) (cf. Scheme 1). In 1957, Barton and Cohen[6] suggested oxidative phenol coupling as the mechanism by which a suitable 1-benzyltetrahydroisoquinoline alkaloid could be converted to a hydrophenanthrene derivative.

Battersby and Harper[7] were among the first to test these ideas on the biosynthesis of morphine (2) by feeding experiments. $[\alpha-^{14}C]$-DL-tyrosine was fed to mature Papaver somniferum plants. After a suitable incubation time morphine was isolated, purified and found to be radioactive. Degradation of the recovered morphine showed that about half the radioactivity resided at position 16 of the morphine molecule. The remaining activity was shown by a separate degradative study to be located at carbon 9.[8,9] These experimental data were in agreement with the independent study of a similar nature by Leete.[10,11] It was thus established that morphine is biosynthesized from two molecules of tyrosine.

The role of 1-benzylisoquinolines as intermediates in the biosynthesis of morphine alkaloids was first studied by Battersby et al. in 1960.[12] $[3-^{14}C]$-Norlaudanosoline (3) was shown to be incorporated into morphine after incubation in the opium poppy (P. somniferum). Thin-layer chromatography of the remaining alkaloids showed that codeine (37), thebaine (26), and papaverine (17) were also radioactive. In a later study done in 1963[13], several methylated $[3-^{14}C]$-($\pm$)-1-benzyltetrahydroisoquinoline analogs were fed separately to opium poppies. Incorporations of nor-reticuline (4) and reticuline (5) into morphine was 3.2% and 7.3%, respectively, while

incorporation of norlaudanosoline (3) in a parallel experiment was 2.2%.

Tetrahydropapaverine (6) did not show significant incorporation into morphine. It is generally believed that precursors which are closer to the end product being studied give better incorporation into the product than those which are further removed on the biosynthetic pathway. Therefore, it seemed reasonable to conclude that, in the opium poppy, norlaudanosoline (3) is biosynthesized first, and then O-methylated to give nor-reticuline (4), which in turn is N-methylated to give reticuline (5) prior to further biotransformations into morphine (cf. Scheme I). Methylation of the two remaining phenolic hydroxyl groups as in tetrahydropapaverine prevents the oxidative coupling reaction necessary to give morphine.[14]

Incorporation of (+)-reticuline into morphine was also demonstrated by Barton et al.[15] with the doubly labeled [N-methyl-^{14}C, 1-^{3}H]-(+)-reticuline. The observation that reticuline is the precursor which undergoes phenolic coupling to give the morphine skeleton is in line with Barton and Cohen's original proposal[6] in that the 1-benzyltetrahydroisoquinoline precursor should carry protective groups, and in reticuline these are the two O-methyl groups.

Based on the oxygenation pattern of the 1-benzyltetrahydroisoquinoline intermediates in the biosynthesis of morphine, it was concluded[46,16] that tyrosine must be further hydroxylated in the plant to yield two 3,4-dihydroxy derivatives, which are joined by a condensation reaction to generate the benzylisoquinoline skeleton. The two units directly involved in the condensation were shown to be different.

Leete and Murrill[17] demonstrated that administration of [1-^{14}C]-dopamine to Papaver somniferum plants resulted in the formation of radioactive morphine labeled only at C-16. That dopamine or some closely related compound is one of the two units involved in the biosynthesis of 1-benzyl-

tetrahydroisoquinolines was also established by Battersby and his coworkers.[16,18] They showed the incorporation into morphine of both 3,4-dihydroxyphenyl[2-^{14}C]alanine (dopa) (8) and 3,4-dihydroxy[1-^{14}C]phenethylamine (dopamine), with the label found only in the ethanamine bridge in the morphine molecule. It seemed that tyrosine gives rise to two different units for the condensation by independent pathways, as had been suggested.[19]

A Pictet-Spengler type condensation of a β-arylethylamine with a carbonyl derivative had long been cited as the probable mechanism for genesis of the tetrahydroisoquinoline skeleton. Both 3,4-dihydroxyphenylacetaldehyde (9) and 3,4-dihydroxyphenylpyruvic acid (10) are likely candidates as the carbonyl donor.

Condensation of dopamine with the former (9) would result in direct formation of norlaudanosoline (3), while with the latter (10) norlaudanosolinecarboxylic acid (11) would be formed first. Recently, Wilson and Coscia[20] established the role of 3,4-dihydroxyphenylpyruvic acid as the most likely carbonyl donor in the biosynthesis of 1-benzyltetrahydro-isoquinolines. Feeding experiments were done with Papaver orientale (cf. Chapter 3) seedlings as well as with latex expressed from the capsules of this plant. Good incorporations of labeled dopamine and dopa into norlauda-nosolinecarboxylic acid (11) was observed. Besides, norlaudanosoline-carboxylic acid was found to be readily converted to norlaudanosoline (3) in the plant. 1,2-Dehydronorlaudanosoline (12) was suggested as the inter-mediate between 11 and 3, based on the simultaneous incorporation of dopa into 12, although the possibility of chemical decarboxylation during the isolation procedure cannot be excluded due to the low incorporation (0.02%). These results were supported by similar tracer experiments with intact P. somniferum plants by Battersby et al.[21]
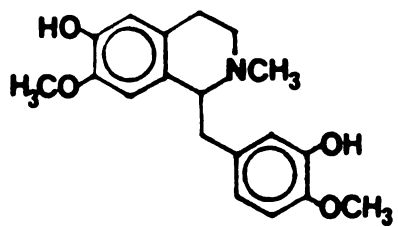
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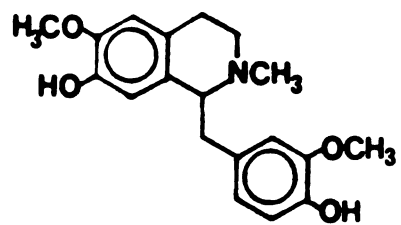
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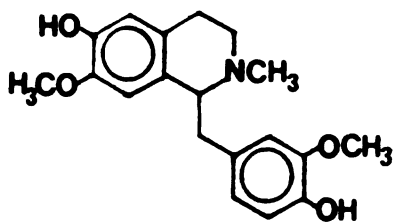
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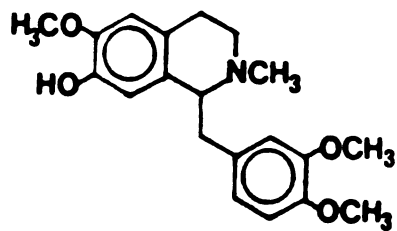
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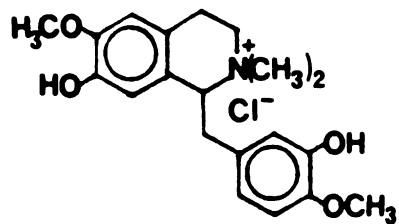
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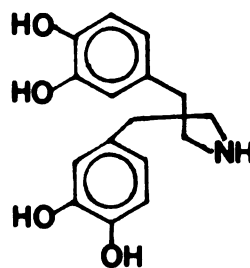
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Due to the presence of an asymmetric center at position 1, norlaudano-
solinecarboxylic acid may exist in two enantiomeric forms. Only one
enantiomer would be expected to be biologically converted since most
enzyme-catalyzed reactions show stereospecificity. The subsequently formed
nor-reticuline and reticuline carry the same asymmetric center, and should
also have two enantiomeric forms. Therefore, the interesting aspect of
stereoselectivity associated with the biotransformations of these 1-benzyl-
tetrahydroisoquinolines attracted the attention of several researchers.

The absolute configuration of morphine was firmly established by both
chemical[22] and X-ray[23] evidence. It follows that R-(-)-reticuline (5b)
should be the biosynthetic precursor of morphine.[24] Reticuline was first
isolated from <u>Anona reticulata</u> as the S-(+)-isomer (5a).[25]

In 1964, Brochmann-Hanssen and Furuya[26] reported the isolation of
(±)-reticuline from crude opium and from the mother liquor obtained in the
commercial production of morphine. It was established by Battersby <u>et al.</u>[24]
that natural norlaudanosoline, the first 1-benzyltetrahydroisoquinoline
alkaloid biosynthesized in the opium poppy, is the S-(-)-isomer. It then
undergoes <u>O</u>-methylation to S-(-)-nor-reticuline, which is further methylated
to give S-(+)-reticuline. Since both isomers of reticuline are present in
opium, there must exist a mechanism for racemization of the (+)-isomer.
This process was found to involve 1,2-dehydroreticulinium ion (13) as the
intermediate.[24] The role of 13 in the racemization of reticuline was also
established by Borkowski, Horn, and Rapoport,[27] in their feeding experiments
with authentic [3-^{14}C]-1,2-dehydroreticulinium chloride. They also demons-
trated the natural presence of this ion in <u>Papaver somniferum</u>.

Since only the (-)-isomer of reticuline can be directly biotransformed
to the morphine alkaloids, it would seem reasonable that (+)-reticuline
should exist in excess of the (-)-isomer in the flowering plants during

the rapid formation of morphine. This was found to be the case, by isolation experiments with opium[28] and mature poppy plants.[29] In the poppy seedlings, where the biosynthesis of morphine is not active, both isomers were found in about equal amounts.[29]

Based on the experimental results described above, the early steps in the biosynthetic pathway for opium alkaloids are summarized in Scheme 1. The detailed mechanism of the oxidative decarboxylation of norlaudanosolinecarboxylic acid (11) deserves further investigation.

R-(-)-reticuline was demonstrated to be the biosynthetic precursor of hydrophenanthrene alkaloids (thebaine, codeine, and morphine)[24]. On the other hand, the S-(+)-isomer does accumulate in the plant. Besides undergoing racemization to generate the R-(-)-isomer, it is biosynthetically converted to methylated 1-benzylisoquinolines ((+)-laudanidine (14), codamine (15), laudanosine (16)), as well as aporphines (isoboldine (18), corytuberine (19), magnoflorine (20)), protoberberines (stepholidine (23), scoulerine (21), isocorypalmine (22), coreximine (24)), and papaverrubines (porphyroxine (25)).

Scheme 2 shows the role of (±)-reticuline as key intermediate in the biosynthesis of opium alkaloids. The details of the biotransformations of S-(+)-reticuline are not described here.

The second part of this introduction concerns the biosynthetic sequence from (-)-reticuline to morphine.

In a study by Martin, Warren, and Rapoport,[30,31] both the seedlings and budding plants of P somniferum L. were exposed to $^{14}CO_2$ for short periods of time, and incorporation of radioactivity into reticuline (15) and thebaine (26) was followed. Positive evidence was obtained for the biosynthetic relationship: $CO_2 \rightarrow$ reticuline $\rightarrow$ thebaine. Battersby et al.[24,32] synthesized the three diphenolic structural isomers of reticuline, i.e.

Scheme I

18 $R_1 = H$, $R_2 = OH$
19 $R_1 = OH$, $R_2 = H$

21 : $R_1 = OH$, $R_2 = OCH_3$, $R_3 = H$
22 : $R_1 = OCH_3$, $R_2 = OCH_3$, $R_3 = H$
23 : $R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$
24 : $R_1 = H$, $R_2 = OCH_3$
$R_3 = OH$

20

5a

25

15

14

5b

16

26

37

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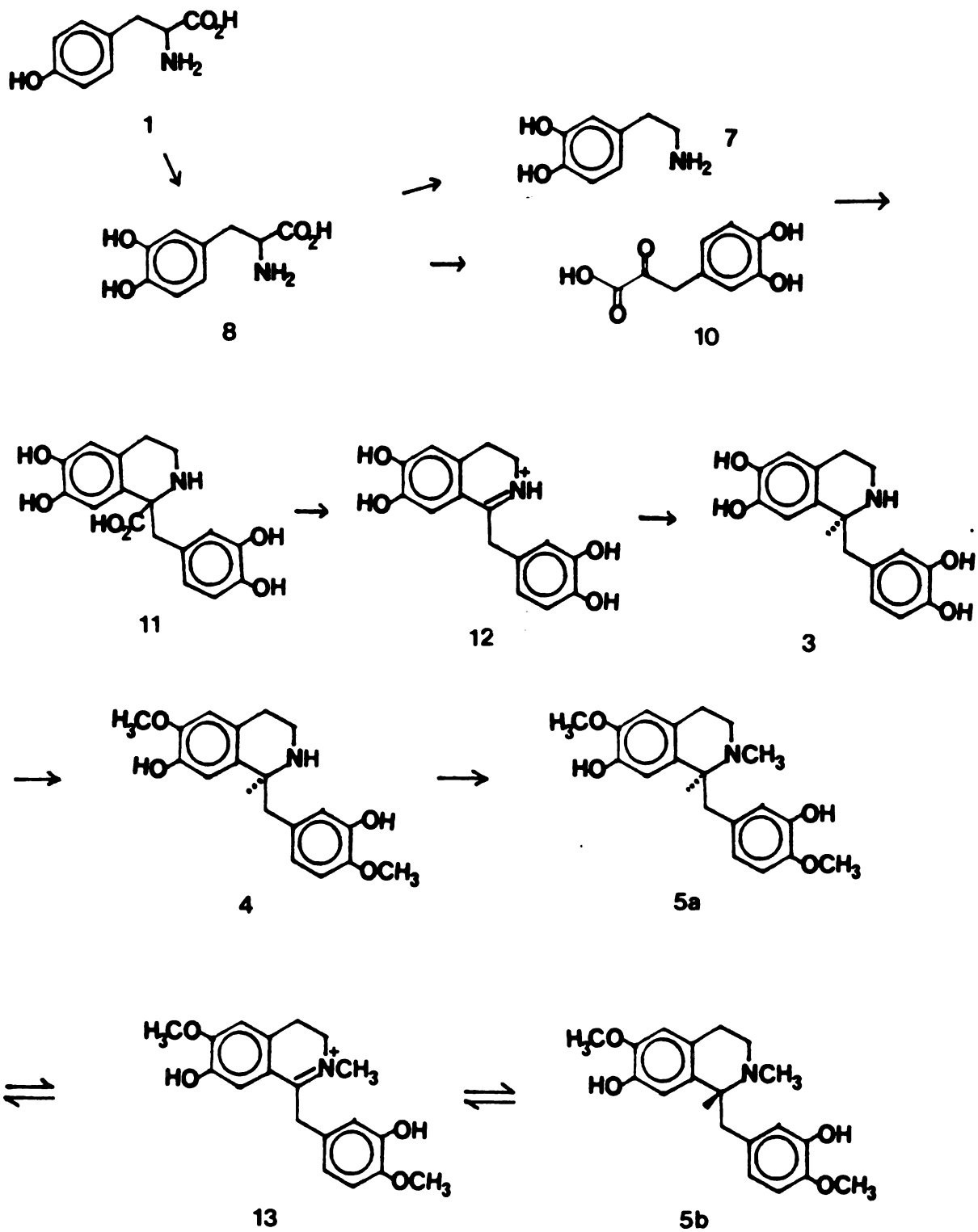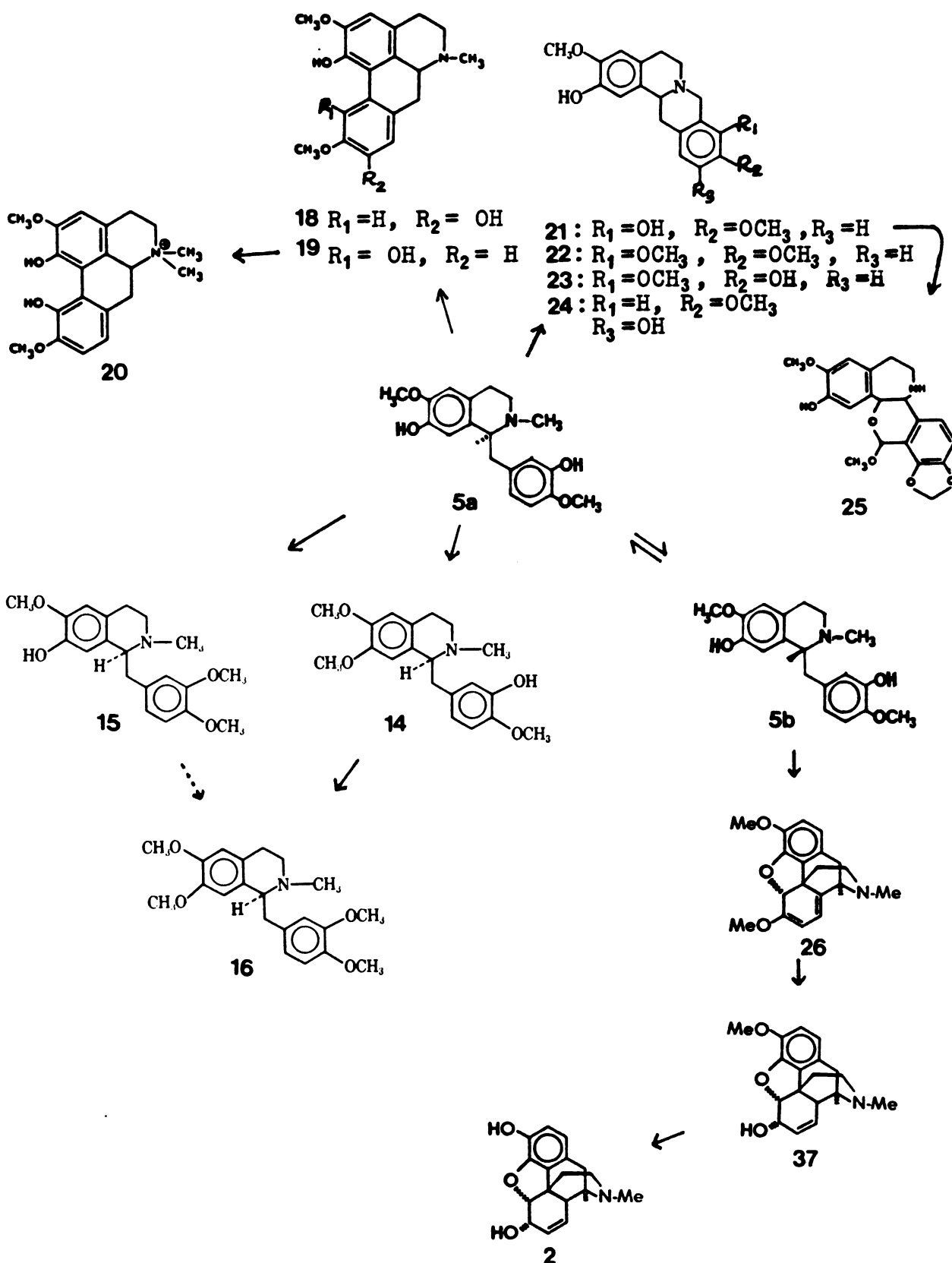
Scheme II   Biosynthetic transformations of reticuline
in the opium poppy

protosinomenine (27), orientaline (28), and iso-orientaline (29), as
well as some reticuline derivatives, including (+)- and (-)-codamine
(15), (±)-reticuline methochloride (=tembetarine chloride) (30),and bis-3,4-
dihydroxyphenethylamine (31), in labeled forms. Feeding experiments with
these analogs showed no incorporation into morphine. These results firmly
established that reticuline is the true biosynthetic benzyltetrahydroiso-
quinoline precursor of the hydrophenanthrene alkaloids.

Barton, Battersby, and coworkers[33] demonstrated that reticuline does
not undergo demethylation prior to its bioconversion to the morphine
skeleton since short-term feeding experiments with quintuply labeled (±)-
reticuline, with ^{14}C in both methoxy groups, in the N-methyl group, and
at C-3, and with ^{3}H at C-1, gave radioactive thebaine (26) with retention
of the methoxyl and N-methyl activities.

According to the original proposal,[6] intramolecular phenol coupling
of reticuline should lead to the dienone salutaridine (32). By analogy
with similar compounds such as Pummerer's ketone (33)[34] and narwedine (34),[35]
this dienone would be expected to cyclize spontaneously to yield the enone
(35), which would give thebaine (26) after reduction and dehydration.

Salutaridine was first isolated from Croton salutaris by Barnes,[36] and
was found to be identical with a sample synthesized from thebaine.[33] In
contrast to the original proposal,[6] ring closure of this dienone did not
take place in either acidic or basic solution. An alternative route
suggested earlier[16,50] involving salutaridinol (36), the alcohol resulting
from the reduction of salutaridine, was found to be operative. It was
shown that both epimers of the alcohol (salutaridinol-I (36a) and salu-
taridinol-II (36b)) readily dehydrate in aq. acidic solution to give
thebaine in 30 - 40% yield.[33] Labeled salutaridine and salutaridinol-I
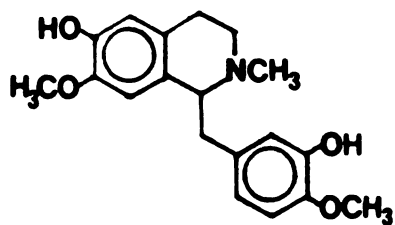
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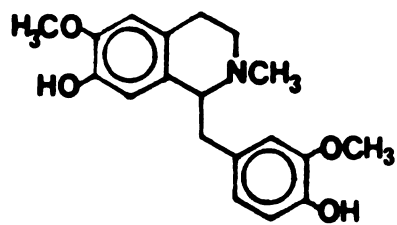
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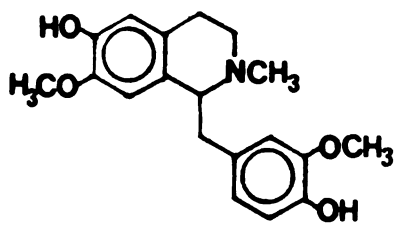
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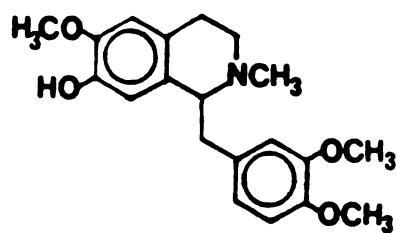
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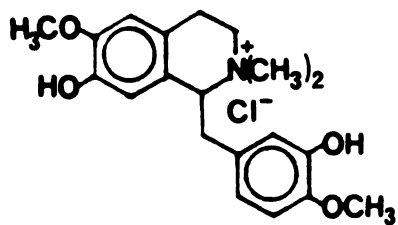
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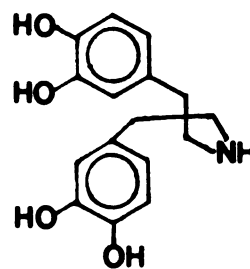
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gave very high incorporation (5-10%) into thebaine, codeine (37), and morphine, while the incorporation of labeled salutaridinol-II was much less efficient (0.5%). These results clearly indicate that salutaridine and salutaridinol are the intermediates between (-)-reticuline and thebaine, and that the cyclization of salutaridinol is enzymatically controlled, with salutaridinol-I being the biological precursor of thebaine[33] (Scheme 3).

The natural presence of salutaridine in $\underline{P}$. somniferum was demonstrated by the isotope dilution method after feeding with either $[3-^{14}C]$ norlaudanosoline or $[2-^{14}C]$tyrosine.[33] Direct isolation, from opium, of this important intermediate was achieved by Brochmann-Hanssen $\underline{et}$ $\underline{al}$.[37]. Barton $\underline{et}$ $\underline{al}$.[15] demonstrated that the phenol coupling of reticuline to generate salutaridine could be carried out in the laboratory, although the yield was very low (0.012%). The absolute configuration of salutaridinol-I was later unambiguously determined[38].

Before the role of salutaridine was established, Rapoport $\underline{et}$ $\underline{al}$.[39] proposed thebaine as the first hydrophenanthrene alkaloid biosynthesized in $\underline{P}$. somniferum, it being converted by successive O-demethylations to codeine and morphine. This proposal was based on their study of the comparative rates of the incorporation of $^{14}CO_2$ into thebaine, codeine, and morphine and was supported by feeding experiments with labeled morphine, codeine and thebaine.[40,41] It could be shown that morphine was not converted to either codeine or thebaine; codeine was converted to morphine only; thebaine was converted to both codeine and morphine.

Supporting evidence for the biosynthetic sequence of thebaine → codeine → morphine was also provided by Battersby and Harper.[42] By feeding $[2-^{14}C]$ tyrosine to $\underline{P}$. somniferum, they observed rapid incorporation of radioactivity into thebaine, followed by a rise of activity in codeine, and then a steady fall in the activities of both thebaine and codeine relative to that of morphine.
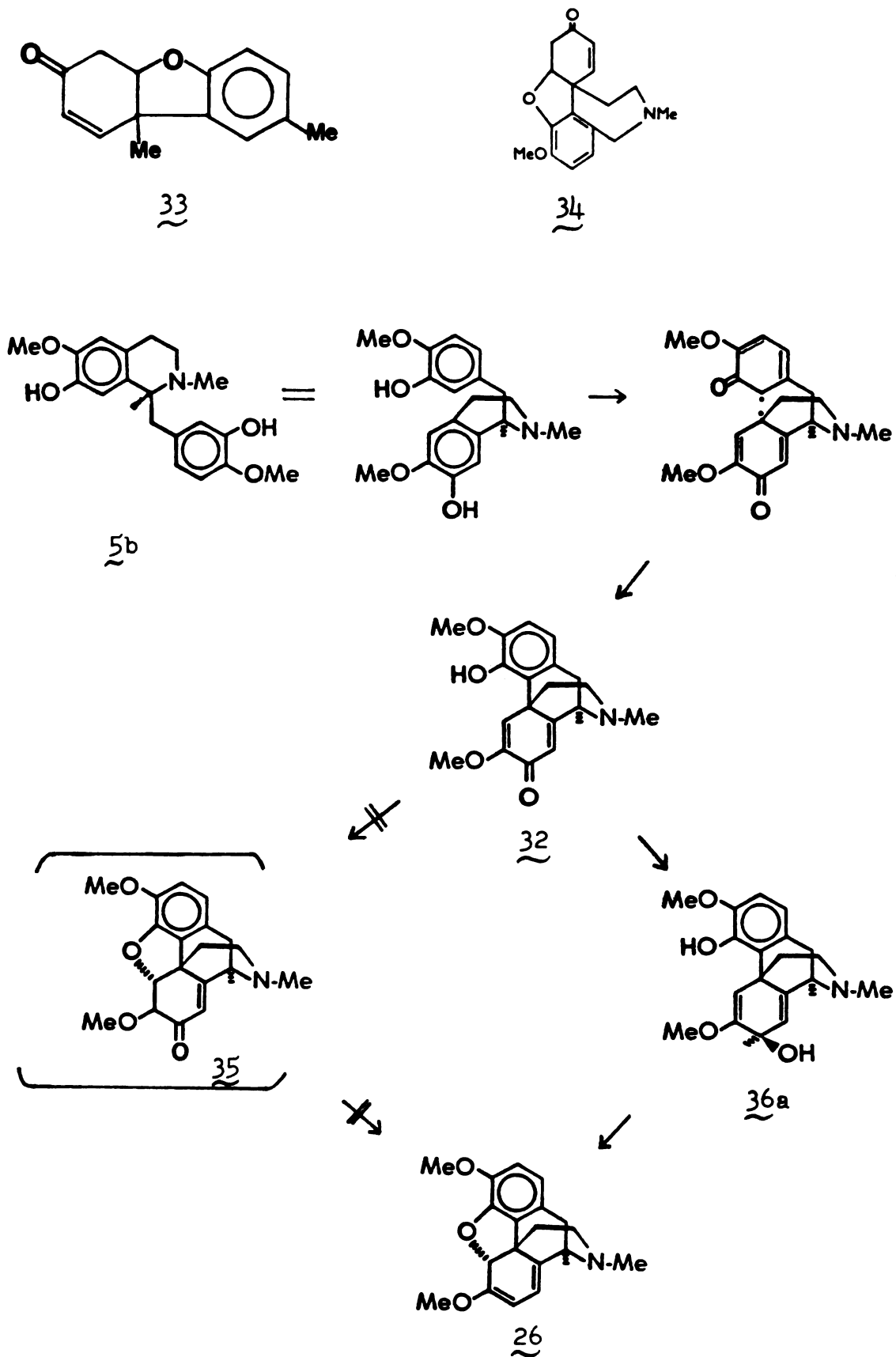
Scheme III

The mechanism by which thebaine is converted to codeine in the opium poppy was postulated[6,16,43] to involve first demethylation to neopinone (38), then migration of the double bond to codeinone (39), followed by reduction to codeine (37). A second possibility would have reduction precede demethylation, with 6-methylcodeine (40) as a potential intermediate.

The first postulate was supported by two independent investigations involving feeding experiments with $[2,6-^3H_2]$codeine and $[2-^3H]$codeinone,[44] and $^{14}CO_2$ exposure experiments.[45] The alternative route involving 6-methylcodeine was found to be not operative in the opium poppy,[45] although this compound had been isolated from the mother liquor obtained during the commercial production of morphine.[47] The enolic demethylation of thebaine was shown by Horn, Paul, and Rapoport[48] to proceed with retention of the 6-oxygen, probably by the same mechanism as aromatic methyl ether cleavage with the participation of an oxygenase (cf. Scheme 4).

Codeine (37) biogenetically derived from thebaine is subsequently O-demethylated by an O-demethylase to give morphine (2). Morphine is generally considered as the end product on the biosynthetic pathway of hydrophenanthrene alkaloids although the irreversible N-demethylation of morphine to normorphine (41) does occur in the plant to an appreciable extent[49]. Scheme 4 shows the detailed biosynthetic sequence from thebaine (26) to morphine (2).

Experimental results described above clearly demonstrate the important positions occupied by reticuline and thebaine in the biosynthesis of opium alkaloids. Reticuline is the intermediate in the formation of practically all known alkaloids in P. somniferum, while thebaine is the first morphine-type alkaloid biosynthesized. The main goal of the study reported in Part I of this dissertation was to explore the substrate specificity associated
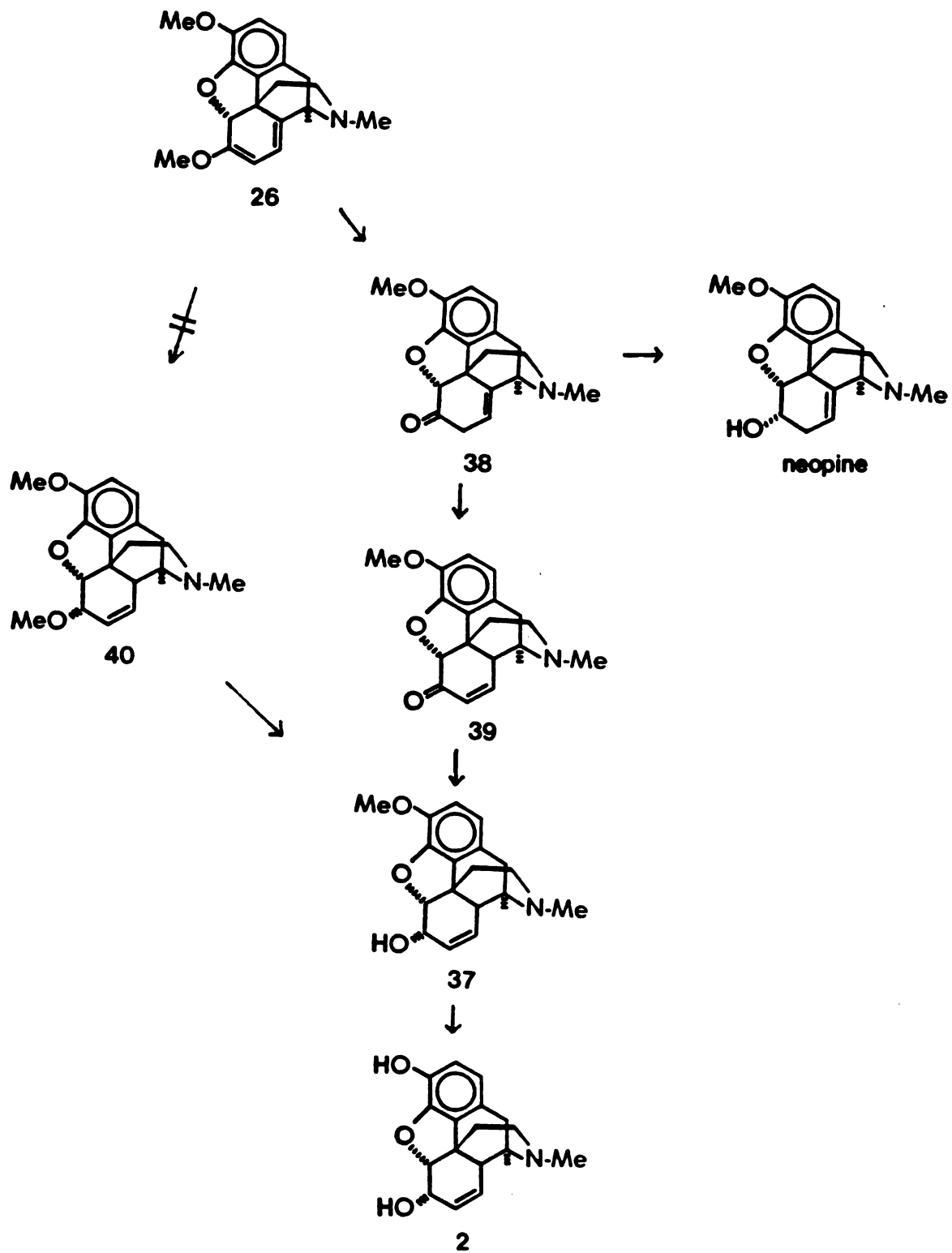
Scheme IV

with the racemization and subsequent biotransformations of reticuline, as well as that associated with the bio-conversion of thebaine to codeine and morphine. The possibility of utilizing the enzyme system in the plant to generate unnatural alkaloids from structurally modified precursors was also examined (Chapters II & III).

Another aspect of the study was to investigate the biosynthesis of hydrophenanthrene alkaloids in Papaver orientale. Only thebaine and its 3-O-demethylated product, oripavine (61), have been found in this poppy plant. The absence of codeine and morphine is probably due to the lack of an enzyme system which is responsible for the cleavage of the enolic methyl ether in thebaine[48] (Chapter IV).

Chapter II.   ABERRANT BIOTRANSFORMATIONS OF THE 6-ETHYL ANALOG OF

THEBAINE TO CODEINE AND MORPHINE IN PAPAVER SOMNIFERUM L.

A.   Introduction:

Biosyntheses of natural products in higher plants are catalyzed by
various enzyme systems. Enzyme-catalyzed reactions are usually stereo-
specific and substrate-specific. Therefore, in biosynthetic studies with
precursor-feeding experiments only natural precursors are expected to
be biotransformed into natural products. The efficient incorporation of
a labeled compound A, without scrambling of the label, into a natural
product B has often been regarded as indication that compound A is a
natural precursor in the biosynthesis of B. However, experimental results
accumulated in the literature have clearly demonstrated that substrate
specificity associated with the biosynthesis of natural products is not
well defined in many cases. Examples of the incorporation of an unnatural
precursor into a natural product have been reported in the opium poppy
(Papaver somniferum),[45,46,51,52] and in the tobacco plant (Nicotiana
glutinosa).[53] Thus, incorporation of radioactivity alone is not sufficient
proof to identify a potential biosynthetic precursor. Necessary supporting
evidence is often provided by (a) experiments to show the natural presence
of the presumptive precursor compound in the plant, either by direct iso-
lation or by the isotope-dilution method (b) feeding experiments to show that the
presumptive precursor is derived in the plant from another established pre-
cursor, which lies earlier on the pathway for the biosynthesis of the natural
end product.

A second type of unnatural biotransformation in higher plants manifests itself in the conversion of an unnatural precursor to the correspondingly modified, unnatural end product. This kind of aberrant biosynthesis has been observed to occur in the tobacco plants (Nicotiana glutinosa,[54] N. tabacum,[55,56] and N. glauca[56]), in the opium poppy[52,57-59] and in the cactus plant Dolichothele sphaerica.[60] Aberrant biosynthetic reactions which have been reported to occur in the opium poppy are described below.

The first example is that of the conversion of an unnatural precursor to a natural product. In 1967, 6-Methylcodeine (40) was found to be efficiently converted to codeine (37) in Papaver somniferum, although the former compound is not a genuine alkaloid in the plant.[45,46] It was suggested that the unnatural formation of codeine might be effected by general or induced demethylating action in the plant.[45] The formation of unnatural alkaloids in the opium poppy from modified precursors was first demonstrated by Kirby et al. in 1972.[57] Table VII shows the results of their feeding experiments with [2-^{3}H]codeine analogs.

Table VII. Incorporation of ^{3}H-Labeled Codeine Derivatives Into Morphine Derivatives

Precursor	Incorpn. %	% Efficiency compared with Codeine → morphine
7,8-Dihydrocodeine	0.59	50
Dihydrodesoxycodeine	9.27	100
Isocodeine	0.27	15
1-Bromocodeine	0.15	5.8
Codeine methyl ether	1.02	69

The following conclusions were drawn from the results: Neither the 6-hydroxy group nor the 7,8-double bond of codeine is important for binding to the demethylating enzyme, based on the efficient conversions of dihydrodesoxycodeine, codeine methyl ether, and dihydrocodeine into their morphine analogs. The less efficient conversion of isocodeine suggests that a hydroxy-group $\underline{cis}$ to the ethanamine bridge may actually hinder approach to the enzyme. The strong inhibition of demethylation observed with 1-bromocodeine could be due to electronic and steric effects exerted by the bromo-substituent close to the site of enzymic attack.

In 1975 Brochmann-Hanssen $\underline{et}$ $\underline{al}$.[51] observed aberrant biotransformations of 1-benzylisoquinoline compounds in the opium poppy during the investigation on the biosynthesis of papaverine (17). To determine the sequence of $\underline{O}$-methylations and the substrate specificity associated with the aromatization of ring B, four labeled isomeric dimethyl ethers (4,42, 43,44) of norlaudanosoline (3) were fed to the plant. Among them, nor-reticuline (4) had been established as an efficient precursor of papa-verine.[13,51] All four isomers were incorporated into papaverine without randomization of the radioactive labels. But, as shown in scheme 5, the conversion of norprotosinomenine (43) and noriso-orientaline (44) into papaverine was found to proceed along an aberrant route, which differs from the conversion of nor-reticuline (4) and nororientaline (42) in that tetrahydropapaverine (6) is not involved as an intermediate. It was also found that (+)-(R)-tetrahydropapaverine (6b), the enantiomer of the natural precursor for the biosynthesis of (+)-(S)-laudanosine (16a), gave good incorporation, although less efficient than the natural process, into the unnatural (-)-(R)-laudanosine (16b), with little loss of the ^{3}H label from the asymmetric center. Thus, it appeared that $\underline{N}$-methylation of tetrahydro-
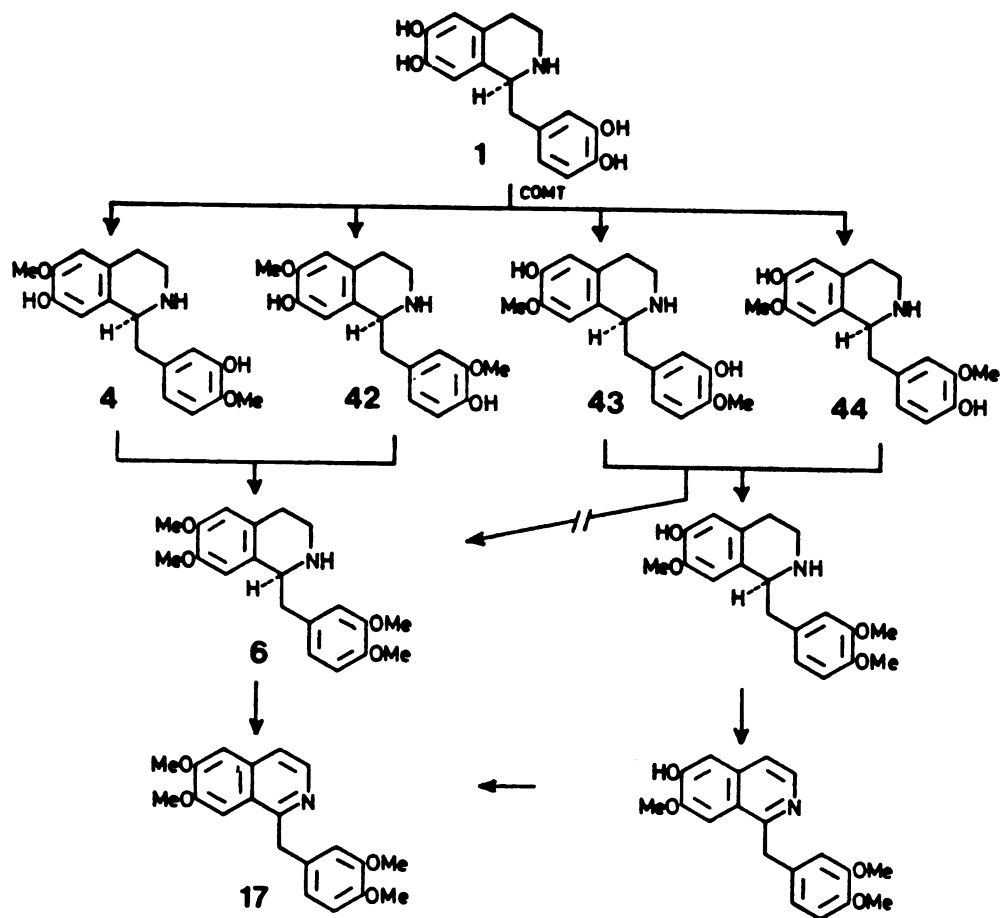
Scheme V
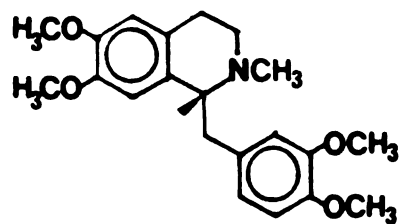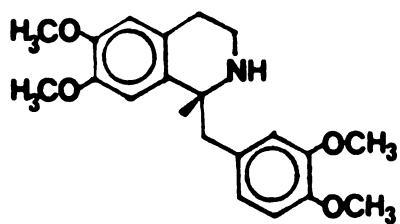
papaverine has only a low degree of stereospecificity. In the same study it was discovered that the labeled "unnatural" (+)-(R)-nor-reticuline (4a) was incorporated into morphine (2) with almost complete retention of ^{3}H at the asymmetric center, although the conversion was much less efficient than that of the (-)-isomer. It was concluded that this unnatural formation of morphine occurs by an aberrant pathway involving the oxidative phenol coupling of (+)-nor-reticuline to norsalutaridine, followed by N-methylation to salutaridine (32) and conversion into morphine (2) in the usual way (cf. scheme 3 for the natural pathway).

In 1980, Brochmann-Hanssen, Chen, and Linn demonstrated the biosynthesis of unnatural papaverine (17) derivatives from modified tetrahydropapaverine (6) analogs.[59] Four labeled analogs (45,46,47,48) formed by the replacement of one of the methoxy substituents of THP by an ethoxy

6; $R^1 = R^2 = R^3 = R^4$ = Me

45; R^1 = Et, $R^2 = R^3 = R^4$ = Me

46; R^2 = Et, $R^1 = R^3 = R^4$ = Me

47; R^3 = Et, $R^1 = R^2 = R^4$ = Me

48; R^4 = Et, $R^1 = R^2 = R^3$ = Me

17; $R^1 = R^2 = R^3 = R^4$ = Me

49; R^1 = Et, $R^2 = R^3 = R^4$ = Me

50; R^2 = Et, $R^1 = R^3 = R^4$ = Me

51; R^3 = Et, $R^1 = R^2 = R^4$ = Me

52; R^4 = Et, $R^1 = R^2 = R^3$ = Me
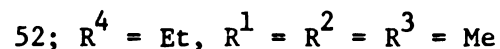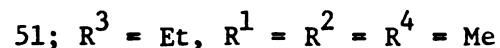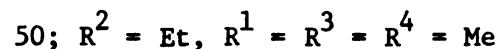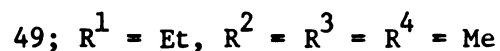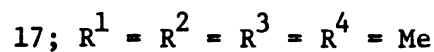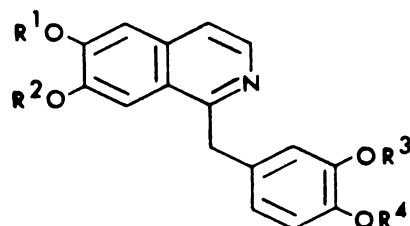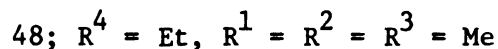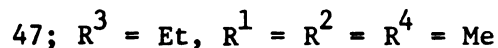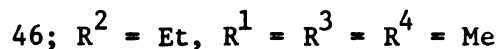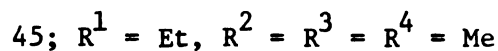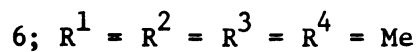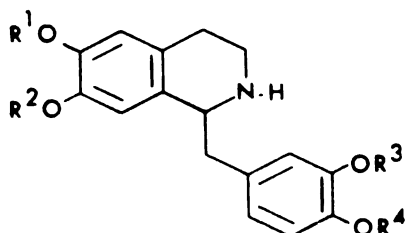
group, were fed to the opium poppy. The efficiencies of the incorporation into the correspondingly modified papaverine analogs (49,50,51,52) are shown in Table VIII.

Table VIII. Efficiencies of the Biosynthesis of Unnatural Papaverine Analogs.

Precursor Compound	Percent incorp. of ^{14}C into compd.			
	49	50	51	52
45	11.25			
46		10.00		
47			1.91	
48				1.50

The results indicated that, "when the modification involved the A-ring of the tetrahydroisoquinoline moiety, dehydrogenation took place with about the same degree of efficiency as with THP itself. However, when the ethoxy group is in the benzylic portion of the molecule, the increased bulkiness seems to prevent optimum interaction with the enzyme."

In 1980 Brochmann-Hanssen and Okamoto[52] reported the biotransformation of an unnatural thebaine analog, namely oripavine 3-ethyl ether (53), to morphine 3-ethyl ether (54) and morphine (2) in the opium poppy. This example of aberrant biosynthesis is interesting in that it involves the conversion of an unnatural precursor first to an unnatural product and subsequently to a natural product. It was concluded that oripavine 3-ethyl ether can enter the natural pathway for the biotransformations of thebaine (26) and the 3-ethyl group does not seem to interfere with

either the 6-O-demethylation or the reduction of the carbonyl group (cf. Scheme 4). The efficient conversion of morphine 3-ethyl ether to morphine indicated that the enzyme responsible for the 3-O-dealkylation is not specific enough to reject the 3-ethyl analog of codeine.
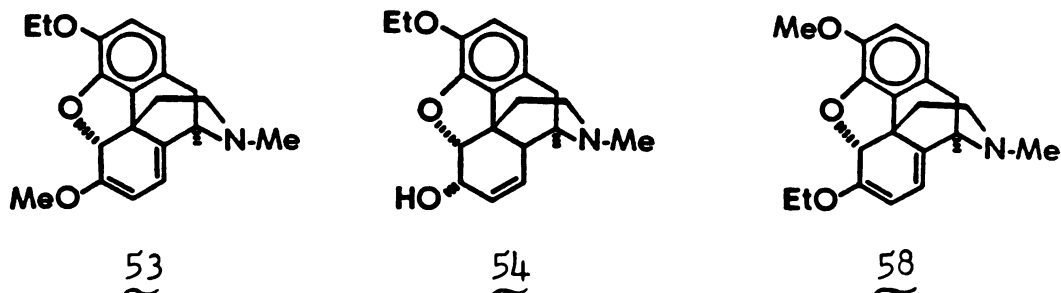
From the above examples, it appears that aberrant biosyntheses in higher plants are important for two main reasons. First, feeding experiments with a series of structurally modified precursors can be employed to study the specificity of the enzyme systems involved in the biotransformations of the natural precursor. Secondly, such experiments might be used for the preparation of analogs of biologically active natural products which are not readily synthesized in the laboratory.

In the present investigation, the substrate specificity associated with the 6-O-dealkylation of thebaine was chosen for study. The 6-ethyl analog of [2-^{3}H]thebaine (58) was fed to P. somniferum plants. Any incorporations of radioactivity into codeine and morphine were examined. The results were used to determine if this unnatural precursor can enter the natural pathway for the biotransformations of thebaine (26) as the unnatural 3-ethyl analog (53) does[52].

B. Results and Discussion:

The results of the feeding experiment are shown in Table IX.

Table IX.

Precursor and its specific activity (µCi/mg)	Amount of precursor fed		Alkaloids isolated and their specific activities			Degree of incorporation[c]	Activity after ^{3}H-proton exchange
	µCi[a]	mg		dpm/mg[b]	µCi/mole		
6-ethyl analog of [2-^{3}H]thebaine (0.599)	23.95	40	Codeine(37):65 mg.	46594	6318	8.84%[d]	------
			Morphine(2):175 mg.	27943	3853	9.28%	morphine (0 dpm/mg)

[a] 1 µCi = 2.2 x 10^6 dpm

[b] dpm = disintegrations/min

[c] degree of incorporation = $\dfrac{A_2 M_2}{A_1 M_1}$ x 100% (A_1 and A_2 are the specific activities of precursor fed and alkaloid isolated respectively; M_1 and M_2 are the amounts of precursor fed and alkaloid isolated (or carried added) respectively.)

[d] 35 mg of cold codeine was added during the purification. So M_2 = 65 mg + 35 mg = 100 mg in calculating the degree of incorporation.

As shown in Table IX, the 6-ethyl analog of thebaine (58) is effect-
ively incorporated into codeine and morphine. Within experimental error,
the tritium label in the precursor remained at position-2 through the
biotransformations to morphine. Radioactive codeine should also be labeled
as in the original [2-^{3}H]thebaine analog since morphine is biosynthetically
derived from codeine. The natural biosynthetic pathway from thebaine (26)
to morphine (2) involves first 6-O-demethylation and then migration of the
double bond to the more stable α,β-unsaturated ketone, codeinone (39), which
in turn is reduced to codeine (37). Codeine is subsequently 3-O-demethy-
lated to morphine (Scheme 4). It appears that the 6-ethyl analog of thebaine
can enter the same pathway, and that the enzyme responsible for 6-O-
dealkylation is not sufficiently specific to reject the 6-ethyl analog.

As described in the introduction, there have been other examples of
aberrant biotransformations along the biosynthetic pathway from thebaine
to morphine. These are the conversion of oripavine 3-ethyl ether (33)
(3-ethyl analog of thebaine) to morphine 3-ethyl ether (54) and morphine[52],
the conversion of codeine methyl ether[45,46,57] to codeine, and the bio-
synthesis of unnatural morphine derivatives from modified codeine analogs[57].
Most of these unnatural biotransformations have efficiences comparable to
the natural process. It may be concluded that the enzyme system involved
in the terminal steps of the biosynthesis of morphine alkaloids is not
specific enough to reject substrates with minor modifications. It was of
interest to determine if this non-specific character can be observed with
enzymes involved in the earlier steps of the biosynthesis of opium alkaloids.
Feeding experiments with ethyl analogs of reticuline are described in
Chapter III.

C. Experimental

1. General.

All melting points were determined with a Thomas-Hoover capillary melting point apparatus and were uncorrected. Progress of reactions and purity of products were tested by thin-layer chromatography (TLC) and in many cases by gas liquid chromatography (GLC). Column chromatography and preparative TLC were used for the purification of compounds synthesized and isolated from plant extracts. TLC was performed on precoated glass plates (silica gel 60 F-254 and aluminum oxide F_{254} (type T), layer thickness 0.25 mm, 0.5 mm and 2 mm obtained from E. Merck). The plates were observed under ultraviolet light or after exposure to iodine vapor. Silica gel (Woelm, activity II) and neutral alumina (aluminum oxide, W200 neutral, activity III) were used for column chromatography. Organic extracts were dried over anhy. Na_2SO_4 (or anhy. $MgSO_4$) and concentrated using a Büchi rotary evaporator under reduced pressure. GLC analyses were carried out on a Varian Aerograph Model 2100 gas chromatograph with a glass column, 1.82 m (6 ft) long, 2 mm i.d.

Confirmation of chemical structures was done by the use of infrared (IR), nuclear magnetic resonance (NMR), and mass (MS) spectrometries. IR spectra were determined on a Perkin Elmer grating infrared spectrometer, model 457. Proton NMR spectra were recorded at 80 MHz. (Varian FT-80 NMR spectrometer) or 100 MHz (Varian XL-100 spectrometer equipped with a Nicolet FT accessory). Chemical shifts are reported in parts per million (δ) relative to Me_4Si (TMS, $\delta = 0.00$) as an internal standard. Electron impact mass spectra were taken on MS-9 and MS-25 Mass Spectrometers (KRATOS) connected to the Berkeley LOGOS II Datasystem (Mass Spectrometry Resource, Space Sciences Laboratory, Univ. of Calif. Berkeley) for high-resolution data treatment.

Spectral data on all compounds were consistent with the structures.

The radioactivity was determined by liquid scintillation counting on a Packard (model 3375) Tri-Carb liquid scintillation spectrometer. Starting materials, reagents and solvents were used as received from suppliers unless otherwise indicated.

2. Synthesis of the 6-Ethyl Analog of [2-^{3}H]Thebaine (58).

The synthetic route to compound 58 is shown in Scheme VI. [2-^{3}H]Morphine was obtained by base-catalyzed nuclear exchange of morphine with tritiated water in dimethylformamide.[61] Methylation of the tritiated morphine with trimethylanilinium sulfate at 125-130° afforded [2-^{3}H]codeine (37). [2-^{3}H]codeine was 6-O-ethylated with EtI/KH to give [2-^{3}H]-6-ethylcodeine, which was oxidized to the target compound, with activated manganese dioxide[62] as described by Barber and Rapoport for the synthesis of thebaine.[63]

[2-^{3}H]-Codeine (37). Anhydrous xylene (12 ml, redistilled and dried over 4 Å molecular sieves) in a two-necked flask was heated to 110°C. KOH (102 mg, 1.1 eq.) was dissolved in anhydrous methanol (12 ml) in a dropping funnel. [2-^{3}H]Morphine (400 mg) was added to the MeOH solution and dissolved by gentle swirling. To this solution was added dried trimethylanilinium sulfate (288 mg, 1.1 eq.) and the mixture was swirled again. The dropping funnel was connected to the two-necked flask. The solution was added dropwise to the hot xylene over a period of 30 min, and the methanol was removed by distillation. The temperature was then raised to 125-130° and held there for 1 h. During this time the dropping funnel was rinsed with xylene (2x2 ml), which was added to the reaction mixture. After cooling, ether was added and the mixture extracted with 1 N HCl$_{(aq.)}$ (4 x 15 ml). The combined acid extract was neutralized with sodium acetate (pH = 3.5 - 4), and washed with ether (2 x 20 ml). The ether washing was shaken with 20 ml of H$_2$O, which was added to the aqueous phase. After adjusting the pH to 14 with

2 → **37** → **65** → **58**
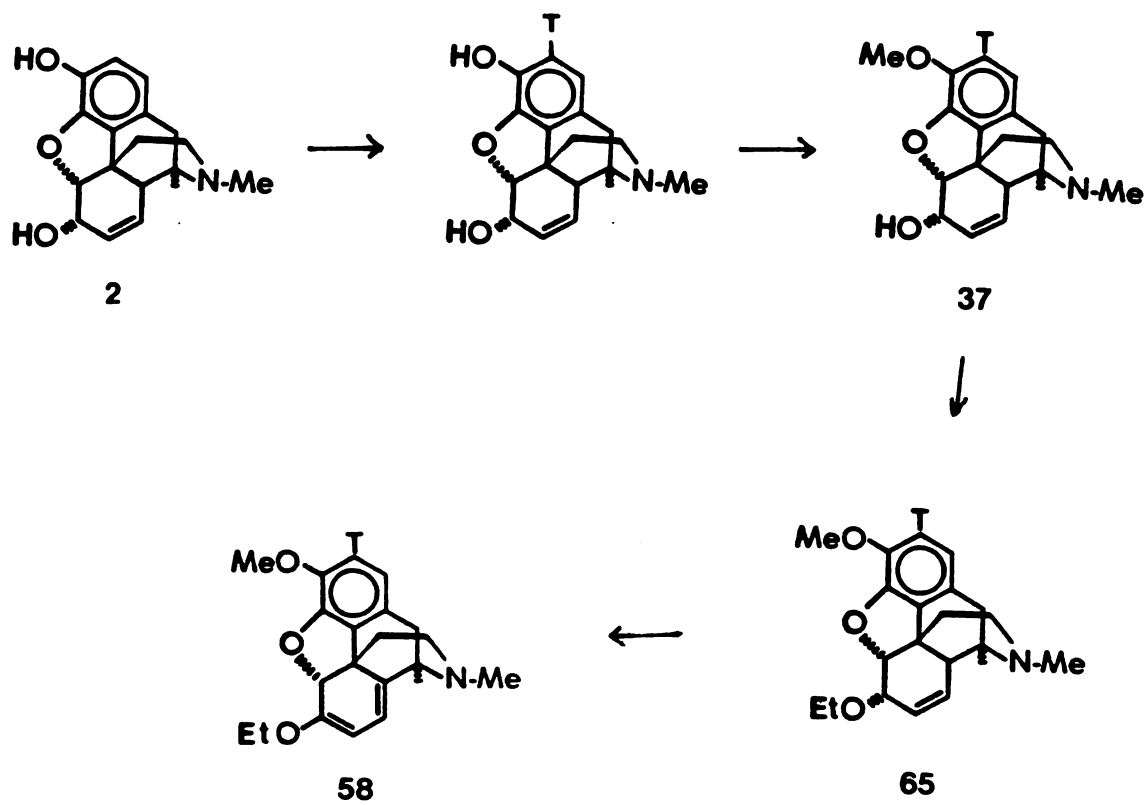
Scheme VI

KOH$_{(aq.)}$, the aqueous phase was extracted with ether. The ether extract was washed with H$_2$O, dried and evaporated to give 341 mg [2-^{3}H]codeine, which was identical with an authentic sample of codeine by TLC.

[2-^{3}H]-6-ethylcodeine (65). An excess of potassium hydride (300 mol %, 22% dispersion in oil) was washed with hexane (3 x 6 ml, distilled from CaH$_2$) and then suspended in anhydrous THF (10 ml). With stirring under a nitrogen atmosphere, a solution of [2-^{3}H]codeine (170 mg, 0.57 mmol) in 4 ml of THF was added to the KH suspension over a period of 30 min and the mixture stirred for an additional 1/2 h . Ethyl iodide (0.15 ml, 2.3 mmol) was added to the mixture rapidly and the reaction was quenched after 2 min with 4 ml of 1 N NaOCH$_3$ in anhydrous MeOH. Water (15 ml) was added and the solution evaporated to remove organic solvents. The resulting aqueous mixture was extracted with CHCl$_3$ (4 x 15 ml). The CHCl$_3$ extracts were washed with water, dried over Na$_2$SO$_4$ and evaporated to give a solid (197 mg) which was identical with a cold sample of 6-ethylcodeine by TLC. ^{1}H NMR of the cold sample: δ 1.26 (t, OCH$_2$C̲H̲$_3$, $J_{1,2}$ = 7 Hz), 2.44 (s, NCH$_3$), 3.70 (q, OC̲H̲$_2$CH$_3$, $J_{1,2}$ = 7 Hz), 3.82 (s, OCH$_3$), 6.46 & 6.62 (2d, H-1 & H-2, $J_{1,2}$ = 8.2 Hz).

6-Ethyl analog of [2-^{3}H]thebaine (58). The [2-^{3}H]-6-ethylcodeine (197 mg) obtained above was dissolved in anhydrous THF (10 ml). The solution was shaken vigorously with γ-MnO$_2$ (250 mg, obtained from Alfa), under a nitrogen atmosphere at room temp. Further portions of γ-MnO$_2$ (250 mg) were added at intervals of 1,3,5, and 10 h. After 48 h the black mixture was filtered through a fine sintered glass funnel, the residue was washed with THF (4 x 30 ml), and then with methanol (4 x 20 ml). The filtrate and washings were combined and evaporated to give 137 mg of crude product.

The crude product was purified by column chromatography on silica gel to give 43 mg of pure 58, which was identical with a cold sample by TLC & GLC. Specific activity: 1,317,285 dpm/mg. ^{1}H NMR (CDCl$_3$) δ 1.33 (t, 6-O-CH$_2$CH$_3$, $J_{1,2}$ = 7.0 Hz), 2.46 (s, NCH$_3$), 3.69 (q, 6-O-CH$_2$CH$_3$, $J_{1,2}$ = 7.0 Hz), 3.85 (s, 3-O-CH$_3$), 5.00 (d, H-7, $J_{1,2}$ = 6.4 Hz), 5.54 (d, H-8, $J_{1,2}$ = 6.4 Hz), 5.26 (s, H-5) 6.60 & 6.62 (2s, H-1, and H-2).

3. Feeding of Precursors.

The method previously described[9,82] for cultivation of plants was followed. The seeds of Papaver somniferum L., variety Noordster, were sown early in February. After 6-8 weeks the seedlings were transplanted into flower pots and grown in a greenhouse. The feeding was done at the end of the flowering season as soon as the petals had fallen. The radio-active precursors were dissolved in 0.1 N sulfuric acid ($\sim$ 1 eq.) and diluted with water to give a concentration of about 3 mg/ml. The solutions were injected into the seed capsule ($\sim$ 0.3 ml to each capsule) with a fine hypodermic needle.[33] After feeding, the plants were allowed to grow normally and were harvested two weeks later. The harvested whole plants were placed in plastic bags and stored in a deep freezer until they were worked up.

4. Isolation and Purification of Alkaloids.

The frozen plants from the freezer were cut with pruning shears and homogenized with methanol in a high-speed blender. The slurry was transferred to a glass percolator and percolated with methanol. The percolation was continued until the extract was almost colorless and gave negative test for alkaloids on TLC. The methanol was evaporated and the resulting aqueous solution was concentrated to about 1 L with a rotary

evaporator at a temperature not above 40°. The aqueous solution was then extracted with ethyl acetate (3 x 200 ml) to remove chlorophyll and other nonbasic materials. The combined ethyl acetate extract was washed with 0.5 N HCl$_{(aq.)}$ (3 x 70 ml), and the washings were combined with the original aqueous solution (total alkaloids). The aqueous solution containing the "total alkaloids" was subjected to the extraction procedure shown in scheme VII.

Fraction II (codeine fraction, 65 mg) Cold codeine (35 mg) was added and the diluted sample (100 mg) was purified by prep. TLC on silica gel (0.5 mm) with chloroform-methanol (9:1) to give 77 mg of codeine. Further purification was done first by column chromatography on neutral alumina (activity IV) with benzene containing increasing amounts of methanol (20% to 100%), and then by prep. TLC on silica gel (0.25 mm) with CHCl$_3$-MeOH (9:1). The pure codeine fraction was crystallized repeatedly from benzene-hexane (1:1) to constant radioactivity.

Fraction IV (morphine fraction, 175 mg) was dissolved in 0.2 N NaOH (25 ml). To the alkaline solution was added ether (50 ml) and enough NH$_4$Cl to give pH 9.0. The solution was shaken vigorously and cooled in a refrigerator. Crystals of morphine were collected, washed with water, and recrystallized from aq. MeOH to constant radioactivity.

Fractions I and III were not purified.

5. Determination of Radioactivity.

The radioactivity was determined by liquid scintillation counting (LSC). The sample, 0.5-2 mg, was weighed accurately on a Cahn Gram Electrobalance and dissolved in 0.2 ml of methanol in an LSC vial. Ten ml of AQUASOL (a universal LSC cocktail from "New England Nuclear") was added. The counting efficiencies were determined with internal standards (toluene-^{14}C for ^{14}C activity; toluene-^{3}H for ^{3}H activity).

Total alkaloids (Aqueous solution, pH ∿ 1.5)

| CHCl$_3$

CHCl$_3$ extract
(Fraction I)   Aqueous

i) Basify with ammonia to pH 9
ii) CHCl$_3$: isopropanol (3:1)

CHCl$_3$:isopropanol   Aqueous (discard)
extract

0.2 N HCl

Organic layer   Aqueous
(discard)

i) Basify with NaOH to pH 13
ii) CHCl$_3$

CHCl$_3$ extract
(Fraction II)   Aqueous

i) Add NH$_4$Cl to pH 9
ii) Ether

Ether extract   Aqueous (pH = 9)
(Fraction III)

CHCl$_3$:isopropanol (3:1)

Aqueous   CHCl$_3$:isopropanol
(discard)   extract (Fraction IV)

Scheme VII

6. Determination of the Position of ^{3}H Label in the Radioactive Morphine

Isolated from Plants Fed with the 6-Ethyl Analog of [2-^{3}H]Thebaine.

The radioactive morphine was subjected to base-catalyzed proton-exchange in aqueous methanolic potassium carbonate as described before[44].

Radioactive morphine (60 mg, specific activity: 27943 dpm/mg) was dissolved in methanol (1 ml) in a 5 ml ampoule. Water (4 ml) and K_2CO_3 (22 mg) were added. The ampoule was sealed with a rubber septum and heated at 100° for 48 h. Morphine was worked up from the mixture and crystallized repeatedly from aqueous methanol (specific activity:0.0 dpm/mg, complete loss of ^{3}H).

Chapter III. ABERRANT BIOTRANSFORMATIONS OF ETHYL ANALOGS OF RETICULINE

IN PAPAVER SOMNIFERUM L. - THE EFFECTS OF ETHYL GROUPS ON

RACEMIZATION AND ALKALOID BIOSYNTHESIS.

A. Introduction:

The significance of unnatural biosynthesis in higher plants and examples of such reactions in the opium poppy have been described (Chap. II, Introduction). In this investigation, the biotransformations of reticuline were chosen for study. Reticuline has been established as the key intermediate in the biosynthesis of opium alkaloids (Chap. I) and is the only 1-benzyltetrahydroisoquinoline alkaloid which has been shown to undergo racemization in the opium poppy[51]. Feeding experiments were performed with three ethyl analogs of (±)-reticuline (55-57), which were doubly labeled with ^3H at position 1 and ^{14}C at position 3. Any incorporations of radioactivity into potential biosynthetic products from these unnatural precursors were examined. Degradative studies were carried out on selected products to test the possibility of randomization of the labels. The $^3H/^{14}C$ ratios were determined to detect possible racemization of the reticuline analogs in the plant. The results were used to define the substrate-specificity associated with the racemization and further biotransformations of reticuline, and to explore the possibility of preparing unnatural analogs of opium alkaloids by the administration of modified reticuline analogs to the plant.
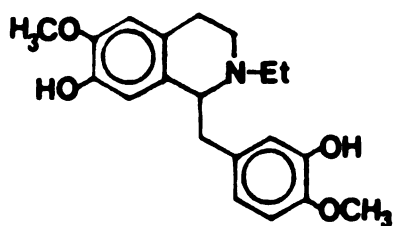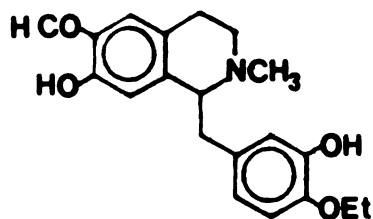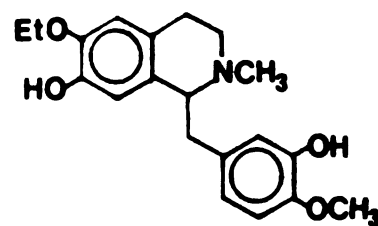
55   57   56

B. Results and Discussion

The results of the feeding experiments with the three ethyl analogs of reticuline are shown in Table X.

Feeding with N-ethylnor-reticuline (55).

No significant incorporation into the alkaloids isolated was observed. Practically all the radioactive precursor administered to the plant could be recovered unchanged. The N-ethyl analog did not undergo racemization during the time of incubation since the established mechanism for racemization via 1,2-dehydroreticulinium ion (13) (Scheme I) would result in loss of ^{3}H activity and a decrease in the ^{3}H/^{14}C ratio. The N-ethyl group prevents O-methylation of N-ethylnor-reticuline to N-ethyltetrahydro-papaverine (59) while O-methylation of (S)-(+)-reticuline (5a) to laudanosine (16) had been established in the opium poppy.[79] N-Ethylnor-reticuline was not N-dealkylated to nor-reticuline (4) which would have given radioactive papaverine and morphine. The N-ethyl analog cannot enter the biosynthetic pathway for the biotransformation of reticuline to morphine (Schemes III and IV) to generate the unnatural N-ethylnormorphine. Since N-ethyl analogs of the intermediate on this pathway were not isolated, it is not clear as to which step was blocked by the N-ethyl group. However, due to the observed substrate-specificity determined by the nitrogen substituent, the original attempt to prepare unnatural N-substituted morphine derivatives (including narcotic antagonists such as N-allylnormorphine) by the feeding of correspondingly modified reticuline analogs is not likely to succeed.

Feeding with 6-ethyl-4'-methyl-laudanosoline (56).

The 6-ethyl analog of reticuline was efficiently incorporated into morphine. The degradation of the radioactive morphine to the formaldehyde-dimedone adduct demonstrated that all the ^{14}C activity resided in the

Table X.

Exp.	Precursor and its specific activity (µCi/mg)	Amount of precursor fed µCi[a]	mg	Amounts of carriers added	Alkaloids isolated and their specific activities	dpm/mg[b]	µCi/mole	$^3H/^{14}C$	Degree of incorporation[c] or recovery (%)	Degradation Product & (%) of radioactivity
1	[1-^{3}H,3-^{14}C,6-OMe-^{14}C]-N-ethyl-nor-reticuline (55) (^{14}C:0.0598, ^{3}H: 0.405 $^3H/^{14}C$: 6.70)	^{14}C:1.148 ^{3}H:7.78	19.2	N-ethylnormorphine (300 mg)	N-ethylnormorphine (60)	<5	---	---	---	---
				N-ethyltetrahydro-papaverine (59) (300 mg)	papaverine (17)	<5	---	---	---	---
					N-ethylnormorphine (60)	~3	---	---	---	---
				N-ethylnor-reticuline (55) (313 mg)	N-ethylnor-reticuline (55)	^{14}C:7837 ^{3}H:53683	1225 8370	6.85	96	---
2	[1-^{3}H,3-^{14}C]-6-(+)-6-ethyl-4'-methyl-laudanosoline (56) (6-ethyl analog of reticuline) (^{14}C:0.370, ^{3}H:10.82, $^3H/^{14}C$:29.26)	^{14}C:6.66 ^{3}H:195	18	6-ethyl-4'-methyl-laudanosoline (56) (473 mg)	6-ethyl-4'-methyl-laudanosoline	---	---	29.12	---	---
					morphine (2) : 780 mg	^{14}C:616 ^{3}H:18295	84.84 2520	29.7	3.28	formaldehyde dimedone (98.9)[e]
3	[1-^{3}H,3-^{14}C]-(±)-4'-ethyl-6-methyl-laudanosoline (57) (4'-ethyl analog of reticuline) (^{14}C:0.893, ^{3}H:11.07, $^3H/^{14}C$:12.40)	^{14}C:7.46 ^{3}H:92.4	8.35	Morphine 3-ethyl ether (54) (300 mg)	Codeine (37)+ morphine[d] 3-ethyl ether (760 mg)	^{14}C:96 ^{3}H:1209	14.44 182	12.59	0.44	---
				4'-ethyl-6-methyl-laudanosoline (57) (358 mg)	Morphine(2):1080 mg 4'-ethyl-6-methyl-laudanosoline	^{14}C:85 ^{3}H:1060	11.71 146	12.47 12.60	0.56	formaldehyde dimedone (100.06)[e]

a, b, and c are as described in Table IX.   [d] obtained from Table IX.   [e] calculated as $\dfrac{\text{specific activity of formaldehyde-dimedone (dpm/mole)}}{\text{specific activity of isolated morphine (dpm/mole)}}$

C-16 position as expected. The ^{3}H/^{14}C ratios in the isolated morphine and in the recovered precursor are the same as that of the administered precursor within experimental error, indicating that racemization of the 6-ethyl analog of reticuline did not take place during incubation. Since only the (-)-isomer of the administered (±)-reticuline analog has the correct stereochemistry to be converted to morphine, the actual efficiency of this unnatural biosynthesis should be close to 6.5%, twice the observed value of 3.28%. Since the incorporation into morphine of 6-ethyl-4'-methyl-laudanosoline is comparable to that of reticuline (the incorporation of (±)-reticuline into morphine has been reported to be 7.3% by Battersby et al.[13] and 0.14% by Barton et al.[15]), it may be concluded that the 6-ethyl analog of reticuline enters the same biosynthetic pathway as is operative for the transformation of (-)-reticuline to morphine (Chap. I) and that the 6-ethyl group does not seem to interfere with the enzymatic reactions involved in this pathway. The 6-ethyl analog of thebaine (58) would be expected to be formed first, which in turn is converted to codeine and morphine as has been discussed in Chap. II.

Feeding with 4'-ethyl-6-methyl-laudanosoline (57).

Significant incorporations of the 4'-ethyl analog of reticuline into 3-ethylmorphine (54) and morphine (2) were observed. Practically all the ^{14}C activity in morphine was shown by chemical degradation to be located in the expected position. Again, racemization of the precursor was prevented by the ethyl group, and the actual degree of incorporation should be twice the observed value, or around 1%. It appears that 4'-ethyl-6-methyl-laudanosoline can be converted by the plant enzyme system, but less efficiently, than the 6-ethyl analog to 3-ethyloripavine (53), which in turn can be converted to 3-ethylmorphine (54) and morphine as has been

demonstrated previously[52]. Based on the incorporations observed with the 6-ethyl and 4'-ethyl analogs of reticuline and the negative results with N-ethylnor-reticuline, the substrate specificity associated with the enzyme systems involved in the biotransformation of (-)-reticuline to morphine may be partially defined. When the modification involved the A-ring of the tetrahydroisoquinoline moiety, the transformation to morphine took place with about the same degree of efficiency as with reticuline itself. However, when the ethyl group was in the benzylic portion of the molecule, some interference with the conversion was observed. This is in line with the observation of differential incorporations of O-ethyl analogs of tetra-hydropapaverine into papaverine analogs[62]. The enzyme systems appear to be very sensitive to modifications on the nitrogen. Replacement of the N-methyl group of reticuline with an ethyl group completely prevented further biotransformations. This kind of substrate specificity might be determined by steric interactions between peripheral groups on the substrate molecule and specific functions on the enzyme molecule.

The racemization of reticuline appears to be a process of strict substrate specificity. None of the ethyl analogs administered could be shown to have undergone racemization during the time of incubation. This adds strength to the observation that reticuline is the only 1-benzyltetrahydro-isoquinoline alkaloid which undergoes racemization in the opium poppy[51].

C. Experimental

    1. General (see Chap. II, p. 26)

    2. Synthesis of Labeled Precursors:

      (a) [1-^{3}H, 3-^{14}C, 6-OMe-^{14}C]-($\pm$)-N-Ethylnor-reticuline (55).

The triply labeled N-ethylnor-reticuline (55) was prepared according to Scheme VIII. The starting material, [1-^{3}H, 3-^{14}C, 6-OMe-^{14}C]-nor-reticuline

Scheme VIII
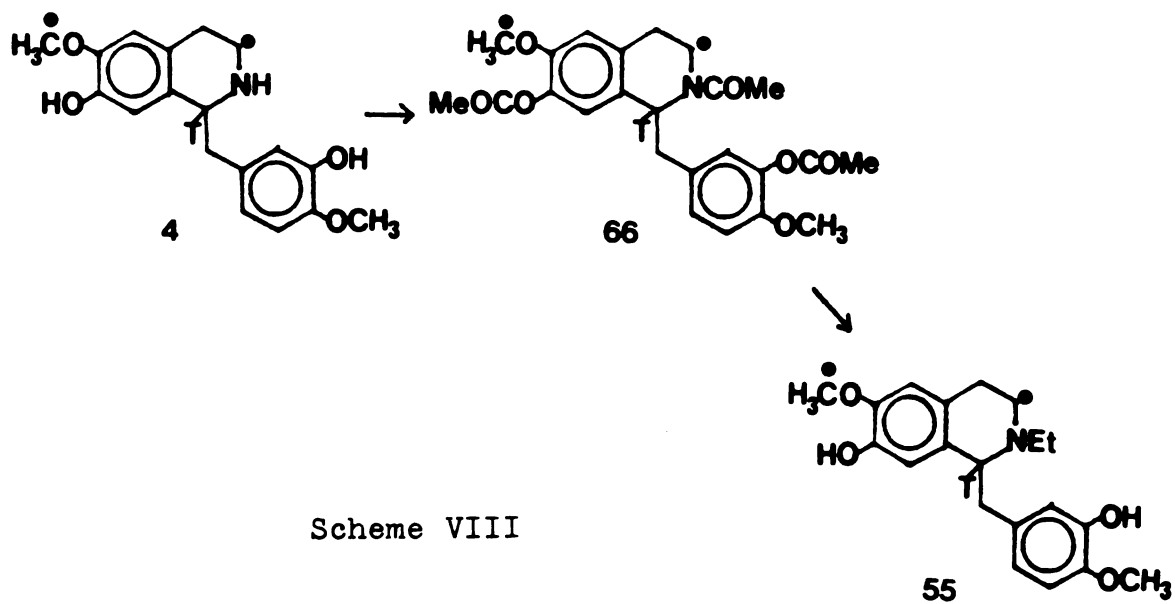
(4) was synthesized by Dr. C.-H. Chen.

[1-^{3}H, 3-^{14}C, 6-OMe-^{14}C]-($\pm$)-N,7-O,3'-O-Triacetylnor-reticuline (66).

[1-^{3}H,3-^{14}C,6-OMe-^{14}C]-($\pm$)nor-reticuline (42.2 mg), obtained from its

hydrochloride salt with aqueous sodium hydrogen carbonate and ether

extraction, was dissolved in acetic anhydride (3 ml). Pyridine (0.3 ml)

was added, and the mixture was stirred at 120° under nitrogen for 3 h.

Water was added to decompose the excess acetic anhydride. The aqueous

mixture was then extracted with CHCl$_3$. The CHCl$_3$ extract was washed succes-

sively with dil. HCl$_{(aq.)}$, water, dil. NH$_4$OH, and water again. The organic

layer was dried and evaporated. The residue showed a single spot on TLC

(silica gel, CHCl$_3$:MeOH = 9:1).

[1-^{3}H,3-^{14}C,6-OMe-^{14}C]-($\pm$)-N-Ethylnor-reticuline (55).

The residue obtained above was dissolved in 4 ml of anhydrous tetra-

hydrofuran (THF). The solution was added to a stirred suspension of LiAlH$_4$

(25 mg) in anhydrous THF (3 ml) from a dropping funnel. The mixture was

stirred overnight at room temperature. The reaction was quenched with

water saturated with Na,K-tartrate. The precipitate was removed by

filtration and washed with CHCl$_3$. The filtrate and CHCl$_3$ washings were

combined and evaporated to dryness. The residue was dissolved in 0.1 N

HCl$_{(aq.)}$. The aqueous solution was washed with CHCl$_3$, and then basified

with KOH$_{(aq.)}$ (pH ~ 14). The alkaline solution was washed with CHCl$_3$,

and then neutralized with NH$_4$Cl$_{(s)}$ until the pH = 8.5 - 9. The aqueous

solution was extracted several times with ether. The combined ether

extract was washed with H$_2$O, dried over MgSO$_4$, and evaporated to give

19.2 mg of triply labeled target compound (55). It was shown to be identical

with a cold sample of N-ethylnor-reticuline (See 3(a), p. 45) by TLC.

The specific activities were determined to be 132,868 dpm/mg for ^{14}C

and 890,215 dpm/mg for ^{3}H, with a ^{3}H/^{14}C ratio of 6.70 (a value of 6.80

was obtained for the starting material).

(b) [1-^{3}H,3-^{14}C]-(±)-6-Ethyl-4'-methyl-laudanosoline (56).
———————————————————————————————

The synthesis of this doubly-labeled 6-ethyl analog of reticuline is

shown in Scheme IX, based on the methods described previously for the

synthesis of (±)-reticuline (cf. 3(a), p. 43 & Scheme X). K^{14}CN and

NaB^{3}H$_4$ (obtained from "New England Nuclear") were used to introduce the

3-^{14}C and 1-^{3}H labels, respectively. Most of the synthetic steps were

carried out by Dr. H.-C. Chiang. The feeding sample was prepared by mixing

appropriate amounts of the [1-^{3}H] and [3-^{14}C] singly labeled compounds.

The physical constants and spectral data were obtained with the non-radioactive

compound prepared for use as a cold carrier.

m.p. = 87-89°C; ^{1}H NMR (CDCl$_3$) δ 1.40 (t, 3H, 6-OCH$_2$CH$_3$, $J_{1,2}$ = 7.2 Hz),

2.43 (s, 3H, N-CH$_3$), 2.61-3.25 (m, 6H), 3.65 (t 1H, H-1, $J_{1,2}$ = 6.0 Hz),

3.82 (s, 3H, 4'-OCH$_3$), 4.05 (q, 2H, 6-OCH$_2$CH$_3$, $J_{1,2}$ = 7.2 Hz), 6.40-6.77

(m, 5H, aromatic H's). high resolution MS (ei), m/e 341.1609 (M-2$^+$,

C$_{20}$H$_{23}$O$_4$N, 10% BP), 206.1176 (C$_{12}$H$_{16}$O$_2$N, 100%). It was found that M$^+$ peaks

from reticuline analogs (including an authentic sample of reticuline itself)

cannot be observed under ei conditions. Invariably, (M-2)$^+$ peaks were the

highest m/e's observed. But the dibenzyl derivatives gave the correct M$^+$

peaks. Besides, the BP's on the mass spectra of these reticuline derivatives

apparently resulted from cleavage of the benzylic portion of the molecule.

(c) [1-^{3}H,3-^{14}C]-(±)-4'-Ethyl-6-methyl-laudanosoline (57).
———————————————————————————————

This compound was prepared by Dr. H.-C. Chiang by a synthetic sequence
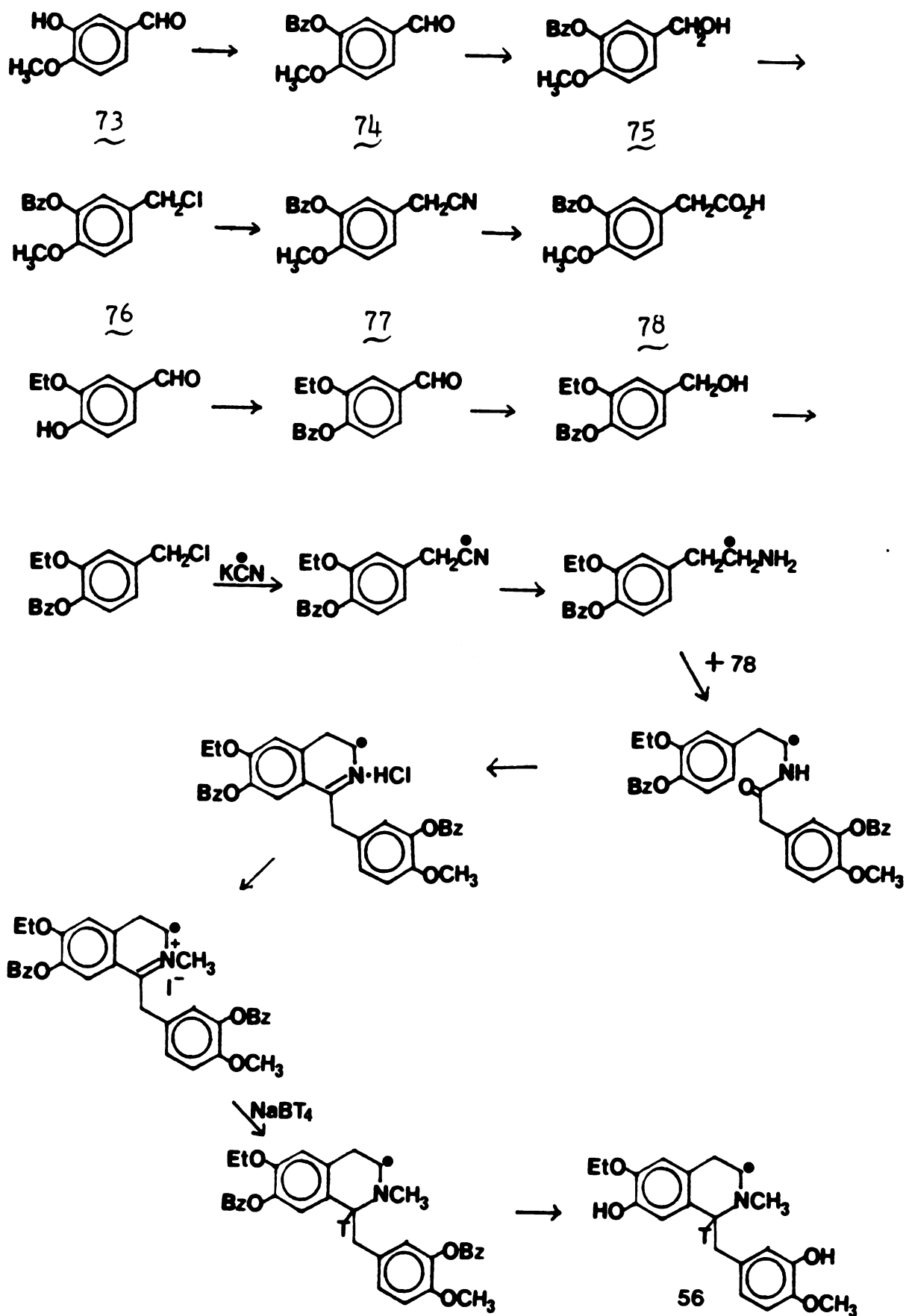
Scheme IX

similar to Scheme IX.

m.p. = 58-61°C; ^{1}H NMR (CDCl$_3$) δ 1.32 (t, 4'-OCH$_2$CH$_3$,J$_{1,2}$ = 7. Hz)

2.36 (S, N-CH$_3$), 3.60 (t, H-1, J$_{1,2}$ = 7.2 Hz), 3.74 (s, 6-O-CH$_3$), 3.93

(q, 4'-OCH$_2$CH$_3$, J$_{1,2}$ = 7.0 Hz), 6.29-6.70 (m, aromatic H's). high resolu-

tion MS (ei), m/e 341.1623 (M-2$^+$, C$_{20}$H$_{23}$O$_4$N, 1.53% BP), 192.1018

(C$_{11}$H$_{14}$O$_2$N, 100%).

3. Synthesis of Unnatural Alkaloids for Reverse Isotope Dilution.

(a) (±)-N-Ethylnor-reticuline (55).

Methods for the synthesis of (±)-reticuline have been described[64-67].

The sequence worked out by Jain[67] was followed with modifications for the

preparation of the N-ethyl analog of reticuline (Scheme X). The starting

materials were vanillin (67) and isovanillin (73). The phenolic groups

were protected by reacting with benzyl chloride under mildly basic condition

to yield the corresponding O-benzyl ethers (68 & 74). The aldehyde groups of

68 & 74 were reduced with NaBH$_4$ to the corresponding alcohols (69 & 75) which

were converted to the chlorides (70 & 76) with thionyl chloride and

reacted with potassium cyanide in DMSO to give the corresponding nitriles

(71 & 77). 4-Benzyloxy-3-methoxyphenylacetonitrile (71) was reduced

with LiAlH$_4$ to the corresponding amine (72), while 3-benzyloxy-4-methoxy-

phenylacetonitrile (77) was hydrolyzed with KOH in ethylene glycol to

the acid (78). The amine (72) and the acid (78) was condensed by fusion

at 160-170° under reduced pressure to give 3-benzyloxy-N-(4-benzyloxy-3-

methoxyphenethyl)-4-methoxyphenylacetamide (79). Bischler-Napieralski cycli-

zation with phosphorous oxychloride then afforded a 3,4-dihydroisoquinoline

hydrochloride (80). The imine hydrochloride (80) was reduced with NaBH$_4$

to give O,O-dibenzylnor-reticuline (81). ^{1}H NMR (CDCl$_3$) δ 2.60-3.20

(m, 6H), 3.85 (S, OCH$_3$), 4.22 (broad S, NH), 5.03 & 5.07 (2S, OCH$_2$C$_6$H$_5$),

6.50-7.42 (m, aromatic H's).
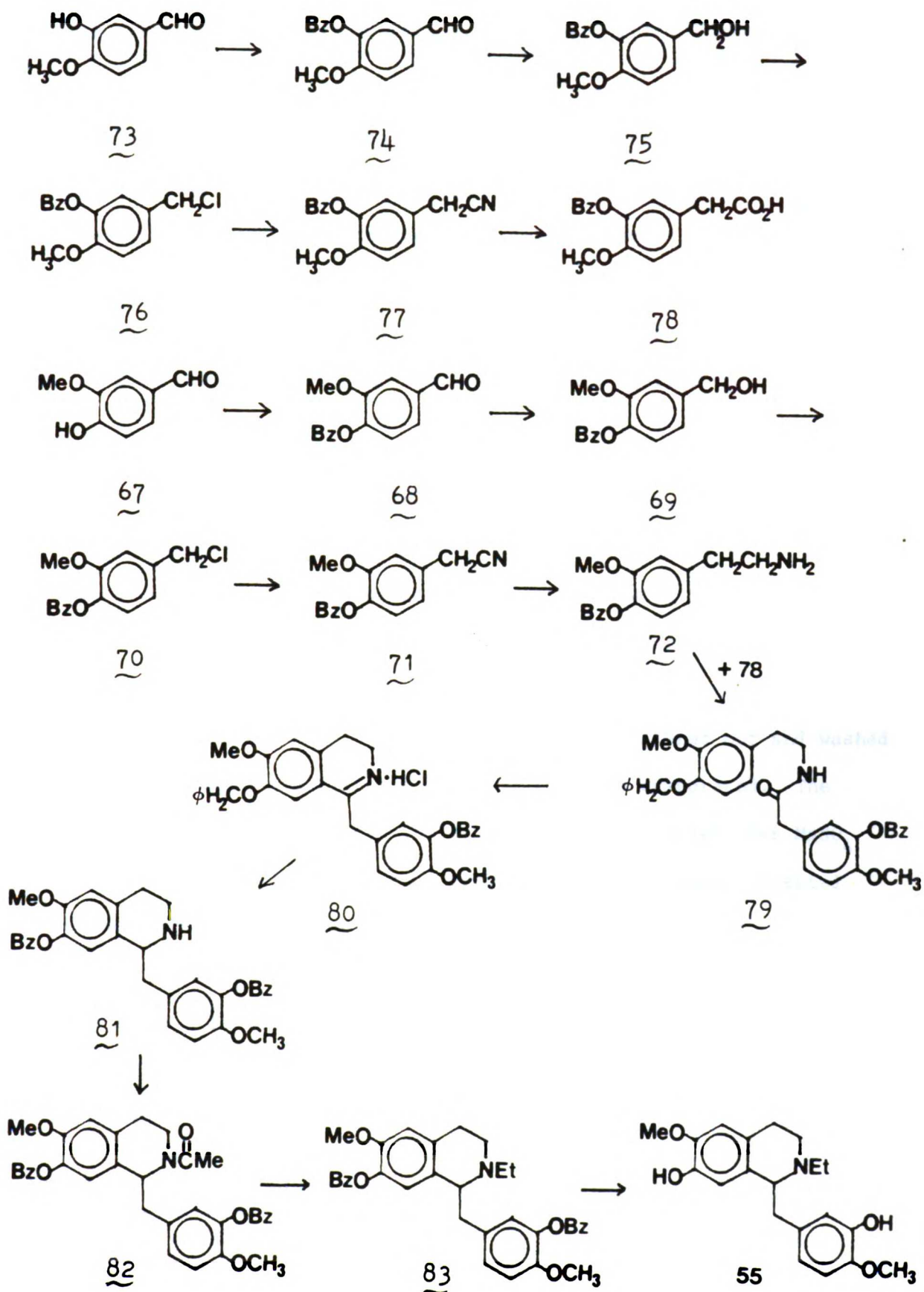
Scheme X

(±)-N-Acetyl-$\underline{O}$,$\underline{O}$-dibenzylnor-reticuline (82).   (±)-$\underline{O}$,$\underline{O}$-Diben-

sylnor-reticuline (192 mg) was dissolved in acetic anhydride (3 ml) 0.5 ml

of pyridine was added, and the mixture was stirred under nitrogen at 120°

for 3 h. Water (15 ml) was added to decompose the excess acetic anhydride.

The aqueous mixture was then extracted with $CHCl_3$. The extract was washed

with dil $HCl_{(aq.)}$, water, dil. NH_4OH, and water again. The $CHCl_3$ layer

was dried and evaporated. The residue was crystallized from absolute

alcohol to give 162 mg of 82.   mp 128-129.5°C; pure by TLC.

(±)-$\underline{N}$-Ethyl-$\underline{O}$,$\underline{O}$-dibenzylnor-reticuline (83).   (±)-$\underline{N}$-Acetyl-$\underline{O}$,$\underline{O}$-diben-

zylnor-reticuline (82, 200 mg) in THF (5 ml, anhydrous 99.9%, from Aldrich)

was added dropwise to a stirred suspension of $LiAlH_4$ (150 mg) in THF (15 ml).

The stirring was continued and the reaction monitored with TLC. After

5 h, TLC showed complete disappearance of the starting material, and the

reaction was terminated. Drops of water were added to the reaction mixture

to decompose the excess $LiAlH_4$. The precipitate was filtered out and washed

with THF. The washings and filtrate were combined and evaporated. The

residue was extracted with ether. The ether extract was dried over $MgSO_4$

and evaporated to give 173 mg of crude product (83), which was subjected

to the subsequent debenzylation without further purification. A portion

of the sample was purified by column chromatography.

^{1}H NMR ($CDCl_3$) δ 1.13 (t, N-CH$_2$CH$_3$ $J_{1,2}$ = 7 Hz), 2.70 (q, $\underline{N}$-CH$_2$CH$_3$, $J_{1,2}$ =

7 Hz), 3.83 (S, OCH$_3$), 4.75 (s, 7-OCH$_2$C$_6$H$_5$), 5.07 (S, 3'-OCH$_2$C$_6$H$_5$), 5.94-

7.45 (m, aromatic H's); MS (ei) m/e 523 (M$^+$, $C_{34}H_{37}O_4N$).

(±)-$\underline{N}$-ethylnor-reticuline (55). The product above (173 mg) was dissolved

in conc. $HCl_{(aq.)}$ (10 ml), and benzene (10 ml) was added to form a two-phased

system. The mixture was vigorously stirred under nitrogen at room tem-

perature[81]. The benzene layer was monitored by TLC for disappearance

of the starting material. The reaction was terminated after 10 h, water

was added (20 ml), and the aqueous layer separated from the benzene layer.

While cooled in ice-water, the aqueous solution was neutralized with $NH_4OH_{(aq.)}$

until the pH = 8.5-9.0. The resulting solution was extracted several

times with $CHCl_3$. The $CHCl_3$ extract was dried and evaporated to give

132 mg of the crude product (55). Further purification was done by column

chromatography on silica gel with MeOH in $CHCl_3$ (1%, 2%, and 4%) as eluent

and recrystallization from benzene-hexane. m.p. 85-88°; ^1H NMR ($CDCl_3$)

δ 1.12 (t, N-C$\underline{H}_2$CH$_3$, 2.73 (q, N-C$\underline{H}_2$CH$_3$), 3.84 & 3.86 (2S, OCH$_3$), 6.26-

6.75 (m, aromatic H's); MS (ei), m/e 341 ($M-2^+$, 13% BP), 206 (100%) (cf.

2b p. 41).

b. (+)-N-Ethyltetrahydropapaverine (59).

     This N-ethyl analog of laudanosine was prepared from tetrahydropapa-

verine hydrochloride synthesized by Dr. C.-H. Chen. Liberation of the base

THP (6), followed by N-acetylation, gave N-acetylnorlaudanosine (84). Reduct-

ion of 84 with LiAlH$_4$ resulted in the target compound (59) (Scheme XI).

     (+)-N-acetylnorlaudanosine (84). Tetrahydropapaverine (1.065 gm,

obtained from its hydrochloride salt by treatment with dil. NH$_4$OH and

$CHCl_3$ extraction, was dissolved in acetic anhydride (5 ml), and 1 ml of

pyridine was added. The mixture was stirred under nitrogen at 120° for

3 h. Water (20 ml) was added to decompose the excess acetic anhydride,

and the mixture extracted with $CHCl_3$ (3 x 2 ml). The combined extract

was washed with dil. $HCl_{(aq.)}$, water, dil. NH$_4$OH, and water again. The

$CHCl_3$ layer was dried and evaporated to give 0.880 gm of 84, which was

pure by TLC and was subjected to the subsequent reduction.

(±)-N-ethyltetrahydropapaverine (59).   (±)-N-acetylnorlaudanosine

(880 mg) obtained above was dissolved in anhydrous THF (5 ml) and the

solution added dropwise to a cooled stirred suspension of LiAlH$_4$ (200 mg)

in THF (10 ml).   Stirring was continued at room temperature overnight.

The excess LiAlH$_4$ was decomposed cautiously by adding water. The mixture

was filtered and the precipitate washed several times with CHCl$_3$. The

filtrate and washings were combined and evaporated. The residue was

dissolved in dil. HCl$_{(aq.)}$. The acidic solution was washed with ether and

basified with NH$_4$OH$_{(aq.)}$ (pH = 9). The mixture was then extracted with

CHCl$_3$ (3 x 30 ml). The CHCl$_3$ extract was washed with H$_2$O, dried and evapo-

rated to give 867 mg of crystalline residue. The crude product was recrystal-

lized from aqueous alcohol to give 780 mg of pure 59. m.p. 88-89°; ^{1}H NMR

(CDCl$_3$) δ 1.17 (t, N-CH$_2$CH$_3$), 2.60-3.30 (m, including NCH$_2$CH$_3$), 3.55, 3.80,

3.83, and 3.84 (4S, OCH$_3$'s), 5.97-6.82 (m, aromatic H's); MS (ei) m/e 371

(M$^+$), 220 (BP).

(c)   N-Ethylnormorphine (60).

The synthetic sequence for the preparation of N-ethylnormorphine (60)

was shown in Scheme XII. The N-demethylation of morphine was carried out

with the use of 2,2,2-trichloroethyl chloroformate followed by zinc reduction,

a procedure developed by Montzka et al[68]. The normorphine (41) thus obtained

was reacted with acetic anhydride to yield N-acetylnormorphine (85), which

was reduced with LiAlH$_4$ to give N-ethylnormorphine (60).

N-2,2,2-Trichlorocarbethoxynormorphine (85).   To a suspension of

morphine monohydrate (2.00 gm, 6.6 mmol) and finely divided KHCO$_3$ (11.26 g)

in CHCl$_3$ (200 ml) was added a large excess of trichloroethylchloroformate

(12.23 g, 57.7 mmol). The reaction mixture was refluxed for 60 h.

6 → **84**

84 → **59**

Scheme XI

2 → **85** → **41**

41 → **60** → **86**
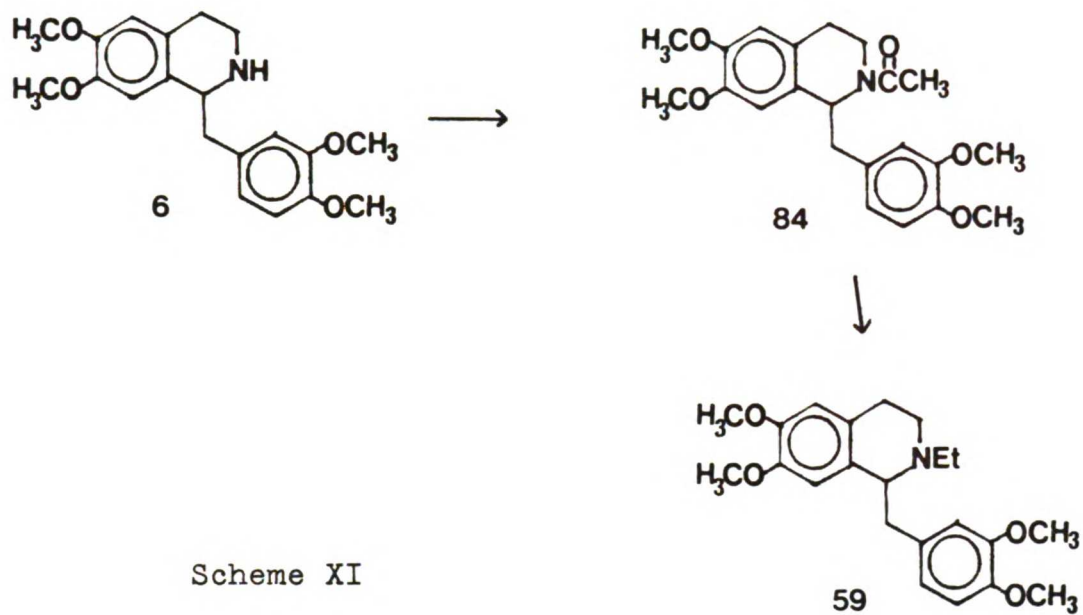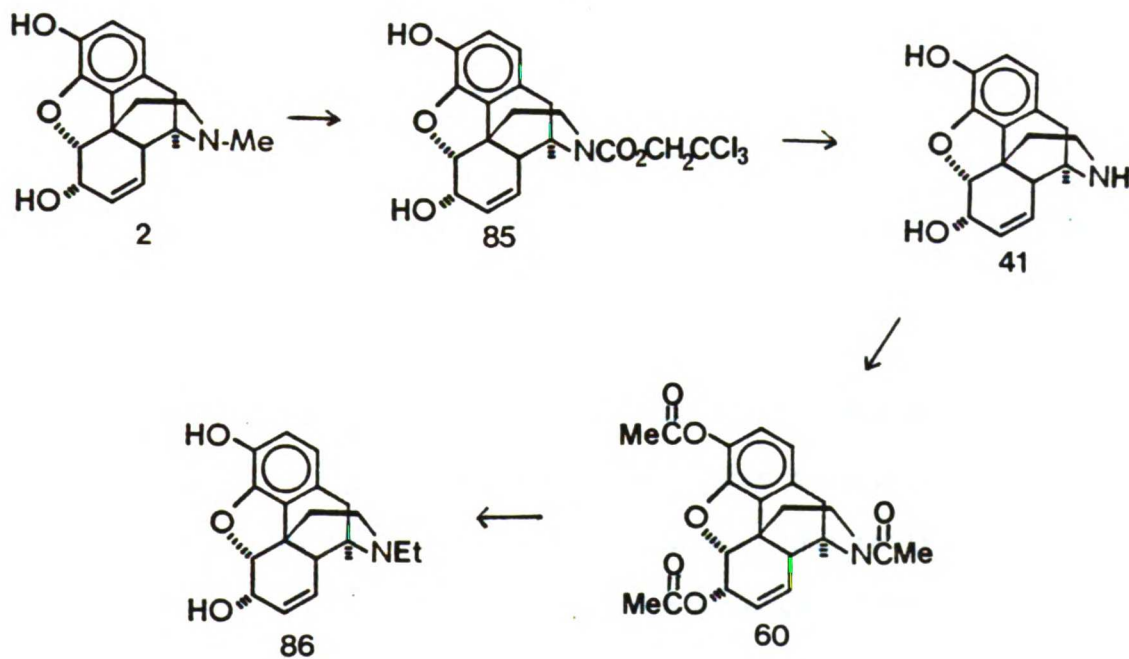
Scheme XII

Water (100 ml) was added, and the $CHCl_3$ layer was separated and

concentrated in vacuo. The residue was dissolved in MeOH (120 ml), and a

solution of KOH (4.2 g) and $KHCO_3$ (7.5 g) in H_2O (75 ml) was added.

The mixture was stirred under nitrogen at room temperature for 24 h. Under

cooling the mixture was acidified with conc. HCl, and evaporated to remove

MeOH. The residue was diluted with H_2O and extracted with CH_2Cl_2. The CH_2Cl_2

extract was washed with water, dried over Na_2SO_4, and evaporated to give

a yellowish oily residue (3.07 g). The crude product was chromatographed

on a column of silica gel (50 g, activity II) with $CHCl_3$ containing 3%

MeOH as eluent to give 2.76 g of 85, pure by TLC, m.p. 195-197° (lit.[68]

mp 196-198°).

Normorphine (41). To a solution of the carbamate (85) (1.184 g) in

90% acetic acid (50 ml) was added powdered zinc (1.5 g, in portions), and

the mixture was stirred at room temperature overnight. The reaction mixture

was filtered and the precipitate washed with water (10 ml). The combined

filtrate was evaporated under reduced pressure at 40°. Water (30 ml) was

added to the residue, and the insoluble material was removed by filtration.

The acidic filtrate was washed with ether, and then basified with $NH_4OH_{(aq.)}$

(pH = 8-9) to give a red-colored solution. The aqueous solution was

extracted repeatedly with $CHCl_3$-isopropyl alcohol (3:1). The extracts

were combined, washed with water, dried and evaporated to give 439 mg (61%)

of normorphine (41), pure on TLC, mp 268-270° decomp. (Due to its more polar

nature, normorphine is difficult to extract from aqueous solution. Seventy-

five percent recovery with 6 portions of $CHCl_3$-isopropanol (3:1) at pH 8.6[49]).

N,O,O-triacetylnormorphine (86). Normorphine (1.0 g) was dissolved

in acetic anhydride (5 ml), and pyridine (1 ml) was added. The mixture

was stirred under nitrogen at 120° for 3 h. Water (20 ml) was added to

decompose the excess acetic anhydride. The aqueous solution was extracted

with $CHCl_3$ (3 x 20 ml). The $CHCl_3$ extract was washed successively with H_2O, dil. NH_4OH, dil. $HCl_{(aq.)}$, and H_2O again. Drying over Na_2SO_4 followed by evaporation gave the crude product (1.329 g, homogeneous on TLC), which was used in the next reaction without further purification

N-Ethylnormorphine (60). N,O,O,-triacetylnormorphine (86, 1.329 gm) obtained above was dissolved in anhydrous THF (10 ml). The solution was added dropwise to a stirred suspension of $LiAlH_4$ (550 mg) in THF (10 ml). The stirring was continued at room temperature overnight. The excess $LiAlH_4$ was decomposed by adding water saturated with Na,K-tartrate. The precipitate was removed by filtration and thorougly washed with chloroform-isopropanol (3:1). The orange-colored filtrate and washings were combined and evaporated to dryness. The residue was dissolved in H_2O (pH = ~ 8.5) and extracted with $CHCl_3$/isopropanol (3:1). The extract was washed with a small portion of H_2O, dried, and evaporated to give 652 mg (60% from normorphine) of N-ethylnormorphine (60) which was recrystallized from 50% aqueous alcohol, pure on TLC; m.p. 220-221° dec. ^1H NMR ($CDCl_3$), δ 1.18 (t, NCH_2CH_3, $J_{1,2}$ = 0.72), 1.75-3.10 (m, inclu. NCH_2CH_3) 4.86 & 4.93 (2d, H-5, & H-6, $J_{1,2}$ = 1.0 Hz), 5.25 and 5.54 (2dd, H-7 & H-8), 6.60 (2d, H-1 & H-2, $J_{1,2}$ = 8 Hz); MS (ei) m/e 299 (M^+).

4. Purification of isolated alkaloids.

The cultivation of plants, administration of labeled precursors, isolation of alkaloids, and determination of radioactivity were carried out by the same methods as described in Chap. II, Experimental.

Three batches of plants were used for the separate feedings of the three ethyl analogs of reticuline. The amounts of precursors fed and cold carriers added are shown in Table X. The plants were worked up as described in Chap. II with the addition of cold samples of alkaloids as

carriers to give the "total alkaloids," which were subjected to the
extraction procedure shown in Scheme VII, Chap. II. Purification of
isolated alkaloid fractions is described below.

(a) Plants fed with [1-^{3}H, 3-^{14}C, 6-OMe-^{14}C]-N-ethylnor-reticuline (55):

Fraction 1 (weak bases). 1.278 g. GLC showed the presence of
mainly N-ethyltetrahydropapaverine (59), tetrahydropapaverine (6), papaverine
(17), and thebaine (26). Column chromatography on silica gel with ethyl
acetate containing 1% methanol as eluent gave crude papaverine and N-ethyl-
tetrahydropapaverine. Papaverine was purified by preparative TLC on silica
gel with ethyl acetate-methanol (96:4), and crystallized from ether-methanol
to constant radioactivity. The N-ethyltetrahydropapaverine was purified
by prep. TLC on silica gel (0.5 mm) with CHCl$_3$-MeOH (9:1) to give 165 mg of
pure sample, which was crystallized repeatedly from ethanol.

Fraction III (reticuline fraction). 501 mg. GLC showed the presence
of N-ethylnor-reticuline (55), N-ethylnormorphine (60), and reticuline (5).
The alkaloids were separated by prep. TLC on silica gel first on 2 mm plates,
then on 0.5 mm plates. N-Ethylnormorphine was crystallized from aqueous
alcohol to constant activity. N-Ethylnor-reticuline was further purified
by prep. TLC.

Fractions II (nonphenolic alkaloids, mainly codeine and thebaine) and
IV (morphine fraction) were not purified.

(b) Plants fed with [1-^{3}H,3-^{14}C]-(+)-6-ethyl-4'-methyl-laudanosoline (56):

Fraction I (weak bases), 600 mg. TLC showed the presence of mainly
papaverine with a trace amount of thebaine. Column chromatography on
silica gel with CHCl$_3$ containing 1% MeOH as eluent gave pure papaverine,
which was crystallized repeatedly from ether-methanol and aqueous ethanol.

Fraction II (nonphenolic fraction) was not purified.

Fraction III (reticuline fraction) was purified repeatedly by prepa-
rative TLC on silica gel for the isolation of pure N-ethylnor-reticuline.

Fraction IV (morphine fraction, 780 mg) was dissolved in dil. HCl$_{(aq.)}$.

The solution was saturated with ether, basified to pH 9 with NH$_4$OH, and

cooled in a refrigerator to effect crystallization. The crystals were

collected, washed with water, and recrystallized from aqueous MeOH to

constant radioactivity.

(c)   Plants fed with [1-^{3}H,3-^{14}C]-(±)-4'-ethyl-6-methyl-laudanosoline (57).

Fraction I (weak bases, 830 mg) was purified as before to give

papaverine, which was crystallized to constant radioactivity.

Fraction II (nonphenolic fraction, 760 mg). TLC showed the presence

of mainly codeine, morphine 3-ethyl ether, and thebaine. The separation

was done by column chromatography on alumina (activity III) with benzene

containing increasing amounts of chloroform ranging from 20% to 50%. Further

purification was done by preparative TLC on silica gel (0.25 mm) with

chloroform-methanol (3:1) to give a homogeneous fraction containing a

mixture of codeine and morphine 3-ethyl ether.

Fraction III (reticuline fraction, 1.20 g). The 4'-ethyl analog of

reticuline was isolated and purified by repeated prep. TLC on silica gel

(0.5 mm and 0.25 mm) with chloroform-methanol (8:2).

Fraction IV (morphine fraction, 1.08 g) was purified as described

above and crystallized repeatedly from aqueous MeOH to constant radioactivity.

5.   Determination of the Positions of Labels.

(a)   Degradation of radioactive morphine isolated from plants fed

with [1-^{3}H,3-^{14}C]-(±)-6-ethyl-4'-methyl-laudanosoline (56).

The degradation of morphine followed the sequence shown in Scheme XIII.

The radioactive morphine was methylated by Pschorr and Dickhäuser's

method[83] to yield codeine methyl ether methiodide (87) which was subjected

to Hofmann degradation[83]. The resulting α-dimethylmorphimethine (88)

was reduced by catalytic hydrogenation with platinum oxide to give tetra-

hydrocodeimethine methyl ether, which was converted to the methiodide[9]

2 → **87** → **88** →

89 → → **90** →

91 + H$\overset{\bullet}{\text{C}}$HO

92
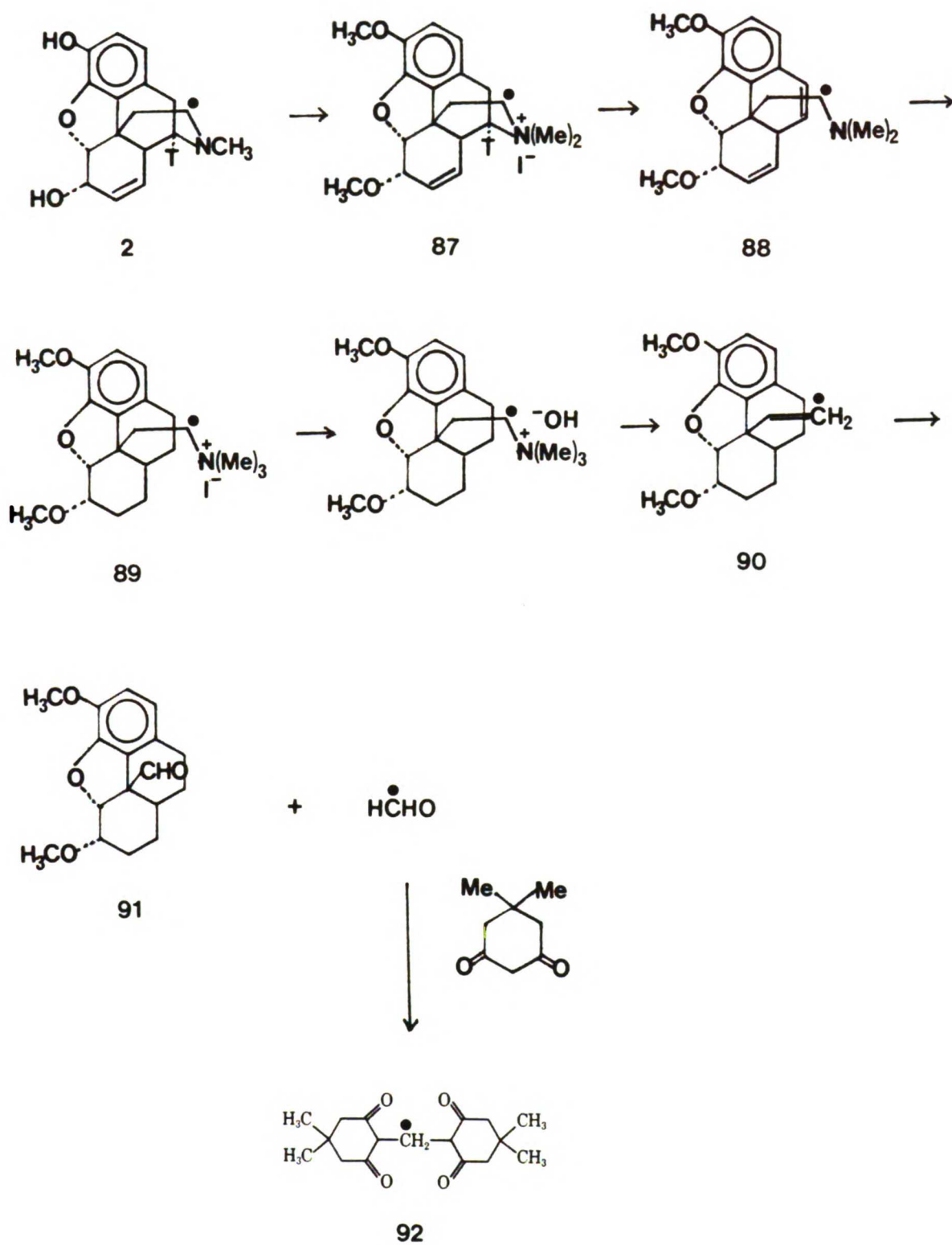
Scheme XIII

(89). The methiodide (89) was converted to the methohydroxide by treatment with Ag_2O and degraded to 6-methoxy-13-vinyloctahydromethylmorphenol (90) and trimethylamine[84]. Ozonolysis of compound 90 by the method of Battersby et al.[14] for the oxidation of 5-vinylphenanthrene gave 6-methoxy-13-formyloctahydromethylmorphenol (91) and formaldehyde. The formaldehyde was converted to the crystalline formaldehyde-dimedone adduct (92) for determination of radioactivity.

Codeine methyl ether methiodide (87). Radioactive morphine (321 mg) was dissolved in 1.5 ml of 1 N NaOH$_{(aq.)}$. Dimethylsulfate (0.2 ml) was added at 0° with stirring until its disappearance. 10 N NaOH (0.1 ml) and dimethylsulfate (0.1 ml) were added three more times, and the stirring was continued at 0° for 4 h. Sufficient conc. KI$_{(aq.)}$ was added, and crystals were formed after cooling in the refrigerator overnight. The crystals were recrystallized from 10 parts of 80% alcohol to give codeine methyl ether methiodide (276 mg). m.p. 240-242°.

α-Dimethylmorphimethine (88). To a heated solution of 87 (276 mg) in 1.5 ml of water was added 25% NaOH (0.5 ml). The separation of a brown-colored oil occurred, and the heating ($\sim$95°) was continued for 10 min. The oil crystallized upon cooling in ice-water. The crystalline residue was washed repeatedly with ice-water to remove alkali. Recrystallization from aqeuous MeOH gave 177 mg of glossy flakes. m. p. 92-93° (lit.[83] 94°).

Tetrahydrocodeimethine methyl ether methiodide (89). A solution of 88 (177 mg) in ethanol (15 ml) was hydrogenated with a Parr hydrogenator (hydrogen pressure:40 psi) at room temperature in the presence of platinum oxide (73 mg). After 5 h the catalyst was removed by filtration and the filtrate was evaporated to give a gum. The reduction was confirmed by NMR (disappearance of signals from the olefinic protons) and mass spectrometry

(m/e 331, M$^+$ of the reduction product). The free base, tetrahydro-codeimethine methyl ether (94 mg), was converted to the methiodide (89) by warming with CH$_3$I in ethanol.

6-Methoxy-13-vinyloctahydromethylmorphenol (90). Silver oxide was freshly prepared by adding 1 ml of 3 N NaOH$_{(aq.)}$ to a solution of silver nitrate (200 mg, 3-4 eq.) in H$_2$O (1 ml). The precipitate was washed with water until the washings were neutral and then with a portion of H$_2$O previously boiled to remove CO$_2$. From this point on, precaution was taken to exclude CO$_2$ from the reaction mixture. Using 2 ml of H$_2$O to effect the transfer, the silver oxide thus prepared was added to a warm soln. of tetrahydrocodeimethine methyl ether methiodide (119 mg) in 4 ml of H$_2$O. The reaction flask was flushed with nitrogen and sealed with a rubber septum, and the mixture was stirred at room temperature for 8 h. Filter aid was added, and the mixture filtered through a sintered glass funnel. The residue was washed with portions of boiling water, and the combined filtrate was evaporated to dryness under reduced pressure at 40°. After drying in a vacuum overnight, the residue (101 mg) was decomposed by cautious heating under vacuum. Decomposition began at 90° (bath temp.) and was substantially complete by 140°, after which the temperature was increased and held at 170° for 15 min. The condenser and the residue was thoroughly washed with ether. The combined ether extract was washed successively with 1 N HCl$_{(aq.)}$ water, half-saturated NaHCO$_{3(aq.)}$, and water again. Evaporation of ether gave an oil (80 mg) which was subjected to ozonolysis without further purification.

Formaldehyde-dimedone adduct (92)/ Ozonized oxygen generated with a Welsbach ozonator was passed through a solution of 6-methoxy-13-vinylocta-hydromethylmorphenol (90) (80 mg) in ethyl acetate (5 ml) at -78°. After 15 min oxygen was passed through the resulting blue solution for 5 min to expel excess of ozone. The solvent was evaporated and water (20 ml), zinc

dust (200 mg), and silver nitrate (∿ 10 mg) were added to the residue.

After the mixture had been heated under reflux for 20 min, the aqueous

solution was distilled into a solution of dimedone (200 mg) in aqueous

alcohol (50%, 20 ml). Water (10 ml) was added and the distillation conti-

nued. Formaldehyde-dimedone adduct (92) separated out as white needles

which were recrystallized from aqueous alcohol (21 mg). m.p. 192-194°

(Lit.[14] 193-194°); identical with an authentic sample prepared from

formaldehyde and dimedone by TLC and by NMR. specific activity (^{14}C : 288

dpm/mg or 173,838 dpm/mmol).

(b) Radioactive morphine (480 mg) isolated from plants fed with

[1-^{3}H, 3-^{13}C]-(±)-4'-ethyl-6-methyl-laudanosoline (57) was degraded in the

same way as described above to give formaldehyde-dimedone adduct (48 mg).

specific activity (^{14}C: 40 dpm/mg or 24,268 dpm/mmol).

Chapter IV. BIOSYNTHESIS OF HYDROPHENANTHRENE ALKALOIDS IN PAPAVER

ORIENTALE L.

A. Introduction

Papaver orientale L. is a perennial wild poppy growing in the north
and northwest of Iran at 2000-2500 m above sea level. The height of the
plant is 30-70 cm (occasionally 1 m), bearing one to four flowering stems
and usually four, rarely six, pale-orange petals with no marking above
the base. It blooms in late June at low elevations, but flowering continues
until early September at higher altitudes and on open slopes.[69] P. orientale
is closely related to P. pseudo-orientale and P. bracteatum. The latter
two plants can be differentiated from P. orientale either by cytological
examination[70] of the epidermis of plants or by chemotaxonomy[71] based on
color reactions of plant latex with a specific reagent.

In contrast to the opium poppy (P. somniferum), no morphine nor codeine
can be detected in these plants. The alkaloid contents of these plants
have been studied after authentic species-determination.[72]

In P. orientale L, oripavine, (61) was either the sole alkaloid, or
the single major alkaloid accompanied by the presence of thebaine (26) iso-
thebaine (62), and alpinigenine (63).[69,73] In Papaver bracteatum, thebaine
is the only major alkaloid, and may account for 98% of the alkaloid con-
tent,[74,75,76] with alpinigenine (63) present in a small amount.[73,76]
P. pseudo-orientale contains isothebaine (62) as the major alkaloids, with
the existence of orientalidine (64) and other minor alkaloids,[73] which
are not pertinent to this study. It appears that thebaine (26) is the most
widely occurring hydrophenanthrene alkaloid in the genus Papaver. In
Papaver somniferum, it is the first morphine-type alkaloid biosynthesized
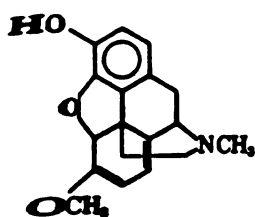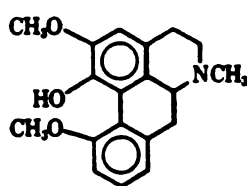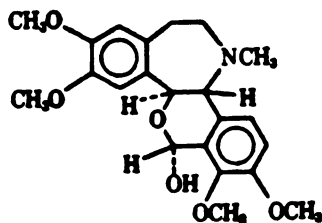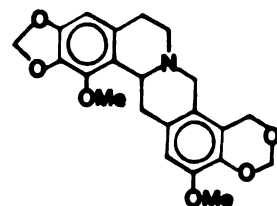
61   62   63   64

from (R)-(-)-reticuline and it is converted by successive enzymatic O-deme-
thylations to codeine (37) and morphine (2). (cf. Chap. I, General
Introduction).

The biosynthetic pathway for hydrophenanthrene alkaloids in Papaver
bracteatum was examined by Brochmann-Hanssen and Wunderly[77]. The good
incorporation (12%) of [1-^{3}H, N-methyl-^{14}C]-(+)-reticuline into thebaine
was observed with a decrease in the ^{3}H/^{14}C ratio indicative of racemization.
Incorporation of radioactivity into codeine was insignificant (0.002%), and
none at all into codeinone and morphine. But when codeinone was administered
to the living plant, it was efficiently converted to codeine, with no
detectable formation of morphine. Similar results were obtained by Hodges,
Horn, and Rapoport,[78] in their feeding experiments with [3-^{14}C]-(+)-reticuline
[16-^{3}H]-codeinone, and [16-^{3}H]-codeine. The same mechanism for the race-
mization of reticuline as occurs in P. somniferum (Scheme 1) was established
by feeding with [3-^{14}C]-1,2-dehydroreticulinium chloride. Fed [16-^{3}H]-thebain
was substantially metabolized (∿50%), with low incorporations into oripavine
(0.06%-0.31%) and northebaine (0.48%). Results from these two independent
studies indicate that the biosynthesis of thebaine in Papaver bracteatum

proceeds by the same pathway as in the opium poppy. But the plant apparently lacks the enzyme to perform the $\underline{O}$-6-demethylation of thebaine (26) to give neopinone (38), which would readily rearrange to codeinone, and be converted to codeine.

Since both $\underline{O}$-3 and $\underline{O}$-6 demethylations occur in $\underline{P}$. somniferum, it seems reasonable to conceive that this species may contain oripavine (61) as a genuine alkaloid. But the attempt by Brochmann-Hanssen and Okamoto[79] to detect this alkaloid in $\underline{P}$. somniferum, based on its presumed biosynthesis from reticuline was unsuccessful. [$\underline{N}$-methyl-^{14}C]-($\pm$)-Reticuline gave only insignificant incorporation (0.003%) into oripavine. It was concluded that oripavine is not a true alkaloid in the opium poppy. If oripavine is biosynthesized from thebaine by 3-$\underline{O}$-demethylation, the enzyme responsible for this cleavage must be different from the $\underline{O}$-demethylase which converts codeine (37) to morphine (2), the former enzyme being absent from $\underline{P}$. somniferum.

Since oripavine is the major alkaloid in $\underline{P}$. orientale L., it would be interesting to see if the biosynthesis of oripavine from reticuline can be established in this plant with radiotracer experiments.

In 1961 Stermitz and Rapoport[40] reported the incorporation in $\underline{P}$. orientale of [14]CO_2 biosynthetically labeled thebaine into two substances, whose behavior on TLC and by U.V. absorptions is such that would be indicative of oripavine (61), although positive identification of either as oripavine was lacking. Later, Battersby et al.[80] showed, in a feeding experiment done in conjunction with the study on the biosynthesis of isothebaine (62), that [3-^{14}C]-($\pm$)-reticuline was incorporated (0.2%) into thebaine (26) in $\underline{P}$. orientale in the same way as previously shown for the opium poppy. On the basis of our present knowledge we may now suspect that Battersby et al. were working with $\underline{P}$. pseudo-orientale rather than $\underline{P}$. orientale.

In the present investigation, doubly labeled $[1-^3H, \underline{N}-\underline{methyl}-^{14}C]-(\underline{+})-$ reticuline and $[2-^3H]$-thebaine were fed separately to P. orientale plants. The incorporation of radioactivity into thebaine and oripavine was examined. The ^3H to ^{14}C ratio was determined to detect possible racemization of reticuline in the plant. The goals of the study were to determine if reticuline is a true biosynthetic precursor of oripavine (61) in P. orientale as had been expected, and to firmly establish the role of thebaine in this biosynthetic pathway.

B - Results and Discussion:

The results of the two separate feeding experiments are shown in Table XI. (+)-Reticuline was incorporated into thebaine and oripavine. Chemical degradation of oripavine showed that all ^{14}C activity was located in the N-methyl group within experimental error. $[2-^3H]$Thebaine was incorporated into oripavine without randomization of the label. A decrease of $^3H/^{14}C$ ratio indicated that racemization of reticuline took place in Papaver orientale plants, and a ^3H loss of more than 50% means that the reaction is reversible. The relatively low incorporations are probably due to the inefficient aborption of the precursor by the injection procedure described, and in the case of thebaine, also due to the fact that thebaine is rapidly converted to oripavine and has a much smaller pool in the plant[52].

These results clearly demonstrated that oripavine is biosynthesized from reticuline via thebaine in P. orientale L.

Table XI.

Exp.	Precursor and its specific activity (µCi/mg)	Amount of precursor fed		Alkaloids isolated and their specific activities				Degree of incorporation	Degradation prouct and activity retained
		µCi	mg		dpm/mg	µCi/mole	$^3H/^{14}C$		
1	$[1-^3H,N\text{-methyl-}^{14}C]$-(±)-reticuline ($^{14}C$:1.389; ^3H:2.056; $^3H/^{14}C$:1.48)	^{14}C:23.61 ^3H:34.95	17	Oripavine(16): 98 mg	^{14}C:1659 ^3H:1211	223.4 163.1	0.73 (51% ^3H loss)	0.31%	Benzyltrime-thylammonium bromide (98.2%)
				Thebaine(26): 109 mg	^{14}C:229 ^3H:165	32.30 23.27	0.72 (51% ^3H loss)	0.05%	
2	$[2-^3H]$thebaine (0.586)	31.08	53	Oripavine(61): 203 mg	577	77.71	----	0.17%	Proton-exchange (98% loss of ^3H)

C. Experimental

1. General (see Chap. II, p. 26).

2. Preparation of Labeled Precursors.

   (a) $[1-^3H, \underline{N}\text{-}\underline{\text{methyl}}\text{-}^{14}C]\text{-}(\pm)$-reticuline.

The same precursor compound was used before in our laboratory for the study of the biosynthesis of morphine alkaloids in Papaver bracteatum L.[77]. The synthetic sequence from 1-(3-benzyloxy-4-methoxy-benzyl)-6-methoxy-7-benzyloxy-3,4-dihydroisoquinoline hydrochloride (80) is shown in Scheme XIV . The preparation of compound XVI has been described in Chap. II (Scheme VIII).

The liberated base from the hydrochloride (80) was treated with $[^{14}C]$-iodomethane in benzene in a sealed tube at room temperature. The $[\underline{N}\text{-}\underline{\text{methyl}}\text{-}^{14}C]$-methiodide (*93) thus obtained was reduced and debenzylated to give $[\underline{N}\text{-}\underline{\text{methyl}}\text{-}^{14}C]\text{-}(\pm)$-reticuline[82]. The $[1-^3H]\text{-}(\pm)$-reticuline was prepared by reducing the cold methiodide (93) with sodium ^3H-borohydride ($NaBT_4$), followed by debenzylation. The feeding sample was prepared by mixing appropriate amounts of $[1-^3H]\text{-}(\pm)$-reticuline and $[\underline{N}\text{-}\underline{\text{methyl}}\text{-}^{14}C]\text{-}(\pm)$-reticuline (specific activities: ^{14}C : 1.389 Ci/mg; ^3H = 2.056 Ci/mg).

   (b) $[2-^3H]$thebaine (26).

The synthetic route to $[2-^3H]$thebaine is similar to that for the synthesis of its 6-ethyl analog (58) (cf. Scheme VI, p. 28). $[2-^3H]$Morphine as obtained by base-catalyzed nuclear exchange of morphine with tritiated water in dimethylformamide[61]. Methylation of the tritiated morphine with dimethylanilinium sulfate afforded $[2-^3H]$codeine. $[2-^3H]$Codeine was O-methylated with MeI/KH to give $[2-^3H]$-6-methylcodeine, which was idized with active manganese dioxide[62] as described by Barber and Rapoport[63] $[2-^3H]$thebaine (identical with an authentic sample of thebaine by TLC and GLC).

● : ^{14}C

T : ^{3}H

80

$\overset{\bullet}{C}H_3I$

NaBT$_4$

●93

93

5-^{14}C

5-T

mixture:
(1- ^{3}H, <u>N-methyl</u>-^{14}C)-(±)-reticuline
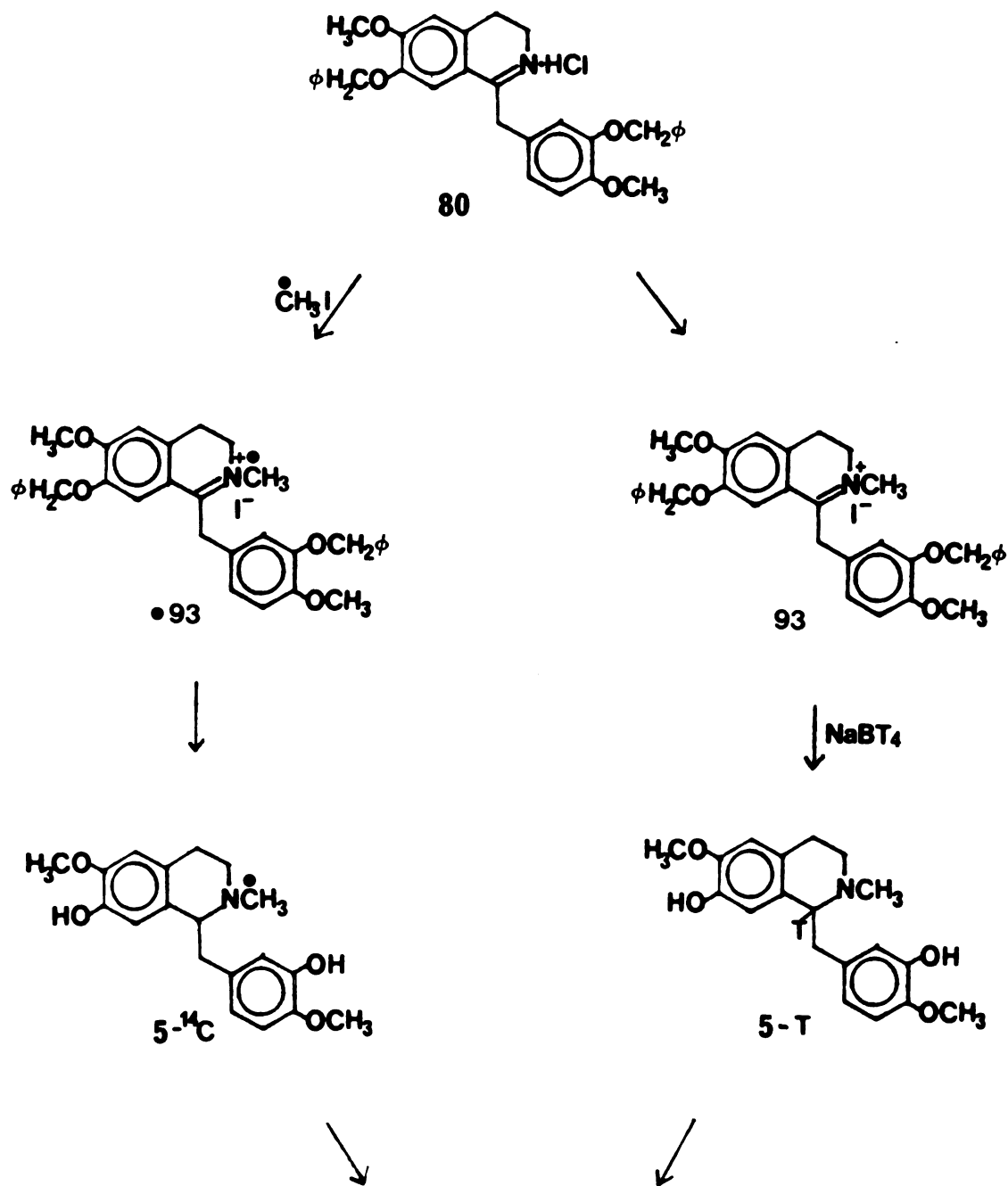
Scheme XIV

3. Feeding of Precursors.

The seeds of _Papaver orientale_ L. were obtained from Iran after authentic species-determination[69,72]. The plants were grown in flower pots in a greenhouse. The feeding was done on blooming or budding plants. The radioactive precursor was dissolved in an equivalent amount of 0.1 N H_2SO_4 and diluted with water to a concentration of 2 mg/ml. 0.5 ml portions of the sample were injected into the plants between root and stem at one to two day intervals. The plants were harvested after 10 days and placed in a freezer until they could be extracted.

4. Isolation and Purification of Alkaloids.

The frozen plants were worked up in the same way as described in Chap. II for the isolation of alkaloids from _P. somniferum_ plants. The "total alkaloids" solution was subjected to the extraction sequence shown in Scheme XV , which is similar to Scheme XI.

(a) From plants fed with $[1-^3H$, _N_-methyl-$^{14}C]$-(+)-reticuline.

Fractions I (weak bases) & II (nonphenolic bases). TLC showed the presence of mainly thebaine in both fractions. The two fractions were combined and dissolved in chloroform. The $CHCl_3$ soln was extracted with 0.1 N $NaOH_{(aq.)}$ to remove any traces of oripavine present. The organic layer was washed with water, dried, and evaporated to give 109 mg of crude thebaine. Further purification was done by column chromatography first on neutral alumina (act. III) with chloroform and then on silica gel (act. II) with chloroform containing 2-3% of methanol. The pure thebaine (27 mg) thus obtained was crystallized repeatedly from benzene-hexane to constant radioactivity.

Fraction III (98 mg) was recrystallized repeatedly from aqueous alcohol to oripavine of constant radioactivity.

Total alkaloids (Aqueous solution, pH ~ 1.5)

| CHCl₃

CHCl₃
(Fraction I)

Aqueous

  i) Basify with ammonia to pH 9
 ii) CHCl₃

CHCl₃

   i) Evapd. to dryness
  ii) Dissolve in 0.2 N HCl
 iii) Basify with NaOH to pH 13
  iv) CHCl₃

CHCl₃ extract

(Fraction II)

Aqueous

  i) Add NH₄Cl to pH 9
 ii) CHCl₃:isopropanol (3:1)

CHCl₃ : isopropanol extract

(Fraction III).

Scheme XV
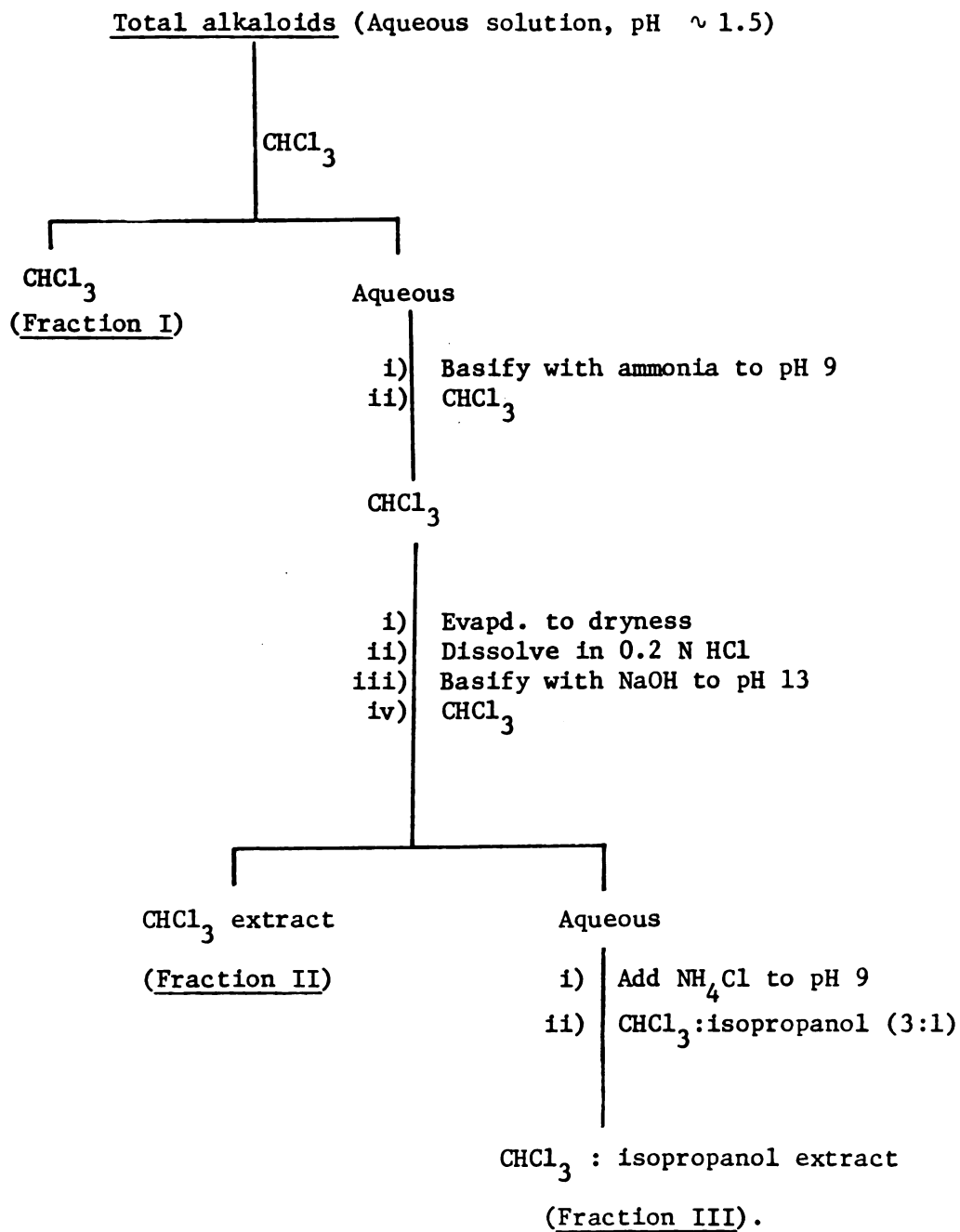
(b) From plants fed with [2-^{3}H]thebaine.

Fraction III (203 mg) was purified by passing it through a column

of silica gel with chloroform containing increasing amounts of methanol

(0-5%) to give 164 mg of pure oripavine, which was crystallized from

aqueous EtOH to constant radioactivity.

5. Determination of Radioactivity.

This was done by liquid scintillation counting as described in Chap.

II, p. 31.

6. Determination of the Positions of Labels.

(a) Degradation of radioactive oripavine isolated from P. orientale

plants fed with [1-^{3}H, N-methyl-^{14}C]-($\pm$)-reticuline.

The degradation of oripavine followed the sequence shown in Scheme XVI.

The radioactive oripavine was O-methylated with trimethylanilinium sulfate

(cf. the methylation of [2-^{3}H]morphine to [2-^{3}H]codeine described in Chap

II, p.27) to give thebaine (58). Thebaine thus obtained was degraded

by the method of Battersby et al.[14] Vigorous treatment of thebaine with a

solution of dry HCl in methanol gave rise to methebenine hydrochloride (94),

an exotic rearrangement reaction known for many years[85]. The hydrochloride

(94) was converted to N,O-dimethylmethebenine methosulphate (95) by

treatment with dimethyl sulfate. Hofmann degradation of compound 95 gave

3,4,8-trimethoxy-5-vinylphenanthrene (96) and trimethylamine which was

trapped with benzyl bromide to give crystalline benzyltrimethylammonium

bromide (97).[77]

Methebenine hydrochloride (94). Thebaine (42 mg) derived from

radioactive oripavine was dissolved in anhydrous methanol (1 ml) in a 5 ml

ampoule. Methanol (1 ml) saturated with dry gaseous hydrogen chloride was

● : ^{14}C

61 → 58 →

94 → 95 →

96 + $H_3\overset{\bullet}{C}N(CH_3)_2$

benzyl bromide

$H\overset{\bullet}{\underset{3}{C}}\overset{+}{N}(CH_3)_2(CH_2C_6H_5)\cdot Br^-$

97
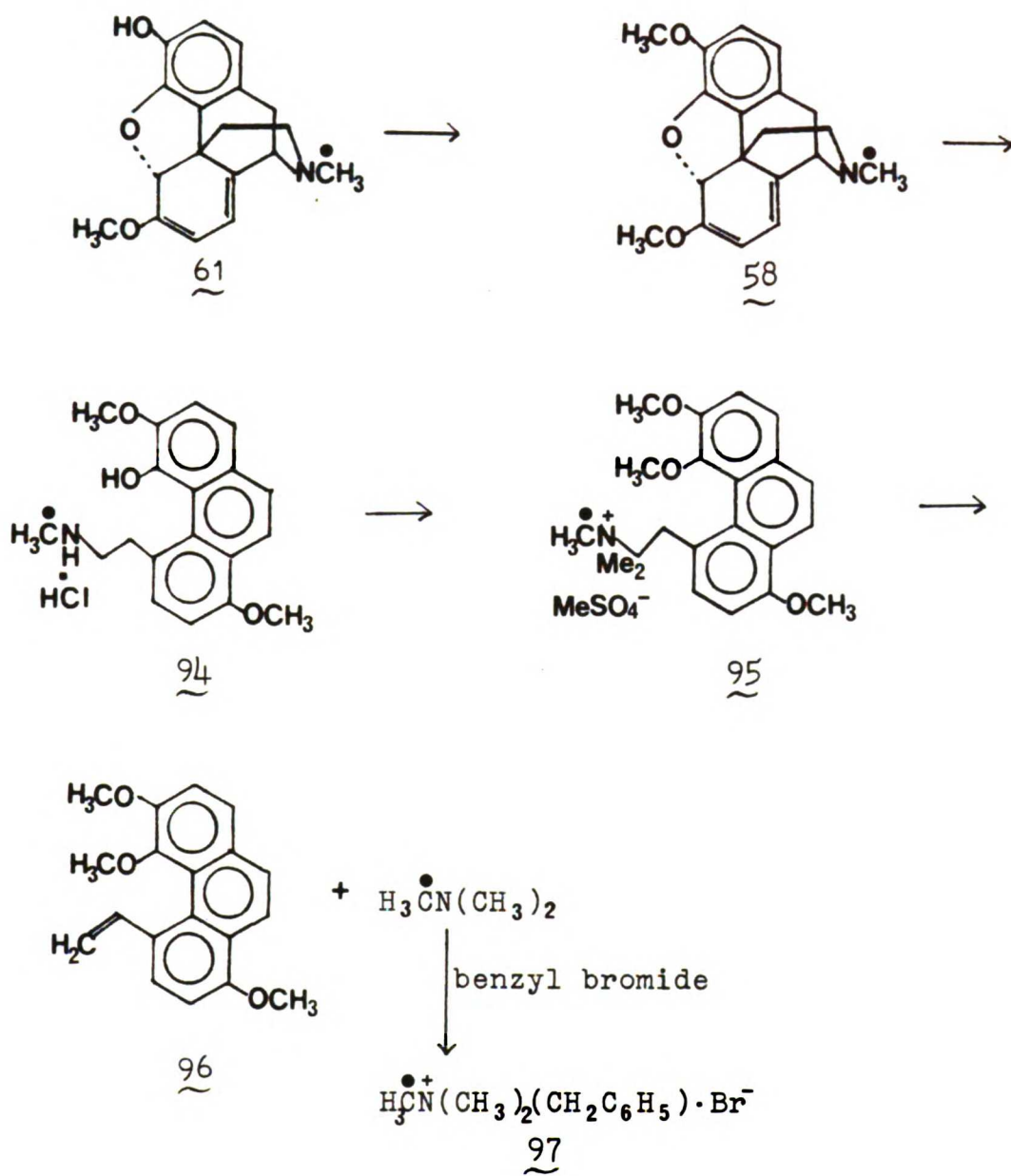
Scheme XVI

added to the thebaine solution. The ampoule was sealed under nitrogen

and heated at 100° for 3 h. Yellowish crystals (10 mg) were obtained

from the solution cooled at 0°C. m.p. 226-230° (lit.[14] 228-231°).

N,O-dimethylmethebenine methosulfate (95). Aqueous sodium hydroxide

(30%, 0.4 ml) was added to a solution of methebenine hydrochloride (10 mg)

in water (1 ml). Dimethyl sulfate (0.4 ml) was added and the mixture stirred

vigorously until a homogeneous solution was formed. (pH maintained at 10).

The methosulfate (95) (13 mg) crystallized in the cooled solution.

m.p. 273° (lit.[14] 273-275°).

Benzyltrimethylammonium bromide (97). The methosulfate obtained

above was dissolved in 6 N NaOH (2 ml) in a 10 ml two-necked flask fitted

with a reflux condenser and a nitrogen inlet tube. The top of the condenser

was connected to a flask containing 0.2 ml of benzyl bromide in 2 ml of

methylene chloride. A slow nitrogen stream was passed into the reaction

flask, bubbling through the solution of benzyl bromide, while the solution

was refluxed for 5 h. The methylene chloride was evaporated. Benzyltrime-

thylammonium bromide (4.0 mg) was precipitated with ether and recrystallized

from ether-chloroform; identical with a cold sample by TLC; specific

activity: ^{14}C, 2219 dpm/mg or 510, 140 dpm/mmole.

(b) Determination of the Position of ^{3}H Label in the Radioactive

Oripavine Isolated from Plants Fed with [2-^{3}H]Thebaine.

The method developed for the detritiation of [2-^{3}H]morphine[44] was

followed with modifications for the work-up procedure (cf. Chap. II, p. 33).

Radioactive oripavine (60 mg, specific activity : 577 dpm/mg) was

dissolved in methanol (1 ml) in a 10 ml ampoule. Water (4 ml) and K_2CO_3

(22 mg) were added. The ampoule was sealed under nitrogen and heated at

100°C for 48 h. After cooling there appeared a large amount of yellow precipitate, which turned dark-colored rapidly upon exposure to air. The mixture was filtered and the dark-colored residue extracted with $CHCl_3$-MeOH. Neither the filtrate nor the extract contained oripavine as checked by TLC. Thus, the dark-colored residue was dissolved in 0.5 N HCl. The solution was neutralized with NH_4OH to pH = 8.5. The alkaline solution was extracted repeatedly with $CHCl_3$-isopropanol (4:1). The extract was washed with H_2O, dried, and evaporated to give a crystalline residue which is identical with oripavine by TLC. specific activity: 11 dpm/mg.

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Chapter V.   QSAR (QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS) METHODS

The biological action of an organic compound is the result of several complex processes, which can be classified into the pharmacokinetic phase and the pharmacodynamic phase. The former includes the absorption, distribution, metabolism, and excretion of the drug, while the latter involves the molecular interaction of the agent or its active metabolites with a specific site of action. Most of these processes, e.g. the passage through bio-membranes, the binding with metabolizing enzymes, and the binding with receptors, involve the interaction of a small molecule (the drug) with a bio-macromolecule (a membrane, enzyme, or "receptor") in an aqueous environment. With a few exceptions, e.g. the alkylation of DNA by an alkylating agent, the interaction between a small molecule and a bio-macromolecule is governed by intermolecular binding forces, which are reversible and non-covalent in nature.

The goal of QSAR methods is first to transform the chemical structure of organic compounds into a series of numerical descriptors which reflects the intermolecular forces relevant to biological activity and then to establish quantitative relationships between these descriptors and the biological activity of the compounds.

The idea of developing quantitative relationships between chemical structure and biological action was born around 1870 when Crum-Brown and Frazer[1] proposed the following equation:

$$\phi = f(c) \qquad\qquad (V - 1)$$

In eq. 1, ϕ is a measure of biological response and $f(c)$ is a function of chemical structure. They suggested that it should be possible to develop a calculus of "structure-activity relationship" by making small changes in chemical structure and relating these to biological response. The realization of their bold dream was hampered mainly by the lack of means

of describing chemical structure in numerical terms at that time.

The first important contribution was made by Meyer and Overton[2,3] at the turn of the century. They found that the narcotic action of organic molecules parallels their oil/water partition coefficients. The oil/water partition coefficient (as log $\underline{P}$) turned out to be a useful descriptor of hydrophobic force in QSAR studies.

Another important breakthrough came in 1935 when Hammett[4] proposed the famous Hammett equation and formulated σ constants for the electronic effects of substituents on the benzene ring. The Hammett equation was extended by Taft[5] in the 1950's to include substituents on nonaromatic parent compounds and to include the steric effects of substituents (E_s constants). Taft and Lewis[6] made the first attempt to factor the electronic effects of substituents into inductive and resonance components.

Besides the developments in physical organic chemistry, another key factor which contributed to the building of QSAR was the availability of advanced computers in the early 1960's.

The generalization of QSAR would not be possible without the efforts by Hansch and Fujita[17]. Their contributions to the development of QSAR include:

(1) demonstrating that more than one physico-chemical property can be considered simultaneously by making a linear combination of substituent constants, following an approach used by Taft[5].

(2) defining π constants for the hydrophobic effects of substituents and establishing the additive-constitutive character of log $\underline{P}$.[7]

(3) showing that, with the aid of computers, the relationship between physico-chemical properties and biological activity can be examined statistically by regression analysis. Statistics provides the criteria

for accepting or rejecting apparent relationships.

The approach developed by Hansch and Fujita[17] has become the most popular QSAR method. Other QSAR methods include the _de novo_ or Free-Wilson approach[8], factor analysis[9], principal component analysis[10], discriminant analysis[11], cluster analysis[12], pattern recognition[13], simplex method[14], distance geometry method[15], etc. These methods are of some use in "drug design", and their potential in the future might be great, but none of them has received the kind of attention that the Hansch method is receiving from medicinal chemists.

The basic assumption of the Hansch method is that "the effect of substituents on the strength of interactions between a drug and its receptor and other biomolecules is an additive combination of the effects of the substituents on various types of simpler model intermolecular interactions."[16] The significance and methodology of the Hansch approach has been reviewed by several authors[16-23]. A comprehensive listing of substituent constants and their experimental bases have been provided by Hansch and Leo[24].

Since most substituent constants such as σ, π and E_s are derived from model systems based on linear free energy relationships (e.g. the Hammett equation for σ constant), the Hansch approach has been called the linear free energy method. It has also been named the extrathermodynamic method due to the fact that although the relationships are stated in thermodynamic terms (e.g., ΔG values and log k values) there is no thermodynamic principle which says that these relationships should be true.

The physico-chemical parameters (or substituent constants) commonly used in QSAR studies are briefly described below.

(1) Electronic Parameters:

    a. Hammett σ constant[4].

$$\sigma = \log K_X - \log K_H, \qquad\qquad (V - 2)$$

where K_H is the ionization constant for benzoic acid in water at

$25°$ and K_X is the ionization constant for a meta or para derivative

under the same experimental conditions. Positive σ values

represent electron withdrawal by the substituent from the aromatic

ring ($\sigma_{4-NO_2} = 0.78$); negative σ values indicate electron release

to the ring ($\sigma_{4-OMe} = -0.27$).

    b. Special sigma values to include the effect of "through resonance."

These are:

σ^{-}[25] for substituents which are capable of accepting lone-pair

electrons from groups such as OH and NH_2 <u>via</u> resonance, σ^{+}[26] for

the situation where a positive charge is generated in the course of

a reaction; $\sigma°$[27-29],a normalized constant in which all through-

resonance would be eliminated.

    c. σ^{*} for aliphatic systems.

Taft[5] defined this polar constant as:

$$\sigma^{*} = 1/2.48 \; [\log (k/k_o)_B - \log (k/k_o)_A] \qquad (V - 3), \text{ in which}$$

(k/k_o) refer to the hydrolysis rate constants of substituted

(XCH_2COOR) and unsubstituted (CH_3COOR) acetic acid esters respect-

ively. B indicates basic conditions; A indicates acidic conditions.

Charton[30] has redefined σ for aliphatic systems using aliphatic

acids.

    d. Separation of inductive and resonance effects.

Taft and Lewis[6] first suggested that σ values can be expressed

as a linear combination of inductive and resonance effects:

$$\sigma = a\sigma_I + b\sigma_R \qquad\qquad (V - 4)$$

(σ_I represents the inductive-field effect; σ_R the resonance effect)
Swain and Lupton[31] later established the "F and R" sytem by a more statistical approach.

d. pKa values.

e. Values from quantum chemical calculations: e.g.

HOMO : energy of the highest occupied molecular orbital.

LUMO : energy of the lowest unoccupied molecular orbital.

(2) Hydrophobic parameters:

Hydrophobicity is probably the most important driving force for non-covalent intermolecular interactions in an aqueous environment[32].

a. log $\underline{P}$ ($\underline{P}$ is the partition coefficient)

$$\underline{P} = \frac{[D]n}{[D]a} \qquad\qquad (V - 5)$$

In the above expression, [D]n is the equilibrium concentration of the monomeric species of a compound in the non-aqueous phase;

[D]a is the concentration of the neutral form in the aqueous phase. The octanol/water system is the most widely used solvent system in measuring log $\underline{P}$ since the character of octanol of having a terminal hydroxyl group and a long hydrophobic alkyl chain approximates that of a biological membrane.

b. Hansch's π constants[7].

The definition is analogous to that of σ :

$$\pi_x = \log \underline{P}_{C_6H_5X} - \log \underline{P}_{C_6H_6} \qquad\qquad (V - 6)$$

(X = substituents on the benzene ring).

A positive value for π indicates that, relative to H, the substituent X favors the octanol phase. A negative π value indicates the hydrophilic character of the substituent relative to H. The measurement of π values has been extended to compounds with other

parent structures. For example, π^- was devised by Fujita et al.[7] for substituents on phenols and anilines. If log $\underline{P}$ of a parent compound is known, π constants can be used to calculate the log $\underline{P}$ values of its derivatives. In 1973, Nys and Rekker[33] undertook a statistical survey of available data on partition coefficients and developed a set of fragment values which could be used in an additive fashion according to the following equation:

$$\log \underline{P} = \sum_{1}^{n} a_n f_n, \qquad (V - 7)$$

where a is the number of occurrences for fragment f of the structural type n. This fragment approach is very useful in the calculation of log $\underline{P}$ values for aliphatic molecules. Recently chromatography (HPLC, TLC, etc.) has also been employed to obtain hydrophobic scale for organic compounds. The use of chromatography in QSAR studies has been reviewed by Kaliszan[34].

(3) Steric parameters:

Of all the physiochemical parameters used in QSAR, those for steric effects present the greatest problem.

a. Taft's E_s values[5].

$$E_{s,X} = \log k_{XCH_2CO_2Me} - \log K_{CH_3CO_2Me}, \qquad (V - 8)$$

where k is the rate constant for hydrolysis of the ester. The derivation of E_s values is based on the assumption that the rate of acid-catalysed hydrolysis is controlled mainly by steric factors.

b. E_s^c values.

Hancock et al.[35] suggested the correction of E_s values for hyper-conjugative effects by the following equation:

$$E_s^c = E_s + 0.306 \ (n - 3) \qquad (V - 9)$$

(n is the number of α-hydrogen atoms).

c. Verloop's Sterimol values[36].

In this approach by Verloop, Hoogenstraaten, and Tipker, five

dimensions were chosen for each substituent and a computer program

was developed to calculate values for the five dimensions

(L, B_1, B_2, B_3, and B_4). The calculation was based on van der Waals

radii, standard bond angles and lengths, and presumed "reasonable"

conformations for flexible substituents. Of the five values, L is

the length of a substituent along the axis of the bond connecting

the first atom of the substituent and the parent molecule; B_1 is

the smallest width, and B_4 the largest width of the substituent.

It has been observed that the B_1 parameter shows some correlation

with E_s ($r^2 = 0.719$)[24].

(4) Parameters for dispersion bonding.

The dispersion force between two organic molecules is governed by their

polarizabilities. The polarizability α can be represented by MR, the molar

refractivity[37]:

$$\alpha = MR = \frac{(n^2-1) \cdot M}{(n^2+2) \cdot d} \qquad (V - 10)$$

In this expression, n is the refractive index, M the molecular weight, and

d the density. MR is an additive-constitutive property of a compound, and

can be readily measured. MR has been widely used in QSAR studies, but it

often shows correlation with the hydrophobic parameter log $\underline{P}$. Therefore,

the use of MR is significant only when it does not show colinearity with

other additive parameters included in the same QSAR equation.

(5) Hydrogen-bonding:

Although the energy contribution from hydrogen-bonding is generally

small ($\Delta G°$ value from 0 to $\sim$ -0.5 kcal/mole, hydrogen bonds are important in determining the specificity of biological interactions. The effect of hydrogen-bonding has been successfully included in QSAR studies by the use of indicator variables[38-40] (see (6) below). In Chap VI is described an attempt to quantitize this effect based on a thermodynamic model.

(6) Indicator (Dummy) variables:

Indicator variables are useful in QSAR studies when the presence or absence in the molecule of certain groups or features cannot be attributed to the physico-chemical parameters considered. In this approach, substituents with a certain feature are given a value of 1, while those without this feature are given a value of 0. An example is the use of $HB_{ind.}$, with values of 1 or 0, to denote hydrogen-bond acceptors and substituents incapable of accepting a hydrogen-bond, respectively. The exclusive use of indicator variables results in the Free-Wilson method of QSAR[8].

In a Hansch equation, the biological activity is usually expressed as log $1/ED_{50}$. The best biological data for use in QSAR are those expressing the concentrations (c) of members of a series which give a standard response in a standard time interval, measured under uniform conditions (e.g., ED_{50}, etc.). Precise measurement of the biological activity is highly desirable, although not always obtainable, since any deficiencies in this respect also reflect on the QSAR results.

Correlation of the linear combination of physico-chemical parameters to the biological activity leads to the general expression of the Hansch equation:

log 1/C = a + b (hydrophobic parameter) + c (electronic parameter)

   + d (steric parameter) + e (polarizability) + f (indeterminate

   property). (V-11)

Nonlinear terms have also been used in a Hansch equation, e.g. π^2 for the parabolic dependence on hydrophobicity[17] and terms in the bilinear model for hydrophobic effects[41].

Cross-product terms such as "$\pi\sigma$" have been used occasionally. But the inclusion of such terms in the equation can not be reasonably interpreted, and is probably not meaningful.

Computer programs are available for carrying out the multiple regression analysis to get the relationship between physico-chemical parameters and biological activity. Examples are the HANSCH program from the Pomona College Medicinal Chemistry Project and the MULREG (superseded by FIT MULTIPLE) program from the PROPHET system.

For the assessment of the significance of a structure-activity correlation, minimal statistical data are required[42]. Among them:

n is the number of compounds in a given equation.

s is the standard deviation of the correlation.

r is the correlation coefficient (r^2 represents the percentage of variance explained by the equation).

F test: In comparing two equations which differ only by one term, the F test is applied to test the significance of adding the new term to the simpler equation.

Student's t test: applied to the regression coefficient of each term in the correlation.

It has been observed by Topliss et al.[43] that when the number of variables screened for possible inclusion in a QSAR equation is large as compared to the number of observations, there is a substantial risk of obtaining a chance correlation. The probabilities of chance correlation at specified r^2 values were given for various combinations of observations and screened variables. This serves as a guideline to determine the number of data points required for obtaining a significant correlation.

A major limitation of the Hansch method of QSAR is that it can usually only be applied successfully to a series of structurally similar compounds. This is due to our inadequacy in describing molecules. Since substituent constants are used in most cases to describe the variation in chemical structure, a parent structure present in the compounds studied is necessary.

A second limitation is the difficulty commonly encountered in obtaining the precise measurement of the biological property of interest. If the biological response measured is a complex result of several processes, as is the case with most in vivo data, it is often difficult to interpret the QSAR results without ambiguity. Despite the limitations and sometimes disappointments of QSAR, the Hansch method has resulted in numerous equations which correctly predicted the potency of untested analogues[16]. The method has also been successfully employed to study substrate-enzyme[21] and other drug-receptor interactions[16].

With continued progress in physical organic chemistry and the development of better techniques for measuring biological response, QSAR methods will become an indispensable part in the planning and evaluation of any synthetic medicinal chemistry project. Its application in drug-design can be further broadened by the integration with other methods such as computer graphics, which explicitly consider the three-dimensional aspects of molecules.

Chapter VI. QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS OF AROMATIC

ESTERS OF 1-METHYL-4-PIPERIDINOL AS ANALGESICS.

A. Introduction

Continued effort has been made to the development of new analgesics

that are devoid of undesirable side effects and to the understanding of

their interactions with specific receptors. Recently an interesting group

of compounds, namely substituted benzoic acid esters of 1-methyl-4-

piperidinol, was synthesized by Waters[44,45]. These compounds possess

analgesic activity by the hot-plate assay, with the more potent ones in

the range of morphine and codeine but, in general, they display no

morphine-like physical dependence liability in monkeys.

These esters have the main structural features of many synthetic

analgesics, namely a benzene ring and a piperidine ring. But they lack

the quaternary phenyl substitution at C-4 of the piperidine ring, which

is present in meperidine, prodine, and other 4-phenylpiperidine analgesics.

Qualitative structure-activity correlations of some 3- and 4-monosubsti-

tuted and 3,4-disubstituted benzoate esters were made with regard to

substituent constants E_s^c and π. No significant conclusions were drawn from

this limited study[45], and it became apparent that a quantitative study

applying multiple-regression analysis would be necessary in order to gain

insight into the involvement of the aromatic ring in determining the

analgesic potency.

Over the years, several publications have appeared[46-51] on the

quantitative structure-activity relationships of narcotic analgesics; but

none has dealt extensively with the effects of substituents on the

aromatic moiety.

Preliminary analysis of available compounds showed an insufficient spread of substituent parameter values. Therefore, additional mono-substituted compounds were synthesized based on Craig's plots[52], as illustrated for para-substituted compounds in Fig. 1. The structure and analgesic potency of all 48 benzoic acid esters of 1-methyl-4-piperidinol are shown in Table 1. The analgesic potencies ranged from an average hot-plate ED_{50} of 3.9 mg/kg to 74 mg/kg (0.012 mmol/kg to 0.23 mmol/kg), with the exception of a few which were marginally active or inactive at 100 mg/kg.

B. RESULTS AND DISCUSSION

Among the various physicochemical parameters included in the study (cf. Experimental Section), those found to be significant in correlating the structure with activity are listed in Table I. The statistically significant regression equations are given below, where n is the number of compounds, r the multiple regression coefficient, s the standard deviation of the regression, and f the value of the F test. The number in parenthesis under each coefficient is the value of Student's t test for that coefficient.

Four inactive compounds, namely 2-cyano (45), 3-methyl-(46), 4-iodo (47), and 2,3,5-triiodo (48) derivatives, are not included in the calculation, because no ED_{50} values can be assigned to them.

Since additivity cannot be readily assumed for many physico-chemical parameters, i.e. multiple substituents might not exert an influence equal to the sum of the individual substituents, the regression analysis was started with subgroups of mono-substituted compounds in order to simplify the process of selecting potential parameters. This approach might also minimize the risk of chance correlation[43] by reducing the number of variables screened in the last stage when all the compounds are included. Examples of subgrouping are not uncommon in the literature of QSAR[38,53-55]
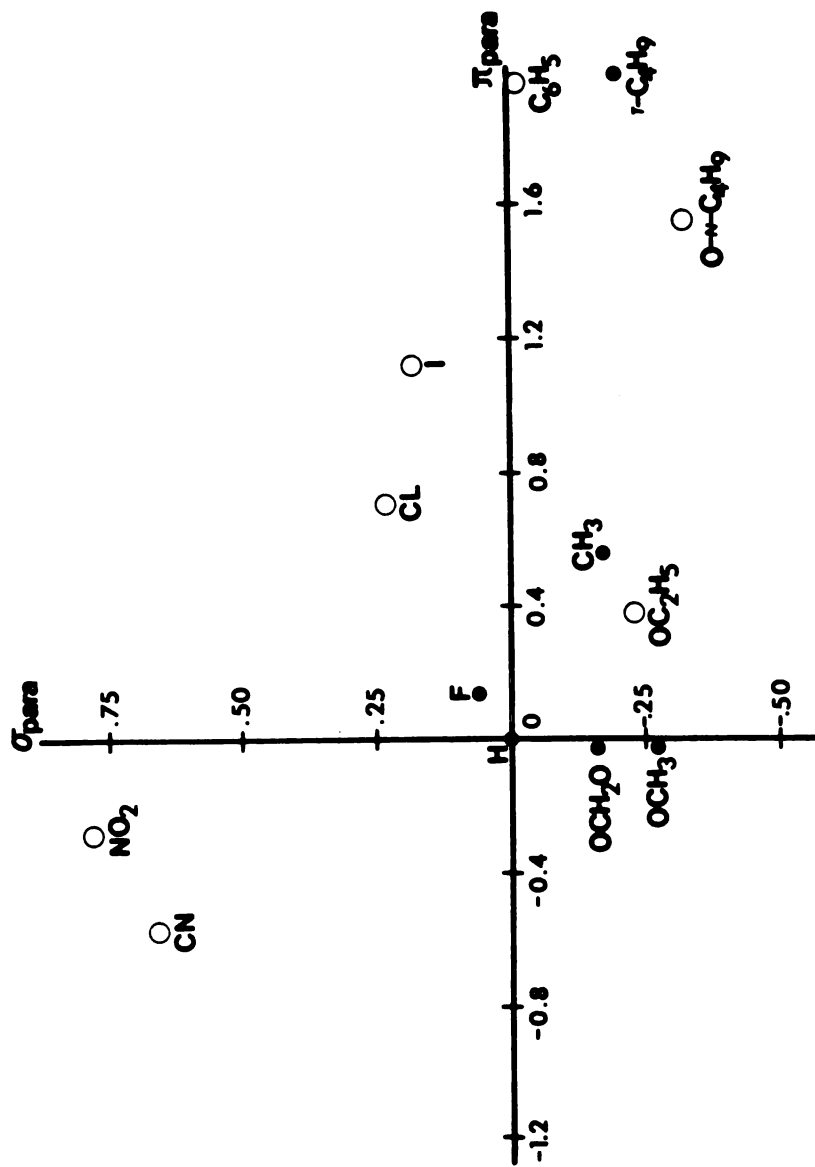
Figure 1. Application of Craig's plot for the selection of additional substituents. The purpose is to avoid the use of only those substituents which lie on or near a straight line, i.e. those which are highly correlated. Those marked by open circles are the substituents of additional compounds synthesized.

(I) <u>Ortho</u>-substituted derivatives (10-12, 14, 16, 17, 24, 25, 27, 29, 38-42, 44):

Equation 1 was obtained with the entire data set.

1) $\log 1/C = -(0.14\pm0.037) \ L_{ortho} + (0.18\pm0.08) \ HB_{ortho} + (0.15\pm0.07)\pi_{ortho}$

   (3.81)   (2.14)   (2.08)

   $+ 1.38$

$n = 16; \quad r = 0.77; \quad s = 0.17; \quad f = 5.84 \ (f_{\alpha(1) = 0.01,3,12} = 5.95).$

Examination of the residuals revealed that the calculated potency of the 2-phenyl derivative (39) was much lower than the observed potency, with a residual of 0.44. The reason for the higher observed potency of this compound is not clear. But this compound is unusual in that its activity is only marginal (Footnote n, Table I) and toxicity was observed at 20 mg/kg.

Omission of compound 39 resulted in a much better correlation with the same parameters:

(2) $\log 1/C = -(0.16\pm0.02) \ L_{ortho} + (0.185\pm0.05) \ HB_{ortho} + (0.13\pm0.04)\pi_{ortho}$

   (7.25)   (3.75)   (2.91)

   $+ 1.41$

$n = 15; \ r = 0.93; \ s = 0.10; \quad f = 21.77 \ (f_{\alpha(1) = 0.0005,3,11} = 13.7)$

If all five marginally active <u>ortho</u>-compounds (39, 40, 41, 42, 44) were omitted from the regression, eq. 3 was obtained in which the π_{ortho} term is no longer significant.

(3) $\log 1/C = -(0.21\pm0.03) \ L_{ortho} + (0.144\pm0.046) \ HB_{ortho} + 1.57$

   (6.66)   (3.13)

   $n = 11; \quad r = 0.93; \quad s = 0.098; \quad f = 27.32 \ (f_{(1) = 0.0005,2,8} = 22.7).$

It has been observed that $HB_{\underline{ortho}}$ can be replaced by $HB_{\underline{ortho-ind}}$ in the above equations with little change in the statistics since the two variables are highly correlated ($r^2 = 0.92$).

(II) $\underline{Meta}$-substituted derivatives (3, 9, 14, 19, 26, 28, 36):

The following equations were obtained:

(4) $\log 1/C = (0.49\pm0.12) HB_{\underline{meta}} - (0.95\pm0.24)B_{1\ \underline{meta}} + (0.61\pm0.18)_{\underline{meta}}$

$\quad\quad\quad (4.1) \quad\quad\quad\quad\quad (4.0) \quad\quad\quad\quad\quad (3.3)$

$\quad\quad + 1.46$

$n = 7; \quad r = 0.94; \quad s = 0.10; \quad f = 7.08 \ (f_{\alpha(1)} = 0.05,3,3 = 9.28)$

(5) $\log 1/C = (0.54\pm0.06)HB_{\underline{meta-ind}} + (0.42\pm0.05)E_{s\ \underline{meta}} + (0.67\pm0.09)\pi_{\underline{meta}}$

$\quad\quad\quad (8.28) \quad\quad\quad\quad\quad (8.34) \quad\quad\quad\quad\quad (7.20)$

$\quad\quad + 1.40.$

$n = 7; \quad r = 0.98; \quad s = 0.05; \quad f = 32.04 \ (f_{\alpha(1)} = 0.01,3,3 = 2.95).$

As shown in the squared correlation matrix (Table II), $B_{1\ \underline{meta}}$ and $E_{s\ \underline{meta}}$ are highly correlated ($r^2 = 0.84$), and so are $HB_{\underline{meta}}$ and $HB_{\underline{meta-ind}}$ ($r^2 = 0.85$). It is, therefore, not surprising that a combination of either "$B_{1\ \underline{meta}}$, $HB_{\underline{meta}}$, and $\pi_{\underline{meta}}$" or $E_{s\ \underline{meta}}$, $HB_{\underline{meta-ind}}$, and $\pi_{\underline{meta}}$" may be used to give significant correlation. Equation 5 is statistically superior to eq. 4, although $E_{s\ \underline{meta}}$ and $\pi_{\underline{meta}}$ in eq. 5 are marginally correlated ($r^2 = 0.59$), while $B_{1\ \underline{meta}}$ and $_{\underline{meta}}$ in eq. 4 show little correlation ($r^2 = 0.30$). Since three parameters are included with only seven data points, the possibility that eq. 4 and 5 are the results of chance correlation cannot be excluded. However, the same parameters

continue to give significant correlations in equations 7-13, where the number of meta-substituted derivatives is increased with the inclusion of multi-substituted compounds. There are sixteen compounds with meta-substituents in the combined data set.

(III) Para-substituted derivatives (2, 4, 6, 14, 15, 20, 30, 32-34, 37):

With the entire data set, eq. 6 gave the best correlation:

$$(6) \quad \log 1/C = (0.15 \pm 0.03) \, E_{s \, para} + (0.25 \pm 0.07) \, HB_{para-ind.} + 1.41.$$

$$(4.52) \qquad\qquad (3.24)$$

$$n = 11; \quad r = 0.91; \quad s = 0.123; \quad f = 19.78 \, (f_{\alpha(1)} = 0.001, 2, 8 = 18.5)$$

(IV) Ortho- and/or meta-substituted derivatives (8, 21 and compounds from I and II):

Regression analysis on the complete data set gave the following equation:

$$(7) \quad \log 1/C = (-0.14 \pm 0.03) \, L_{ortho} + (0.48 \pm 0.16) \, HB_{meta} + (-0.82 \pm 0.30) B_{1 \, meta}$$

$$(4.44) \qquad\qquad (3.04) \qquad\qquad (2.67)$$

$$+(0.17 \pm 0.07) \, HB_{ortho} + (0.59 \pm 0.25) \, _{meta} + (0.15 \pm 0.06) \pi_{ortho}$$

$$(2.35) \qquad\qquad (2.33) \qquad\qquad (2.28)$$

$$+ 1.40$$

$$n = 24; \quad r = 0.815; \quad s = 0.155; \quad f = 5.60 \, (f_{\alpha(1)} = 0.0025, 6, 17 = 5.51).$$

As in eq. 1, poor prediction was obtained for the 2-phenyl derivative (residual = 0.438). A much better correlation was obtained with the omission of this compound from the calculation.

(8) $\log 1/C = -(0.17\pm0.02)L_{\underline{ortho}} + (0.49\pm0.10)HB_{\underline{meta}} - (0.87\pm0.20)B_{1\ \underline{meta}} +$

$(7.54) \qquad\qquad (4.66) \qquad\qquad (4.30)$

$(0.18\pm0.04)HB_{\underline{ortho}} + (0.62\pm0.17)\pi_{\underline{meta}} + (0.12\pm0.04)\pi_{\underline{ortho}} +$

$(3.78) \qquad\qquad (3.69) \qquad\qquad (2.88)$

$+ 1.43$

$n = 23; \quad r = 0.925; \quad s = 0.103; \qquad f = 15.73 \ (f_{\alpha(1)=0.0005,6,16} = 7.74).$

(V) <u>Meta</u>- and/or <u>para</u>-substituted derivatives (1, 5, 18, 22, 23, 35, 43, and compounds from II and III):

Equation 9 was obtained from regression analysis on the entire data set.

(9) $\log 1/C = (0.39\pm0.07)HB_{\underline{meta}} + (0.15\pm0.03)E_{s\ \underline{para}} - (0.70\pm0.14)B_{1\ \underline{meta}}$

$(5.19) \qquad\qquad (5.14) \qquad\qquad (5.04)$

$+ (0.25\pm0.05)HB_{\underline{para-ind.}} + (0.48\pm0.13)_{\underline{meta}} + 1.42.$

$(4.51) \qquad\qquad (3.77)$

$n = 24; \quad r = 0.91; \quad s = 0.117; \quad f = 17.72 \ (f_{\alpha(1)} = 0.0005,5,18 = 7.71).$

(VI) The combined data set (1 - 44):

Stepwise regression analysis on the complete data set including all the active compounds resulted in eq. 10.

(10) $\log 1/C = (0.14\pm0.03)E_{s\ \underline{para}} + (0.40\pm0.1)HB_{\underline{meta}} - (0.72\pm0.18)B_{1\ \underline{meta}}$

$(4.04) \qquad\qquad (3.97) \qquad\qquad (3.88)$

$+ (0.25\pm0.07)HB_{\underline{para-ind.}} - (0.07\pm0.02)L_{\underline{ortho}} +$

$(3.64) \qquad\qquad (3.35)$

$(0.51\pm0.17)\pi_{\underline{meta}} + 1.44$

(2.93)

$n = 44; \quad r = 0.77; \quad s = 0.166; \quad f = 9.14 \ (f_{\alpha(1)} = 0.0005,6,35 = 5.39)$

(8) $\log 1/C = -(0.17\pm0.02)L_{ortho} + (0.49\pm0.10)HB_{meta} - (0.87\pm0.20)B_{1\ meta}^+$

(7.54)　　　　　(4.66)　　　　　(4.30)

$(0.18\pm0.04)HB_{ortho} + (0.62\pm0.17)\pi_{meta} + (0.12\pm0.04)_{ortho} +$

(3.78)　　　　　(3.69)　　　　　(2.88)

$+ 1.43$

$n = 23; \quad r = 0.925; \quad s = 0.103; \quad f = 15.73 \ (f_{\alpha(1)=0.0005,6,16} = 7.74).$

V. <u>Meta</u>- and/or <u>para</u>-substituted derivatives (1, 5, 18, 22, 23, 35, 43, and compounds from II and III):

Equation 9 was obtained from regression analysis on the entire data set.

(9) $\log 1/C = (0.39\pm0.07)HB_{meta} + (0.15\pm0.03)E_{s\ para} - (0.70\pm0.14)B_{1\ meta}$

(5.19)　　　　　(5.14)　　　　　(5.04)

$+ (0.25\pm0.05)HB_{para-ind.} + (0.48\pm0.13)\pi_{meta} + 1.42.$

(4.51)　　　　　(3.77)

$n = 24; \ r = 0.91; \ s = 0.117; \ f = 17.72 \ (f_{\alpha(1)} = 0.0005,5,18 = 7.71).$

(IV) The combined data set (1 - 44):

Stepwise regression analysis on the complete data set including all the active compounds resulted in eq. 10.

(10) $\log 1/C = (0.14\pm0.03)E_{s\ para} + (0.40\pm0.1)HB_{meta} - (0.72\pm0.18)B_{1\ meta}$

(4.04)　　　　　(3.97)　　　　　(3.88)

$+ (0.25\pm0.07)HB_{para-ind.} - (0.07\pm0.02)L_{ortho} +$

(3.64)　　　　　(3.35)

$(0.51\pm0.17)\pi_{meta} + 1.44$

(2.93)

$n = 44; \ r = 0.77; \ s = 0.166; \ f = 9.14 \ (f_{\alpha(1)} = 0.0005,6,35 = 5.39).$

The 2,4,6-trimethyl (7), 2,6-dimethoxy (38), and 2-phenyl (39)
derivatives were poorly predicted, with residuals of 0.415, 0.536, and
0.381 respectively. The higher observed activity of compound 7 might
be the result of a large increase in lipophilicity, which is not sig-
nificant in the correlation due to predominant steric effects.

The unusual behavior of the 2-phenyl derivative (39) has been observed
in eq. 1 and 10.

Omission of compounds 7 and 39 from the regression resulted in
eq. 11.

$$(11) \quad \log 1/C = (-0.10\underline{+}0.02)L_{\underline{ortho}} + (0.15\underline{+}0.03)E_{s\ \underline{para}} + (0.28\underline{+}0.05)HB_{\underline{para-ind}}.$$

$$(5.73) \qquad\qquad (5.44) \qquad\qquad (5.36)$$

$$+ (0.39\underline{+}0.08)HB_{\underline{meta}} - (0.70\underline{+}0.14)B_{1\ \underline{meta}} + (0.51\underline{+}0.13)\pi_{meta}$$

$$(5.05) \qquad\qquad (4.75) \qquad\qquad (3.84)$$

$$+ (0.11\underline{+}0.05)HB_{\underline{ortho}} + 1.42.$$

$$(2.31)$$

$$n = 42; \quad r = 0.88, \quad s = 0.128, \quad f = 16.51 \ (f_{\alpha(1)=0.0005,7,30} = 5.31)$$

The correlation here is much better than in eq. 10; but the 2,6-dimethoxy
derivative (38) is still poorly predicted, with a residual value of 0.408.
The low activity of this compound is probably due to the steric effects of
its di-ortho-substituents. It appears that this effect cannot be approxi-
mated by twice the steric effect of a single substituent (in Table I, $L_{\underline{ortho}}$,
$2,6-(OCH_3)_2 = 2 \ X \ L_{\underline{ortho},\ 2-OCH_3}$).

Omission of compound 38 from the calculation gave eq. 12

$$(12) \quad \log 1/C = (0.15 \pm 0.02) E_{s \ \underline{para}} + (0.40 \pm 0.07) HB_{\underline{meta}} - (0.72 \pm 0.12) B_{1 \underline{meta}}$$

$$(6.72) \qquad\qquad (6.04) \qquad\qquad (5.86)$$

$$+ (0.26 \pm 0.04) HB_{\underline{para-ind.}} - (0.08 \pm 0.02) L_{\underline{ortho}} + (0.52 \pm 0.11) \pi_{\underline{meta}}$$

$$(5.77) \qquad\qquad (4.85) \qquad\qquad (4.53)$$

$$+1.44$$

$n = 41$; $r = 0.89$; $s = 0.110$; $f = 21.43$ $(f_{\alpha(1)=0.0005,6,30} = 5.66)$

Replacing $HB_{\underline{meta}}$ and $B_{1 \ \underline{meta}}$ in eq. 12 with $HB_{\underline{meta-ind.}}$ and $E_{s \ \underline{meta}}$ resulted in a similar correlation. However, addition of the $HB_{\underline{ortho}}$ (or $HB_{\underline{ortho-ind.}}$) term to the equation resulted in slight improvement of the correlation:

$$(13) \quad \log 1/C = (0.15 \pm 0.02) E_{s \ \underline{para}} + (0.27 \pm 0.04) HB_{\underline{para-ind.}} + (0.39 \pm 0.06) HB_{\underline{meta}}$$

$$(6.63) \qquad\qquad (6.32) \qquad\qquad (6.18)$$

$$- (0.68 \pm 0.12) B_{1 \ \underline{meta}} - (0.08 \pm 0.02) L_{\underline{ortho}} + (0.50 \pm 0.11) \pi_{\underline{meta}}$$

$$(5.80) \qquad\qquad (5.34) \qquad\qquad (4.63)$$

$$+ (0.09 \pm 0.04) HB_{\underline{ortho}} + 1.42$$

$$(2.15)$$

$n = 41$; $r = 0.90$; $s = 0.105$; $f = 21.00$ $(f_{\alpha(1)=0.0005,7,30} = 5.31)$.

Equation 12 was used to give the calculated log 1/C values listed in Table I. Table III shows the squared correlation matrix for the variables of eq. 12. The small numbers indicate that these variables are reasonably independent. It was observed that the two parameters for hydrogen-bonding effect, HB and $HB_{\underline{ind.}}$ can be interchanged in the above equations with little change in the statistics. The indicator variable $HB_{\underline{ind.}}$ has been used successfully for hydrogen-bonding effect in QSAR studies[38-40] although the real situation is conceivably more complicated.

No earlier attempts have been made to use a continuous parameter for this stereospecific interaction. Our effort to partially quantitize it by the HB parameter did not result in significantly better equations. We also observed the correlation between the B_1 parameter and the E_s parameter. This correlation has been discovered as a general phenomenon[24,36], and where the minimum width of substituents is important, the choice between these parameters seems to depend on the spcific congeneric series at hand[36].

In QSAR studies, in order to discover a meaningful correlation, oftentimes, a large number of independent variables have to be screened. But as the number of variables becomes large, as compared to the number of observations, the risk of obtaining a chance correlation will be substantial. This phenomenon has been observed by Topliss et al.,[41,56] based on simulated QSAR studies employing random numbers. In our study, 30 variables (cf. Experimental Section) were screened for possible inclusion in the correlation. Taking into account the high correlation between E_s and B_1, and that between the two hydrogen-bonding parameters, the actual number of independent variables reduces to 24. In equation 12 six independent parameters were entered with a total of 41 observations. According to Fig. 7, in ref. 11, with 24 variables and 41 observations, the probability of encountering a chance correlation with $r^2 = 0.7$ is 1% or less. It is therefore, safe to conclude that the correlation represented by eq. 12 is not spurious. A correlation coefficient of 0.89, i.e. about 80% of the total variance explained, is generally considered reasonable for QSAR studies based on in vivo data.

Of the four inactive derivatives (45-48), only the 2,3,5-triiodo derivative was correctly predicted to have very low potency. The reasons for the inactivity of the other three compounds are not clear at this

moment. The 2-cyano substituent might make the ester linkage in compound

45 less stable through its strong electron-withdrawing effect, while the

4-iodo-substituent might impart some special property to compound 47. The

inactivity of the 3-methyl derivative (46) was rather surprising because

the 3,5-dimethyl derivative (36) showed activity, but the possibility of

an experimental error cannot be excluded.

The behavior of different $\underline{N}$-substituents on the piperidine ring

was also examined by regression analysis. A correlation was obtained

between analgesic potency (log 1/C) and the length of $\underline{N}$-substituents measured

by Verloop's sterimol value of L.

(14) log 1/C = -(0.087$\pm$0.028)L + 2.06

$$(3.15)$$

n = 4;   r = 0.912;   s = 0.116;   f = 9.89 $(f_{\alpha(1)=0.10,1,2} = 8.53)$

Although the data are from substituted pyrrolecarboxylates (Table IV)

instead of benzoates, it is apparent from previous work that a suitable

heterocyclic or aromatic ring can be interchanged in esters of this type[44].

The QSAR results may be summarized as follows:

(1)   Increasing the length of <u>ortho</u>-substituents results in a decrease

of potency.

(2)   Substituents capable of accepting a hydrogen-bond tend to increase

potency. A hydrogen-bond acceptor can function at all three positions

on the phenyl ring, but the energy contribution is the greatest at

the <u>meta</u>-position.

(3)   The <u>meta</u>- and <u>para</u>-positions are sensitive to steric hindrance, which

may be represented by either the minimal width (B_1) or the E_s parameter

of a substituent.

(4) Sensitivity to increase in lipophilicity, which enhances the potency, is observed at meta- and ortho-positions; but the effect is less significant at the ortho-position, possibly due to the predominant steric effect.

(5) There appears to be a steady trend toward decreasing potency with increasing length of N-substituents.

Based on the QSAR, we can define a desirable substitution pattern of the phenyl ring for this type of benzoate. A compound of optimal potency should have (1) no ortho-substitution; (2) meta- and para-substituents of good hydrogen-bond accepting ability, but of minimal width to minimize steric hindrance; (3) good lipophilicity associated with the meta-substituent; and (4) N-substituent not bulkier than a methyl group.

The most potent derivative synthesized so far in this series, namely 3,4-dimethoxybenzoic acid ester of 1-methyl-4-piperidinol (1), seems to have met the above criteria, and further modification of the phenyl ring is unlikely to generate compounds of higher potency.

Since the regression-analysis was done on data from in vivo assays, an interpretation of the structure-activity relationships should preferably include events in both the pharmacokinetic phase (absorption, distribution, metabolism, and excretion of the drug) and the pharmacodynamic phase (molecular interaction of the active agent with its specific site of action). Good correlations between opiate receptor affinity and analgesic potency have been reported for homologous series of meperidines[57-59] prodines[60], N-alkylnorbenzazocines[61] and N-alkylketobemidones[62]. This suggests that the observed differences in potency among homologous compounds of the 4-phenylpiperidine type are mainly due to receptor-related events rather than differential access to the receptor[57], and the in vivo ED_{50} potencies

Chart 1:   3-Tropanol 2,4,5-Trimethylpyrrole-3-carboxylate

β-isomer   α-isomer
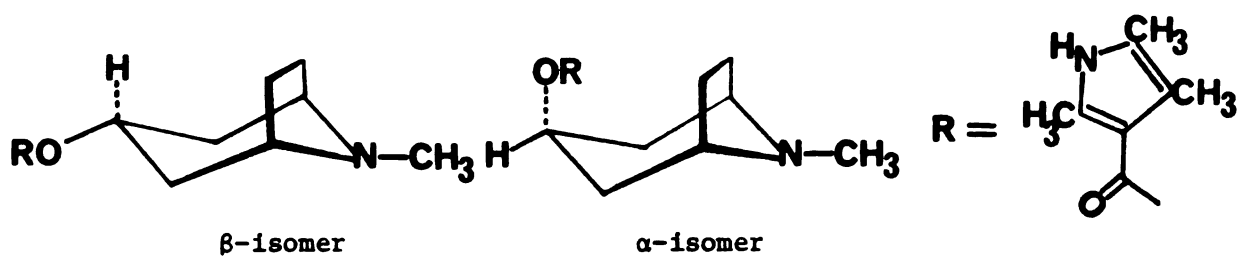
Hot-plate ED_{50} 21.3 mg/kg   50 mg/kg (marginal)

appear to provide a fair approximation of the relative receptor affinities[58].

On the other hand, it has been shown[47], with a group of structurally diverse analgesics, that lipophilicity combined with receptor binding affinity are adequate for explaining in vivo antinociceptive activity. Regression equations described above have demonstrated that the overall lipophilicity ($\Sigma\pi$) is not significant in correlating the in vivo hot-plate potency, indicating that these aromatic esters of 4-piperidinol might behave like other homologous 4-phenylpiperidines. Thus, the QSAR results can be rationalized a priori, in terms of drug-receptor interactions. There are two ways by which the length of an ortho-substituent can decrease the potency: one is through unfavorable steric interaction with the receptor; the other is through intramolecular steric hindrance, which creates an energy barrier to the adoption of a favorable binding conformation with the receptor. The latter appears to be more likely because the length of adjacent meta-substituents is not significant in correlating the activity. In order to visualize this intramolecular steric interaction, it is necessary to determine whether the ester linkage prefers the equatorial or the axial position. Previous work[63] with aromatic esters of 3α- and 3β-tropanol (Chart I), where the orientation of the ester linkage is fixed by a two-carbon bridge between C-2 and C-6, showed that the activity resides mainly in the β-isomer, in which the ester group is in the equatorial position, assuming a chair conformation of the piperidine ring. It is therefore, reasonable to assume that the preferred binding conformation of the benzoic acid esters of 4-piperidinol also has the ester group in the equatorial position. The steric hindrance exerted by the ortho-substituent may be caused by the phenyl ring tilting up towards the piperidine ring in such a way that the dihedral angle formed by C-4 of the piperidine ring, the

oxygen atc the carbonyl carbon and C-1 of the benzene ring approaches 0°. This would result in a conformation approaching that assumed by rings A, D, and E of the morphine molecule (Fig. 2), although a complete overlap cannot be achieved due to steric interactions between ortho-hydrogens or other ortho-substituents on the benzene ring and hydrogen atoms on the piperidine ring. Therefore, the ortho-position on the phenyl ring of the 4-piperidinol benzoates does not correspond to the phenolic position in morphine.

The relative energy contribution from hydrogen-bonding, in the order of meta >para >and ortho, indicates that in the preferred binding conformation, hydrogen-bond accepting substituents at the meta-position are more favorably oriented to accept a hydrogen bond, whereas hydrogen-bond acceptors at para-or ortho-position, being less favorably oriented, can only form weaker hydrogen bonds.

The relatively high values of the regression coefficient associated with the π_{meta} term might indicate the presence of a hydrophobic area in the vicinity of the meta-position when these aromatic esters are bound to the receptor. Hypothetical models[64-67] of the opiate receptor propose a planar area, or a lipophilic site, on the receptor to accommodate the aromatic ring of morphine-like compounds. The negative correlation with the B_1 (or E_s) parameter of a meta- or para-substituent indicates steric hindrance to such an interaction.

Variation of N-substituents has been used as a means of comparing modes of drug-receptor interactions of several classes of opiates[66]. Table V shows the relative analgesic potency of N-substituted analogs of the aromatic ester, meperidine, and morphine. At first glance, the variation of activity in the aromatic ester series does not resemble that of either

the meperidine homologues or the morphine homologues. However, if the trend shown in equation 12 is extrapolated, i.e. if the activity continues to decrease with further increase in the length of the N-substituent, then, the behaviour of N-substituents of the aromatic esters appears to resemble that of morphine derivatives more than that of meperidine derivatives. In the meperidine series, the potency is dramatically increased when the N-methyl group is replaced by bulky aralkyl groups, e.g., phenylbutyl and cinnamyl, while the potency of morphine derivatives is greatly reduced.

A non-opiate type receptor was previously proposed for these aromatic acid esters of non-quaternary C-4 piperidinol[44,45] because of their atypical behaviour in the testing for physical dependence liability and their marginal binding affinity towards the opiate receptor in rat brain homogenate. However, the QSAR results presented thus far seem to favor a binding mode approximating that of morphine-like compounds, at least in binding to the μ-receptor which mediates heat-antinociception[68], with morphine being a prototype agonist. The marginal binding affinity of these flexible esters to the opiate receptor may be compensated by their rapid and efficient penetration into the CNS, as has been shown to be the case with 4-phenylpiperidines[58,69]. Further receptor binding studies in rat brain homogenates and other systems would be needed to verify the proposal based on the QSAR results.

C. EXPERIMENTAL SECTION

1. QSAR Methods: (cf. Chap. V)

The calculation was performed with the MULREG program, which carries out an unweighted step-wise multiple regression analysis and is available in the PROPHET system, a time-sharing computer resource sponsored by the

National Institutes of Health.

The physico-chemical parameters included in the regression analysis are described briefly as follows:

(a) Electronic effects:

σ, Hammett's sigma constant for substituents on an aromatic ring[4].

F and R, values for inductive and resonance effects respectively, according to Swain and Lupton[31].

(b) Steric effects:

E_s, Taft's steric parameter[5].

MR, the molar refractivity of the substituent.

L, B_1, and B_4, Verloop's Sterimol values (in Å) for the length, the smallest width, and the largest width, respectively, of a substituent[36].

(c) Hydrophobic effects:

π, the log of the octanol-water partition coefficient for a substituent[7].

$$(\pi_X = \log P_{C_6H_5X} - \log P_{C_6H_6})$$

(d) Hydrogen-bonding:

HB-ind., the indicator variable. Substituents capable of accepting a hydrogen-bond are given a value of one and those unable to form hydrogen-bond are given a value of zero.

HB, a thermodynamically derived parameter indicating the relative hydrogen-bond accepting ability of a substituent. The development of the HB parameter is shown in Table VI.

With the exception of the HB parameter, all parameter values are obtained from or estimated according to the literature[24,70]. The analgesic activity is represented as log 1/C, where C is the hot-plate ED_{50} in mmol/kg (Table I).

2. Synthesis and Biological Assay:

Forty-eight substituted benzoic acid esters of 1-methyl-4-piperidinol are presented in Table I. The synthesis and biological assay of these compounds were carried out by Dr. James A. Waters at NIAMDD, National Institutes of Health. Compounds 2, 4, 6, 10, 12, 19, 24, 27, 29, 32, 34, 37, 39, 42, 45 and 47 were synthesized for this study, the details of which are summarized in Table VII. The remaining esters in Table I were synthesized previously[44,45]. All of the esters were prepared via the acid chloride. The substituted benzoic acid was converted to the acid chloride with thionyl chloride. Without isolation and purification, the acid chloride was reacted with 1-methyl-4-piperidinol in dry pyridine. The reaction mixture was worked up appropriately. Further purification by column chromatography on silica gel or recrystallization from appropriate solvent then gave the desired benzoic acid ester[45].

These compounds were tested subcutaneously as water soluble HCl salts for analgesic activity by the hot-plate method as described by Jacobson and May[71].

Table I. Hot-plate analgesic potency and physico-chemical parameters of substituted benzoic acid esters of 1-methyl-4-piperidinol.
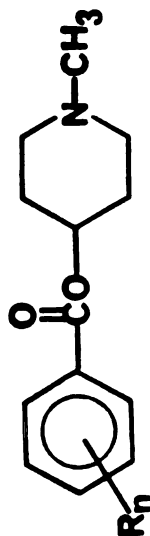
No.	Rn	$\Sigma\pi$	π_{meta}	π_{ortho}	L_{ortho}^a	$B_1 meta^a$	$E_s meta^a$	$B_1 para^a$	$E_s para$	HB_{ortho}	HB_{meta}	HB_{para}	Log 1/C[b] Obsd.	Log 1/C[b] Calcd[c]	Δ
1	3,4-$(OCH_3)_2$	0.08	0.04	0.	0.	0.35	-0.55	0.35	-0.55	0.	1.128	1.128	1.91	1.84	0.07
2[d]	4-OC_2H_5	0.38	0.	0.	0.	0.	0.	0.35	-0.62[e]	0.	0.	1.248	1.72	1.61	0.11
3	3-OCH_3	-0.02	-0.02	0.	0.	0.35	-0.55	0.	0.	0.	1.128	0.	1.67	1.63	0.04
4[d]	4-CN	-0.57	0.	0.	0.	0.	0.	0.6	-0.51	0.	0.	1.898	1.65	1.62	0.03
5	3,4-(OCH_2O)	-0.05	-0.025	0.	0.	0.2[f]	-0.55	0.2	-0.55	0.	1.128	0.	1.61	1.65	-0.04
6[d]	4-O-n-C_4H_9	1.55	0.	0.	0.	0.	0.	0.35	-0.94[g]	0.	0.	1.248	1.58	1.56	0.02
7[h]	2,4,6-$(CH_3)_3$	1.29	0.	0.86	1.88	0.	0.	0.52	-1.24	0.	0.	0.	1.55	1.11	-0.44
8	2,3-$(OCH_3)_2$	0.08	-0.02	-0.02	1.92	0.35	-0.55	0.	0.	1.128	1.128	0.	1.55	1.49	0.06
9	3,5-$(OCH_3)_2$	0.08	0.08	0.	0.	0.7	-1.1	0.	0.	0.	1.128	0.	1.53	1.43	0.10
10[d]	2-CF_3	0.88	0.	0.88	1.24	0.	0.	0.	0.	1.078	0.	0.	1.49	1.35	0.14
11	2-CH_3	0.56	0.	0.56	0.94	0.	0.	0.	0.	0.	0.	0.	1.48	1.37	0.11
12[d]	2-NO_2	-0.28	0.	-0.28	1.38	0.	0.	0.	0.	1.918	0.	0.	1.48	1.34	0.14

Table I (continued -2)

No.	Rn	$\Sigma\pi$	π_{meta}	π_{ortho}	L_{ortho}[a]	$B_{1\ meta}$[a]	$B_{s\ meta}$[a]	$B_{1\ para}$[a]	$E_{s\ para}$[a]	HB_{ortho}	HB_{meta}	HB_{para}	Obsd.	Calcd[c]	Δ
13	2,4,6-(OCH$_3$)$_3$	0.06[l]	0.	0.04	3.84	0.	0.	0.35	-0.55	0.[j]	0.	1.128	1.47	1.33	0.14
14	H	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1.43	1.44	-0.01
15	4-OCH$_3$	-0.02	0.	0.	0.	0.	0.	0.35	-0.55	0.	0.	1.128	1.43	1.62	-0.19
16	2-OCH$_3$	-0.02	0.	-0.02	1.92	0.	0.	0.	0.	1.128	0.	0.	1.43	1.30	0.13
17	2-F	0.14	0.	0.14	0.59	0.	0.	0.	0.	0.	0.	0.	1.41	1.40	0.01
18	3-OCH$_3$,4-CH$_3$	0.54	-0.02	0.	0.	0.35	-0.55	0.52	-1.24	0.	1.128	0.	1.4	1.44	-0.04
19[d]	3-CN	-0.57	-0.57	0.	0.	0.6	-0.51	0.	0.	0.	1.898	0.	1.39	1.48	-0.09
20	4-F	0.14	0.0	0.	0.	0.	0.	0.35	-0.46	0.	0.	0.	1.38	1.37	0.01
21	2,5-(CH$_3$)$_2$	1.07[k]	0.56	0.56	0.94	0.52	-1.24	0.	0.	0.	0.	0.	1.36	1.29	0.07
22	3,4,5-(OCH$_3$)$_3$	-0.6	-0.4	0.	0.	0.7	-1.1	0.35	-0.55	0.	1.128	1.128	1.35	1.36	-0.01
23	3-F,4-CH$_3$	0.7	0.14	0.	0.	0.35	-0.46	0.52	-1.24	0.	0.	0.	1.32	1.07	0.25
24[d]	2-CH$_2$C$_6$H$_5$	2.01	0.	2.01	1.57	0.	0.	0.	0.	0.	0.	0.	1.28	1.32	-0.04
25	2,6-(CH$_3$)$_2$	1.07	0.	1.07	1.88	0.	0.	0.	0.	0.	0.	0.	1.27	1.30	-0.03
26	3-F	0.14	0.14	0.	0.	0.35	-0.46	0.	0.	0.	0.	0.	1.26	1.26	0.00
27[d]	2-Cl	0.71	0.	0.71	1.46	0.	0.	0.	0.	0.	0.	0.	1.25	1.33	-0.08
28	3-OH	-0.67	-0.67	0.	0.	0.35	-0.55	0.	0.	0.	1.0	0.	1.21	1.24	-0.03
29[d]	2-Br	0.86	0.	0.86	1.77	0.	0.	0.	0.	0.	0.	0.	1.21	1.31	-0.10

Table I (continued -3)

No.	Rn	Σπ	π meta	π ortho	L_{ortho}[a]	B_{1} meta[a]	E_{s} meta	B_{1} para[a]	E_{s} para	HB ortho	HB meta	HB para	Obsd.	Calcd[c]	Δ
30	4-CH$_3$	0.56	0.	0.	0.	0.	0.	0.52	-1.24	0.	0.	0.	1.17	1.25	-0.08
31	2,4,5-(CH$_3$)$_3$	1.5	0.56	0.56	0.94	0.52	-1.24	0.52	-1.24	0.	0.	0.	1.17	1.10	0.07
32[d,h]	4-NO$_2$	-0.28	0.	0.	0.	0.	0.	0.7	-2.52	0.	0.	1.918	1.14	1.31	-0.07
33	4-C(CH$_3$)$_3$	1.98	0.	0.	0.	0.	0.	1.59	-2.78	0.	0.	0.	1.13	1.01	0.12
34[d]	4-Cl	0.71	0.	0.	0.	0.	0.	0.8	-0.97	0.	0.	0.	1.11	1.29	-0.18
35	3,4-Cl$_2$	1.25	0.71	0.	0.	0.8	-0.97	0.8	-0.97	0.	0.	0.	1.08	1.08	0.00
36	3,5-(CH$_3$)$_2$	1.07	1.07	0.	0.	1.04	-2.48	0.8	-0.97	0.	0	0.	1.07	1.25	-0.18
37[d]	4-C$_6$H$_5$	1.96	0.	0.	0.	0.	0.	2.11[l]	-3.82[l]	0.	0.	0.	0.85	0.85	0.00
38[h]	2,6-(OCH$_3$)$_2$	0.08	0.	0.08	3.84	0.	0.	0.	0.	0.[j]	0.	0.	0.63	1.15	-0.52
39[d,h]	2-C$_6$H$_5$	1.96	0.	1.96	4.22	0.	0.	0.	0.	0.	0.	0.	1.52[n]	1.12	0.40
40	2-OC$_6$H$_5$	2.08	0.	2.08	2.45	0.	0.	0.	0.	0.■	0.	0.	1.24[n]	1.26	-0.02
41	2-OC$_2$H$_5$	0.38	0.	0.38	2.86	0.	0.	0.	0.	1.248	0.	0.	1.18[n]	1.23	-0.05
42[d]	2-C$_2$H$_5$	1.02	0.	1.02	2.05	0.	0.	0.	0.	0.	0.	0.	1.15[n]	1.29	-0.14
43	3,4-(CH$_3$)$_2$	0.99	0.56	0.	0.	0.52	-1.24	0.52	-1.24	0.	0.	0.	1.15[n]	1.17	-0.02
44[d]	2-C$_2$H$_4$C$_6$H$_5$	2.66	0.	2.66	6.27	0.	0.	0.	0.	0.	0.	0.	0.86[n]	0.97	-0.11
45[d]	2-CN	-0.57	0.	-0.57	2.17	0.	0.	0.	0.	1.898	0.	0.	inact.	1.27	--
46	3-CH$_3$	0.56	0.56	0.	0.	0.52	-1.24	0.	0.	0.	0.	0.	"	1.35	--
47[d]	4-I	1.12	0.	0.	0.	0.	0.	1.15	-1.4	0.	0.	0.	"	1.23	--
48	2,3,5-I$_3$	3.	2.00	1.12	2.17	2.3	-2.8	0.	0.	0.	0.	0.	"	0.65	--

Table I (Footnotes)

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[a] The listed values are the actual values minus the value for "H" so that the unsubstituted compound (14) can have zero value for all the parameters.

[b] $C = ED_{50}$ (mmol/kg). Tested subcutaneously as water soluble HCl salts by the hot-plate method; cf. A.E. Jacobson and E.L. May, $\underline{J}$. $\underline{Med}$. $\underline{Chem}$., 8, 563 (1965).

[c] Derived from Eq. 11.

[d] These derivatives were synthesized following the Craig's Plot analysis.

[e] $-0.62 = E_{s,OCH_3} + (E_{s,C_2H_5} - E_{s,CH_3}) = -0.55 + ((-1.31)-(1.24))$.

[f] Estimated from B_{1,OCH_3}.

[g] $-0.94 = E_{s,OCH_3} + (E_{s,\underline{n}-C_4H_9} - E_{s,CH_3}) = -0.55 + (1.63)-(-1.24))$.

[h] Omitted in deriving Eq. 11.

[i] $0.06 = \pi_{3,5-(OCH_3)_2} + \pi_{OCH_3} = 0.08 + (-0.02)$.

[j] Assuming that a hydrogen-bond can not be formed because of unfavorable conformation imposed by the di-ortho-substituents.

[k] The value of $\pi_{3,5-(CH_3)_2}$ was used.

[l] Because of the preferred perpendicular conformation of the biphenyl, maximum dimensions were used for steric effect, i.e. $E_s(L)$ and B_4 were used instead of $E_s(S)$ and B_1.

[m] Assuming the bulky phenyl group prevents "OC_6H_5" to be a hydrogen-bond acceptor.

[n] Marginally active. Activity was observed in 4 to 5 out of 10 mice tested.

Table II. Squared Correlation Matrix for Variables of Eq. 4 and 5.

	$B_{\underline{1m}}$	$E_{\underline{sm}}$	$\pi_{\underline{m}}$	$HB_{\underline{m}}$	$HB_{\underline{m\text{-ind}}}$
$B_{\underline{1m}}$	1.00	0.84	0.30	0.014	0.004
$E_{\underline{sm}}$		1.00	0.59	0.047	0.04
$\pi_{\underline{m}}$			1.00	0.44	0.43
$HB_{\underline{m}}$				1.00	0.85
$HB_{\underline{m\text{-ind}}}$					1.00

Table III. SQUARED CORRELATION MATRIX FOR VARIABLES OF EQ. 12

	L_{ortho}	HB_o	Bl_m	HB_m	π_m	$E_{s\,p}$	$HB_{p\text{-ind}}$
L_{ortho}	1.00	0.08	0.11	0.06	0.00	0.12	0.02
HB_o		1.00	0.03	0.00	0.00	0.06	0.03
Bl_m			1.00	0.22	0.18	0.01	0.01
HB_m				1.00	0.20	0.02	0.00
π_m					1.00	0.01	0.03
$E_{s\,p}$						1.00	0.03
$HB_{p\text{-ind}}$							1.00
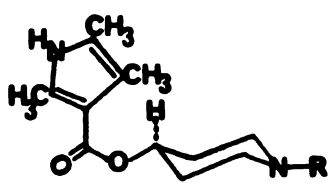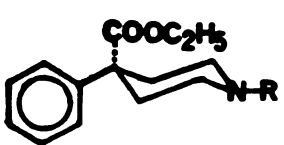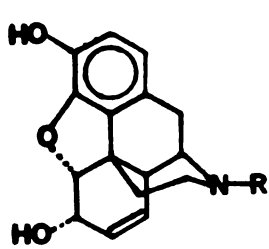
Table IV. 2,4,5-Trimethylpyrrole-3-carboxylic acid esters of $\underline{N}$-substituted 4-piperidinol.
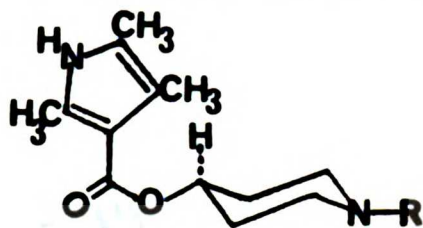
R	L (Å)[a]	ED_{50}(mg/kg)[b]	Formula	Log $1/C$[c]
CH_3	3.0	4.9	$C_{14}H_{22}N_2O_2 \cdot F_3CCOOH$	1.871
$CH_2C_6H_5$	3.63	6.3	$C_{20}H_{26}N_2O_2 \cdot HCl\ H_2O$	1.781
CH_2CH_3	4.11	10.4	$C_{15}H_{24}N_2O_2 \cdot F_3CCOOH$	1.561
$CH_2CH_2C_6H_5$	8.33	20 (marginal)	$C_{21}H_{28}N_2O_2 \cdot F_3CCOOH$	1.356

[a]Length of R.   [b]From ref. 26   [c]ED_{50} in mmol/kg.

Table V. Relative analgesic activity[a] of N-substituted homologues of

aromatic esters of 4-piperidinol, meperidine, and morphine.

R	N-substituted 4-piperidinol pyrrole carboxylate[b]	N-substituted normeperidine[c]	N-substituted normorphine[c]
Me	1.0	1.0	1.0
$C_6H_5CH_2$	0.8	<0.5	<0.1
Et	0.5	0.5	<0.1
$C_6H_5(CH_2)_2$	0.3	2.6	6
$C_6H_5(CH_2)_4$	---	2.8	∿0.3[d]
$C_6H_5CH=CHCH_2$	---	40	<0.1

[a]Activity relative to the N-methyl homologue in each series.

[b]From Table IV.

[c]From Table II in ref. 29.

[d]The activity of the morphinan analog was used.

Table VI. The derivation of HB parameter.

Substituent[a]	$K_{25°C,}$[b]	$-\Delta G_{25°C,}$[c]	HB[d]
	1/mole	kcal/mole	
OH	0.74	-0.178	1.000
OC_6H_5	0.79	-0.14	1.038
CF_3	0.84	-0.1[e]	1.078
OCH_3	0.92	-0.05	1.128
OC_2H_5	1.04	0.07	1.248
CN	3.4	0.72	1.898
NO_2	3.5	0.74	1.918

[a]Substituents capable of accepting a hydrogen-bond.

[b]Equilibrium constant of hydrogen-bonding between substituted benzene and

phenol as a hydrogen-bond donor in carbon tetrachloride.

Values from the compilation by Joesten & Schaad.[36]

[c]$-\Delta G_{25°C}$ = RT ln $K_{25°C}$.

[d]Hydrogen-bond accepting abilities relative to -OH.

[e]$\Delta G_{,CF_3} \simeq \Delta G_{,CH_2F} = \Delta G_{CH_2Cl} + (\Delta G_{C_6H_{13}F} - \Delta G_{C_6H_{13}Cl})$
$= 0.4 + (0.372 - 0.673) = 0.4 + (-0.3) = 0.1$

Table VII. 1-Methyl-4-piperidinol Esters

No.	Rn	Mp, °C[a]	Formula	Recrystn. Solv.[b]	Yield,%	Analyses[c]
2	4-OC$_2$H$_5$	228-229	C$_{15}$H$_{21}$NO$_3$·HCl	C	58	C,H,N
4	4-CN	247-249	C$_{14}$H$_{16}$N$_2$O$_2$·HCl	C	37	C,H,N
6	4-O-n-C$_4$H$_9$	215-216	C$_{17}$H$_{25}$NO$_3$·HCl	C	61	C,H,N
10	2-CF$_3$	213-214	C$_{14}$H$_{16}$F$_3$NO$_2$·HCl	C	19	C,H,N
12	2-NO$_2$	172-175	C$_{13}$H$_{16}$N$_2$O$_2$·HCl	C	20	C,H,N
19	3-CN	239-241	C$_{14}$H$_{16}$N$_2$O$_2$·HCl	A	45	C,H,N
24	2-CH$_2$C$_6$H$_5$	193-200	C$_{20}$H$_{23}$NO$_2$·HCl	C	26	H,N,C[d]
27	2-Cl	175-176	C$_{13}$H$_{16}$ClNO$_2$·HCl	C	37	C,H,N
29	2-Br	190-193	C$_{13}$H$_{16}$BrNO$_2$·HCl	C	61	C,H,N
32	4-NO$_2$	215-217	C$_{13}$H$_{16}$N$_2$O$_4$·HCl·1.5H$_2$O	B	25	C,H,N
34	4-Cl	229-229.5	C$_{13}$H$_{16}$ClNO$_2$·HCl	C	64	C,H,N
37	4-C$_6$H$_5$	272-275	C$_{19}$H$_{21}$NO$_2$·HCl	D	48	C,H,N
42	2-C$_2$H$_5$	179.5-180.5	C$_{15}$H$_{21}$NO$_2$·HCl	C	26	C,H,N
45	2-CN	195-197	C$_{14}$H$_{16}$N$_2$O$_2$·HCl	C	3	C,H,N
47	4-I	267-268.5	C$_{13}$H$_{16}$INO$_2$·HCl	E	83	C,H,N
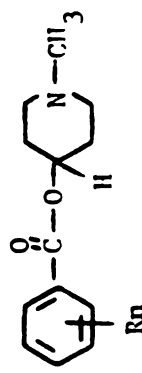
[a] Melting points were taken on a Kofler hot stage and are corrected.

[b] A = acetone, B = EtOH, C = acetone-EtOH, D = EtOH-H$_2$O, E = Acetone-EtOH-H$_2$O.

[c] Analytical results obtained were within ± 0.4 % of the theoretical values, unless otherwise noted.
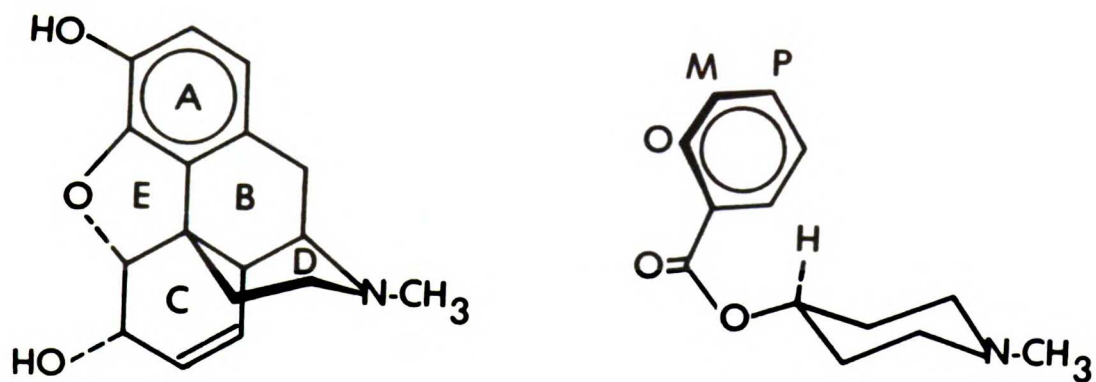
[d] C : Calcd., 69.45; found, 70.00.

Figure 2. The proposed binding conformation of aromatic esters of
1-methyl-4-piperidinol approximates A, D, and E rings of
the morphine molecule.

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