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Sponge Derived Marine Natural Products as Pharmaceutical Leads



**Author**
Carroll, Jennifer A.

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# SPONGE DERIVED MARINE NATURAL PRODUCTS AS PHARMACEUTICAL LEADS

A dissertation submitted in partial satisfaction
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

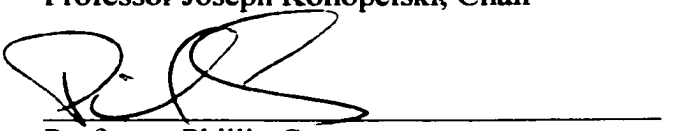
by

**Jennifer Carroll**

December 2001

The Dissertation of Jennifer Carroll
is approved:

Professor Joseph Konopelski, Chair



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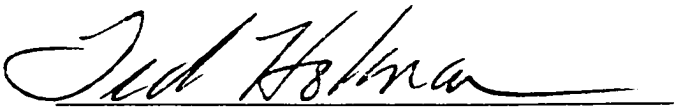

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**Abstract**

**SPONGE DERIVED MARINE NATURAL PRODUCTS AS PHARMACEUTICAL LEADS**

**Jennifer Carroll**

The field of marine natural products continues to produce novel bioactive agents. This work describes the investigation of three different marine sponge species that have yielded interesting new chemistry. Also covered are the isolation and structural characterization techniques used in this study.

Two sponge samples from Papua New Guinea; a *Suberea* sp. and *Cosinoderma* sp. have yielded the new terpenoid compounds subersin, subersic acid, and a sulfonated terpene, halisulfate 8. These compounds were tested for the efficacy in anti-ischemic assays and an assay for inhibitors of 15-Human Lipoxygenase. Inhibitors of these processes might be useful as pharmaceuticals, or as molecular tools to add to the understanding of cellular processes. Another sponge sample, *Cacospongia mycofijiensis*, collected off Vanuatu led to the isolation of the microtubule inhibitors fijianolide A and B and a new microtubule inhibitor fijianolide C. The mechanism of action of these compounds has been determined by collaborators to be similar to the commercial anticancer drug paclitaxel.

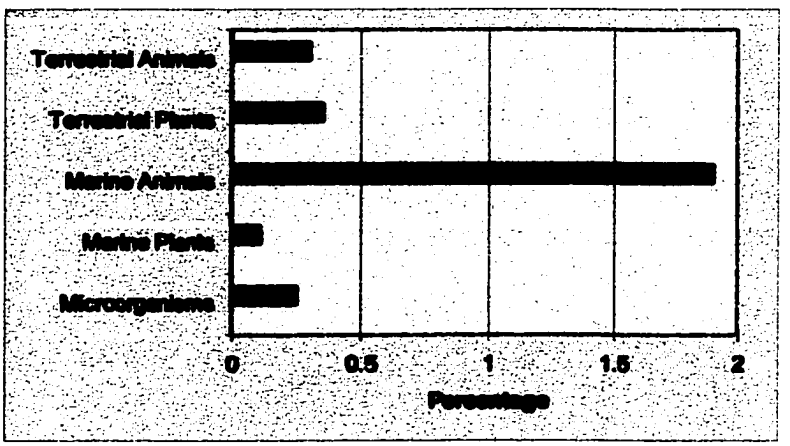
# Chapter 1. Introduction: Relevance and Techniques in the Study of Marine Natural Products

## Background

The origins of marine natural products chemistry can be traced back to the early 1950's.[1] Since that time the field has flourished and even matured. Perhaps reflecting the latter is the many literature reviews that have appeared over the years.[2,3,4,5] This is mostly due to the incredibly high number of biologically interesting molecules isolated from the marine environment. The marine ecosystem has promoted a much higher percentage of therapeutic leads with significant selective cyctotoxic activity than any other category. This point can be illustrated by the large number of compounds active against cancer cells that have been reported from the National Cancer Institute (NCI). In Figure 1 the percentage of NCI "hits" from marine animals is close to 2% (from a total of 6,540 samples) whereas the percentage of active terrestrial animals (434 samples), plants (18,293 samples) and microorganisms (8,246 samples) are in each case less than 1%.[6] Although the overall number of plant samples that have been tested is overwhelmingly higher than that of marine animals, one must consider that unlike terrestrial natural products, marine natural products have only been truly accessible for the last 50 years. During this time an overwhelming 13,176 structures have been published in the literature.[7] Thus the

importance of marine animals as sources for bioactive compounds cannot be underestimated.

**Figure 1** Percent of bioactive "Hits" from plants, animals and microorganisms.[6]

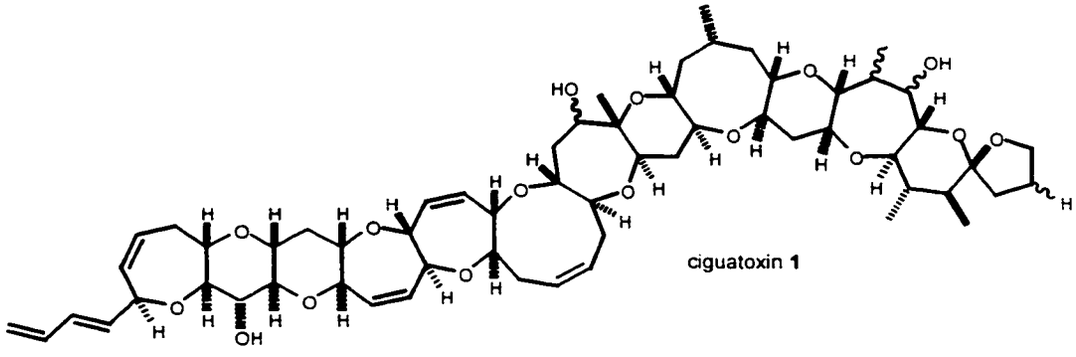



**Relevance**

The pioneering marine natural product chemists structured their research on the already long-standing fields of insect chemical ecology and phytochemistry. Early on, the marine natural products discipline became subdivided into three main areas: marine chemical ecology, marine toxins and marine pharmaceuticals. These three areas have become important contributors to the knowledge of bioorganic and medicinal chemistry.

The marine toxins field has been primarily driven by the health problems associated with toxic algal blooms and shellfish contamination. Occasionally, the agents responsible for this blight are relatively small molecules such as domoic acid,

a water-soluble amino acid.[8] More often they are large molecules, which are isolated in very low yield and require a plethora of spectroscopic techniques to fully characterize. As an example, ciguatoxin (**1**), is responsible for ciguatera poisoning that occurs from eating coral reef fish. This large toxin was identified from both the Pacific moray eel, *Gymnothorax javanicus*[9] and from the dinoflagellate *Gamierdiscus toxicus*,[10] indicating the importance of the food web on the concentration of toxins in seafood. The dinoflagellate toxins are transferred through the food chain among coral species and accumulated mostly in carnivorous fish. The tropical, worldwide occurrence of ciguatera endangers both public health and fishing industries. Amazingly, the structure of **1** was determined on only 0.74 mg of pure compound.

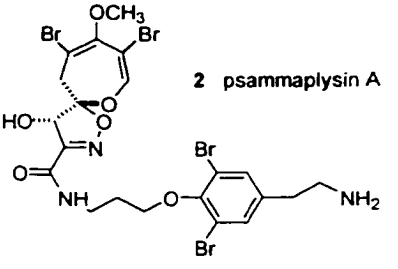


ciguatoxin **1**

The exciting field of marine chemical ecology focuses on three main points. First, which organism is ultimately responsible for the production of highly bioactive compounds, second, why are compounds so important to the ecosystem in which the

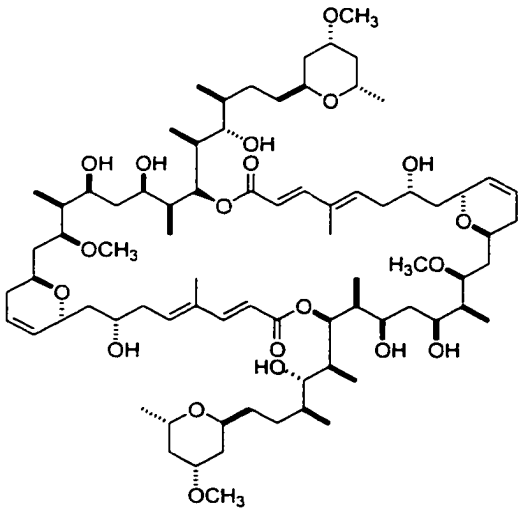
organism resides and third, how are the compounds produced in the organism. The following specific examples are often used to highlight such questions.

The hypothesis that compounds serve as inhibitors to fouling organisms has been contemplated.[11] One of the many compounds isolated from marine sponges that inhibit fouling is psammaplysin A (**2**). This cytotoxic compound was isolated from a Japanese sample of *Pseudoceratina purpurea*.[12] Psammaplysin A inhibits the settlement and metamorphosis of the barnacle *Balanus amphitrite*, and inhibits the larval metamorphosis of the ascidian *Halocynthia roretzi* at levels of 0.27 μg/mL and 1.2 μg/mL respectively.

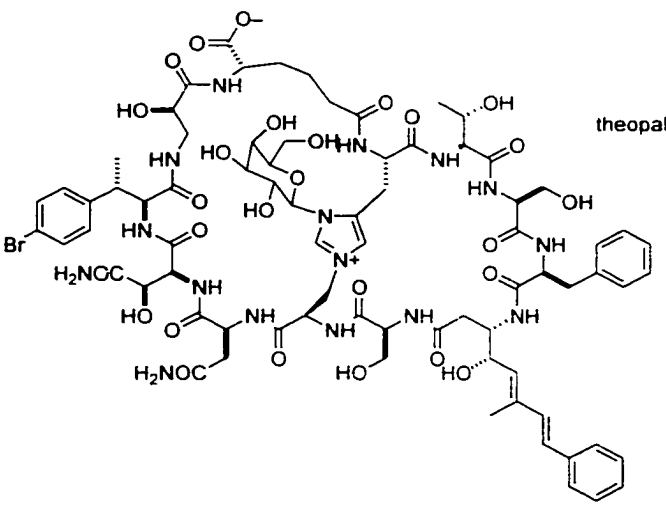



**2** psammaplysin A

A second interesting example of a marine chemical ecology is the marine sponge, *Theonella swinhoei*, known to contain large cyclic peptides similar to those found in blue-green algae. Initially it was theorized that these cyannobacteria were the producers of the biologically active compounds. Interestingly, two such active compounds; swinholide A, **3** and theopalauamide, **4** were found from a mixed bacterial and a δ-proteobacterium fraction respectively[13] and not from the ectosomal cyanobacteria.

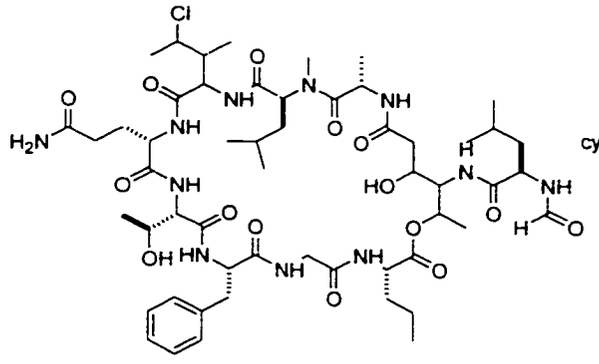


swinholide A **3**



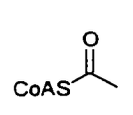
theopalauamide **4**

In another study, a large cyclic peptide, cyclolithistide A, **5** was found from a *Theonella swinhoei* sample that was collected in a completely dark environment, thus indicating that a non-chlorophyll (non-cyanobacterial) cell type was possibly responsible for the interesting chemistry.[14]

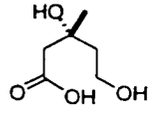


cyclolithistide A **5**

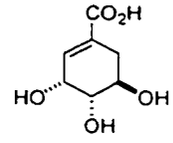
The biosynthesis of natural products is also of fundamental interest. All organisms, including marine invertebrates, have an intake of building blocks (food) that allow them to create a variety of primary and secondary metabolites. The secondary metabolites produced are created from the by-products of the Krebs cycle and/or glycolysis. The most common building blocks are acetyl coenzyme A (acetyl-CoA **6**), mevalonic acid (**7**) and shikimic acid (**8**). These are involved in biosynthetic systems named the acetate, mevalonate and shikimate pathways respectively.[15]



**6** acetyl-CoA



**7** melvalonic acid



**8** shikimic acid

The formation of acetyl-CoA is through either the β-oxidation of fatty acids or from the glycolysis of pyruvic acid. Important products of this pathway include phenols, prostaglandins and marcrolide antibiotics. Acetyl-CoA supplies a two-

carbon segment to the molecule, either in the form of a simple acetyl group, a long alkyl chain, or part of an aromatic phenol group.

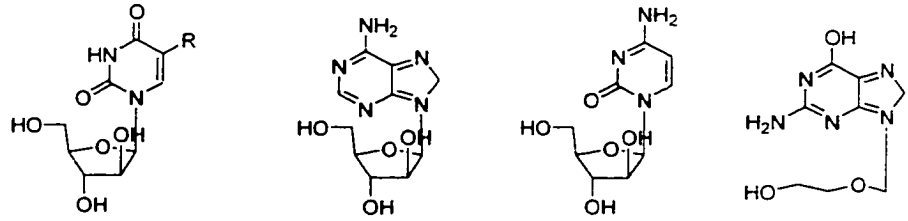
Shikimic acid is produced by a combination of phosphoenolpryuvate, erythrose 4-phosphate and another intermediate of glycolysis. This pathway leads to many structural types such as phenols, cinnamic acid derivatives, and alkaloids. The amino acid products of shikimic acids are the aromatic L-phenylalanine, L-tyrosine and L-tryptophan.

The pathway responsible for the vast number of terpenoid and steroid metabolites is the mevalonic acid pathway. This acid is formed through the condensation of three molecules of Acetyl-CoA, but its products are very different from that of the acetate pathway. The branched C5 'isoprene' units are a feature of compounds formed though the mevalonate pathway. One of mevalonic acid's carbons is lost in this production.

Molecules can contain any number of building blocks of the same type, or a combination of several different types. This expands the breadth of structural diversity available in natural products. Most natural products contain one or more of these pathways in addition to other accessorizing components such as sugars or amino acids. The biosynthesis of marine natural products has been reviewed,[16] and is largely spurred on by the incredible bioactivity of many of the compounds isolated.

The largest and by far most significant arena of marine natural products chemistry has been in the discovery and development of bio-pharmaceuticals. This began with Werner Bergman's discovery of two compounds from the marine sponge

*Cryptotethya crypta* collected off the Elliot Key, Florida.[17,18] Spongouridine (**9**) and spongothymidine (**10**) were noteworthy in that they were the first nucleosides that used sugars other than ribose or deoxyribose. These two compounds have provided scaffolding from which all the modified nucleoside drugs for the treatment of viruses and tumors were derived.



**9** spongouridine R=H
**10** spongothymidine R=$CH_3$

**11** ara-A

**12** ara-C

**13** acyclovir

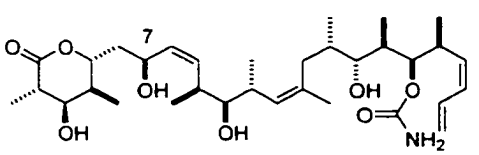
Over the next thirty years a number of synthetic derivatives of **9** and **10** were made and tested. This led to antitumor agents arabinosyladenine[19] (**11**) (ara A or Vidarabine®), ara-C (**12**) and Acyclovir (**13**). Also interesting was that the molecule originally prepared by synthesis, Ara A, was isolated later along with spongouridine from a Mediterranean gorgonian (*Eunicella cavolini*) in 1984.[20]

An intense search for pharmaceutical leads has continued in the area of Cancer research. The National Cancer Institute has developed an excellent screen for anti-cancer compounds, which has led to the discovery of numerous highly active components of marine organisms. Among these are discodermolide (**14**),[21]

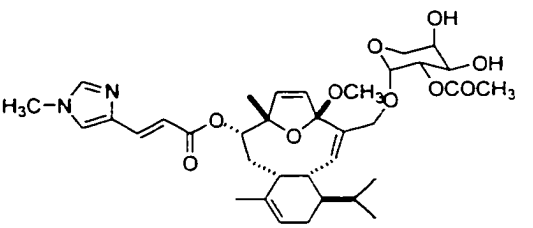
eleutherobin (**15**),[22] dehydrodidemnin B (**16**),[23] bryostatin 1 (**17**),[24] dolastatin 10 (**18**),[25] and ecteinascidin 743 (**19**).[26]

The deep water Bahaman sponge, *Discodermia dissolute*, has yielded the cytotoxic polyhydroxylated discodermolide (**14**),[27] which was determined to have microtubule stabilizing effects.[28] These effects seem to be analogous to that of Paclitaxel.[29] Smith has established the synthetic routes to discodermolide in gram scale allowing for further development of this compound as a therapeutic.[30] Interestingly, SAR studies have shown that the acetylated analogue of discodermolide (7-OAc) is 5 times more potent than Paclitaxel.[31]

Eleutherobin (**15**), and a similar compound, sarcodictyin (not shown)[32] were isolated from the soft corals *Eleutherobia* sp. and *Sarcodictyon roseum*, respectively. These diterpenoid compounds showed microtubule stabilizing effects similar to that of paclitaxel, with eleutherobin showing the greatest activity. Eleutherobin underwent preclinical trials at Bristol-Myers Squibb but is not in clinical trials at this point.

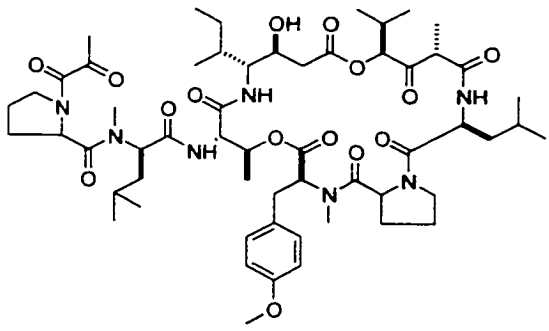



**14** discodermolide

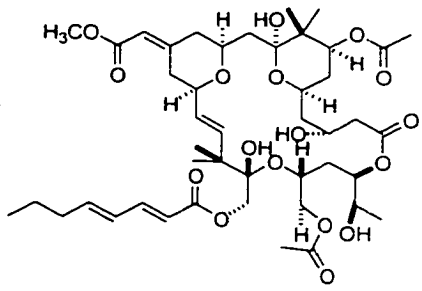



**15** eleutherobin

Dehydrodidemnin B (also known as aplidine[33] **16**),[34] was isolated from the Mediterranean tunicate, *Aplidium albicans*, but can also be produced semi-synthetically from didemnin A (not shown).[19] Dehydrodidemnin B shows similar activity to that of didemnin B (also not shown) that was dropped from clinical trials due to toxicity.[35] Currently Pharma Mar in Madrid, Spain holds rights to this compound.[36]



**16** dehydrodidemnin B

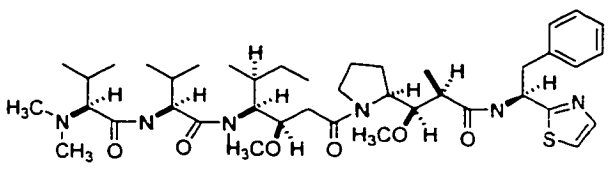


**17** bryostatin 1

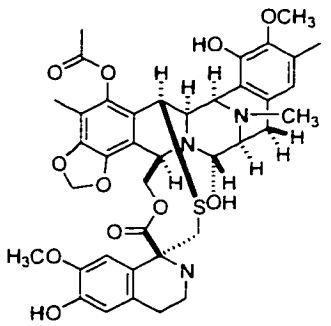
Another compound in clinical trials, bryostatin 1 (**17**), was isolated from a bryozoan, *Bugula neritina*, which was collected in the Sea of Cortez. This compound was found to be a potent protein kinase C inhibitor. Bryostatin 1 recently underwent phase II clinical trials against non-Hodgkin's lymphoma with poor results.[37] Materials for additional clinical trials can be obtained from aquaculture, which has been pioneered by CalBioMarine.

Dolastatin 10 (**18**) was isolated from 1000 kg of the sea hare *Dolabella auricularia* collected in the Indian Ocean.[38] The extremely large quantity of sea hare

collected was considered a crime against the conservation of biodiversity. Dolastatin 10 was synthesized in adequate yield for clinical trials, which are supported by the NCI. Phase II clinical trials have been completed against non-small-cell lung, prostate, colorectal and hepatobiliary carcinomas, melanoma and sarcoma without favorable results.[39] Interesting also is that dolastatin derivatives have been isolated from a *Lyngbya majuscula / Schizothrix calcicola* assemblage indicating that the sea hare might be obtaining its metabolites from a cyannobacterial food source.[40]



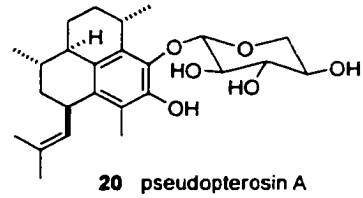
**18** dolastatin 10



**19** ecteinascidin 745

Ecteinascidin 745 (**19**) is a potent antitumor agent isolated from the Caribbean tunicate *Ecteinascidia turbinata*, which has completed phase I clinical trials in the United States and is in phase II clinical trials in Europe. Ecteinascidin 745 is most effective against small cell lung cancer and skin cancer. Although E. J. Corey's group has completed its synthesis in decent yield,[41] its demand for testing is currently being supplied by Pharma Mar (Spain).[42]

Currently, the one marine natural product that is approved by the FDA for use is the diterpene glycoside pseudopterosin (**20**), which was isolated from the Bahaman sea whip *Pseudopterogorgia elisabethae*.[43] This compound was shown to be nontoxic to mice as well as being anti-inflammatory. Its mechanism of action is not well known, but has been shown to inhibit phospholipase A2, cyclooxygenase and lipoxygenase. A partly purified extract of *Pseudopterogorgia elisabethae* is currently sold as an additive to Estee Lauder Resilience skin cream.



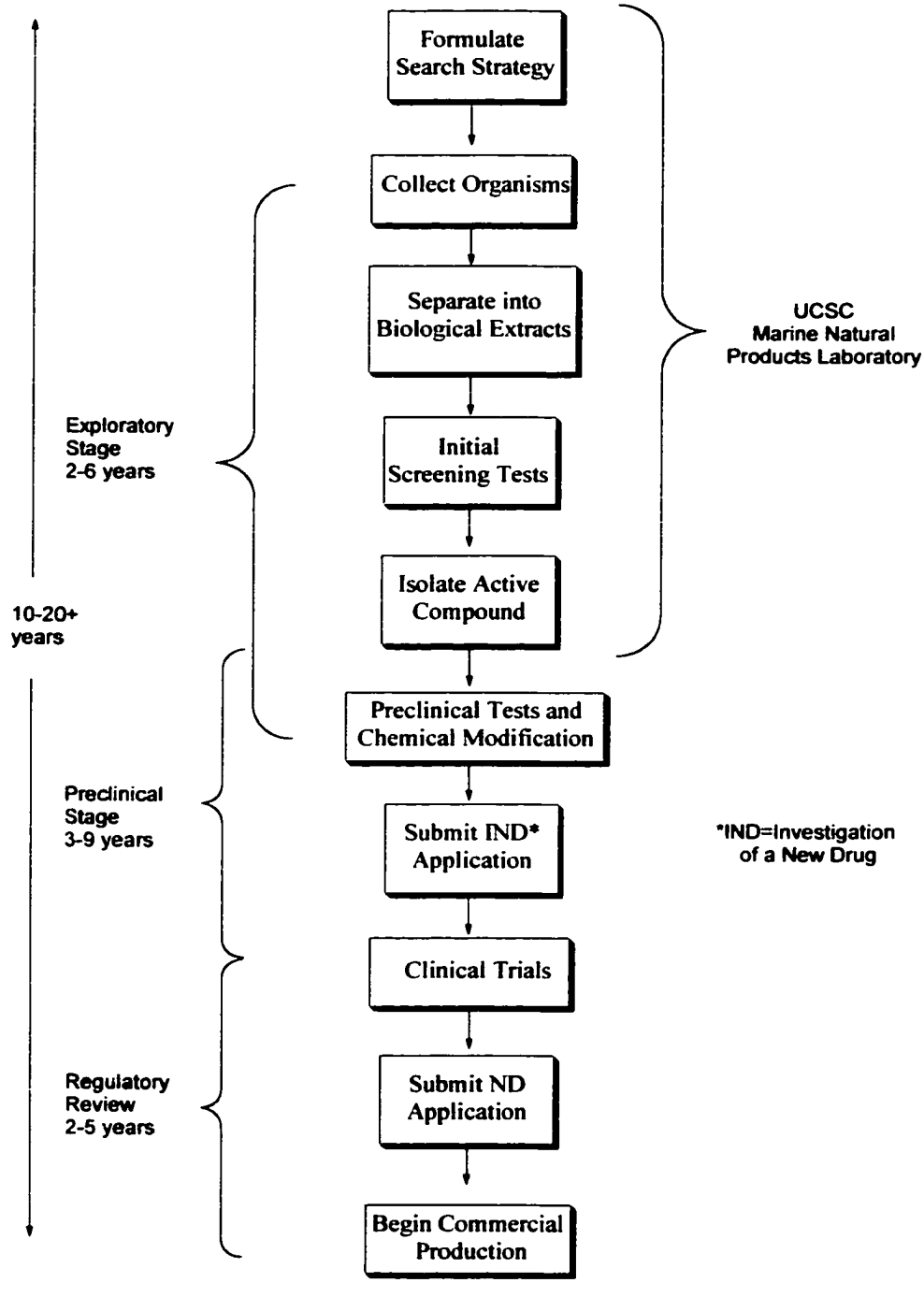

**20** pseudopterosin A

There are currently no marine natural products on the market for the treatment of serious medical conditions such as cancer and heart disease. As can be seen in Scheme 1, the timeline for events that leads up to a marketable drug is often in excess of 20 years. These compounds are often too highly toxic for medicinal use and must therefore be considered as lead compounds for medicinal chemists to further expand upon. As in the case of spongouridine **8** and spongothymidine **9**, the natural product will often have to be synthetically modified and tested to find a highly active, but less toxic compound.

The UCSC Marine Natural Products Laboratory is currently involved with the first five steps of the process in Scheme 1. The formulation of a search strategy is important so that the reisolation of known chemistry can be avoided. The collection, extraction and initial screening tests are performed in the hopes of obtaining an active fraction. The most time consuming and labor intensive of the first five steps is the isolation and characterization of the active compound. An important precursor to this step therefore, is the dereplicaiton of known compounds from the extract. This will be discussed at length later in this work. The length of this exploratory stage can be anywhere from two to six years.

Following the discovery of an active compound, the preclinical stage begins with tests and chemical modifications such as SAR and metabolism studies. Next, applications and clinical trials must be approved during the regulatory review before commercial production of a drug can begin. The overall process from planning strategy to market can take as long as 20 years.

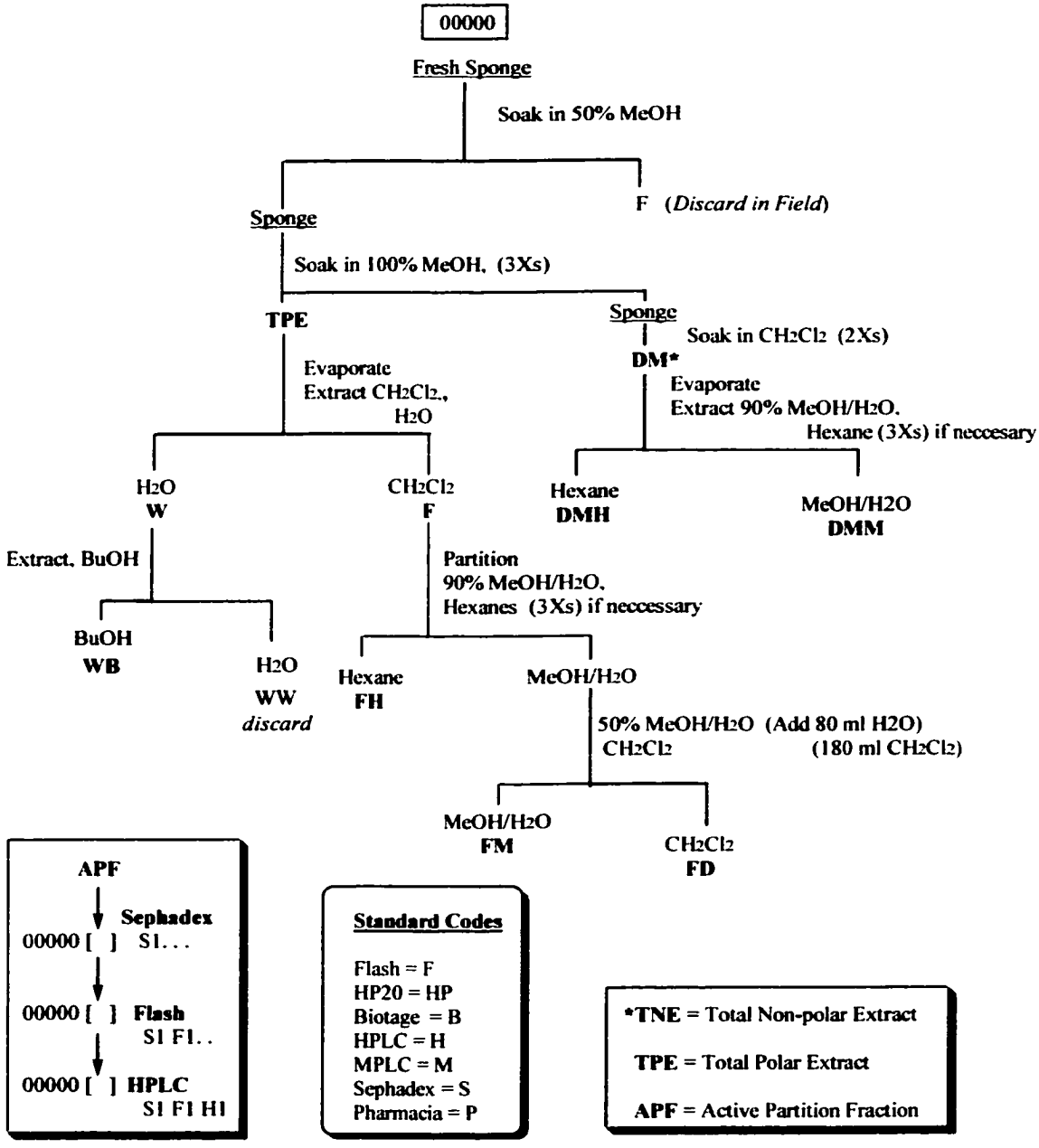
**Scheme 1** Timeline of events leading to a marketable drug.



**Techniques**

Following the completion of a collecting trip, samples are returned to the lab and extracted for bioassay. Scheme 2 shows the solvent partition scheme adapted from the Kupchan method[44] for the fractionation of the crude sponge extract into six oils of differing polarity. These are then submitted to various collaborators for whole cell and/or enzymatic biological screening, or are submitted to an in house assay such as the brine shrimp cytotoxicity assay.[45] When a fraction is deemed active it is next subjected to one of the separation techniques shown in Scheme 2, such as Sephadex™, flash or HP-20. The fractionation technique chosen depends on the polarity of the fraction selected. High performance liquid chromatography is the final means to obtaining a pure compound. These are either run "normal phase" with non-polar solvents such as hexanes and ethyl acetate, or " reversed phase" with highly polar solvents such as methanol and water.

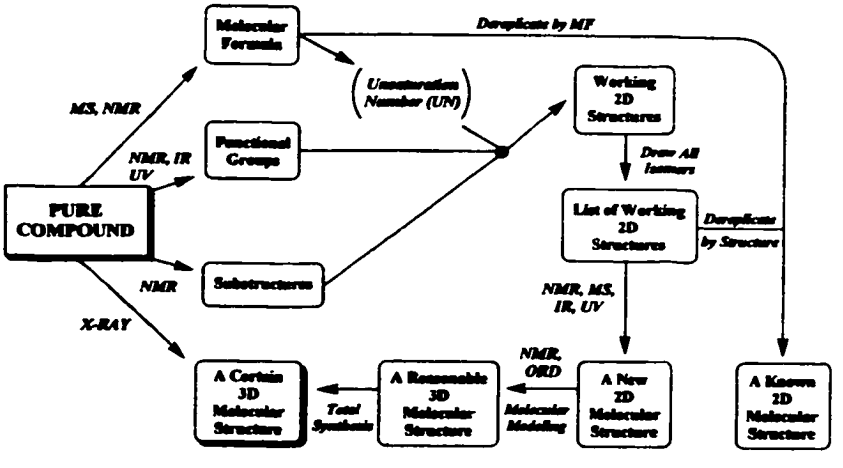
**Scheme 2** UCSC extraction scheme



By following this procedure, a pure compound can be obtained with a minimum of chromatographic steps. At each point during the separation, fractions are returned to the biological assay for testing along with a sample of the original active crude fraction. In this way, a true "bioassay guided" isolation of the pure compounds can be pursued.

Identifying the molecular structure of the active pure compounds follows the steps in Figure 2. In order to eliminate costly and time-consuming spectroscopy of known compounds, a high-resolution mass spectrum is one of the first data acquired. From this a molecular formula and unsaturation number can be obtained. Combined with $^{1}H$ and $^{13}C$ NMR data, these can be used to search out known compounds from various databases such as Marinlit[7] or Scifinder Scholar.[46] If it is established that the compound is indeed new, additional spectroscopy is then aggressively pursued.

**Figure 2** Steps in establishing a molecular structure.[47]

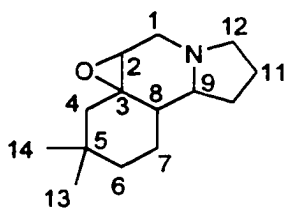


Although there are a large number of basic NMR experiments to choose from,[48] the standard UCSC routine involves the following four pulse sequences. DEPT (Distortionless Enhancement by Polarization Transfer) determines the number of protons on each carbon. gHMQC (gradient strength Heteronuclear Multiple Quantum Coherence) is used for the determination of one bond C to H connections. gCOSY (gradient strength Correlation Spectroscopy) gives data in which signals from the normal $^{1}$H NMR are coupled through $^{2}J$ and $^{3}J$(HH). The gHMBC (gradient strength Heteronuclear Multiple Bond Correlation) suppresses $^{1}J$(CH) so that signals from $^{2/3}J$(CH) can be distinguished. From these four basic experiments, along with a detailed comparison of literature values from known compounds, a 2-dimensional working structure can usually be established.

The relative stereochemistry can be established through a variety of 1-dimensional and 2-dimensional NOE (Nuclear Overhauser Enhancement) experiments, while the absolute stereochemistry is best left to methods such as a modified Mosher's esterification,[49] CD analysis,[50] or a comparison of optical rotation data. Mosher's modified method is to couple a secondary alcohol with that of an enantiomeric pair of $^{19}$F triflouro acids. From the resulting pair of diasteromeric α-methoxy-α-trifluoromethylphenylacetate esters, a difference in the fluorine shifts provides information on the stereochemistry about the secondary alcohol in question. Adaptations of this method have been developed in which the fluorine enantiomeric acids are replaced with acids containing a mandelate and *O*-methylmandelate

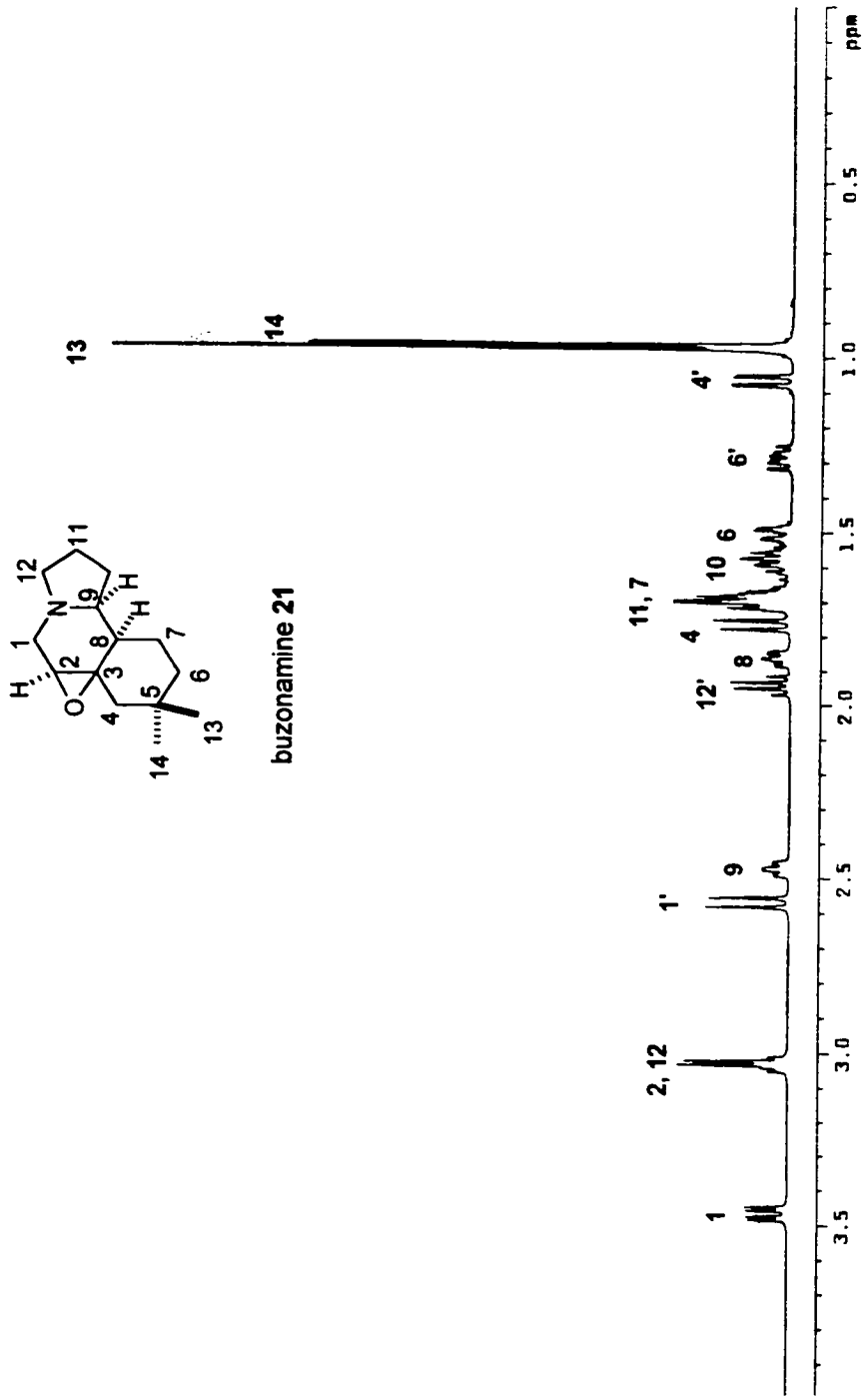
esters.[51,52,53,54,55] In this technique the resulting diastereomeric esters can be distinguished by the shielding or deshielding effect of the phenyl group, which is held in place by the conformational rigidity of the ester. The advantages of the MTPA esters are that they reduce the epimerization at the hydrogen alpha to the carbonyl by replacing it with a $CF_3$ group.

A pertinent example of structure analysis by these NMR methods can be seen in the collaborative characterization of buzonamine (**21**), a defensive secretion from a northern California millipede.[56] From the following experiments performed at California State University Humboldt, Professor William Wood established a two dimensional, working structure of buzonamine. A high-resolution mass spectrum showed the molecular ion at $m/z$ = 221.1785, indicating a molecular formula of $C_{14}H_{23}NO$. From the $^{13}C$ NMR data, buzonamine had 14 carbons while DEPT experiments indicated that there were 23 hydrogens ($2CH_3$, $7CH_2$, 3CH and 2C). The $^1H$ NMR spectrum of buzonamine was compressed, with all resonances between 0.9 and 3.5 ppm (Figure 3 retaken at UCSC). The correlating of $^1H$ resonances was accomplished by COSY experiments.




buzonamine working structure **21**

**Figure 3** Proton NMR of buzonamine (**21**) rerun at UCSC (500Mhz, CDCl3).



From this initial NMR data collected at Humboldt, it appeared that in addition to the structure of buzonamine, there were three possible working structures that could be considered (Figure 4). These have a 5,6,6-ring system (**i**), a 7,6,4-ring system (**ii**), and a 6,7,4-ring system (**iii**) respectively. To verify the two dimensional structure of buzonamine and its relative stereochemistry, I conducted high field NMR experiments such as the HMBC (Figure 5), and NOE (Figures 6 and 7) experiments.

Working structure **i** was considered unsuitable because of COSY correlations observed between H6, H6' and H7. The HMBC correlations that ruled out the possibility of working structure **ii** was from the methylene protons H6 to the methine, C8. Working structure **iii** was ruled out due to the HMBC correlation of H1 to C9 and the one bond $^{1}J$C-H coupling of C1, C9 and C12 of 132-134 Hz, which is characteristic of carbons adjacent to nitrogen.

**Figure 4** Alternate buzonamine working structures.

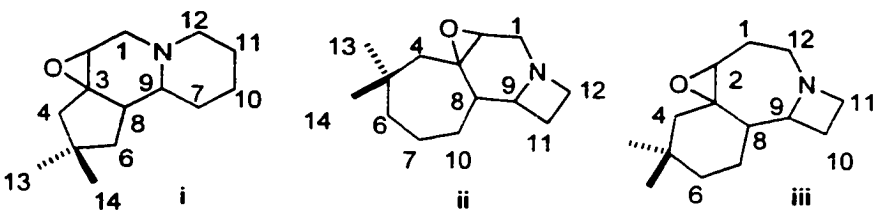
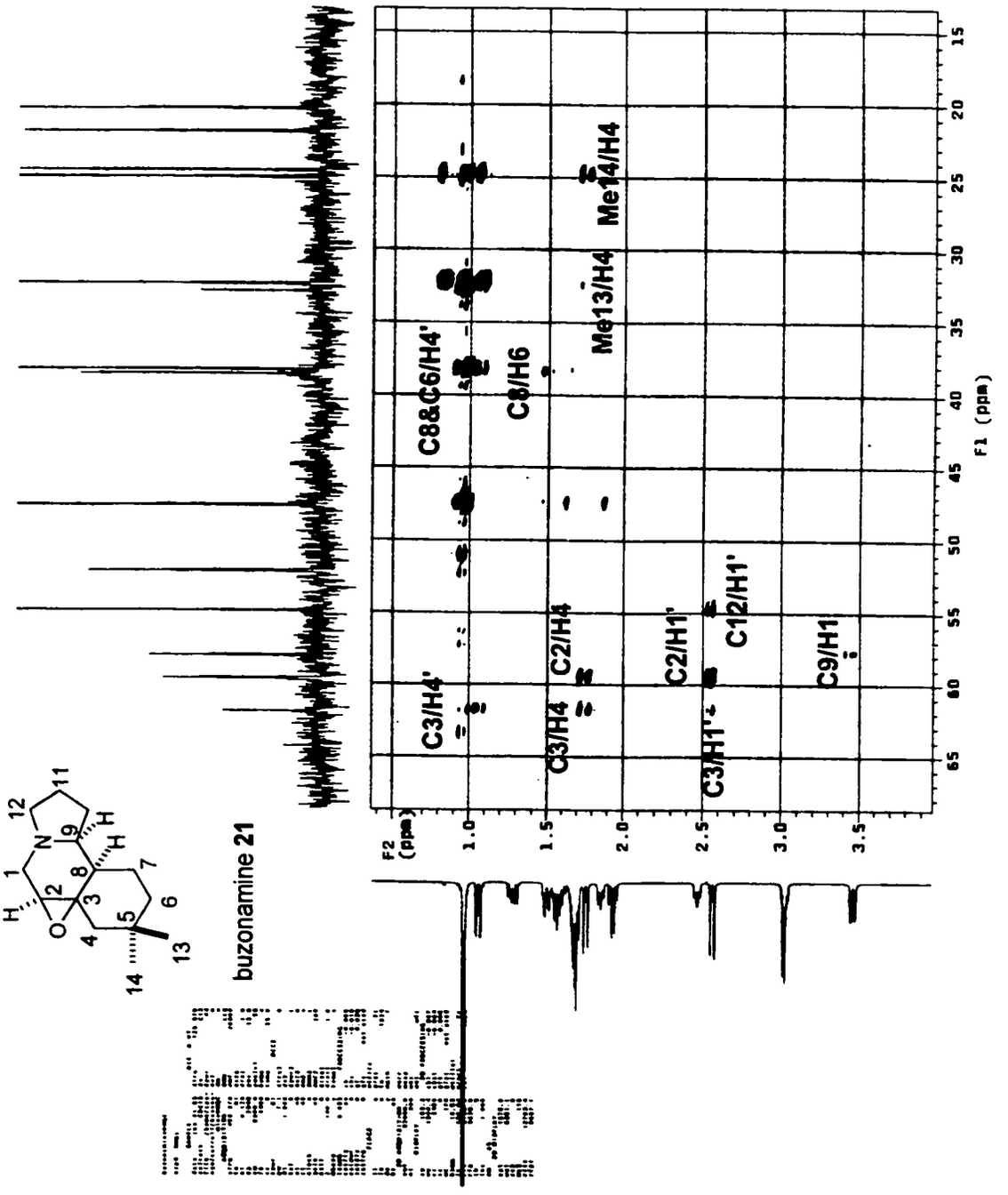
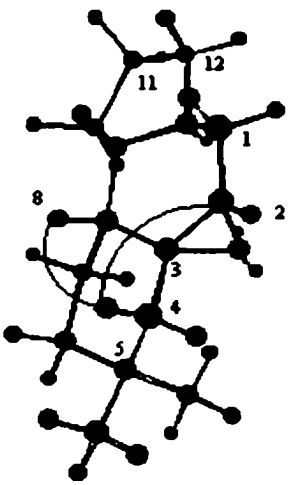


**Figure 5** HMBC of buzonamine (**21**) (500 MHz, $CDCl_3$).



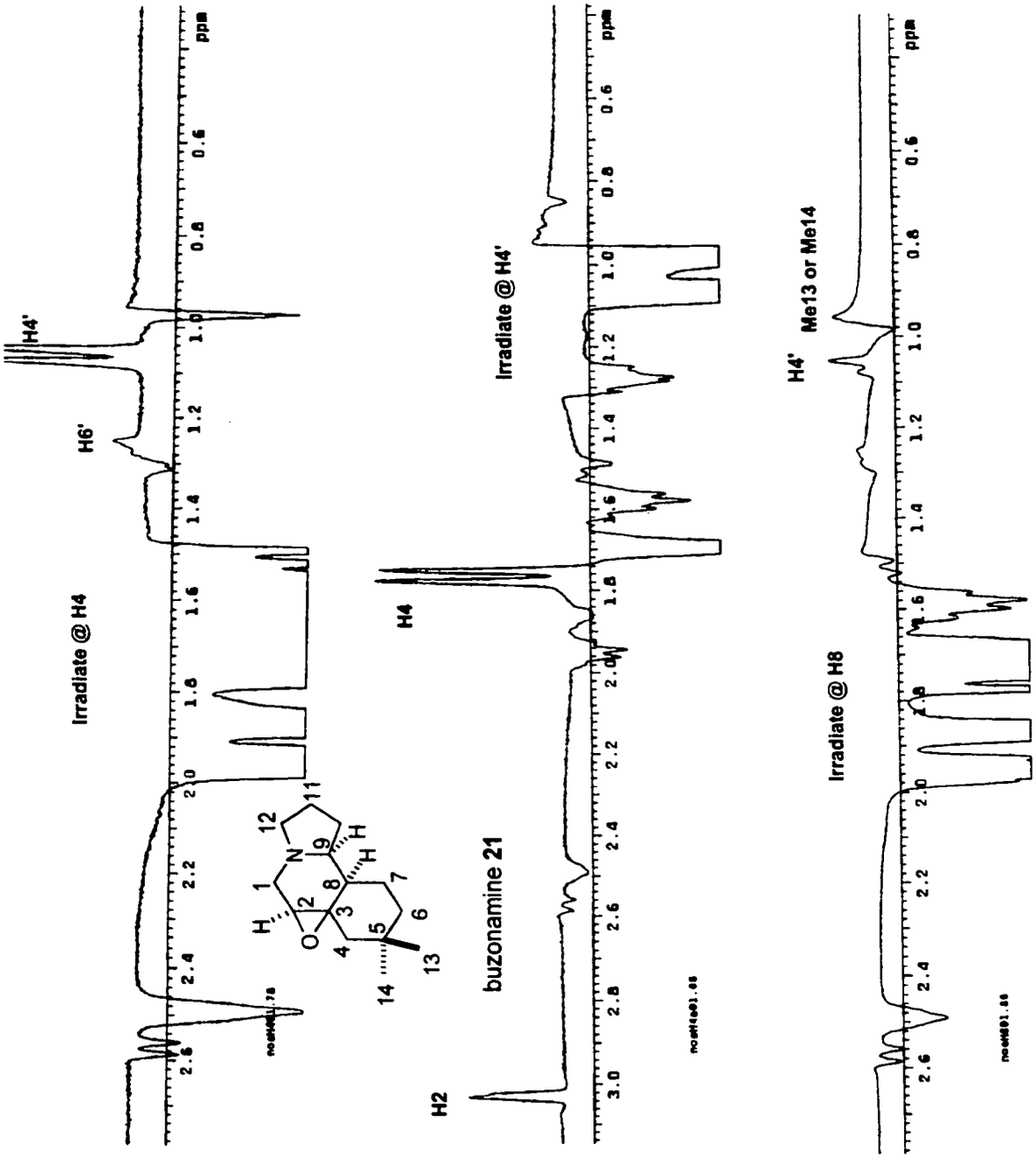
Next, relative stereochemistry of **21** was determined by the use of $^{3}J_{H\text{-}H}$ coupling constant data and NOE experiments. Protons H8 and H9 have $^{3}J$ = 3.3 Hz which is equivalent to literature values for two axial protons on 5,6 ring junctions.[57] The spatial relation of the epoxide proton was determined through the use of NOE correlations which placed H8, H4' and H2 on the same plane as shown in Figure 6.

**Figure 6** Important NOE correlations for buzonamine (**21**).




With this data, the structure of buzonamine was confirmed. The working structures and relative stereochemistry of buzonamine would have remained unanswered questions without the use of these high field experiments. The important techniques learned from this collaboration were used for the structure elucidation of molecules that make up the next three chapters.

**Figure 7** Selected NOE spectra of buzonamine (**21**) (500MHz, $CDCl_3$)



**Future Prospects**

The biosynthetic bioengineering of natural products is the current "hot" topic in natural products. As mentioned previously, the biosynthetic mechanisms of bioactive compounds have been studied intensively. At the same time the techniques of molecular biology have improved considerably. The combination of these two fields will be the driving force for the future of natural products chemistry. Many articles on this subject have appeared recently.[58, 59, 60]

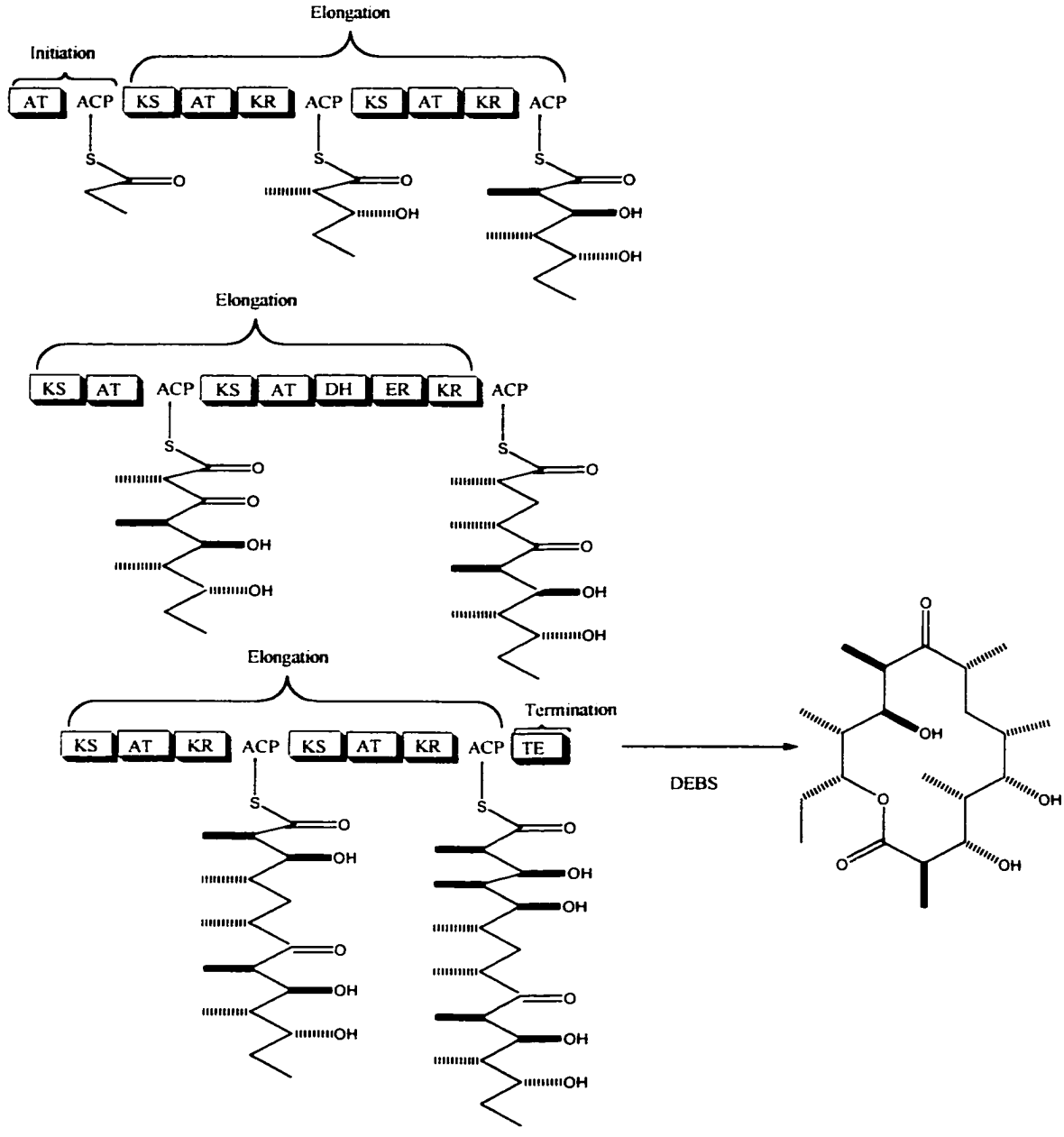
The biosynthesis of peptide and polyketide natural products occurs by two genetic paths. The acetate pathway, as mentioned earlier in this work, is associated with the biosynthetic genes called polyketide synthases (PKS). The amino acids, which originate from either shikimate or melvalonate pathways, are analogously elaborated by nonribosomal peptide synthetases (NRPS). The technology for sequencing of the gene clusters responsible for the biosynthesis, regulation, and precursor formation of natural products has only been available in the last two decades. Since this time a large number of genetic pathways have been identified from both prokaryotes and eukaryotes, the number of which can be seen in Table 1. Because of the advances in automated gene sequencers, this number is expected to grow exponentially in the next few years.

**Table 1** Gene Clusters for the Biosynthesis of Natural Products from Microorganisms. [61]

| DNA source encoding | Number of gene clusters |
|---|---|
| *Prokaryotic Pathways* | |
| Total | 115 |
| Polyketide synthetases | 47 |
| Nonribosomal peptide synthetases | 40 |
| Aminoglycosides | 11 |
| Other | 17 |
| *Eukaryotic Pathways* | |
| Total | 41 |
| Polyketide synthases | 15 |
| Nonribosomal peptide synthetases | 13 |
| Isoprenoid | 12 |
| Other | 1 |

The PKS and NRPS work in a similar fashion, by chain elongation and condensation reactions that occur on proteins. For example, PKS undergo a Claisen-like condensation reaction that adds acetate to the starter material, and then can undergo further elongation, reduction, epimerization and/or cyclization steps. This system, shown in Figure 7, shows the "upstream" initiation acyl group attached to acyl transferase (AT) enzyme. The following steps, acyl carrier protein (ACP), keto synthase (KS), keto reductase (KR), dehydrase (DH), enoyl reductase (ER), and epimerase (Epim) function by moving the molecular substrate along a gene sequence, then performing transformations to the structure. These steps are followed by a termination step (TE) and deoxyerythronolide B synthase (DEBS), which results in the 14-membered lactone 6-deoxyerythronolide B.

**Figure 8** Example of a modular polyketide synthase generating the precursor to erythromycin.[62]



The future of natural products is through the combinatorial manipulation of these genes and the expression of these genes in suitable hosts or the native producer. This could expand the diversity of natural products outside of what is possible with current synthetic techniques. If the goal of a medicinal chemistry program is to conduct structure activity relationships, adjusting the modular genes required for biosynthesis can result in a number of modifications. First, inserting additional modules of acyl transferase can alter the lenghth of the polyketide chain. Second, changing the starter and extender units can make adaptations in the final product. Third, the number of reductions can be adjusted, and fourth; the stereochemistry of substituents can be altered. These changes produce a series of compounds that are structurally related. Examples of such compounds have already been the subject of review.[55] Future natural products chemistry will undoubtedly be enhanced by the production of these analogues and from the medicinal understanding that would arise.

The work described in the following chapter will build on the ideas laid out thus far. The compounds from Chapter Two are inhibitors of an important target, 15-human lipoxygenase, an enzyme implicated in inflammatory processes. The focus of Chapter Three is to characterize novel terpenoid compounds for the treatment of conditions such as heart attack and stroke. The final chapter covers polyketide compounds that function as microtubule inhibitors similar to the potent anti-tumor agent Paclitaxel.

**Experimental Section**

**General Experimental Procedures.** HMBC and NOE NMR spectra were recorded in $CDCl_3$ at 500 MHz on a Varian Unity spectrophotometer. For Collection, extraction and purification, please see reference 56.

**Buzonamine (21)**: white powder; m/z = 221.1785 [$M^+$] (4 mmu of calcd. for $C_{14}H_{23}NO$); $^1H$ NMR (500MHz, $CDCl_3$) δ (ppm) 3.44 (dd, 1H, $J$ = 13.2, 4.8, H1), 3.00 (dt, 1H, $J$ = 8.0, 4.0, 4.0, H12), 2.54 (d, 1H, $J$ = 13.2, H1'), 2.47 (ddd, 1H, $J$ = 8.2, 8.2, 3.3, H9), 1.92 (q, 1H, 8.0, 8.0, 3.0, H12'), 1.84 (ddd, 1H, $J$ = 9.0, 6.3, 3.3, H8), 1.74 (d, 2H, $J$ = 12.9), 1.60-1.70 (m, 4H, H11 & H7), 1.50-1.60 (m, 2H, H10), 1.04 ( dd, 1H, $J$ = 12.9, 2.4, H4'), 0.95 (s, 3H, Me13), 0.94 (s, 3H, Me14). $^{13}C$ NMR 125 MHz, $CDCl_3$) δ (ppm) 61.89 (s, C3), 59.67 (d, C2), 58.00 (d, C9), 54.93 (t, C12), 52.23 (t, C1), 47.68 (t, C4), 38.64 (d, C8), 38.34 (t, C6), 32.91 (s, C5), 32.42 (q, C13), 25.02 (q, C14), 24.57 (t, C10), 21.90 (t, C11), 20.28 (t, C7).

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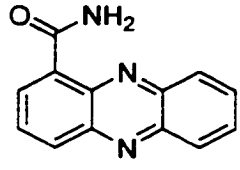
## Chapter 2 Novel Marine Derived Small Molecule Inhibitors of 15-Human Lipoxygenase.

### Background

Several thousand bioactive marine natural products are known,[1] but few are noted as being lipoxygenase (LO) inhibitors.[2] The 5-, 12-, and 15-lipoxygenases, ubiquitous in terrestrial animals, carry out hydroperoxidation of polyenoic fatty acids. The resultant leukotrienes and lipoxins are important classes of signaling molecules that may be involved in a variety of human diseases.[3,4] Thus, the availability of selective inhibitors of the various human lipoxygenase (HLO) isoforms could provide biomolecules useful as pharmacological agents,[5] nutraceuticals,[4] or molecular tools.[6] The recent description of orange peel-derived hexamethoxyflavone, [7] as an inhibitor of soybean lipoxygenase (SLO) ($IC_{50}$ = 49 μM), is an interesting example. More intriguing is that the small number of known marine-derived LO inhibitors have unique molecular structures. Among the first were the pseudopterosins[8a] and the fuscosides,[8b] diterpenoid-glycosides isolated from marine gorgonians. The pseudopterosins putatively possess anti-inflammatory properties derived from the inhibition of both cycloxygenase and LO biosynthetic pathways,[8] while the fuscosides were originally identified as 5-HLO inhibitors.[9] The only sponge-derived 15-HLO inhibitors described to date are the pentabromo biphenyl ethers, isolated from cyanobacterial containing sponges.[10] Believing that marine-derived chemotypes were an untapped source of new LO inhibitors, we began an investigation of the

UCSC repository of marine sponge extracts and pure compounds. The assay design, developed by collaborator Prof. Theodore Holman (UCSC), employed a purified 15-HLO rather than the more common soybean 15-LO screen. Out of an initial set of 100 crude extracts evaluated in the 15-HLO assay, one of these exhibited potent lipoxygenase inhibition activity at a sub μg/mL level. The single potent extract of this set was from a Papua New Guinea sponge, *Suberea* sp. Bergquist, 1995[11] (Porifera, Dictyoceratida, Verongida, Aplysinellidae, coll. no. 97243). Described herein are results on a series of terpenoids discovered from this sponge.

Interestingly, the sponges within the order Verongida are typically characterized by brominated tyrosine metabolites.[12] Due to the relatively new characterization of this genus, only three publications have appeared with remarkable chemistry from *Suberea* sp.[13,14,15] The earliest of these publications is of a pseudomonad isolated from a *Suberea creba* sponge from the Coral Sea. The bacterium in this example gave non-halogenated alkaloids represented by phenazine-a-carboxamide (**1**) and 2-n-heptylquinol-4-one (**2**),[13] whereas the sponge sample yielded typical Verongid type brominated compounds such as (+)-aeroplysinin-1 (**3**), (+)-aerothionin (**4**), and homoaerothionin (**5**). These compounds were tested on the basis of their anti-microbial activity, with **3** showing strong activity against *Pecten maximus* larve.[13]



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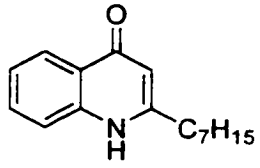
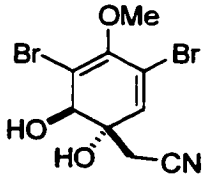


2



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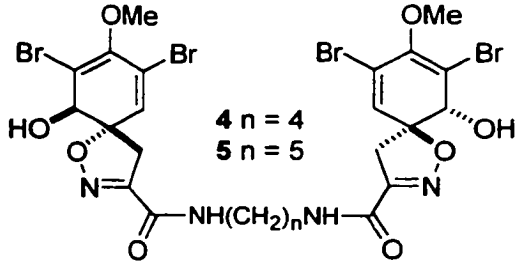
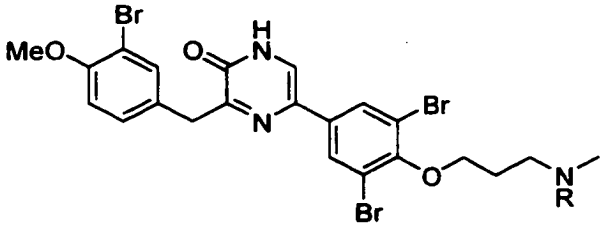
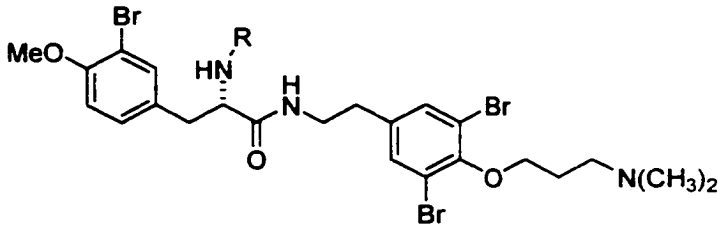


Other *Suberea* sp. compounds include the ma'edamines A (**6**) and B (**7**) and the suberedamines A (**8**) and B (**9**) from Okinawan samples. The ma'edamines A and B contain an unusual pyrazinone ring between two bromotyrosine units. These compounds have exhibited cytotoxicity against murine leukemia L1210 cells ($IC_{50}$, 4.3 and 3.9 μg/mL respectively) and epidermoid carcinoma KB cells ($IC_{50}$, 5.2 and 4.5 μg/mL respectively) in vitro.[14] The suberedamines A and B are rare in that they contain an *N*-methyl amino group at the α-carbon whereas most of the Verongida bromotyrosine alkaloids posses an oxime group for this position.[15] Also, **8** and **9** exhibited cytotoxicity against murine leukemia L1210 cell lines ($IC_{50}$, 8.0 and 8.6 μg/mL respectively) and both showed antibacterial activity against *Micrococcus luteus* with an MIC value of 12.6 μg/mL.




**6** R = $CH_3$
**7** R = H

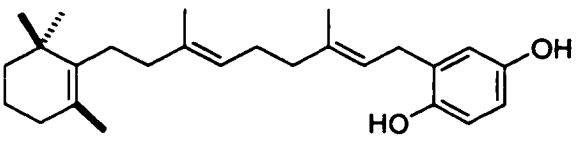



**8** R = H
**9** R = $CH_3$

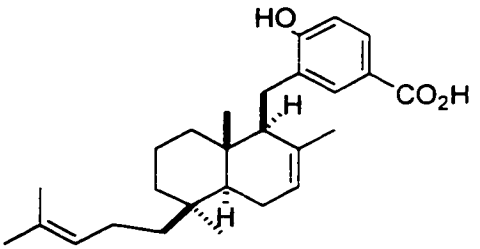
## Results and Discussion

The $IC_{50}$ of sponge 97243, 0.1 μg/mL, merited bioassay guided follow-up using the 15-HLO screen. Inspection of the NMR and MS data for the active crude extract and semi-pure fractions indicated the presence of several compounds possessing aliphatic methyls appearing as intense $^1H$ NMR singlet resonances. At varying stages of the isolation, especially as pure substances were obtained, these were first evaluated in the 15-HLO assay and then via standard structural-dereplication procedures. Two compounds proved to be both known and strikingly potent, including jaspaquinol (**10**), $IC_{50}$ = 0.3 μM, and (-)-jaspic acid (**11**), $IC_{50}$ = 1.4 μM. This same pair of compounds was isolated in 1996 by our group[16] from a very different sponge now characterized as *Jaspis splendens*,[17] the well-known source of (+)-jasplakinolide (**18**). A comparison of the newly isolated **10** and **11** with that of the

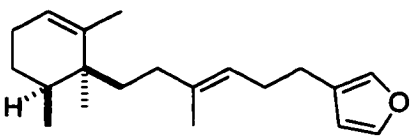
literature values can be seen in Tables 1 and 2. The two other new compounds isolated possessed significantly less potency and included (+)-subersin (**12**), $IC_{50}$ >100 μM, and (-)-subersic acid (**13**), $IC_{50}$ = 15 μM.



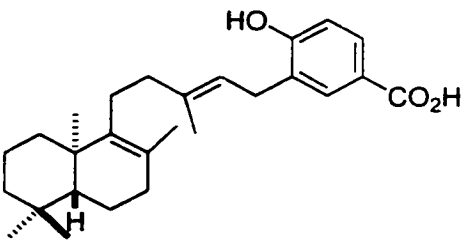

jaspaquinol **10**




(-)-jaspic acid **11**




(+)-subersin **12**




(-)-subersic acid **13**

**Table 1** NMR data comparison for jaspic acid (**11**) ($CDCl_3$, 500MHz)

| | literature | | reisolation | |
|---|---|---|---|---|
| atom no. | $^{13}C$ δ | $^{1}H$ δ (mult, *J* Hz) | $^{13}C$ δ | $^{1}H$ δ (mult, *J* Hz) |
| 1 | 39.7 | 1.92 (br m) | 39.8 | 1.92 (br m) |
| | | 1.24 (m) | | 1.24 (m) |
| 2 | 18.7 | 1.49 (m) | 18.8 | 1.49 (m) |
| 3 | 37.1 | 1.78 (br d, 14.0) | 37.2 | 1.78 (br d, 14.0) |
| 4 | 35.7 | | 35.8 | |
| 5 | 52.5 | 1.38 (t, 8.0) | 52.5 | 1.38 (t, 9.0) |
| 6 | 23.3 | 2.00 (br s) | 23.1 | 1.99 (br s) |
| 7 | 122.8 | 5.40 (br s) | 122.9 | 5.40 (br s) |
| 8 | 135.0 | | 135.0 | |
| 9 | 54.3 | 2.49 (br s) | 54.4 | 2.48 (br s) |
| 10 | 37.1 | | 37.1 | |
| 11 | 28.9 | 0.90 (s) | 29.0 | 0.90 (s) |
| 12 | 32.7 | 1.65 (m) | 32.8 | 1.63 (m) |
| | | 1.24 (m) | | 1.24 (m) |
| 13 | 22.4 | 1.44 (s) | 22.4 | 1.45 (s) |
| 14 | 14.9 | 0.95 (s) | 14.9 | 0.95 (s) |
| 15 | 26.0 | 2.67 (br m) | 25.8 | 2.67 (br m) |
| 16 | 130.1 | | 130.2 | |
| 17 | 132.4 | 8.03 (br s) | 132.5 | 8.04 (br s) |
| 18 | 121.7 | | 121.6 | |
| 19 | 129.4 | 7.83 (br d, 8.0) | 129.2 | 7.83 (br dd, 8.5, 2) |
| 20 | 115.2 | 6.78 (br d, 8.0) | 115.0 | 6.78 (br d, 9.0) |
| 21 | 158.2 | | 158.2 | |
| 22 | 171.7 | | 171.7 | |
| 23 | 23.0 | 1.86 (m) | 23.0 | 1.86 (m) |
| 24 | 125.6 | 5.13 (br t, 7.0) | 125.6 | 5.13 (br t, 7.0) |
| 25 | 131.0 | | 131.0 | |
| 26 | 17.6 | 1.62 (s) | 17.6 | 1.62 (s) |
| 27 | 25.8 | 1.71 (s) | 25.8 | 1.70 (s) |

**Table 2** NMR data comparison for jaspaquinol (**10**) ($CDCl_3$, 500 MHz)

| | literature | | reisolation | |
|---|---|---|---|---|
| atom no. | $^{13}C$ δ | $^{1}H$ δ (mult, J Hz) | $^{13}C$ δ | $^{1}H$ δ (mult, J Hz) |
| 1 | 35.0 | | 35.7 | |
| 2 | 39.9 | 1.43 (m) | 40.1 | 1.43 (m) |
| 3 | 19.6 | 1.70 (m) | 20.4 | 1.70 (m) |
| | | 1.60 (m) | | 1.60 (m) |
| 4 | 32.8 | 1.92 (t, 6.5) | 34.0 | 1.92 (t, 6.5) |
| 5 | 126.9 | | 128.0 | |
| 6 | 137.2 | | 137.2 | |
| 7 | 27.9 | 2.08 (m) | 27.9 | 2.08 (m) |
| 8 | 40.3 | 2.04 (m) | 40.3 | 2.04 (m) |
| 9 | 136.6 | | 138.2 | |
| 10 | 123.3 | 5.13 (br t, 5.5) | 125.0 | 5.13 (br t, 6.0) |
| 11 | 26.4 | 2.14 (m) | 28.0 | 2.14 (m) |
| 12 | 39.7 | 2.12 (m) | 40.8 | 2.12 (m) |
| 13 | 138.7 | | 138.7 | |
| 14 | 121.3 | 5.32 (br t, 7.5) | 124.1 | 5.32 (br t, 8.0) |
| 15 | 29.8 | 3.32 (d, 7.5) | 27.9 | 3.32 (d, 8.0) |
| 16 | 128.2 | | 130.3 | |
| 17 | 148.3 | | 149.5 | |
| 18 | 116.6 | 6.69 (d, 8.5) | 117.0 | 6.69 (d, 8.5) |
| 19 | 113.8 | 6.59 (dd, 8.5, 2.5) | 113.8 | 6.59 (dd, 8.5, 2.5) |
| 20 | 149.3 | | 149.3 | |
| 21 | 116.6 | 6.62 (d, 2.5) | 118.1 | 6.62 (d, 2.5) |
| 22 | 28.7 | 1.01 (s) | 27.9 | 1.01 (s) |
| 23 | 28.7 | 1.01 (s) | 27.9 | 1.01 (s) |
| 24 | 19.9 | 1.61 (s) | 20.8 | 1.61 (s) |
| 25 | 16.1 | 1.65 (s) | 16.1 | 1.65 (s) |
| 26 | 16.3 | 1.78 (s) | 16.3 | 1.78 (s) |

The characterization of (+)-subersin (**12**) commenced once its molecular formula of $C_{20}H_{30}O$, was established. The formula was supported by data including positive ion HREIMS (Figure 1) *m/z* of 286.2267 $[M]^+$ ($\Delta$ 3.0 mmu of calcd.), and the DEPT-135 $^{13}C$ NMR (Figure 2) indicated the carbon types, $4CH_3 + 6CH_2 + 6CH + 4C$, for a count of $C_{20}H_{30}$. A furan moiety was proposed since heteroatom hydrogens were lacking in the $^1H$ NMR spectra (Figure 3) and characteristic $^{13}C$ NMR shifts (Figure 4) could be located ($\delta$ 142.7, d, C15; 139.1, d, C16; 125.1, s, C13; 111.3, d, C14). There were also two double bonds ($\delta$ 139.6, s, C1; 122.1, d, C2; 137.2, s, C9; 123.3, d, C10). The preceding features accounted for five of the six degrees of unsaturation, indicating that a carbocyclic ring was present.

**Figure 1** $EI^+$ Mass spectrum for (+)-subersin (**12**).

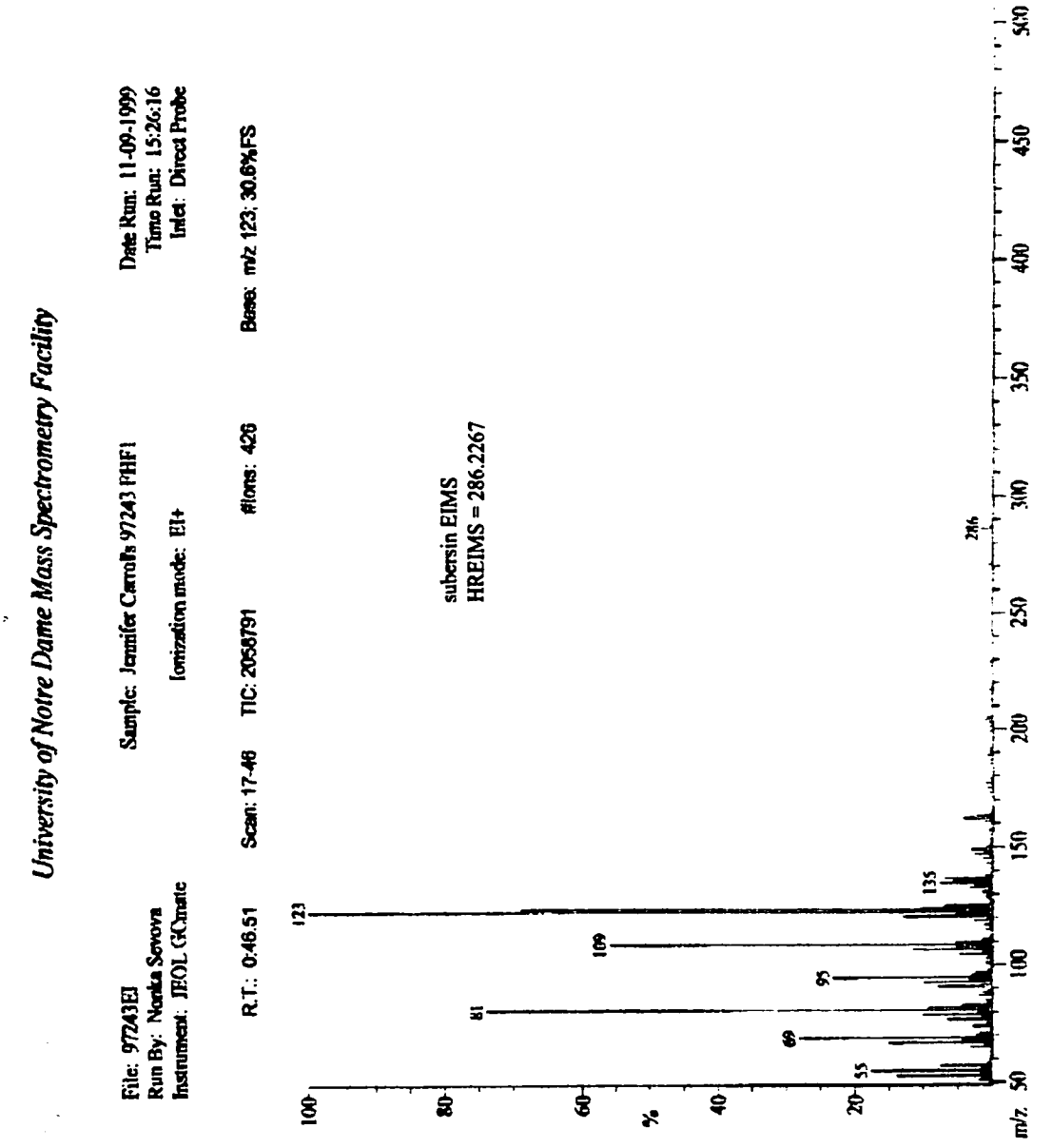


**Figure 2** DEPT NMR spectrum for (+)-subersin (**12**) (125.7 MHz, $CDCl_3$).

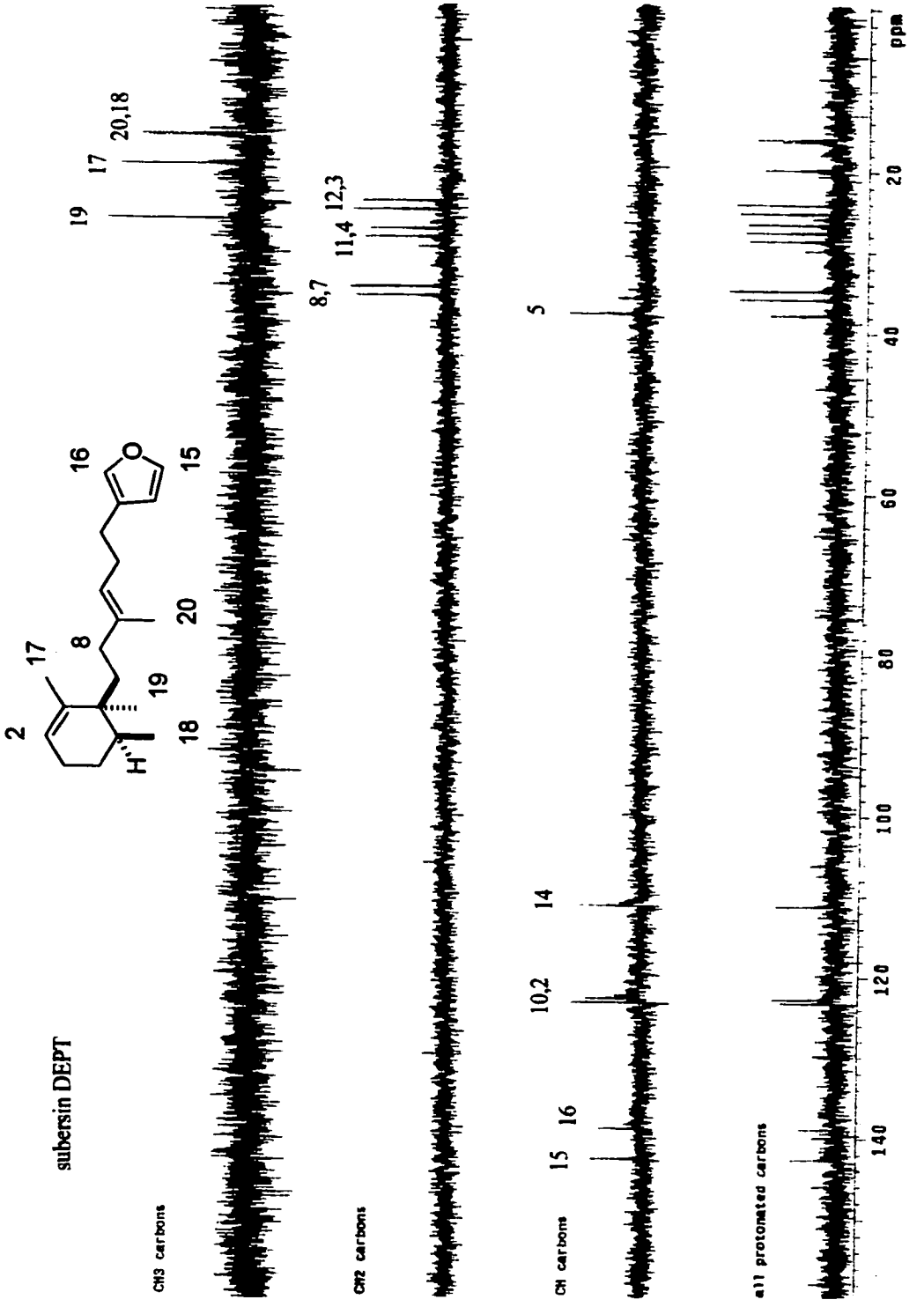


**Figure 3** Proton NMR spectrum of (+)-subersin (**12**) (500MHz, $CDCl_3$).

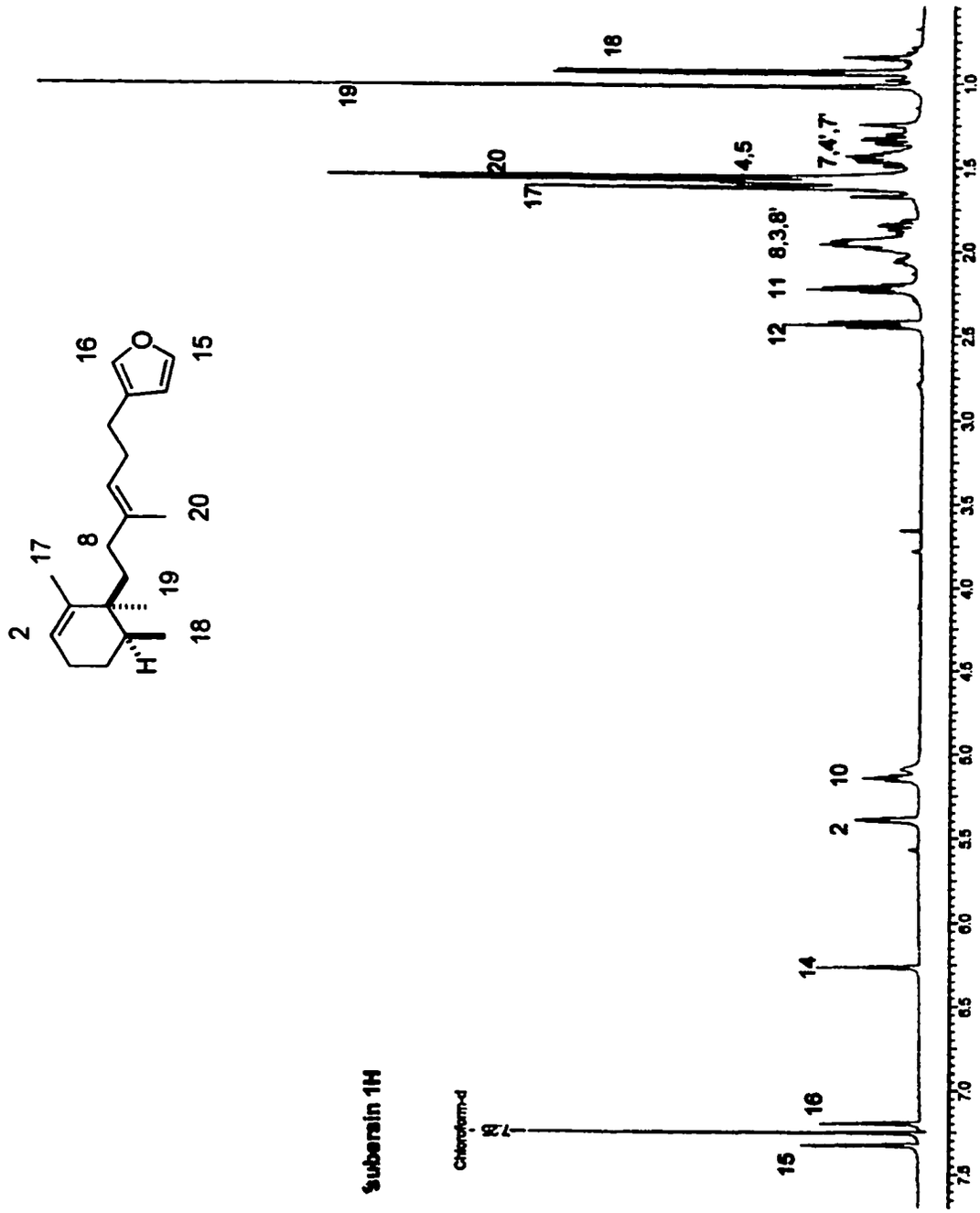


**Figure 4** Carbon NMR spectrum of (+)-subersin (**12**) (125.7 MHz, $CDCl_3$).

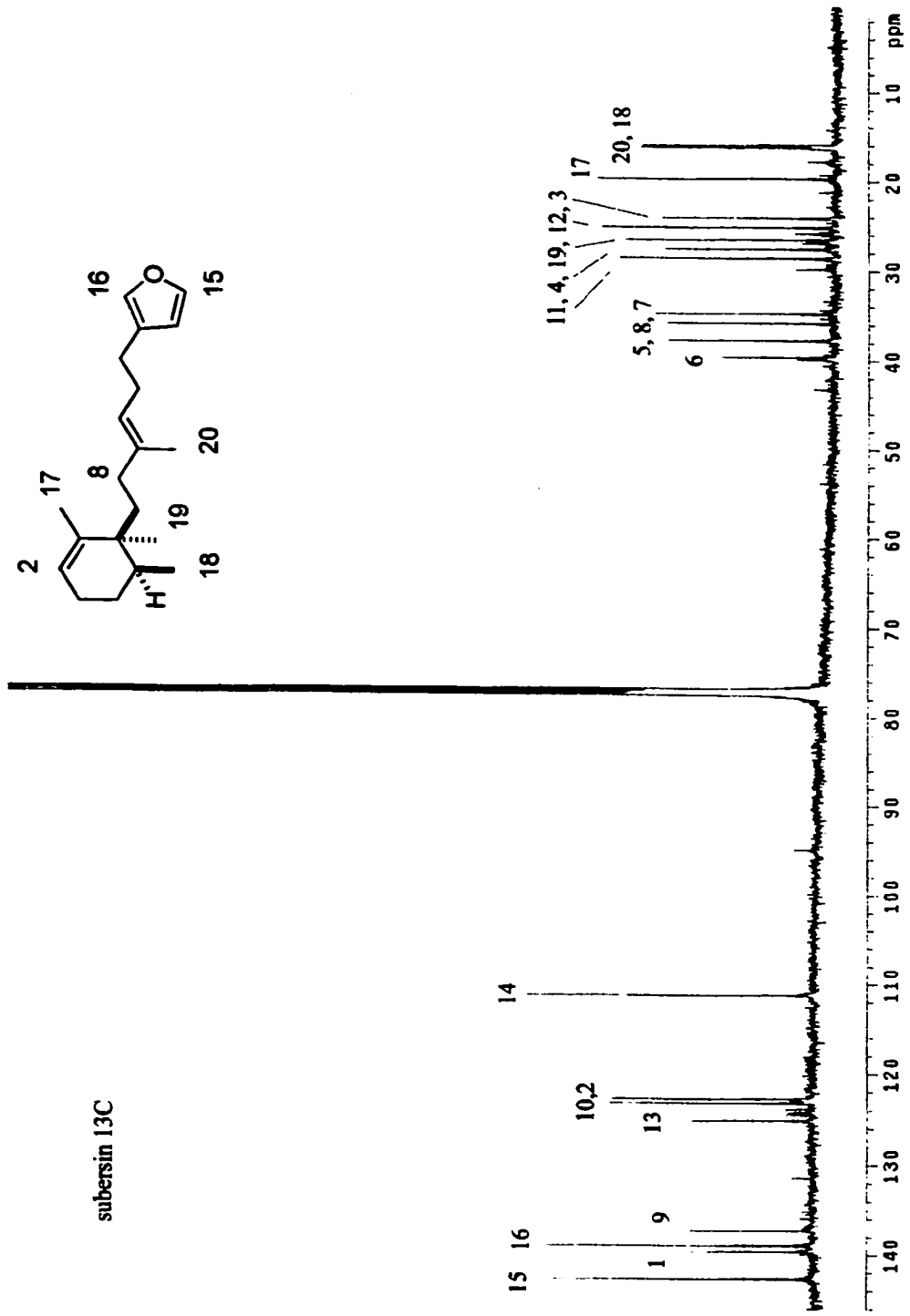
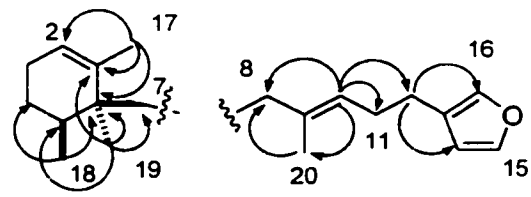


**Figure 5**. Substructures and selected gHMBC correlations for (+)-subersin (**12**). gHMBC correlation not shown is H7 to C8;




**1a** **1b**

Next, the substructures **1a** and **1b**, (Figure 5), were assembled. Following the carbon to hydrogen $^{1}J$ gHSQC experiment (Figures 6 and 7), additional important data came from a set of gCOSY correlations (Figures 8, 9 and 10), which revealed the spin systems H2-H3-H4-H5-$H_3$18, H10-H11-H12, H14-H15. A series of Me-based gHMBC correlations (Figures 11, 12, 13, 14 and 15) could be seen from $H_3$17 (δ 1.63) to C1, C2, and C6; $H_3$18 (δ 0.94) to C4, C5, and C6; $H_3$19 (δ 1.03) to C1, C5, C6, and C7; $H_3$20 (δ 1.59) to C8; and the other gHMBC correlations shown in Figure 5. The two substructures could be interconnected using the gHMBC correlation from H7' (δ1.34) to C8 (Figure 14). Next, the relative stereochemistry was determined through 1D-nOe correlations (Figure 16) from $H_3$19 (δ 1.03) to H5 and from $H_3$18 (δ 0.94) to H7, indicating that methyls 18 and 19 are on either side of the ring. As expected, the $^{13}$C NMR shifts of *trans* methyls 18 and 19 agree with those of a sponge-derived sesterterpene containing substructure **1a**.[18]

**Figure 6** gHSQC spectrum of (+)-subersin (**12**) (500 MHz, $CDCl_3$).

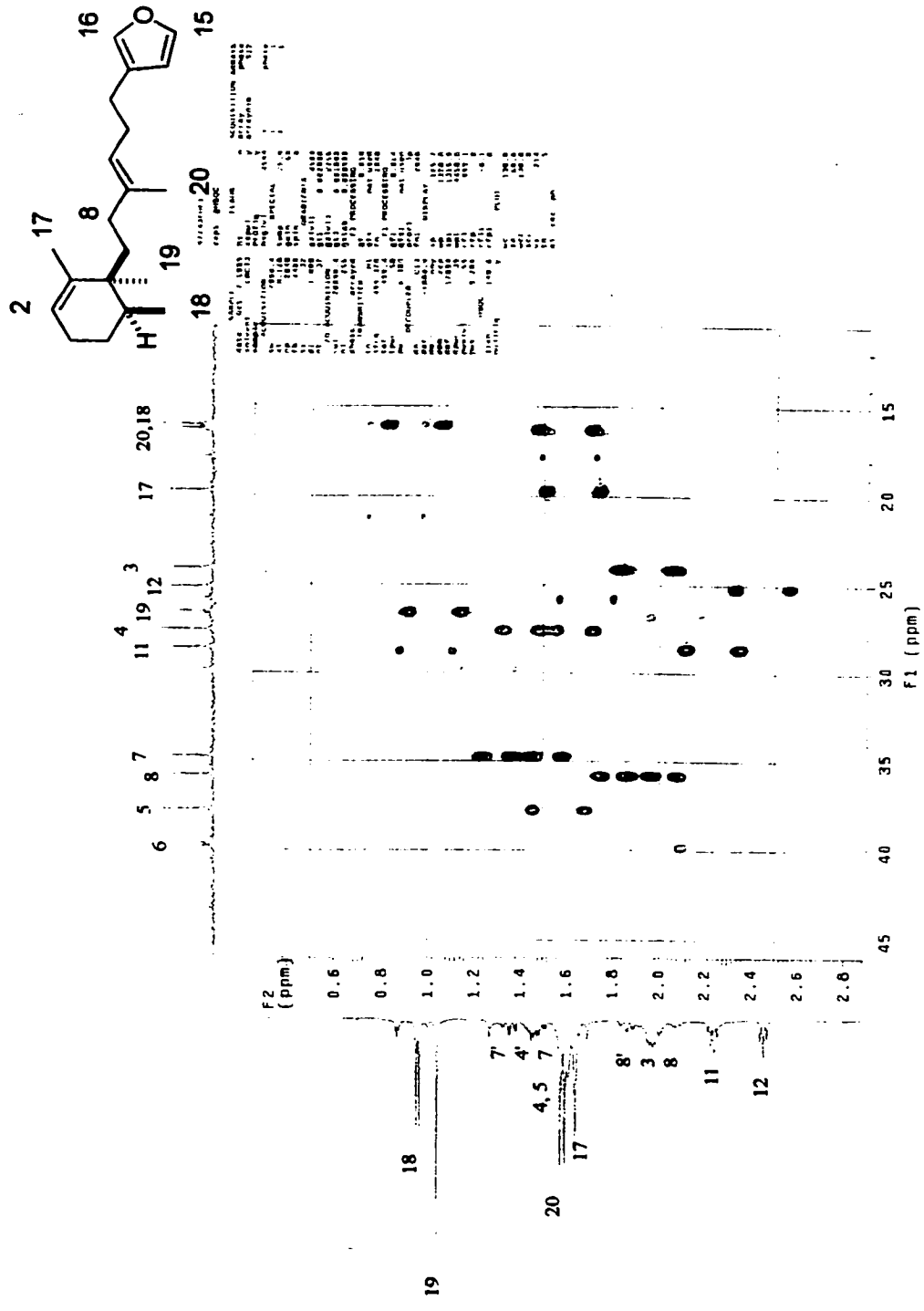


**Figure 7** gHSQC spectrum of (+)-subersin (**12**) (500 MHz, $CDCl_3$).

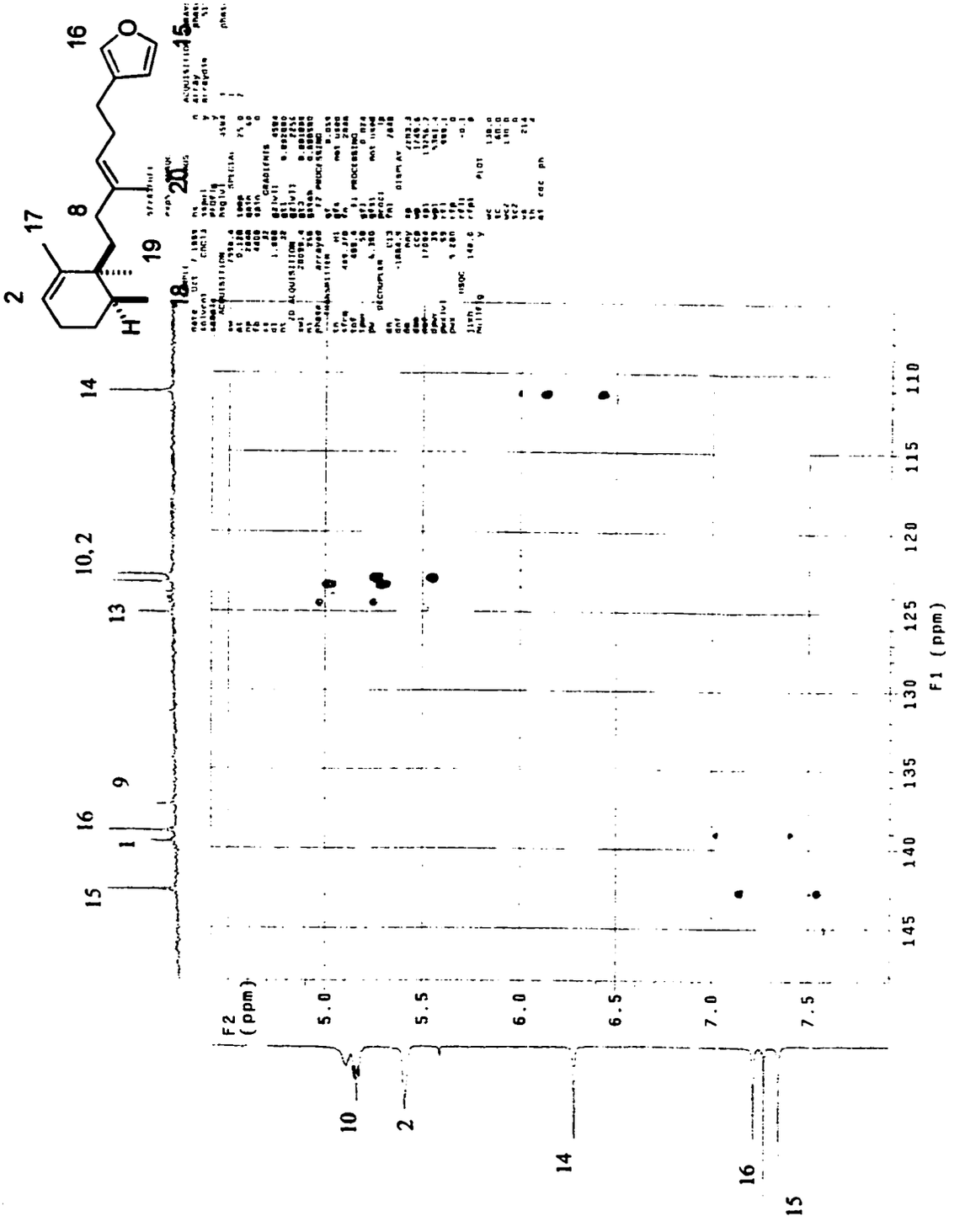


**Figure 8** gCOSY spectrum of (+)-subersin (**12**) (500 MHz, $CDCl_3$).

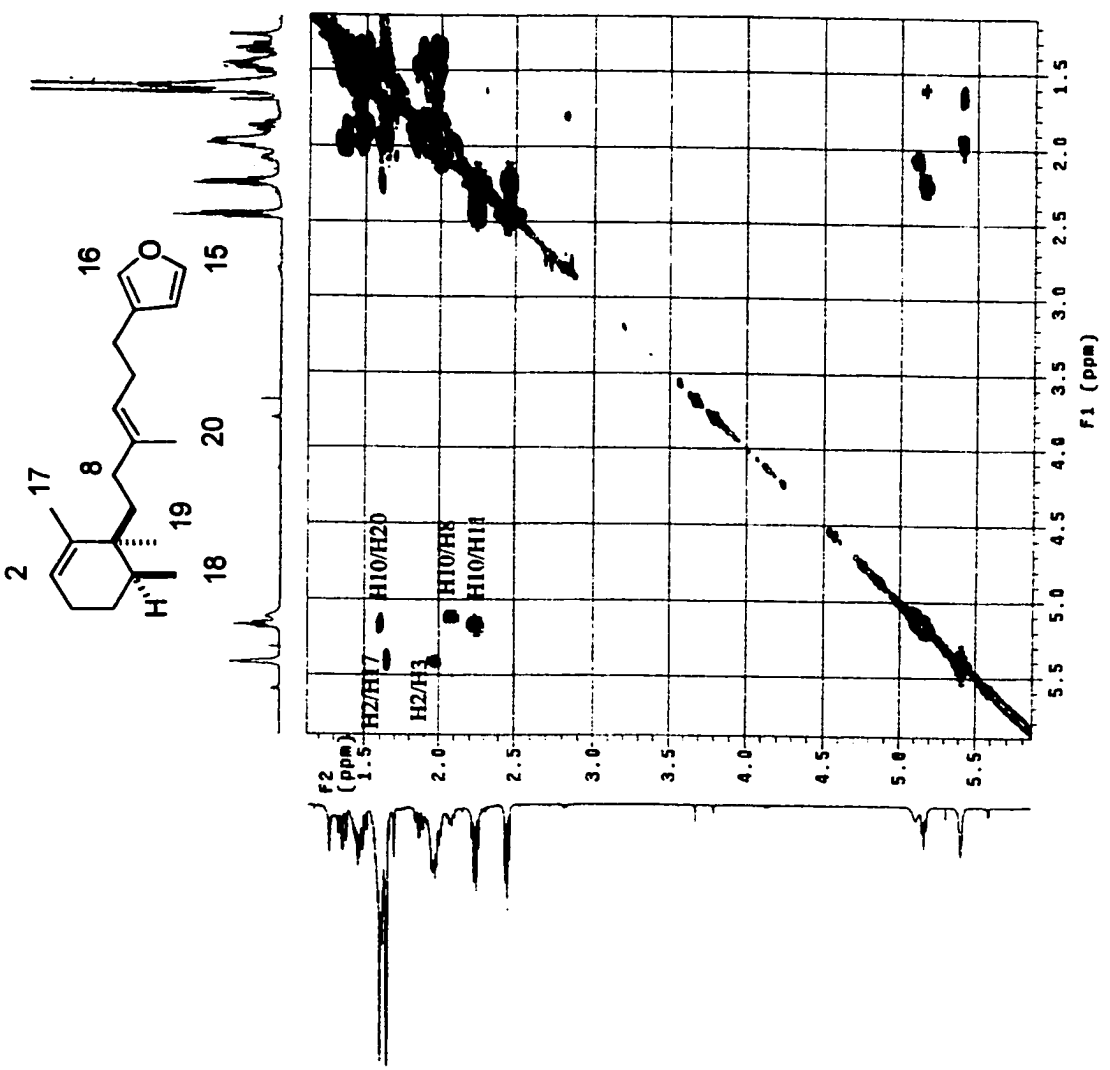


**Figure 9** gCOSY spectrum of (+)-subersin (**12**) (500 MHz, $CDCl_3$).

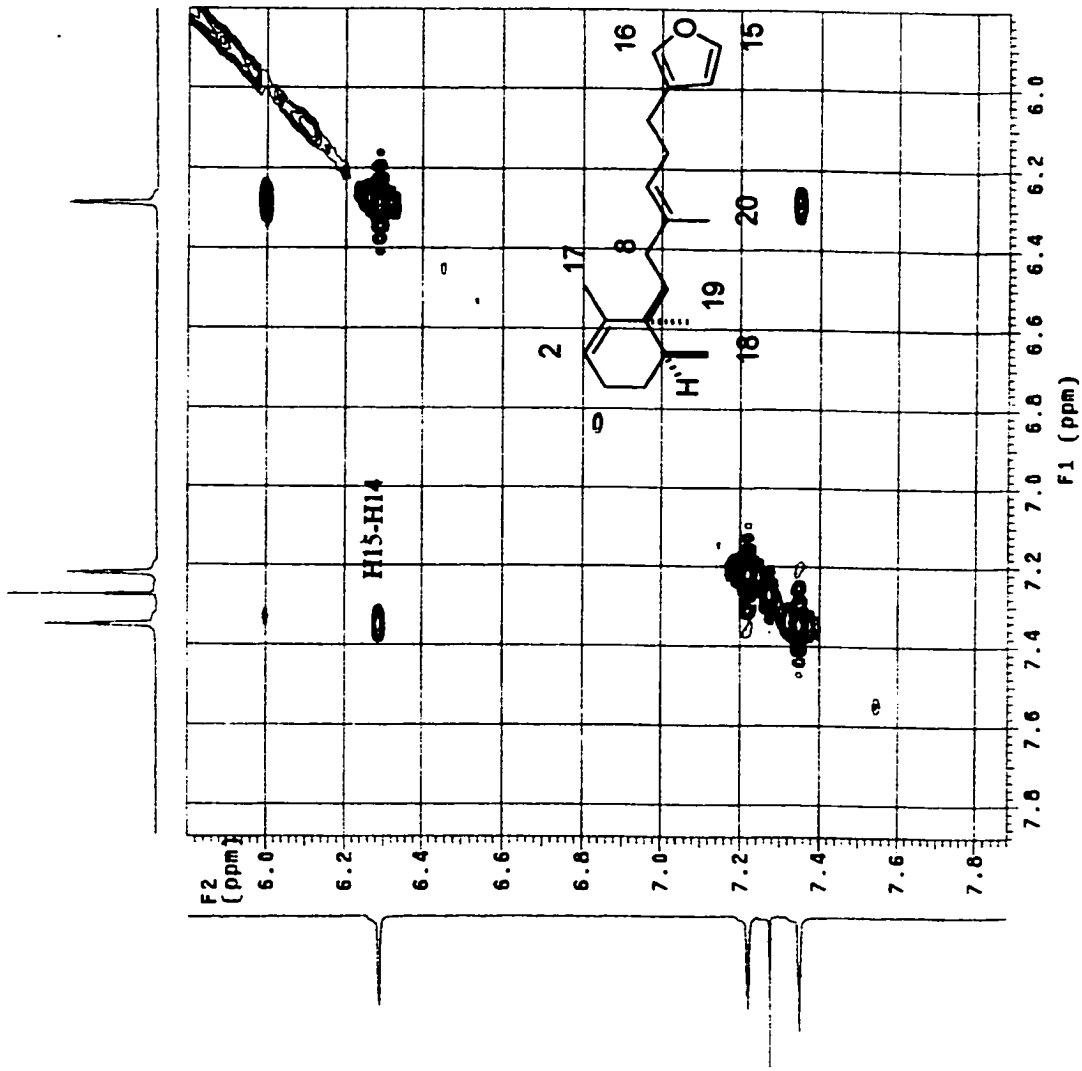


**Figure 10** gCOSY spectrum of (+)-subersin (**12**) (500 MHz, $CDCl_3$).

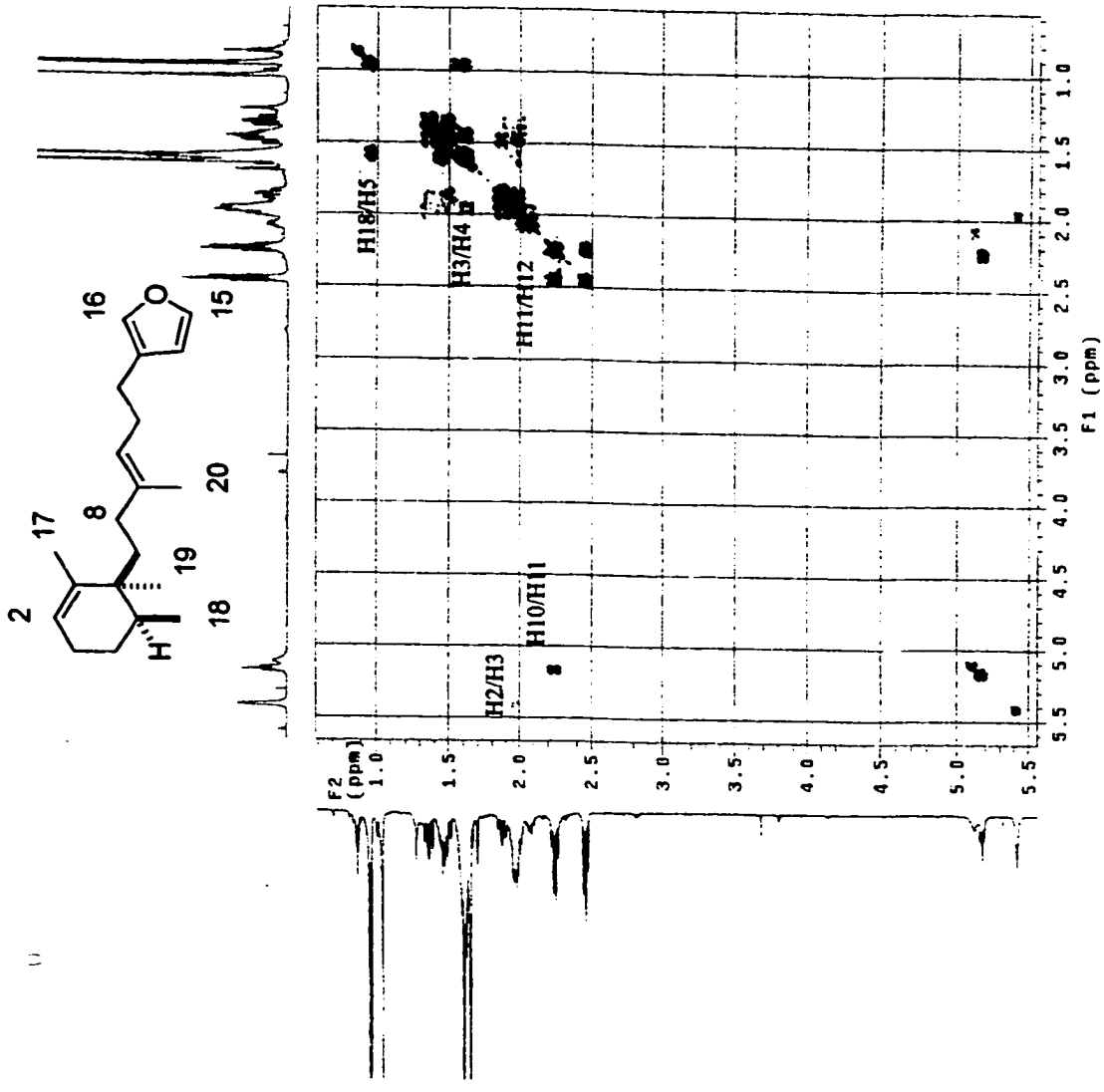


**Figure 11** gHMBC spectrum of (+)-subersin (**12**) (500 MHz, $CDCl_3$).

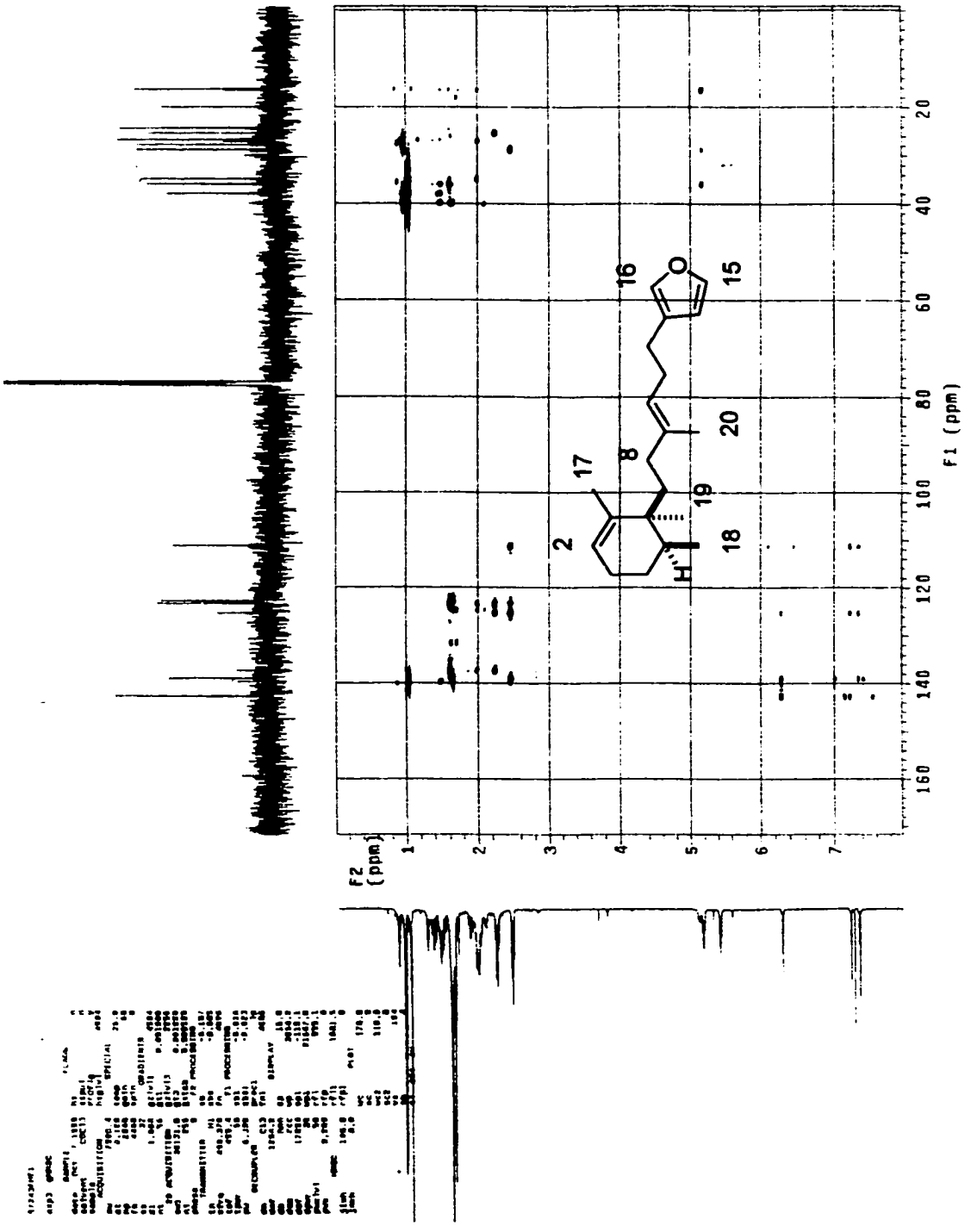


**Figure 12** gHMBC spectrum of (+)-subersin (**12**) (500 MHz, $CDCl_3$).

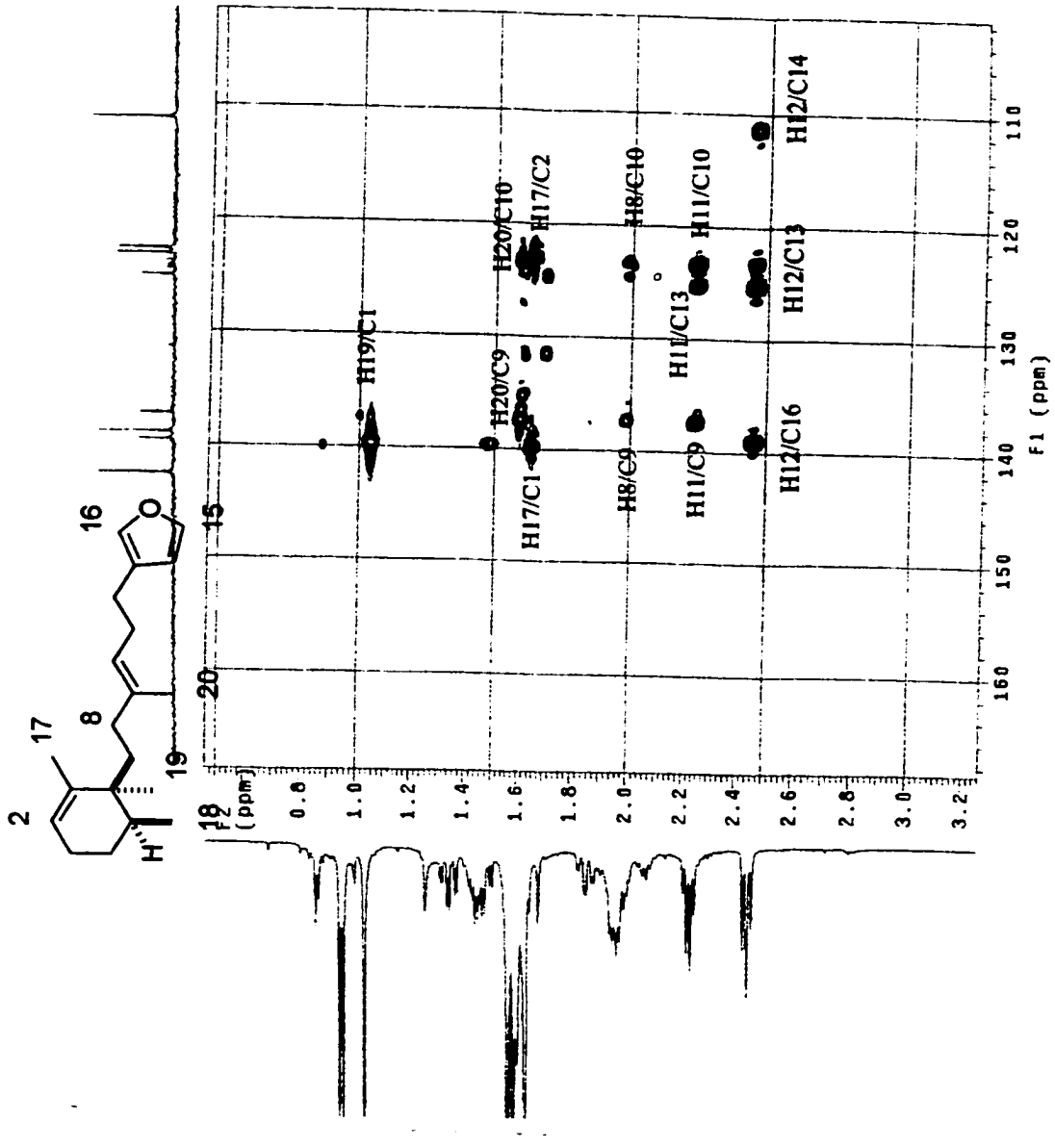


**Figure 13** gHMBC spectrum of (+)-subersin (**12**) (500 MHz, $CDCl_3$).

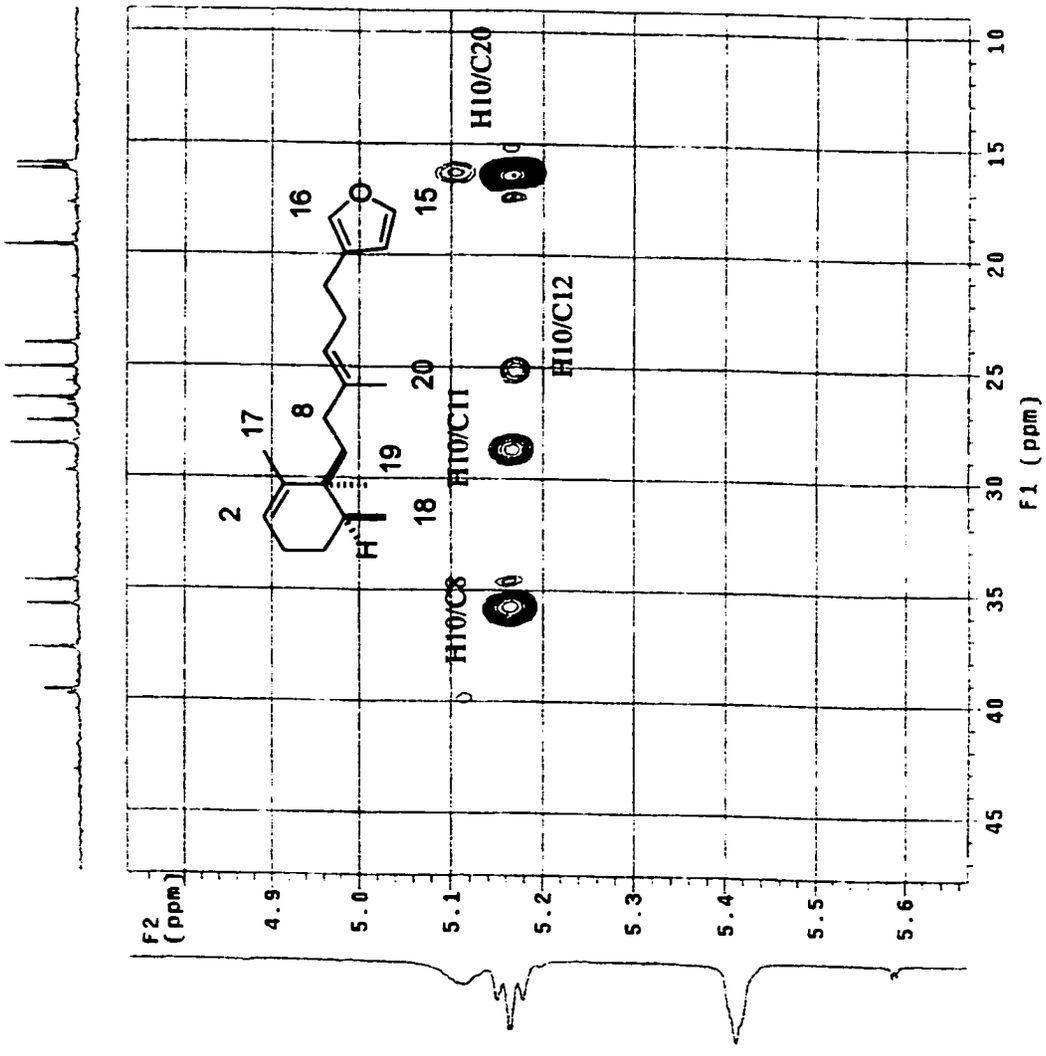


**Figure 14** gHMBC spectrum of (+)-subersin (**12**) (500 MHz, $CDCl_3$).

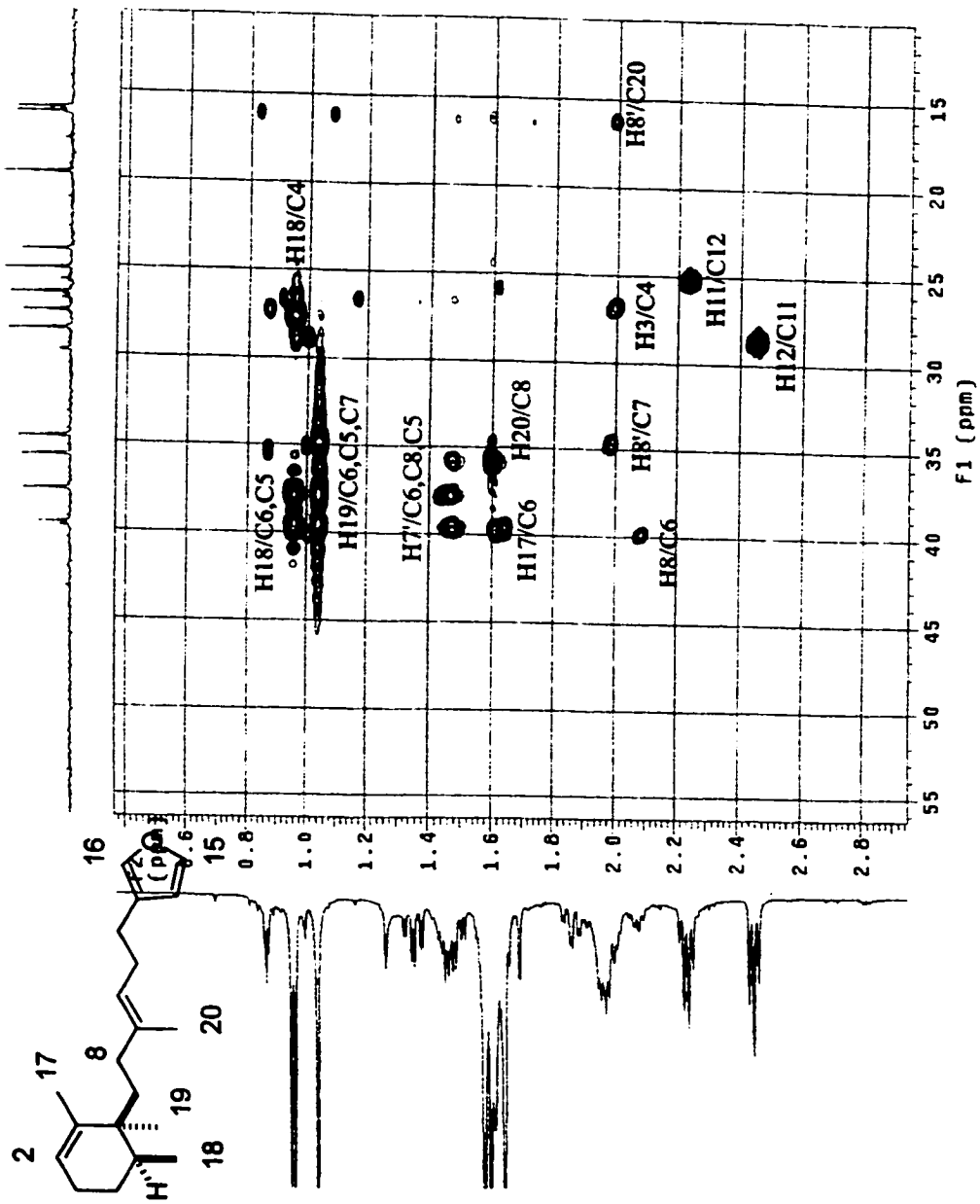


**Figure 15** gHMBC spectrum of (+)-subersin (**12**) (500 MHz, $CDCl_3$).

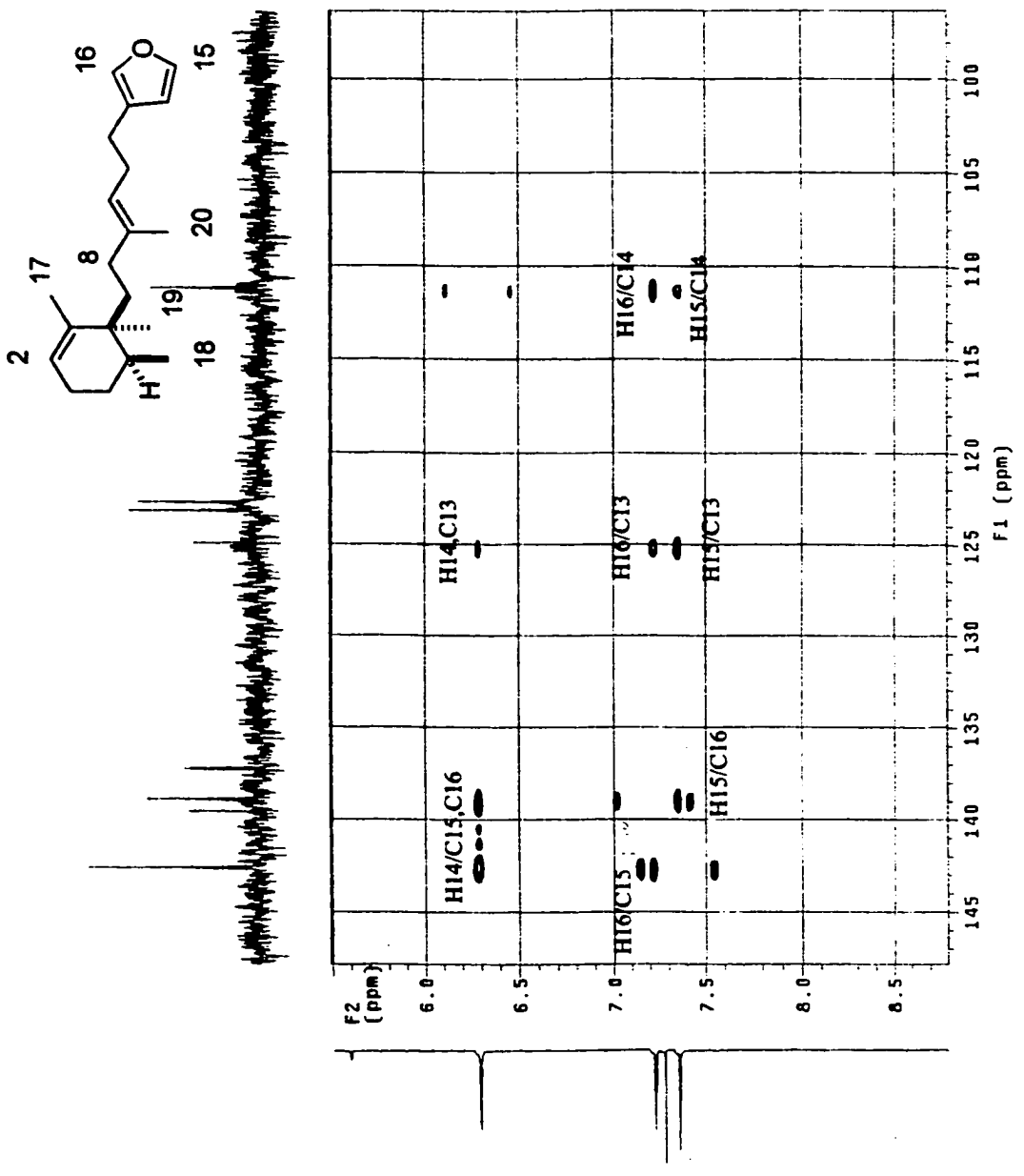
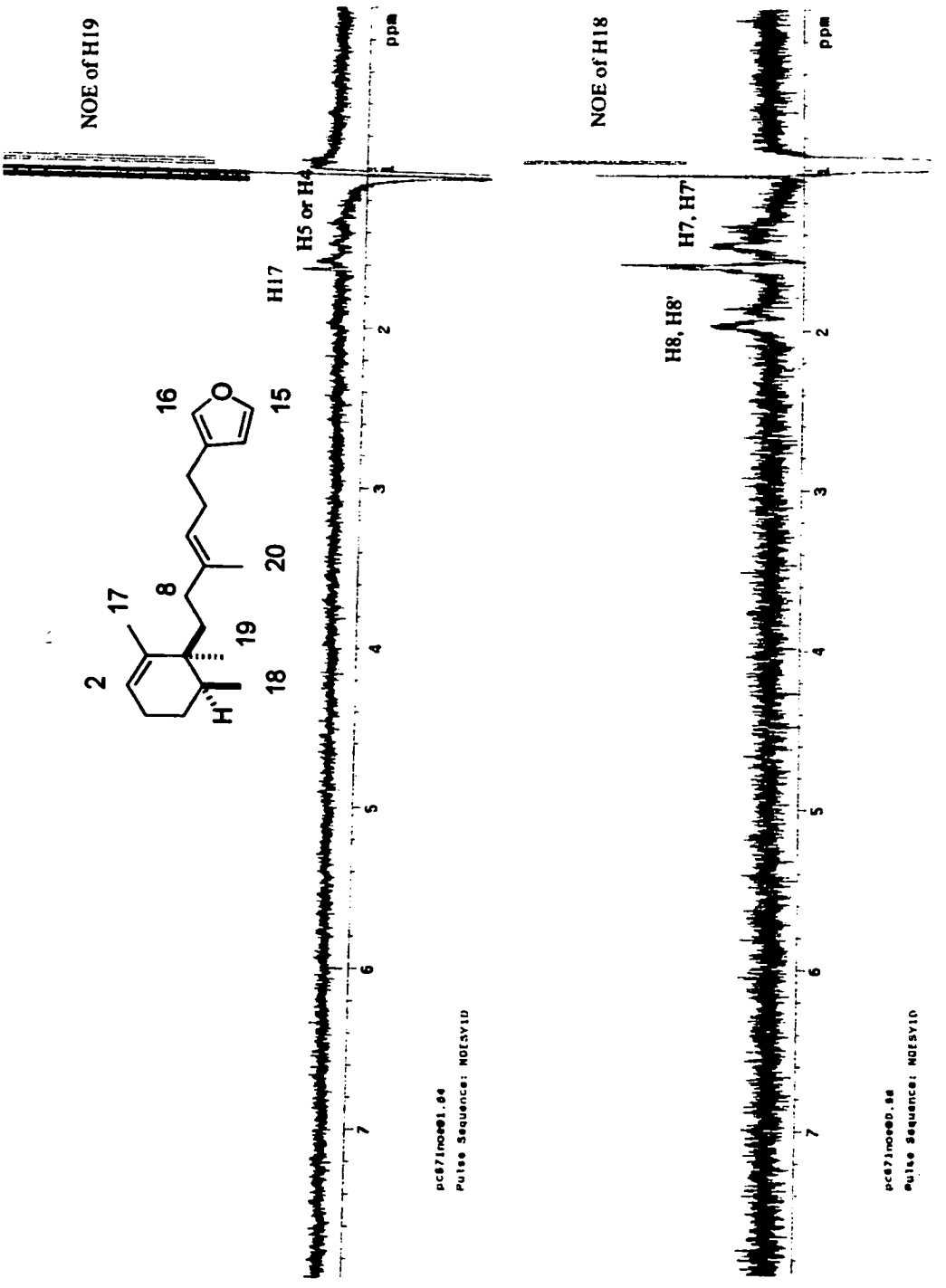
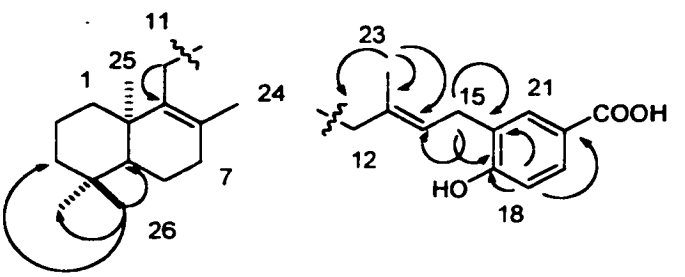


**Figure 16** 1D difference-nOe spectrum of (+)-subersin (**12**) (500 MHz, $CDCl_3$).



Attention was shifted next to the structure elucidation of (-)-subersic acid (**13**), which had a molecular formula $C_{27}H_{38}O_3$. The HRTOFMS data (Figure 17) were identical to that of (-)-jaspic acid (**11**),[13] $[M-H]^-$ *m/z* = 409.2743 (Δ 0.0 mmu of calcd.); whereas the $^1H$ and $^{13}C$ NMR data (Figures 18 and 19) were dissimilar in their vinyl methyl count, showing that **13** and **11** were structural isomers. The DEPT-135 $^{13}C$ NMR data (Figure 20) gave $5CH_3 + 7CH_2 + 7CH + 8C$, totaling $C_{27}H_{36}$, making it evident that two OH residues were present, with one as an aromatic ring OH (159.8, s), and the other as part of a carboxylic acid (δ 171.7, s).




**2a** **2b**

Striking similarities in the $^{13}C$ NMR data between (-)-subersic acid (**13**) and (-)-jaspic acid (**11**) allowed the two substructures **2a** and **2b** in Figure 6 to be assembled. For example, the 4-hydroxybenzoic acid moiety of **2b** had identical shifts to those of **11** between C15 through C22 which were verified by gHSQC (Figures 21

assembled. For example, the 4-hydroxybenzoic acid moiety of **2b** had identical shifts to those of **11** between C15 through C22 which were verified by gHSQC (Figures 21 and 22) and gHMBC data (Figure 5 and Figure 23) to C16 and C20; H15 (δ 3.42) to C13, C14, C16, C17 and C21; H21 &/or H19 (δ 7.91) to C17, C16, and C15; and H23 (δ 1.83) to C12, C13, C14. The $^{13}C$ NMR of the bicyclic C1-C11 portion of **2a** was identical to that of published data for this well-known ring system.[19] The side chain attachment was identified from the gHMBC correlations H11 (δ 2.14) to C8, C9, and C12, with the latter correlation to C12 supporting the proposed interconnection of substructures **2a** and **2b**. The *E* geometry of position $\Delta^{13/14}$ could be deduced from the upfield $^{13}C$ NMR shift of methyl C23 (δ 16.7).

**Figure 17** High Resolution mass spectral data of (-)-subersic acid (**13**)



MAY. 17. 2000 3:08PM CHEMISTRY&BIOCHEM NO. 2009 Page: 1

[ Elemental Composition ]
Data : J.553nhr-001 Date : 16-May-2000 14:07
Sample: 97243FDF1F4H4
Note : - in PBG standard
Inlet : Reserv. Ion Mode : FAB-
RT : 0.47 min Scan#: (1,8)
Elements : C 65/0, H 90/0, O 15/0, N 8/0, S 3/0
Mass Tolerance : 1000ppm, 3mmu if m/z < 3, 5mmu if m/z > 5
Unsaturation (U.S.) : -0.5 - 20.0

| Observed m/z | Int% | Err[ppm / mmu] | U.S. | Composition |
|---|---|---|---|---|
| 409.2751 | 100.0 | +1.9 / +0.8 | 9.5 | C 27 H 37 O 3 |
| | | -4.6 / -1.9 | 14.0 | C 30 H 35 N |
| | | +11.7 / +4.8 | 5.5 | C 22 H 37 O 5 N 2 |
| | | +5.2 / +2.1 | 10.0 | C 25 H 35 O 2 N 3 |
| | | -9.2 / -3.7 | 1.0 | C 18 H 39 O 7 N 3 |
| | | +8.5 / +3.5 | 10.5 | C 23 H 33 O N 6 |
| | | -5.9 / -2.4 | 1.5 | C 16 H 37 O 6 N 6 |
| | | -6.3 / -2.6 | 5.5 | C 24 H 41 O 3 S |
| | | +3.5 / +1.4 | 1.5 | C 19 H 41 O 5 N 2 S |
| | | -3.0 / -1.2 | 6.0 | C 22 H 39 O 2 N 3 S |
| | | +6.8 / +2.8 | 2.0 | C 17 H 39 O 4 N 5 S |
| | | +0.2 / +0.1 | 6.5 | C 20 H 37 O N 6 S |
| | | +10.1 / +4.1 | 2.5 | C 15 H 37 O 3 N S |
| | | +9.6 / +3.9 | 6.5 | C 23 H 41 N 2 S 2 |
| | | -11.3 / -4.6 | 2.0 | C 19 H 43 O 2 N 3 S 2 |
| | | -8.0 / -3.3 | 2.5 | C 17 H 41 O N 6 S 2 |
| | | +1.4 / +0.6 | 2.5 | C 20 H 45 N 2 S 3 |

**Figure 18** Proton NMR spectrum of (-)-subersic acid (**13**) (500 MHz, $CDCl_3$).

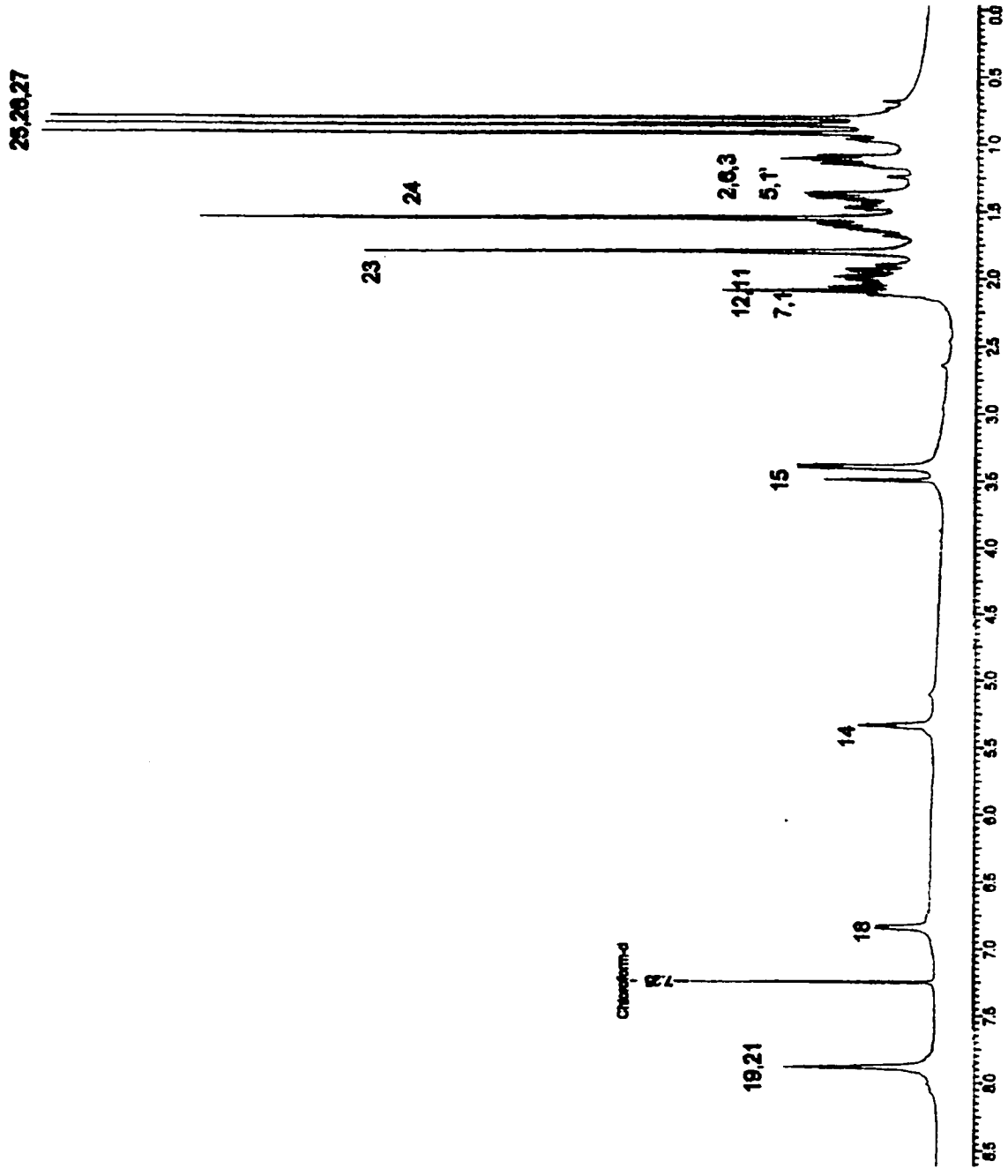


**Figure 19** Carbon NMR spectrum of (-)-subersic acid (**13**) (125.7 MHz, $CDCl_3$)

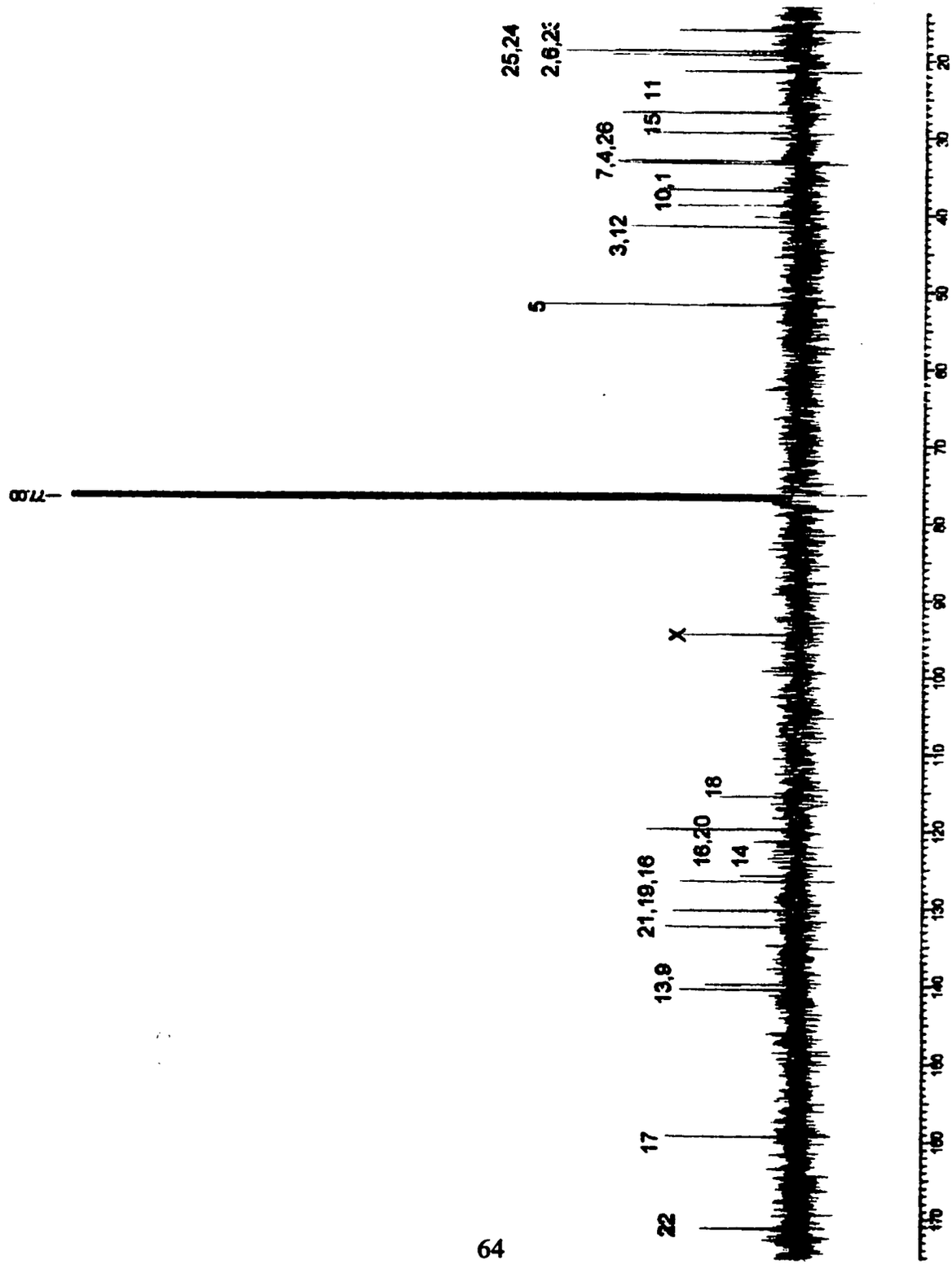


**Figure 20** DEPT NMR spectrum of (-)-subersic acid (**13**) (125.7 MHz, $CDCl_3$)

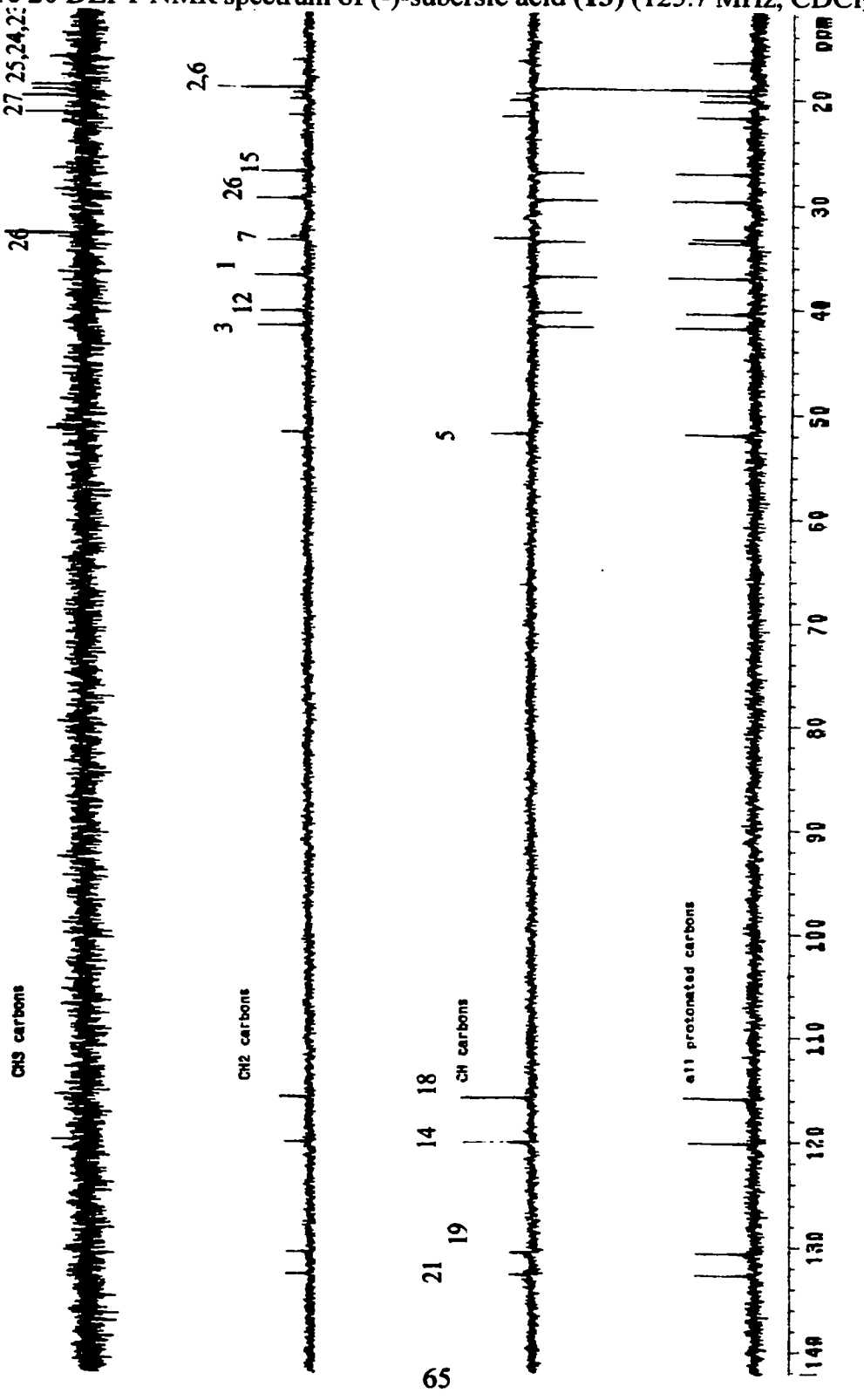


**Figure 21** gHSQC NMR spectrum of (-)-subersic acid (**13**) (500 MHz, $CDCl_3$).

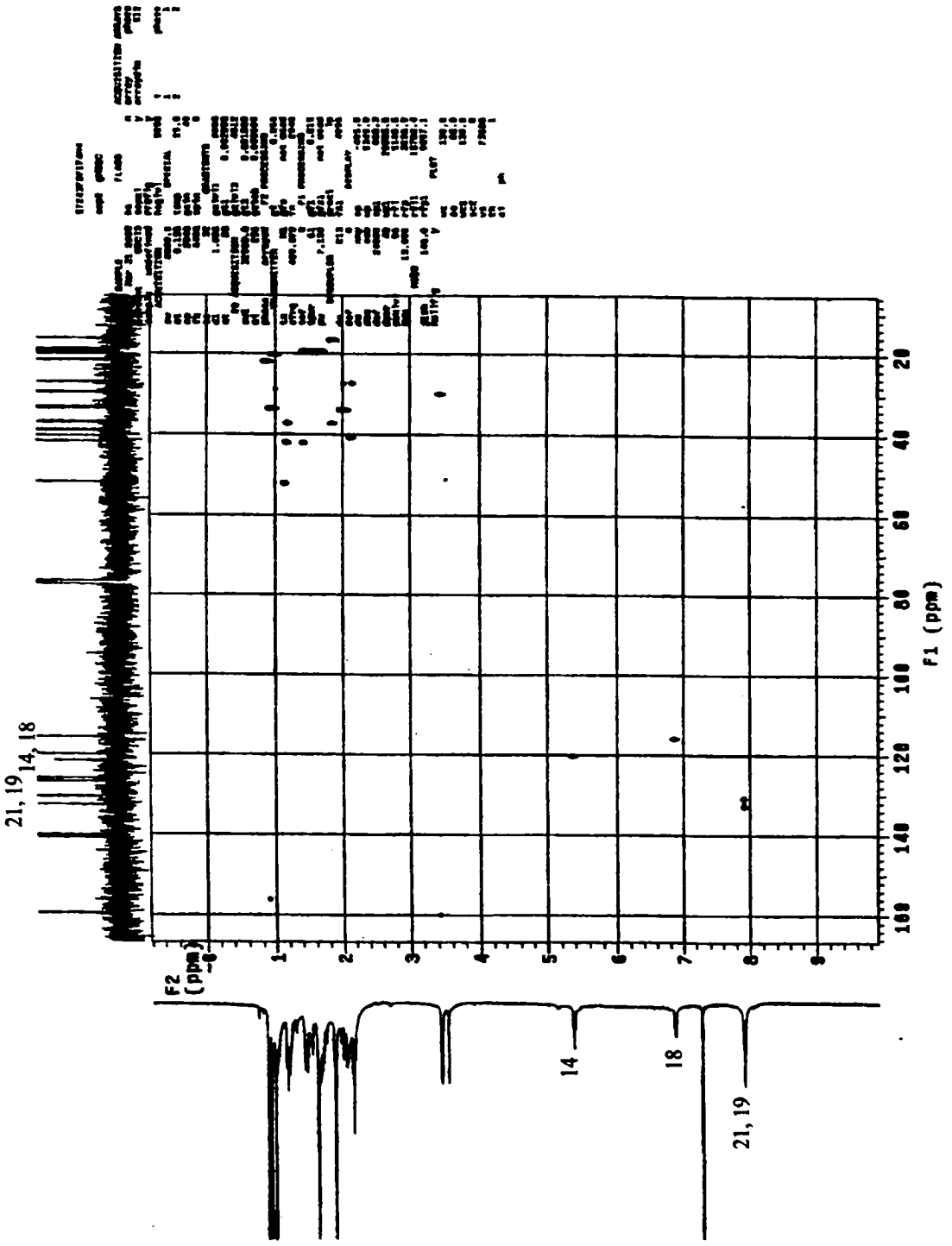


**Figure 22** gHSQC NMR spectrum of (-)-subersic acid (**13**) (500 MHz, $CDCl_3$).

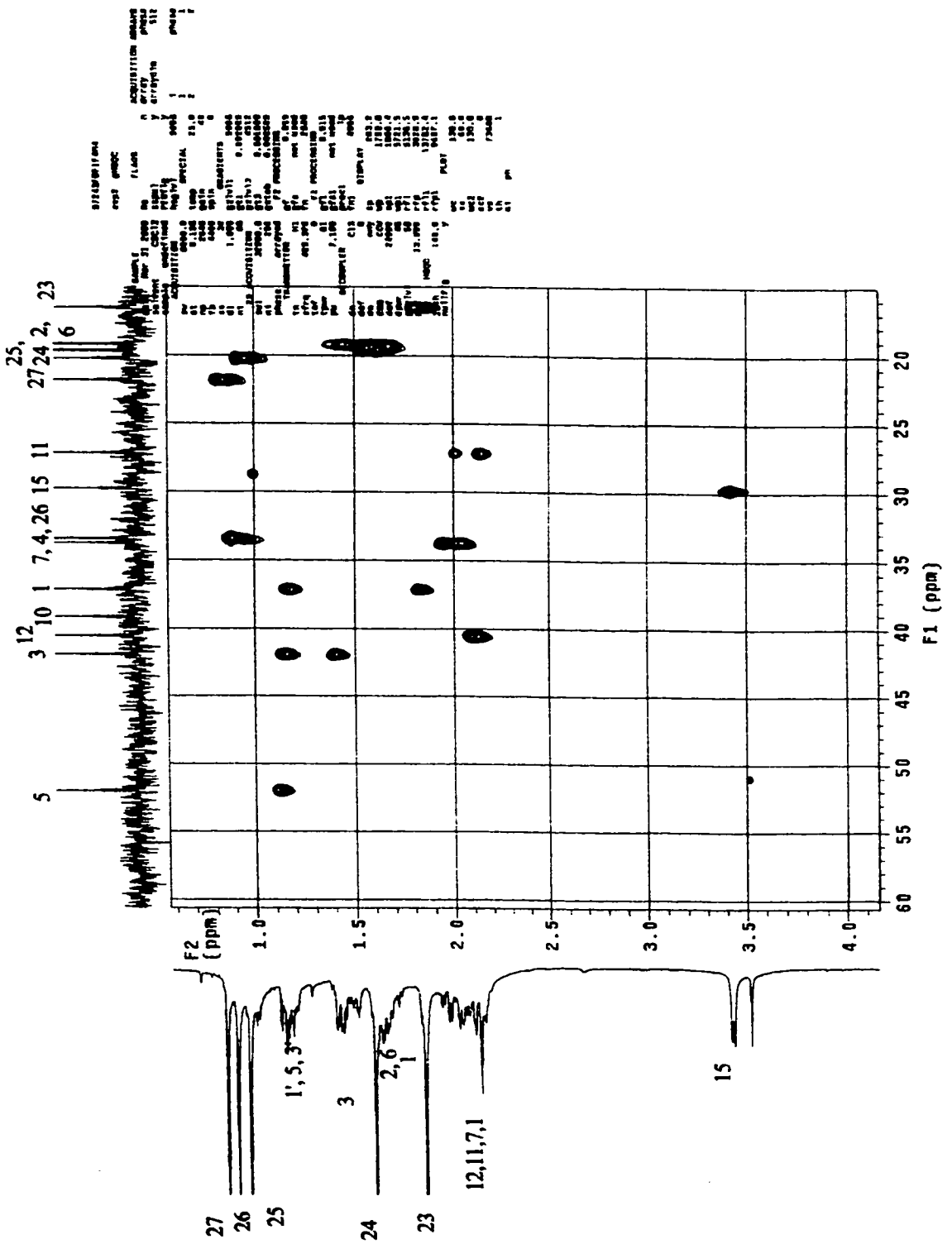
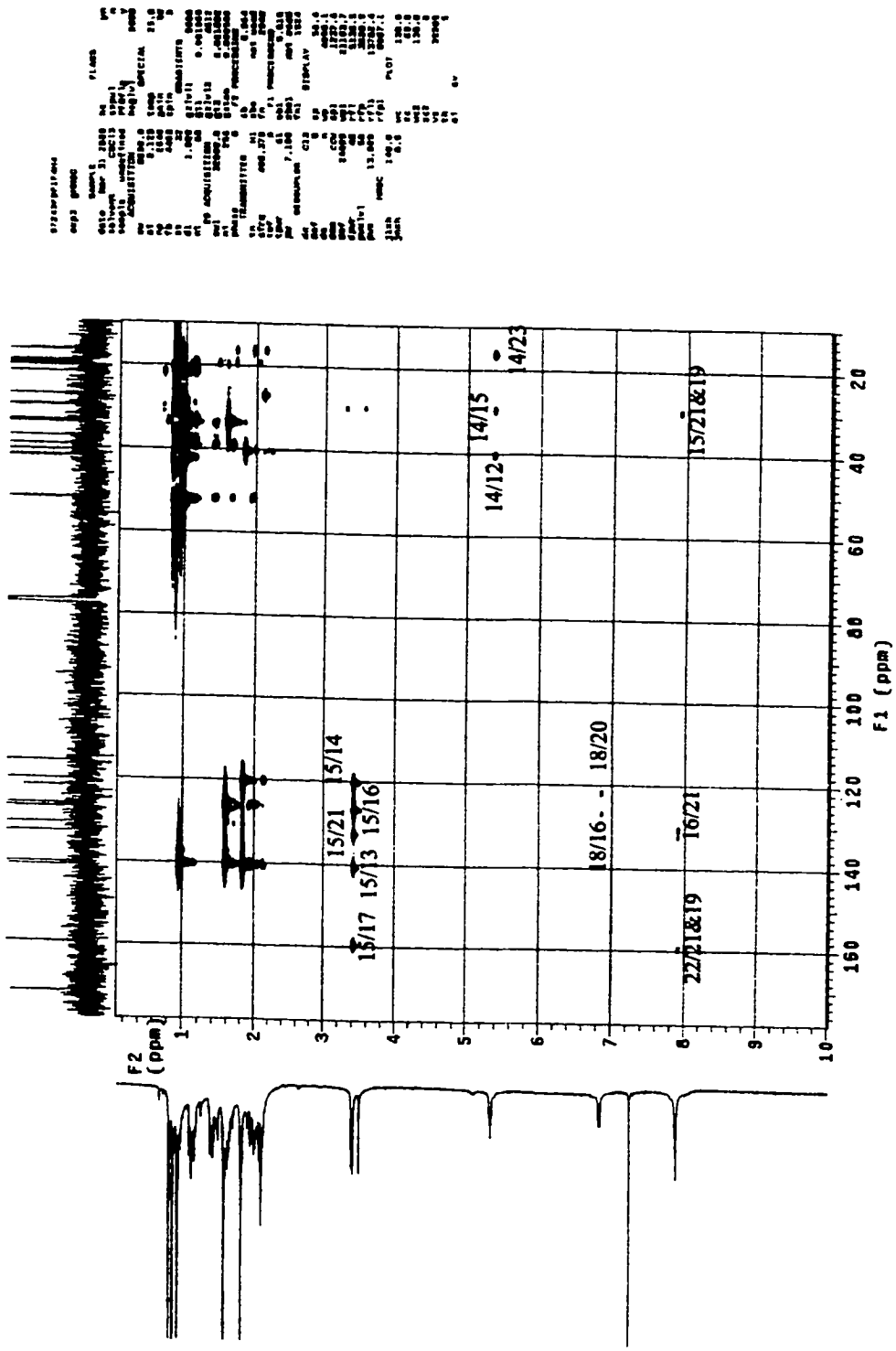
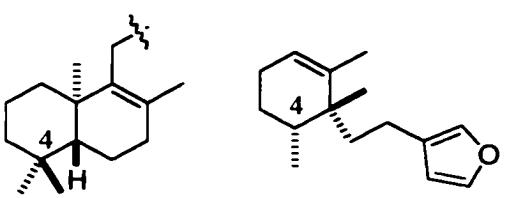


**Figure 23** gHMBC NMR spectrum of (-)-subersic acid (**13**) (500 MHz, $CDCl_3$).



The final point to be established concerned the absolute stereochemistry of the ring substituents in **12** and **13**. This assignment was addressed for **12** by a detailed comparison of its molecular rotation to that of the same ring system, previously determined by Capon.[16] The measured molar rotation ([Φ] = -189) of (-)-subersic acid is comparable with the [Φ] = -191 reported by Capon for substructure (**14**), shown in Figure 24. This analysis then indicates 5*R*, 10*R* stereochemistry for **13**. Using the same approach, the 4*R*, 5*R* absolute stereochemistry known for (-)-microcionin-2 (**15**, Figure 24), assigned by total synthesis, [20] could be used to delineate 5*S*, 6*S* stereochemistry for **12**, given that the molar rotation for **12** ([Φ] = +10), is similar in magnitude but of opposite sign, to that of **11** ([Φ] = -27).[21]

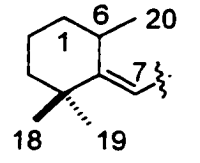
**Figure 24** Compounds used for stereochemical comparison to **12** and **13**.



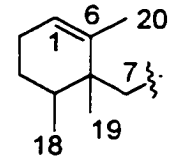

substructure **14** (-)microcionin-2 **15**

It was recently brought to our attention that the exact 2-dimensional structure of **12** has just been seen in the literature as cacospongin A.[22]Although this structure was originally published in 2000 with the substructure **3a**, a review of DQF-COSY and gHMBC data led to the revised structure, containing ring system **3b**, which is

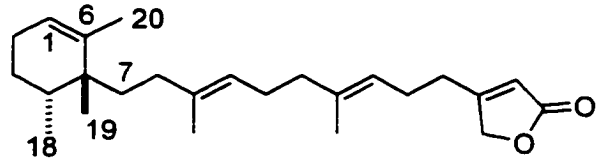
identical in proton and carbon NMR data to that of subersin. This similarity in NMR data indicates that the methyl substituents on subersin and cacospongin A have the same relative conformation, that is, both methyl 18 and 19 are on opposite sides of cyclohexene ring system. To validate this conclusion we compared the $^{13}C$ NMR data of previously published compounds containing this substructure in Table 3. Compound **16**, work by Carotenuto, in which methyls 18 and 19 are trans, fits best with the data from both subersin and cacospongin A. As mentioned previously, the optical rotation for (+)-subersin **12** is $[\alpha]_D$ +30° (*c* 2.6, $CHCl_3$), whereas for (-)-cacospongin A the published optical rotation is $[\alpha]_D$ -14° (*c* 0.16, $CHCl_3$). The $^{13}C$ NMR shifts, in combination with the optical rotation data indicates that these two might be enantiomers, although, enantiomeric compounds from the same species is rarely seen. Ideally the rotation of both molecules should be measured on the same instrument to authenticate their values. Contact is being made with the authors of the cacospongin A publication to exchange material for this purpose.



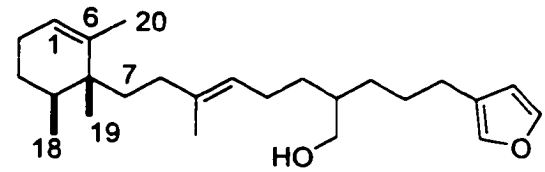

3a




3b




16




17

**Table 3** $^{13}C$ NMR literature comparisons of cyclohexene substructures.

| Carbon no. | **16**[23] | **17**[24] | (+)-subersin | (-)-cacospongin A |
|---|---|---|---|---|
| 1 | 122.7 | 122.4 | 122.1 | 122.6 |
| 2 | 23.9 | 25.5 | 24.1 | 23.9 |
| 3 | 27.4 | 27.0 | 27.5 | 27.4 |
| 4 | 37.6 | 33.1 | 37.8 | 37.6 |
| 5 | 39.5 | 40.3 | 35.8 | 39.5 |
| 6 | 139.4 | 139.5 | 139.6 | 139.5 |
| 7 | 35.7 | 34.1 | 34.1 | 34.6 |
| 18 | 15.9 | 15.8 | 16.0 | 15.9 |
| 19 | 26.4 | 21.0 | 26.6 | 26.4 |
| 20 | 19.6 | 19.7 | 19.7 | 19.5 |

An unexpected outcome represented in the lipoxygenase assay deserves additional comment. The two terpene-benzenoids, jaspaquinol (**10**) and (-)-jaspic acid (**11**), reisolated here were first reported from *Jaspis splendens* (Order: Choristida, Family: Jaspidae). This sponge (collection no. 96117), was chemically distinct from some 18 others of this same species in our repository, though all such extracts

examined contained (+)-jasplakinolide (**18**). In order to further test the efficacy of our assay, we screened five *J. splendens* sponge extracts, including the unique one containing **10** and **11**. In Table 4, three of the five *J. splendens* extracts tested had insignificant inhibition. The most active extract 96117 ($IC_{50}$ = 0.4μg/mL) was the original source of **10** and **11**. Also, as shown in Table 4, the pure compound, (+)-jasplakinolide (**18**) was inactive. Another potent extract was 96555 ($IC_{50}$ = 6.5μg/mL), which appears to contain additional and as yet undescribed constituents. The observation of the identical pair of terpene-benzenoids **10** and **11** from sponges in two distinct orders (Choristida and Verongida, respectively) is unusual and in this case cannot be explained as a taxonomic anomaly.[25]

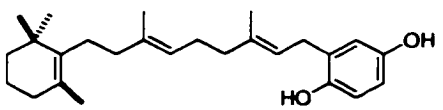
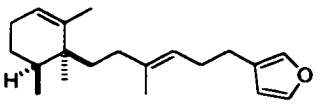
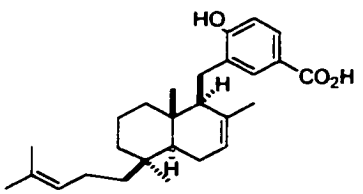
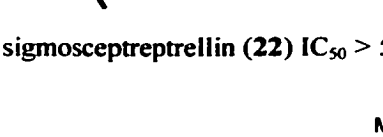
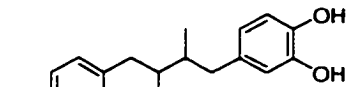
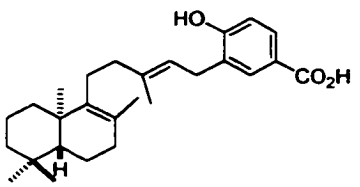
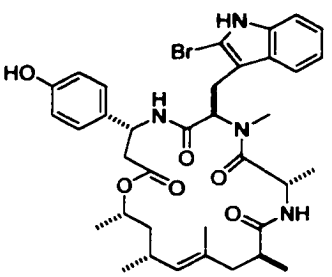
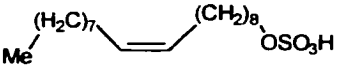
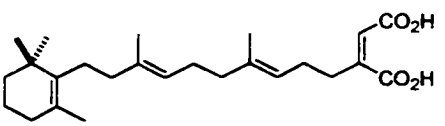
**Table 4** 15-HLO Inhibition Activity of Crude Sponge Extracts.

| *Sponges* | *Coll No.* | *Site* | *$IC_{50}$ (μg/mL)* |
|---|---|---|---|
| *Suberea* sp. | 97243 | Papua New Guinea | 0.1 ± 0.02 |
| *J. splendens* | 94541 | Indonesia | 150 ± 15 |
| " | 95077 | Papua New Guinea | 45 ± 5 |
| " | 96117 | Papua New Guinea | 0.4 ± 0.02 |
| " | 96555 | Indonesia | 6.5 ± 3 |
| " | 97238 | Papua New Guinea | > 300 |

The 15-HLO screen developed for this research is both a robust tool and a reliable reporter of lipoxygenase inhibition. It was important to avoid an inconsistent

behavior in a 5-HLO assay previously encountered with manoalide, a sponge derived sesterterpene.[26] DeVries *et. al.* initially reported manoalide to have an $IC_{50}$ = 0.3μM against 5-HLO (in both RBL-1 and HPMNL cell extracts),[27] while Cabré *et.al.* found it did not inhibit 5-HLO (in HPMNL cell extracts)[22] at concentrations of up to 50 μM, but both observed nordihydroguaiaretic acid (NDGA, **19**) ($IC_{50}$ = 0.3μM), a known LO inhibitor, to be fully potent.[21,28] In order to avoid this problem, the Holman laboratory utilized purified 15-HLO and obtained an $IC_{50}$ of 0.3 ± 0.1 μM for NDGA (**19**), consistent with previous results. Data against 15-HLO was also obtained for another benchmark compound, oleyl sulfate (OS, **20**), previously shown to be an allosteric inhibitor.[29] The Holman labs have observed its $K_i$ to be 0.4 ± 0.05 μM, in good agreement with the $IC_{50}$ of 0.9 μM, shown in Table 5.

**Table 5** 15-HLO Inhibition Activity (μM) of Pure Compounds.

| active natural products | inactive natural products | standards |
|---|---|---|
|  jaspaquinol (**10**) $IC_{50}$ 0.3 ± 0.1 |  (+)-subersin (**12**) $IC_{50}$ > 100 | |
|  (-)-jaspic acid (**11**) $IC_{50}$ 1.4 ± 0.2 |  sigmosceptreptrellin (**22**) $IC_{50}$ > 50 |  NDGA (**19**) $IC_{50}$ 0.3 ± 0.1 |
|  (-)-subersic acid (**13**) $IC_{50}$ 15 ± 3 |  (+)-jasplakinolide (**18**) $IC_{50}$ > 100 |  oleyl sulfate (**20**) $IC_{50}$ 0.9 ± 0.1 |
| |  dehydroluffariellolide diacid (**21**) $IC_{50}$ > 100 | |

**Conclusions**

There are multiple pathways currently known to inactivate lipoxygenases, ranging from competitive,[30] to allosteric,[23] to reductive inhibition.[25] Having a series of LO inhibitors in hand provides an opportunity to explore the requirements of such alternative pathways. On the one hand, NDGA (**18**) is known to be a LO redox inhibitor that reduces the active Fe(III) enzyme to the inactive Fe(II) enzyme.[31] This mode of action is also responsible for the inhibition by jaspaquinol (**10**), the most potent 15-HLO inhibitor isolated in this study ($IC_{50}$ = 0.3), because of the reduction of the active site ferric ion as seen by fluorescence spectroscopy.[32] It is probable that the hydroquinol residue of **10** reduces the iron, but more data will be required to prove this point. A similar mechanism could be operating for sponge derived polybrominated phenols and diphenyl ethers that are known 15-HLO inhibitors ($IC_{50}$ = from 1-7 μM) [10] and these are currently being re-examined.

It would appear likely that the other two 15-HLO inhibitors isolated here, (-)-subersic acid (**13**) and (-)-jaspic acid (**11**) operate by a non-redox inactivation mechanism. This conclusion is based on the observation that these compounds do not cause a change in the fluorescence spectroscopy of 15-HLO (i.e. no reduction of Fe(III)). While (-)-jaspic acid (**11**) and (-)-subersic acid (**13**) are potent inhibitors of 15-HLO with $IC_{50}$ = 1.4 and 15 μM, respectively, they exhibit differential activity of approximately 10-fold. Though the data set is insufficient at this point to draw extensive SAR conclusions; noteworthy is that the polar head groups are the same for

**13** and **11**, indicating their polyunsaturated spacers are critical for inhibition. Furthermore, two other sesterterpene acids evaluated here, dehydroluffarellolide diacid (**21**) sigmosceptreptrellin (**22**), were inactive, as was (+)-subersin (**12**). This data adds further support to the idea that the length of polyunsaturated spacer is important for inhibition activity.

Further research in this area might cover two interesting issues. Firstly, preliminary data indicate these inhibitors may be selective against the different types of human lipoxygenases (12 vs. 15). Secondly, a structural parallelism between **11** and arachidonic acid (AA) may be of significance. Both have a similar spatial separation between the unsaturated functionality that undergoes dioxygen incorporation by 15-HLO and the carboxylic head group. In AA this involves the 14,15 double bond while in the case of **11** our current hypothesis is that the side-chain, tri-substituted double bond is bound in a similar location, but it can not undergo oxygenation possibly due to the lack of a 1,4 diene functionality.

**Experimental Section**

**General Experimental Procedures.** NMR spectra were recorded in $CDCl_3$ and $CD_3OD$ solutions at 500 or 125.7 MHz for $^1H$ and $^{13}C$ respectively. Optical rotation was measured on a JASCO DIP-370 digital polarimeter, UV data were obtained on a Hewlett-Packard 8452A Diode Array Spectrophotometer, ESIMS spectra on a VG Quattro II, HRMS on a PE Biosystems Mariner, and JEOL JMS-AX505HA Mass Spectrometers.

**Biological Material, Collection, and Identification.** The *Suberea* sp. sponge specimen (Photo Figure 26) was collected by SCUBA at a depth of 20 meters in the Madang region of Papua New Guinea (S05°15.762' / E145°13.007'). Preliminary taxonomy was performed by M. Sanders as a Hyrtios sp. (family Thorectidae). Dr. Christina Diaz performed the complete taxonomy. Microscopy of the sponge fibers yielded interesting new taxonomy. Description as follows: Massive globular sponge, dark gray externally and tan internally. The surface is conulose, and the consistency dense and rubbery, very hard in the dry specimen. The skeleton consists only of fibers, (typical of Verongids), which are concentric layed fibers, 250-300 in diameter, with a strong organic pith, 40-50 μm in diameter. The nature of the fibers, with both pith and bark well represented point towards the recently described genus *Suberea*.[12] Taxonomy of the *Jaspis* sp. sponges (Photo Figure 27) was performed by methods previously described.[14]

**Figure 25** Underwater photo of *Suberea* sp.

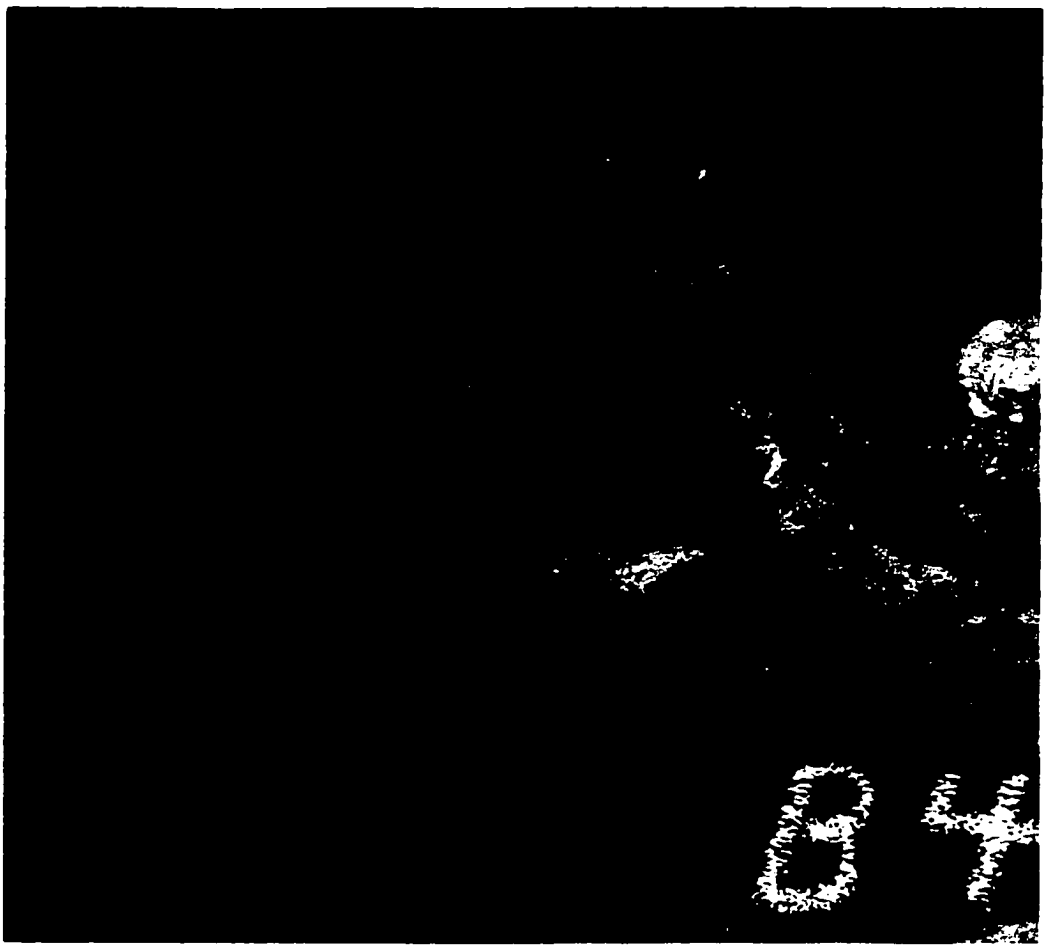
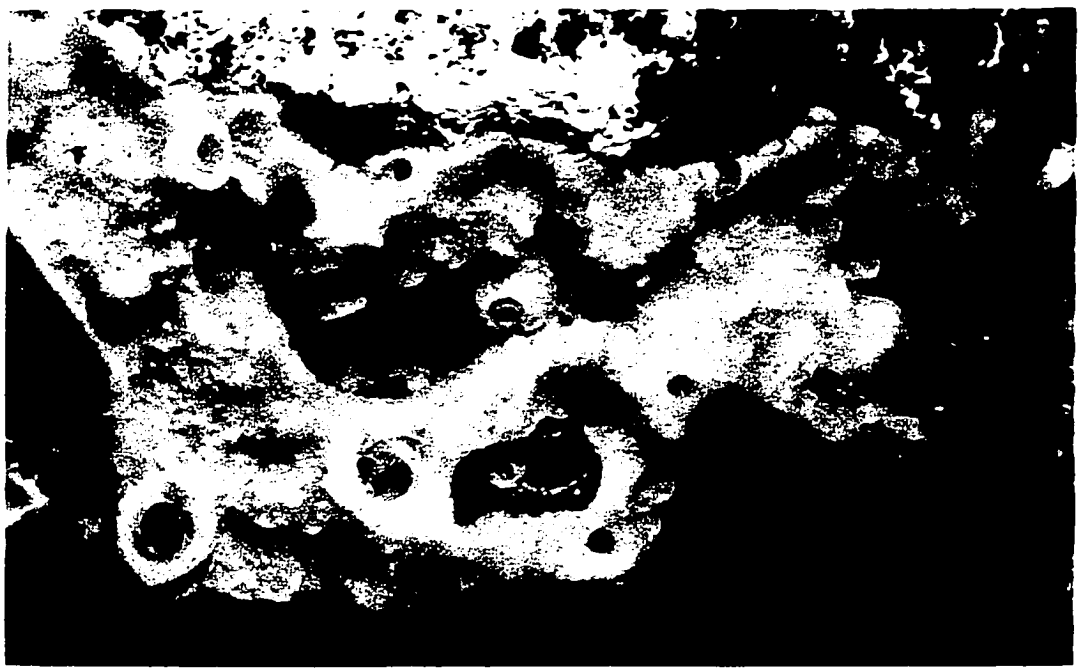


**Figure 26** Underwater photo of *Jaspis splendans*.



**Extraction and Isolation.** The sample was preserved by immersion in a 1:1 alcohol/seawater solution. After 24h this solution was decanted and discarded. The damp organism was then transported in a Nalgene bottle back to the home lab at ambient temperature, was next extracted with 100% MeOH to give a crude oil which was then partitioned between various solvents (Scheme 1). The hexane and methylene chloride fractions were further purified on Biotage Si gel columns (100% $CH_2Cl_2$, and EtOAc/Hexane respectively) to give five fractions each. These were further purified by reverse phase gradient HPLC to give **12** (0.02% dry wt of sponge), **10** (0.01% dry wt of sponge), **11** (0.01% dry wt of sponge) and **13** (0.25% dry wt. of sponge). Pure compounds **18**, **21** and **22** were obtained from the Crews Lab pure compound repository.

**Lipoxygenase Assay.** 15-HLO was expressed and purified as described previously.[33] The enzyme activity was determined by direct measurement of the product formation of a 3μM linoleic acid solution by following the increase of absorbance at 234 nm in 25mM Hepes (pH 7.5), 0.2% (g/L) cholic acid. All reactions were performed in 2 mL of buffer, ≈ 200 nM 15-HLO and constantly stirred with a rotation magnetic bar (≈ 22 °C). $IC_{50}$ values were determined by measuring the enzymatic rate at a variety of inhibitor concentrations (depending on the inhibitor potency) and plotting their values versus inhibitor concentration. The corresponding data was fit to a simple saturation curve and the inhibitor concentration at 50 %

activity was determined. The inhibitors were typically dissolved in methanol at a concentration of ≈ 25 mg/mL.

**(+)-(5*S*, 6*S*)-Subersin (12)**: yellow oil; 24mg; $[\alpha]_D$ +30° (*c* 2.6, $CHCl_3$); HREIMS *m/z* 286.2267 $[M]^+$ (Δ 3.0 mmu of calcd. for $C_{20}H_{30}O$) $^1$HNMR (500MHz, $CDCl_3$) δ (ppm) 7.34 (s, H15), 7.22 (d, *J*=0.5 Hz, H16), 6.28 (s, H14), 5.40 (s, H2), 5.17 (t, *J*=7.5 Hz, H10), 2.45 (t, *J*=8 Hz, 2H, H12), 2.24 (q, *J*=8 Hz, 2H, H11), 1.97 (m, H8), 1.96 (m, 2H, H3), 1.89 (m, H8'), 1.63 (s, $H_3$, H17), 1.60 (m, H4), 1.59 (s, $H_3$, H20), 1.57 (m, H5), 1.47 (m, H7), 1.43 (m, H4'), 1.34, (m, H7'), 1.03 (s, $H_3$, H19), 0.94 (d, *J*=8 Hz, $H_3$, H18). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm) 142.7 (d, C15), 139.6 (s, C1), 139.1 (d, C16), 137.2 (s, C9), 125.1 (s, C13), 123.3 (d, C10), 122.1 (d, C2), 111.3 (d, C14), 39.8 (s, C6), 37.8 (d, C5), 35.8 (t, C8), 34.7 (t, C7), 28.6 (t, C11), 27.5 (t, C4), 26.6 ( q, C19), 25.1 (t, C12), 24.1 (t, C3), 19.7 (q, C17), 16.3 (q, C20), 16.0 (q, C18).

**(-)-(5*R*, 10*R*)-Subersic acid (13)**: yellow oil; 12mg; $[\alpha]_D$ -46° (*c* 0.5, $CHCl_3$); *m/z* = 409.2743 $[M-H]^-$ (Δ 0.0 mmu of calcd. for $C_{27}H_{38}O_3$); $^1$H NMR (500MHz, $CDCl_3$) δ (ppm) 7.91 (s, 2H, H21 & H19), 6.86 (d, *J*=10 Hz, H18), 5.36 (t, *J*=5 Hz, H14), 3.42 (d, *J*=10 Hz, $H_3$, H15), 2.14 (m, 2H, H11), 2.12 (m, 2H, H12), 2.09 (m, 2H, H7), 1.83 (m, H1), 1.83 (s, $H_3$, H23), 1.62 (m, 4H, H6 & H2), 1.58 (s, $H_3$, H24), 1.17 (m, H3'), 1.14 (m, 2H, H1' & H5), 0.95 (s, $H_3$, H25), 0.89 (s, $H_3$, H26), 0.84 (s, $H_3$, H27). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm) 171.7 (s, C22), 159.8 (s, C17), 141.0 (s, C13),

132.9 (d, C21), 130.8 (d, C19), 127.0 (s, C16), 126.3 (s, C8), 121.9 (s, C20), 120.3 (d, C14), 116.0 (d, C18), 52.1 (s, C5), 42.1 (t, C3), 40.7 (t, C12), 39.3 (s, C10), 37.3 (t, C1) 33.9 (t, C7), 33.6 (s, C4), 33.6 (q, C26), 29.9 (t, C15), 27.3 (t, C11), 21.9 (q, C27), 20.4 (q, C25), 19.8 (q, C24), 19.3 (t, C2), 19.3 (t, C6), 16.7 (q, C23).

**Jaspaquinol (10)**: clear oil, 14mg; *m/z* = 381.33 $[M-H]^-$ ($C_{26}H_{38}O_2$, Figure 28); $^1H$ NMR (500MHz, MeOD, Figure 29) $^{13}C$ NMR (125 MHz, MeOD, Figure 30).

**(-)-Jaspic Acid (11)**: yellow oil; 12mg; $[\alpha]_D$ -23° (*c* 0.035, $CHCl_3$, lit $[\alpha]_D$ –23° (*c* 0.08, EtOH); *m/z* = 409.2743 $[M-H]^-$ (Δ 0.0 mmu of calcd. for $C_{27}H_{38}O_3$ Figure 31); $^1H$ NMR (500MHz, $CDCl_3$ Figure 32) $^{13}C$ NMR (125 MHz, MeOD, Figure 33).

**Scheme 1** Isolation of (+) subersin (**12**), (-)-subersic acid (**13**), jaspaquinol (**10**), and (-)-jaspic acid (**11**).

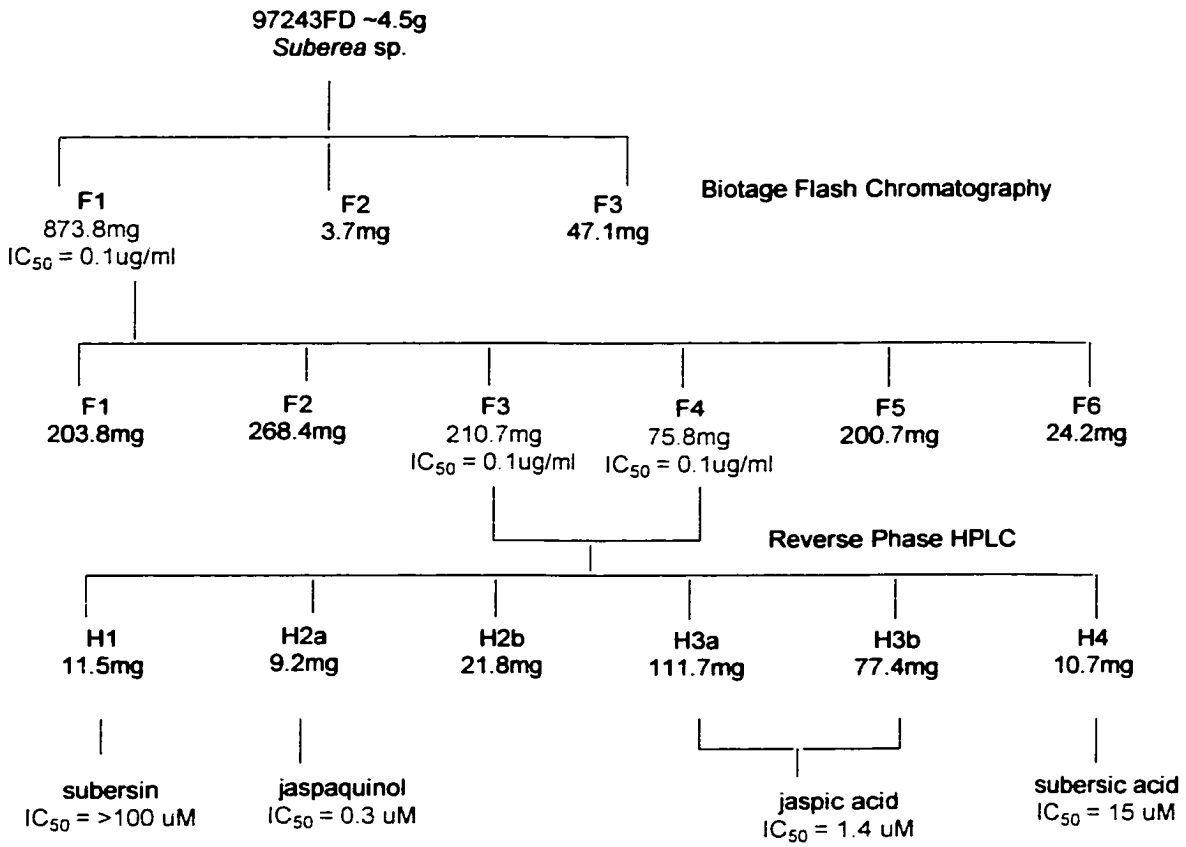


**Figure 28** $ESI^+$ and $ESI^-$ Mass spectrum of jaspaquinol (**10**).

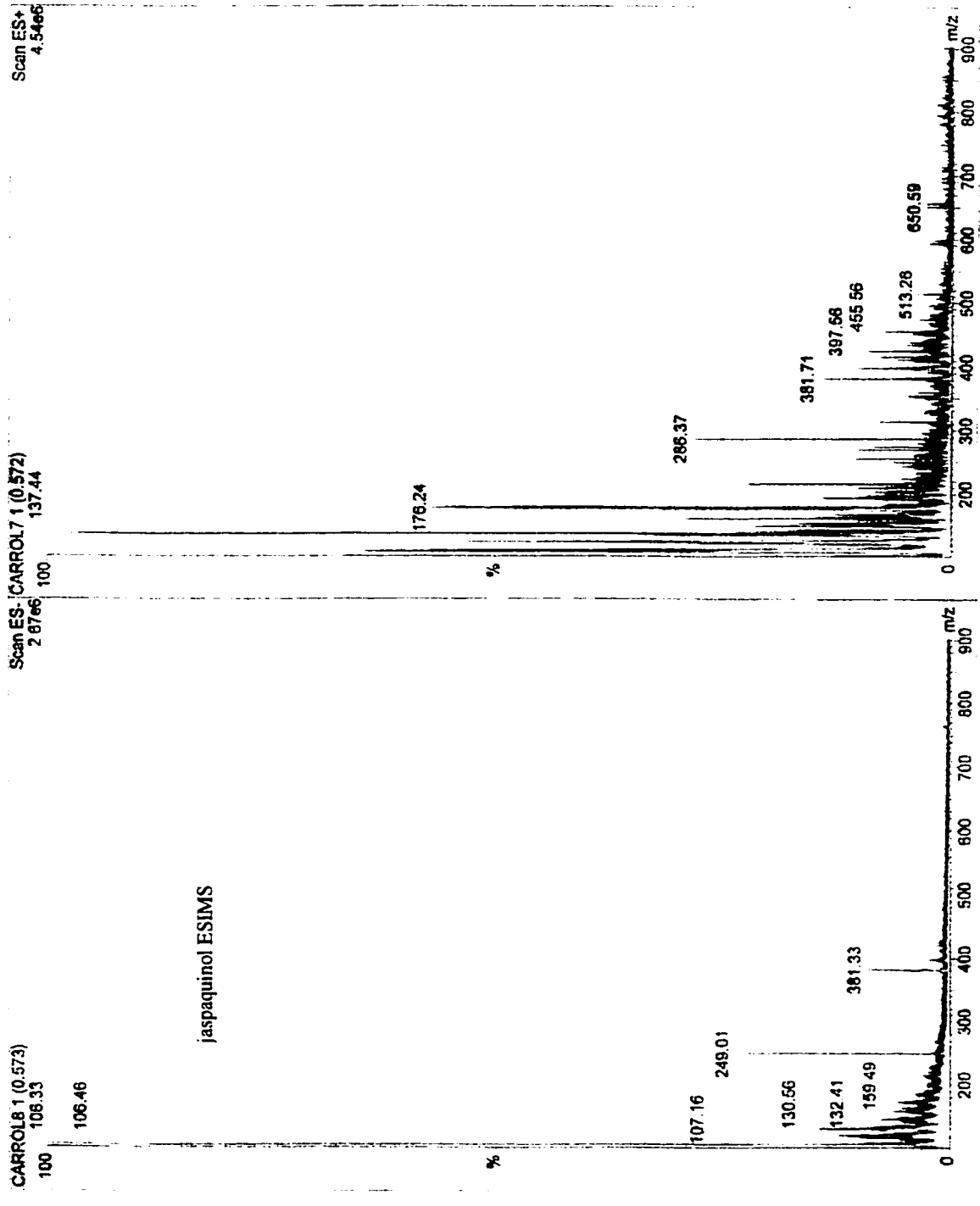


**Figure 29** Proton NMR spectrum of jaspaquinol (**10**) (500 MHz, $CDCl_3$).

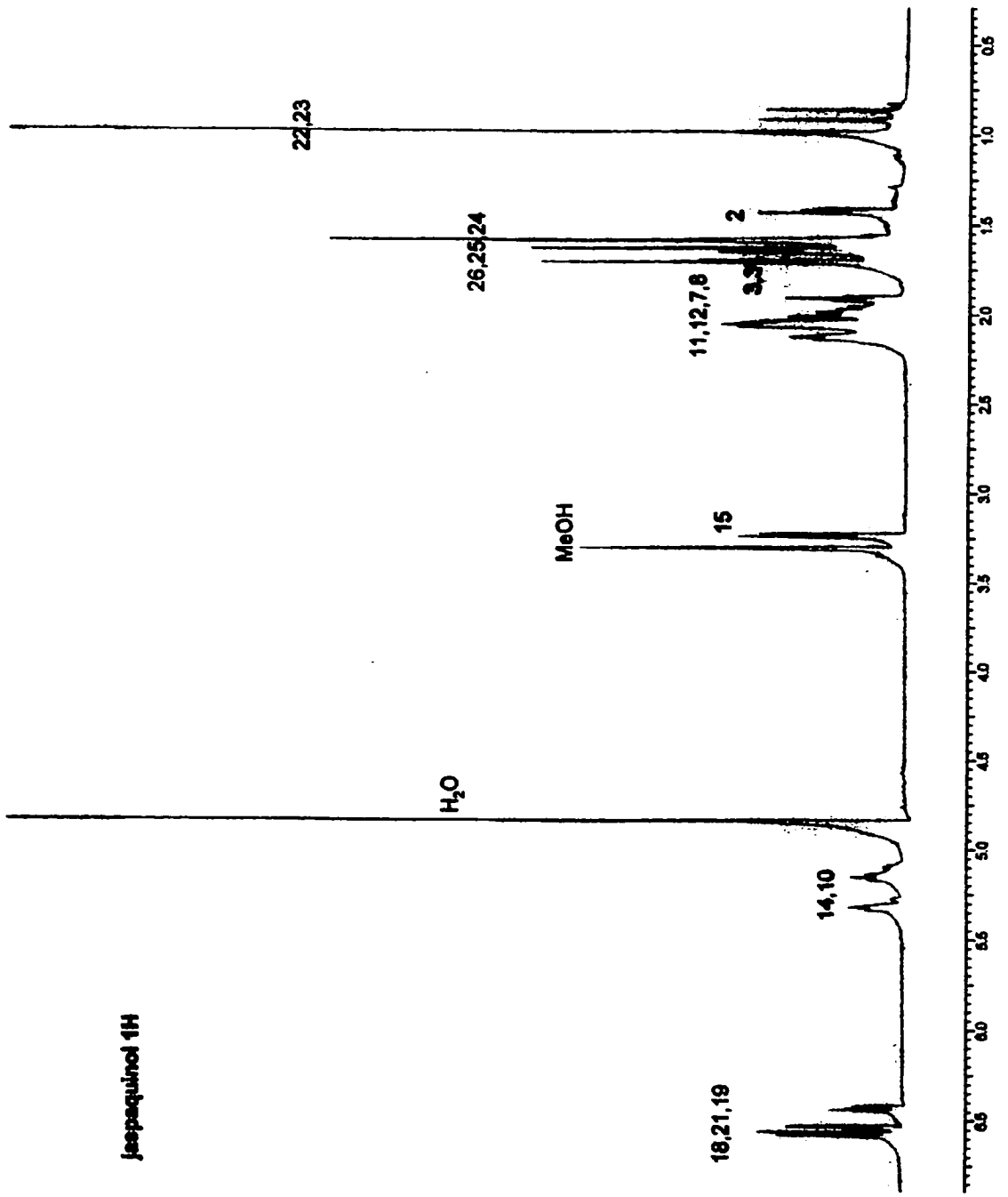


**Figure 30** Carbon NMR spectrum of jaspaquinol (**10**) (125.7 MHz, $CDCl_3$).

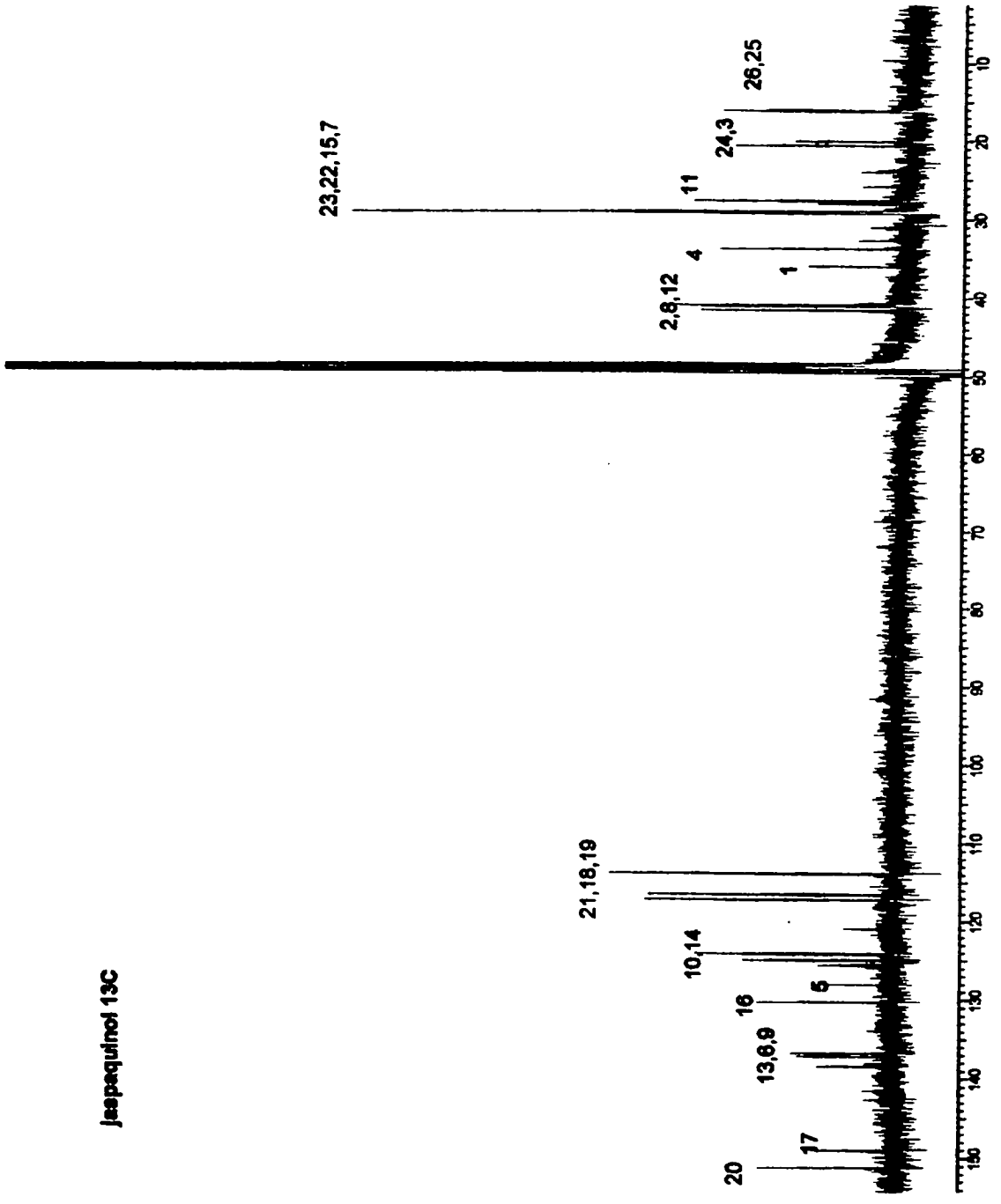


**Figure 31** ESI⁻ Mass spectrum of (-)-jaspic acid (**11**)

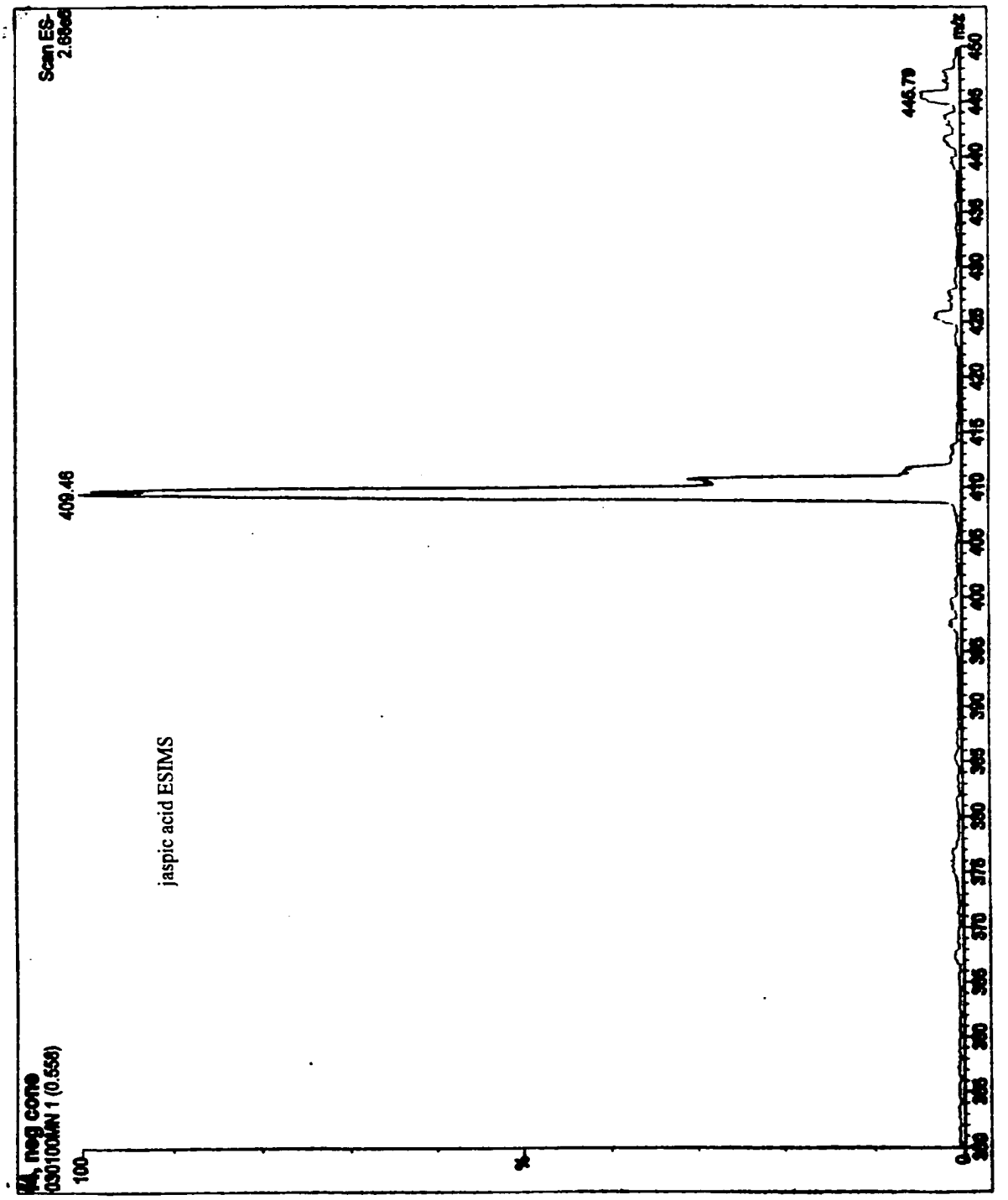


**Figure 31** Proton NMR spectrum of (-)-jaspic acid (**11**) (500 MHz, $CDCl_3$).

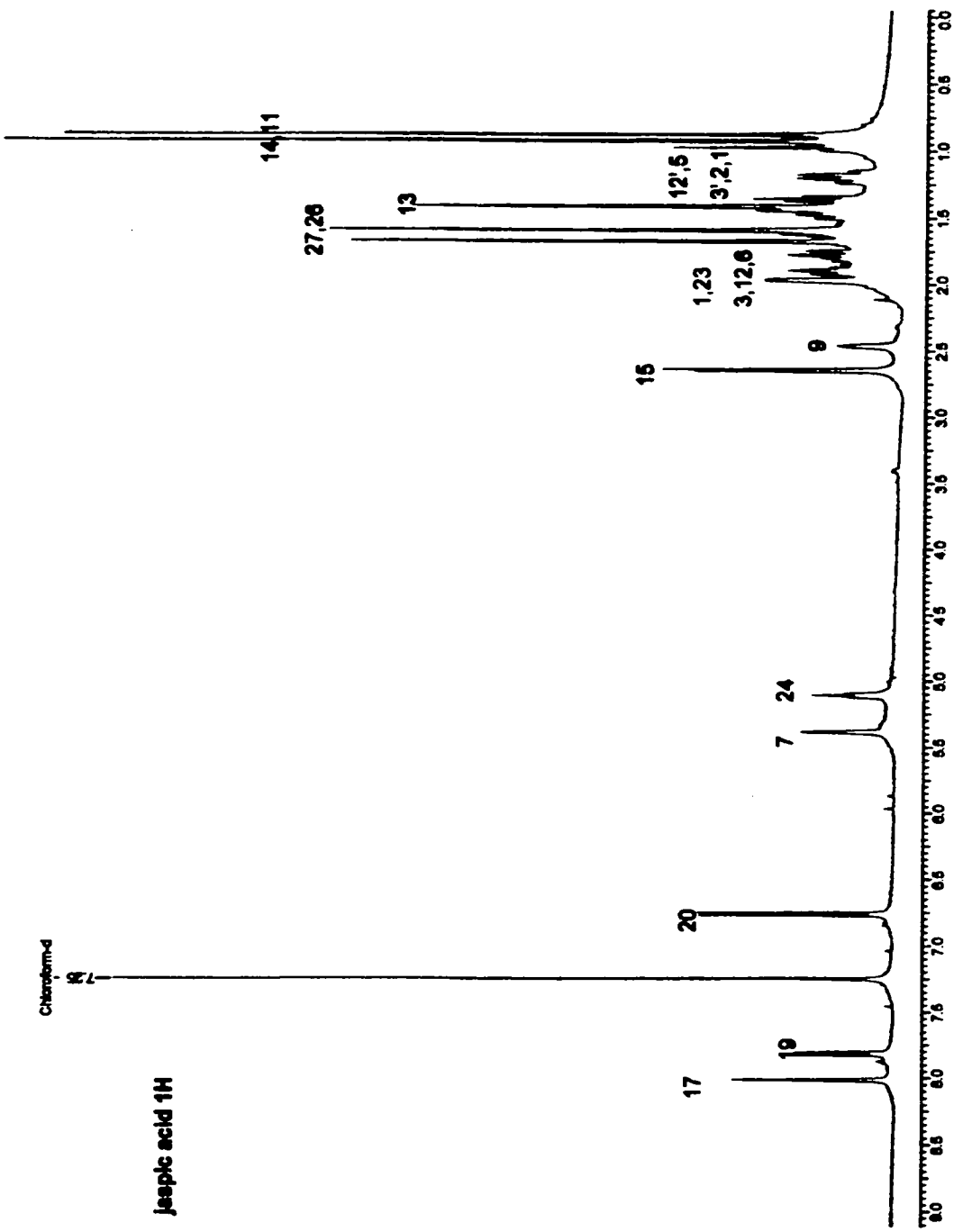
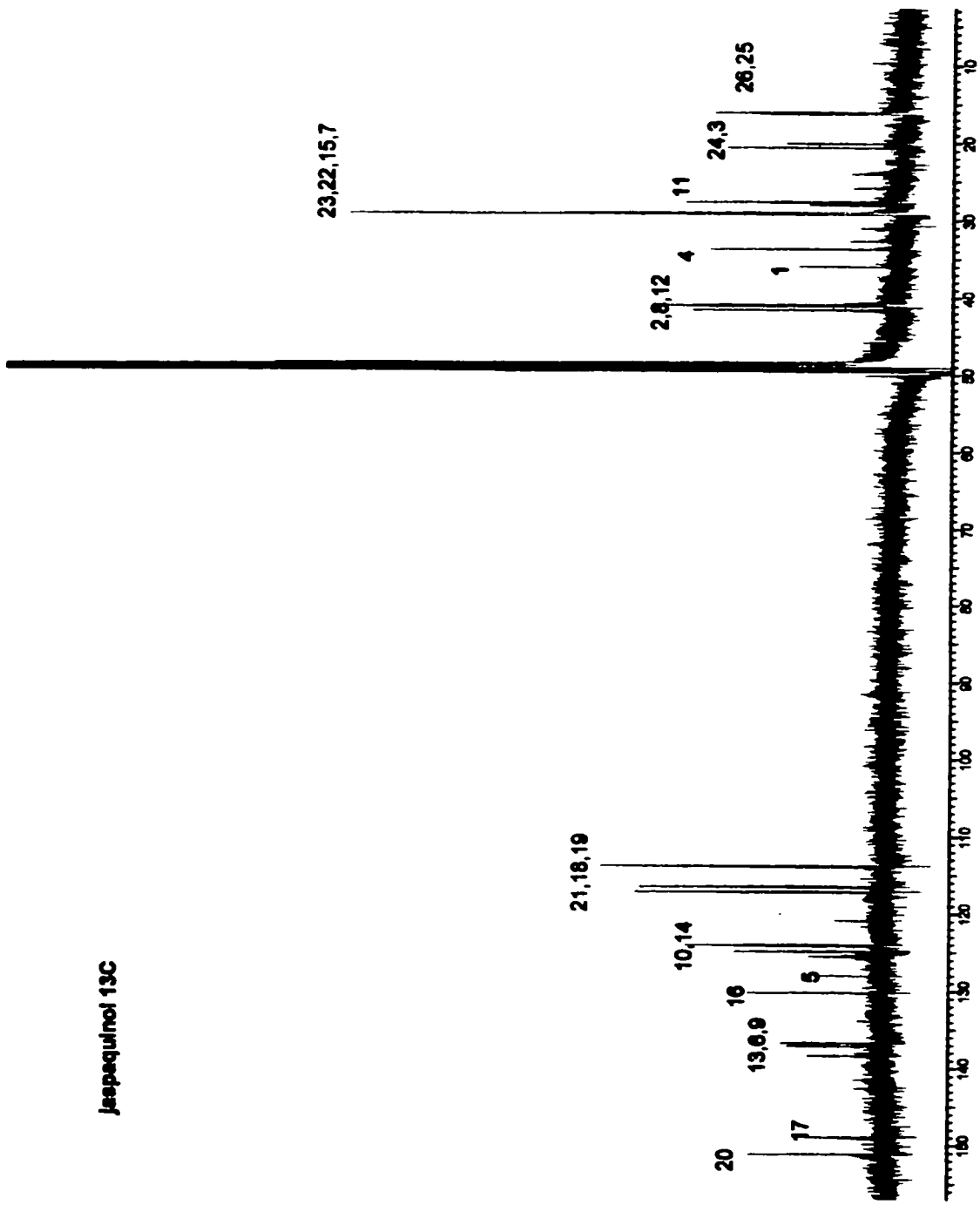


**Figure 32** Carbon NMR spectrum of jaspaquinol (**11**) (125.7 MHz, $CDCl_3$).



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## Chapter 3 Marine Natural Products as Anti-Ischemic Leads

### Background

Ischemia is a condition that results when an organ receives inadequate blood flow. Organs such as the heart and brain are dependent upon the continuous flow of blood and are especially vulnerable to ischemia. Excessive injury results when blood flow is disrupted for even brief periods of time, as in the case of heart attack and stroke. Current estimates are that 60,800,000 Americans have one or more types of cardiovascular disease, and that the cost of these diseases in the United States totaled $298.2 billion last year alone.[1] Currently there are no products available to treat organ injury caused by ischemia.[2] Thus, the goal was to probe the breadth of marine derived natural products that can be used in this regard.

A collaboration was initiated in 1998 between Galileo and UCSC to launch a research effort. The technical association between these two groups, which are in close geographical proximity, is to discover leads for new therapeutic agents by coupling the marine natural product chemistry of the Crews group at UCSC with that of biological expertise of Galileo Laboratories.[3] The UCSC and Galileo partnership has met two notable milestones. Namely, the award of a three-year competitively ranked NOAA Sea Grant Industrial Fellowship, which provided my funding for two years of research, and the submission of a provisional patent for the work discussed in this chapter.

This work utilizes the technology of natural product isolation and anti-ischemia screens to produce a profile of sponge-derived terpenes and their interaction with this important disease target. Out of the first batch of 111 crude sponge extracts tested at Galileo Laboratories, one *Coscinoderma* sp. extract (coll. # 97220, family Spongiidae) indicated a high percent of protection provided for both cardiomyocyte and hippocampal cell lines. From the 97220 sample of Spongiidae; the novel halisulfate 8 (**1**), its corresponding alcohol **5,** the known halisulfate 7 (**2**) and its alcohol **6** were obtained. Other related Spongiidae compounds from the UCSC pure compound repository were tested and include; hipposulfate (**3**), halisulfate 1 (**4**), quinone **9**, as well as alcohols **7** and **8**. Reported herein is the success of these terpenoid compounds as protection against ischemic cell damage.

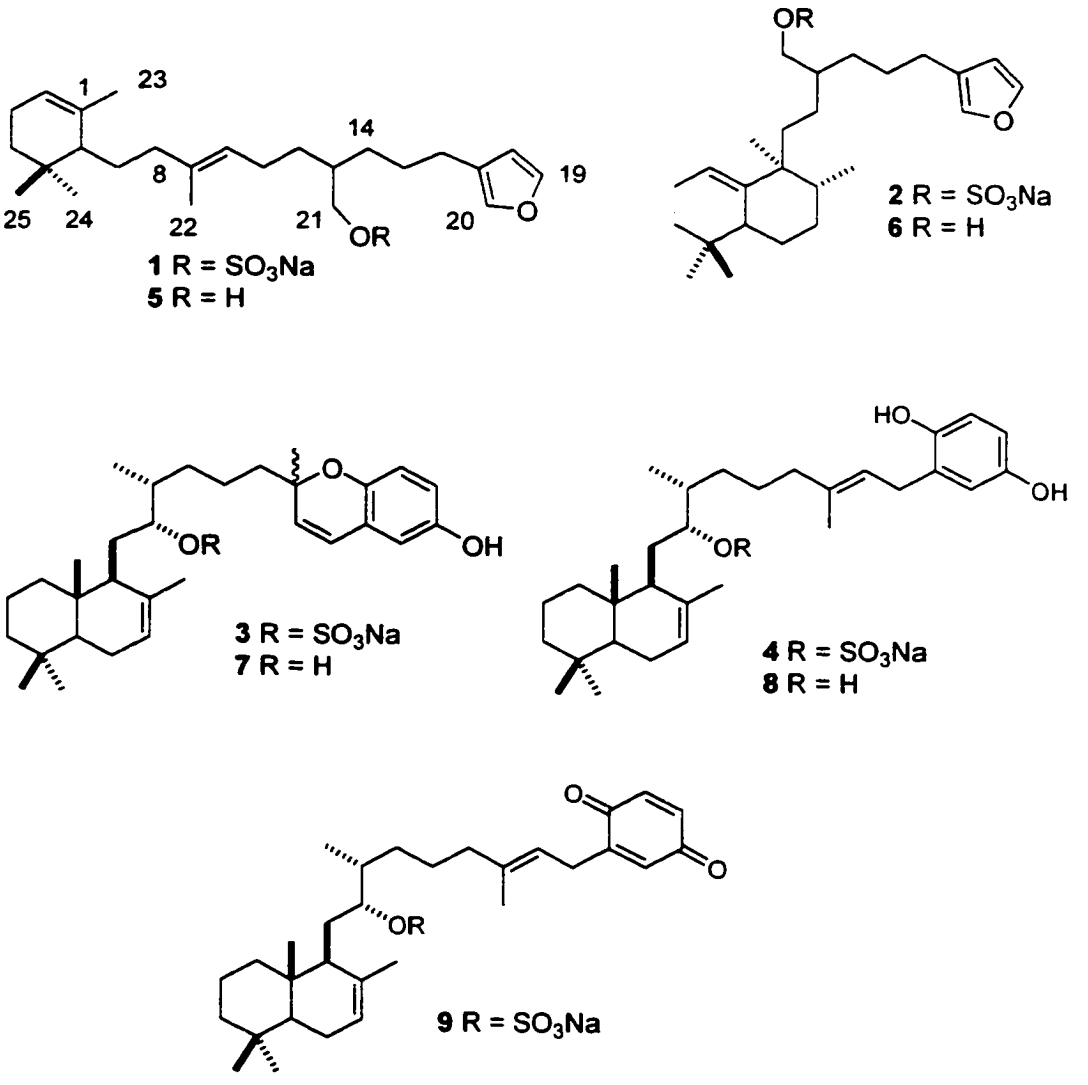
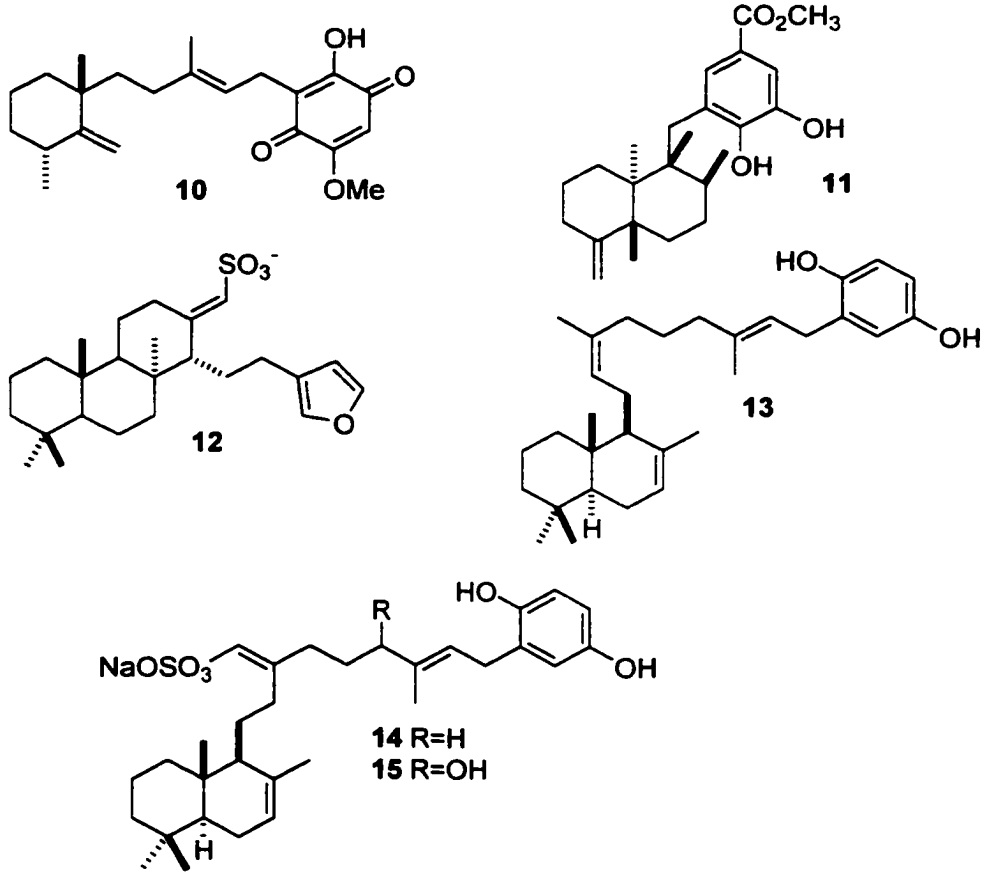


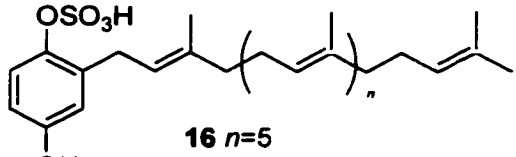

**Chart 1.** Terpenoid Compounds from Spongiidae screened for Anti-Ischemic Activity.

Research by other groups describe the characterization of the sesquiterpenoid metachromins A-(**10**)-H [4,5,6] and the dictyocerratins A (**11**) and B from *Hippospongia* sp.[7] The 1-methylherbipoline[8] and gaunidium[9] salts of suvanine (**12**) have been

previously isolated from *Coscinoderma* sp. and *Coscinoderma mathewsi* respectively. Also from *Coscinoderma* sp. sponges are the cytotoxic sesterterpene, coscinoquinol[10](**13**). Furthermore, sesterterpene sulfates hipposulfates A (**14**) and B (**15**) have been found from *Hippospongia* cf. *metachromia.*[11] All of these compounds are similar to sesterterpene halisulfates 1(**4**)-7 found from within the family Halichondriidae and the genus *Coscinoderma* sp.[13,14] The halisulfates 1-7 have previously been shown to be inhibitors of phospholipase $A_2$ and PMA induced inflammation.

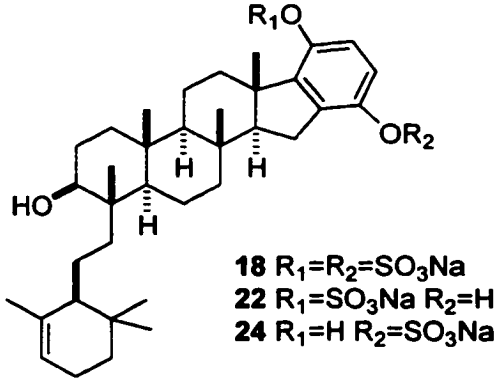


Many articles in the literature report interesting biological activity from sulfonated compounds from the phylum porifera. For example, sulfated terpenes have been discovered from an Australian *Sarcotragus* sp. These two mereoterpenes; hydroquinone sulfate (**16**), and nonaprenylhydroquinone sulfate (**17**), show inhibitory effects towards 1,3-fucosyltransferase VII[12]

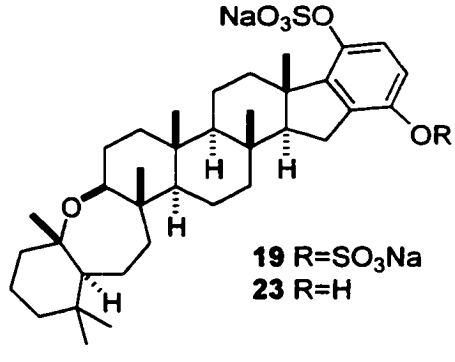



**16** *n*=5
**17** *n*=6

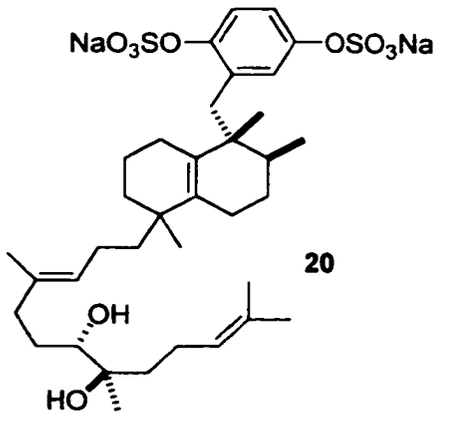
The most diverse sulfonated structures in the literature have arisen from the *Adocia* sp. sponge. The kinesin motor protein inhibitors, adociasulfates 1-6 (**18-24**), were obtained from a Palauan *Adocia* sp. sponge. In addition, adociasulfates 1 (**18**), 7 (**19**) and 8 (**20**) were obtained from an *Adocia* sp. from the Great Barrier Reef and show inhibitory effects against vacuolar $H^+$-ATPase. Additional samples of *Adocia aculeate* also from the Great Barrier Reef contained adociasulfates 5 (**25**) and 9 (**26**).




**18** $R_1$=$R_2$=$SO_3Na$
**22** $R_1$=$SO_3Na$ $R_2$=H
**24** $R_1$=H $R_2$=$SO_3Na$




**19** R=$SO_3Na$
**23** R=H

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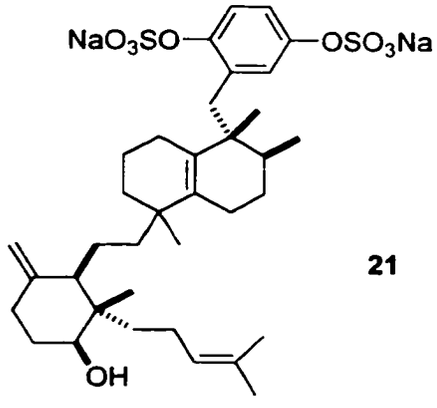


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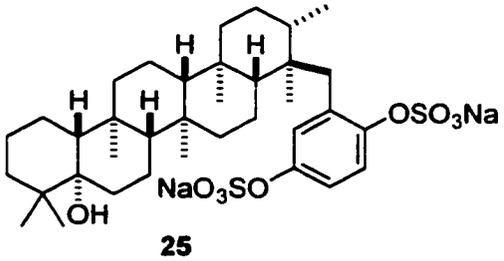


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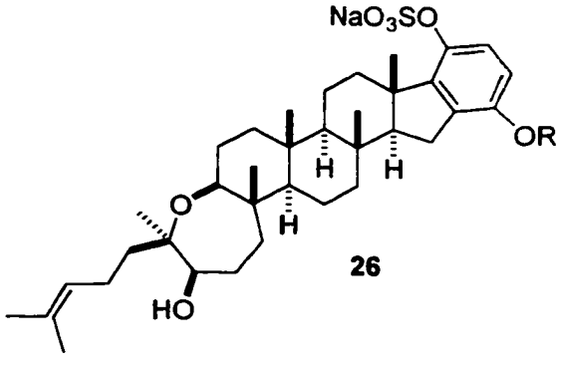



26

Not surprisingly, sulfates are often looked upon as nuisance compounds by pharmacologists. One reason for this is that a major pathway for drug deactivation is through sulfate conjugation.[13] In this process, a drug is made more water-soluble by the addition of a sulfate group. There appear to be a variety of sulfotransferases in the liver, cytoplasm, and other tissues, which carry out this function. Moreover, sulfate conjugates can hydrolyze back to the parent compound by sulfatases. Thus, although the drug may be more soluble in the cytoplasm, it is often difficult to deliver directly into the cell.

**Results and Discussion**

The bioassay-guided fractionation of Spongiidae sample 97220 indicated that the active components were concentrated in the methylene chloride (FD) extract. Step gradient column chromatography (methylene chloride/methanol) of the FD gave four fractions, the first of which showed increased activity in the Galileo assay. This was further purified by RPHPLC using a methanol/water gradient to give mostly pure (~90 %) 16mg halisulfate 8 (**1**) and 25mg halisulfate 7 (**2**).

Halisulfate 8 (**1**), obtained as a viscous oil, had its molecular formula, $C_{25}H_{39}O_5S$ established from high-resolution $FAB^-$ mass measurements *m/z* = 451.2518 (Δ 1.0 mmu calcd. Figure 1). The $^1H$ NMR spectrum (Figure 2) contained three downfield resonances characteristic of a furan ring system (δ 6.25, 7.20, 7.32) two double bonds (δ 5.06, 5.22) and four methyl singlets (δ 0.82, 0.96, 1.55 and 1.60). In addition, a distinguishing pentet, (two overlapping triplets at δ 3.88) indicated the possibility of two diastereotopic protons adjacent to a sulfate group.[14] The $^{13}C$ NMR spectrum (Figure 3) further indicated a furan (δ 124.2, 110.9, 142.3 and 137.0) as well as shifts indicative of a sulfate group (δ 69.8).

**Figure 1** Negative Ion HRFAB Mass spectrum for halisulfate 8 (**1**).

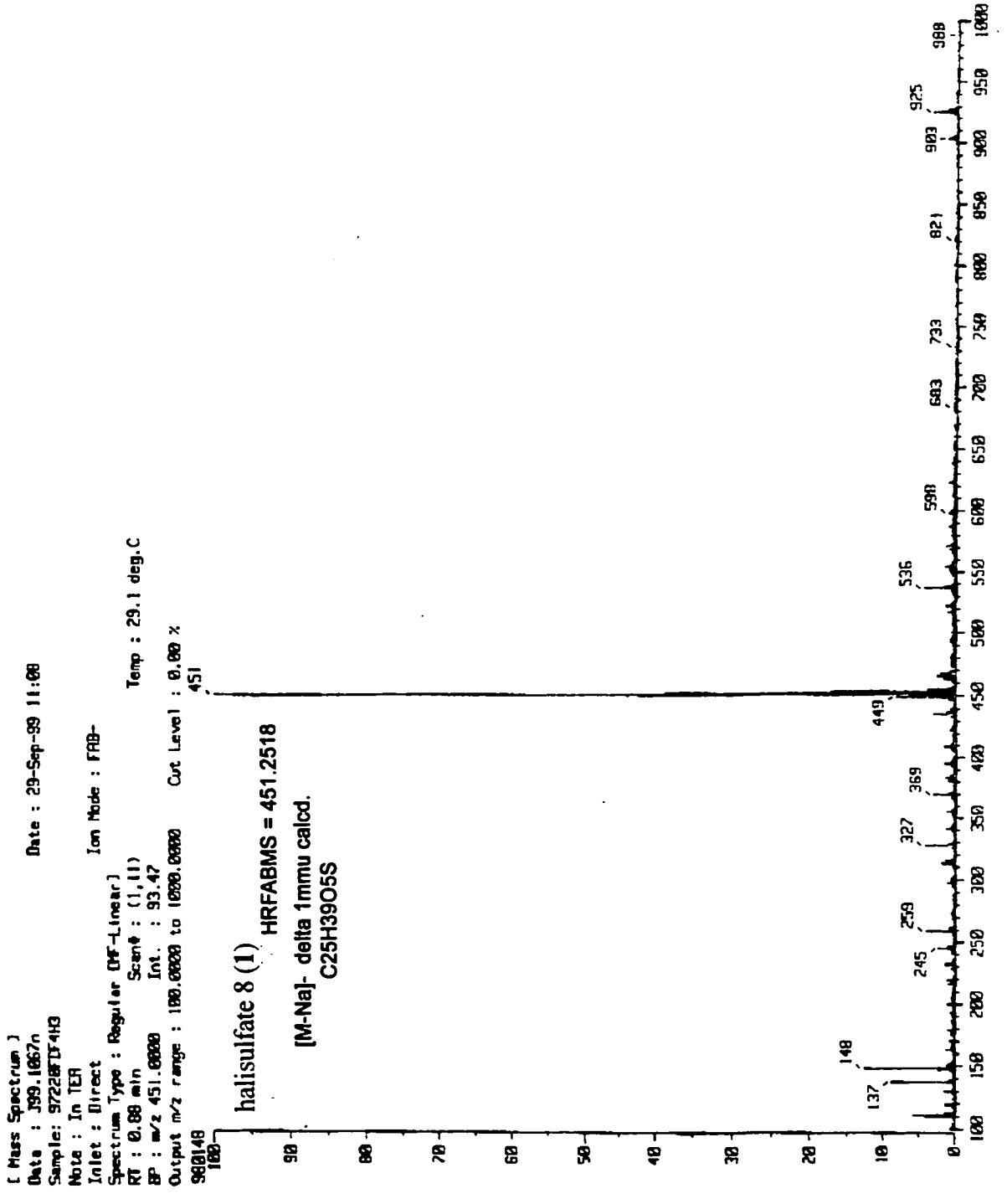


**Figure 2** Proton NMR spectrum for halisulfate 8 (**1**) ($CDCl_3$, 500MHz).

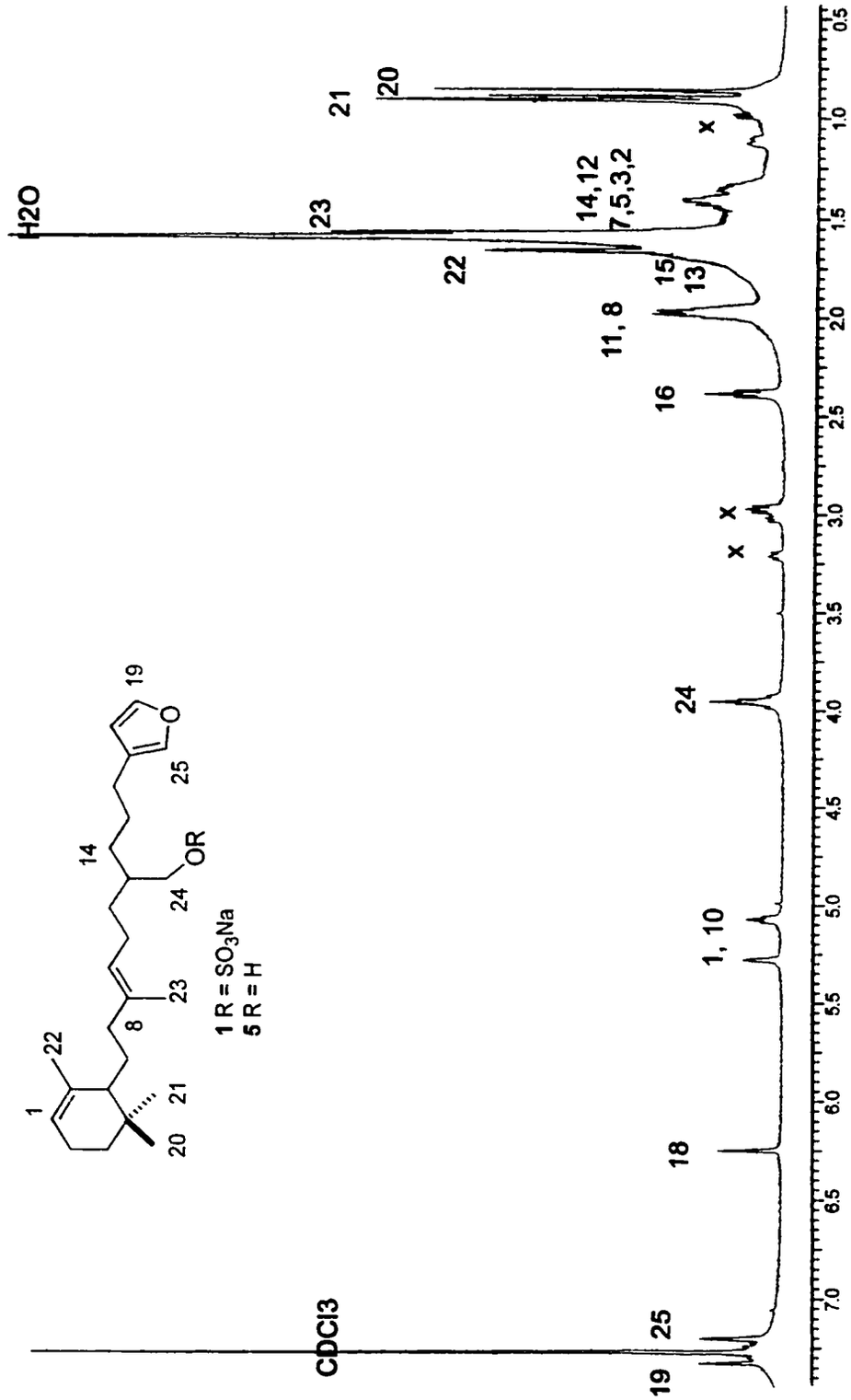


**Figure 3** Carbon NMR spectrum for halisulfate 8 (**1**) ($CDCl_3$, 500MHz).

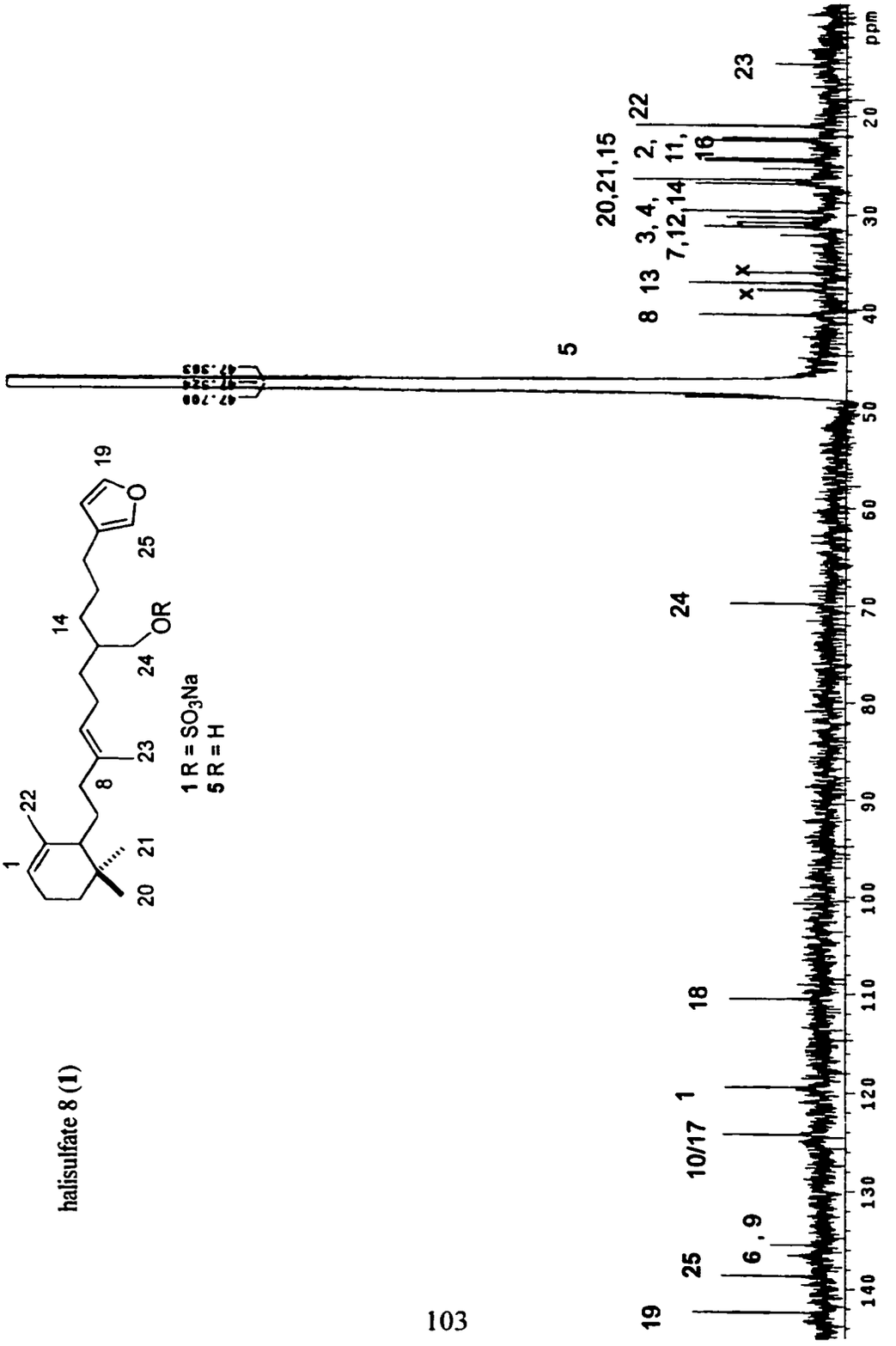


**Figure 4** DEPT spectrum for halisulfate 8 (**1**) ($CDCl_3$, 500MHz).

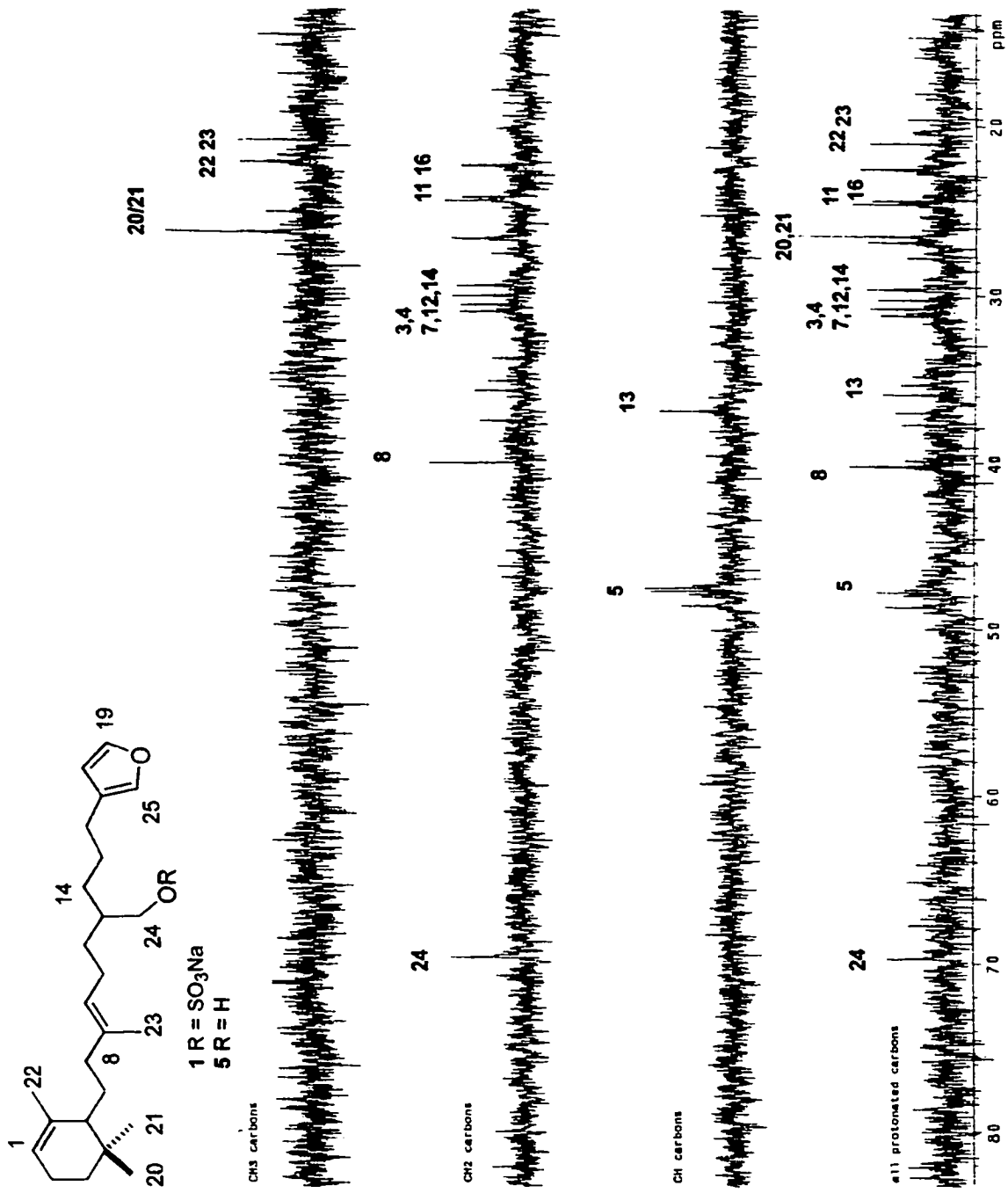


**Figure 5** gHSQC spectrum of halisulfate 8 (**1**) ($CDCl_3$, 500MHz).

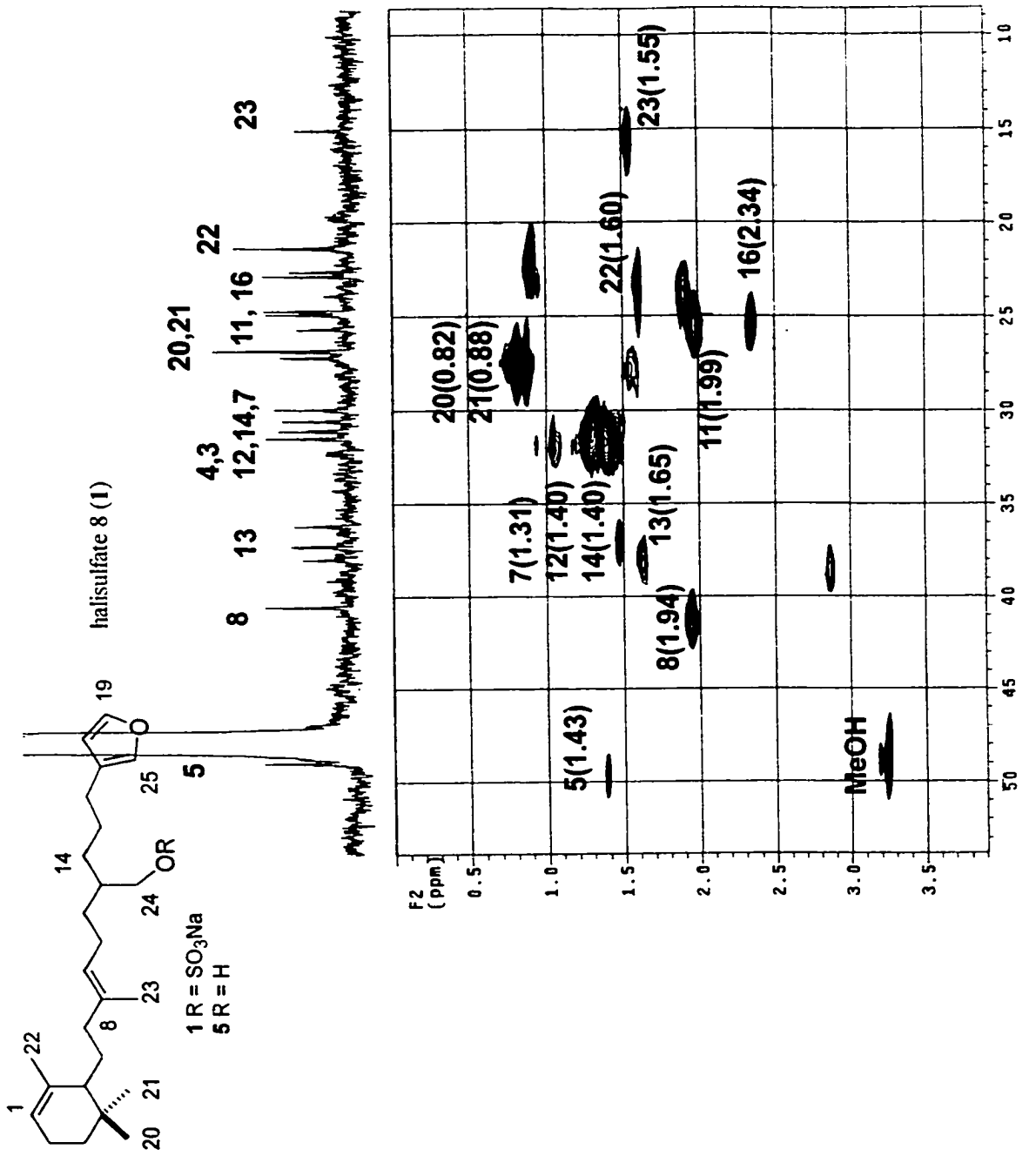


**Figure 6** gCOSY spectrum for halisulfate 8 (**1**) ($CDCl_3$, 500MHz).

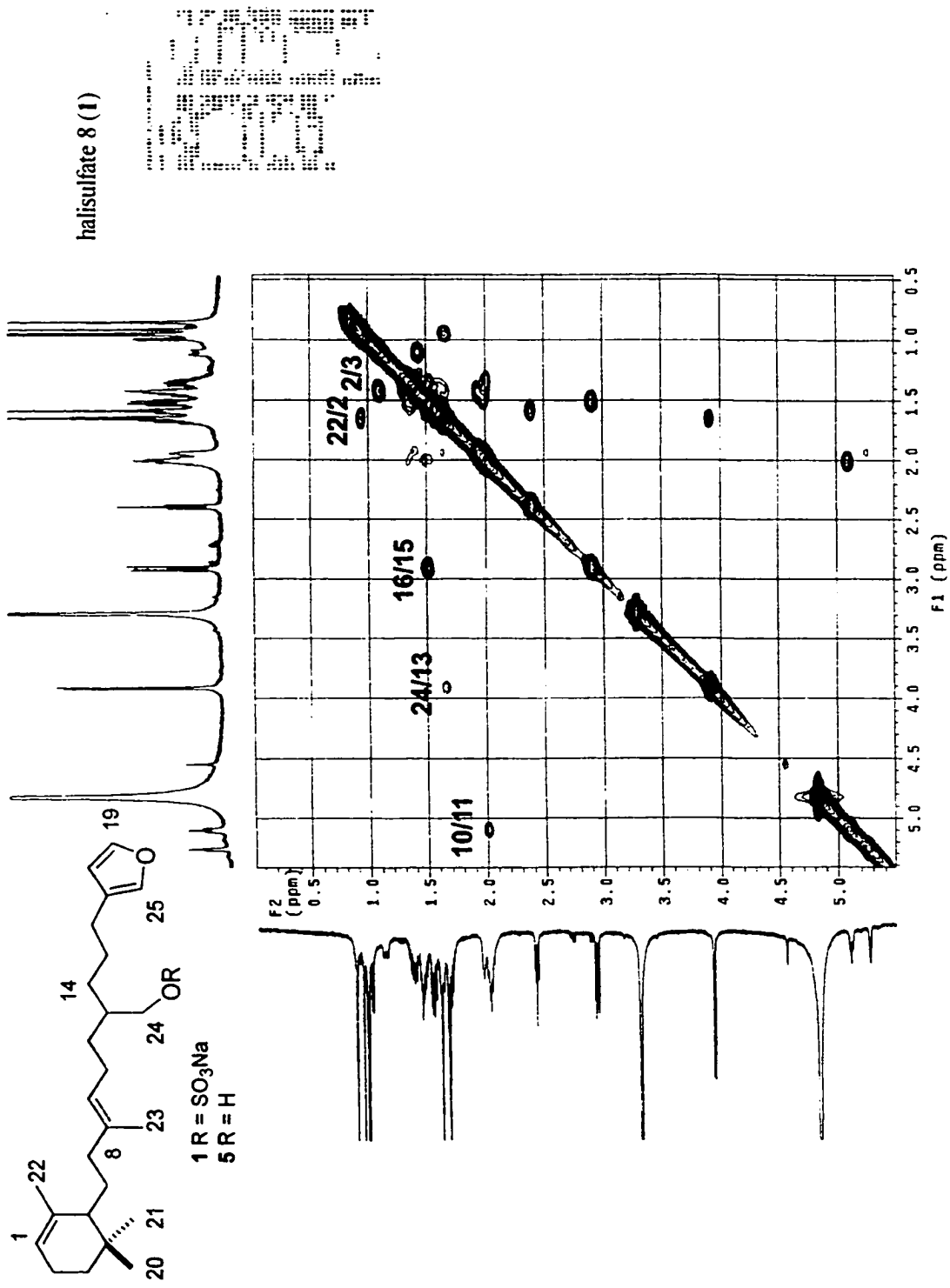


**Figure 7** gHMBC spectrum for halisulfate 8 (**1**) ($CDCl_3$, 500MHz).

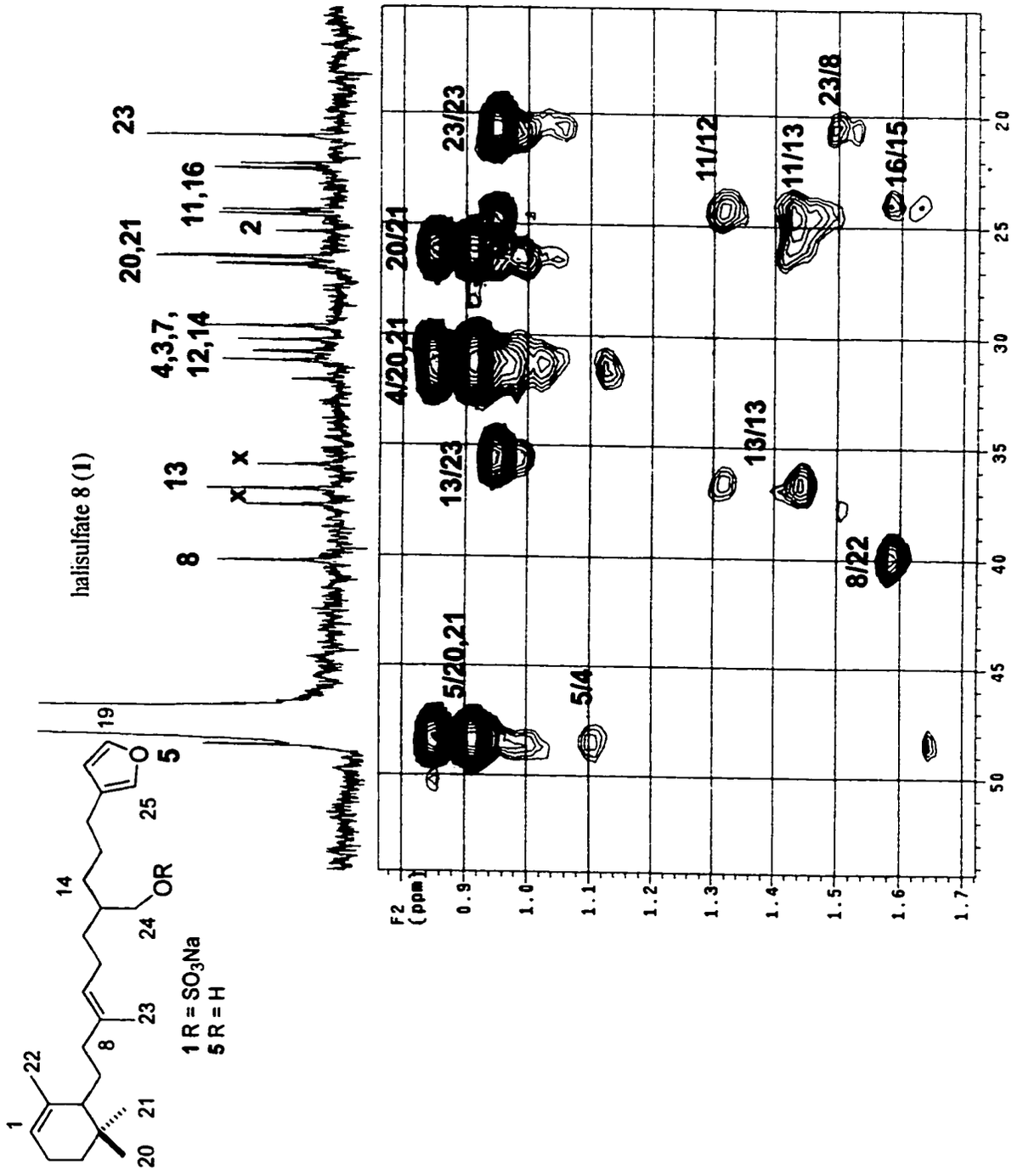
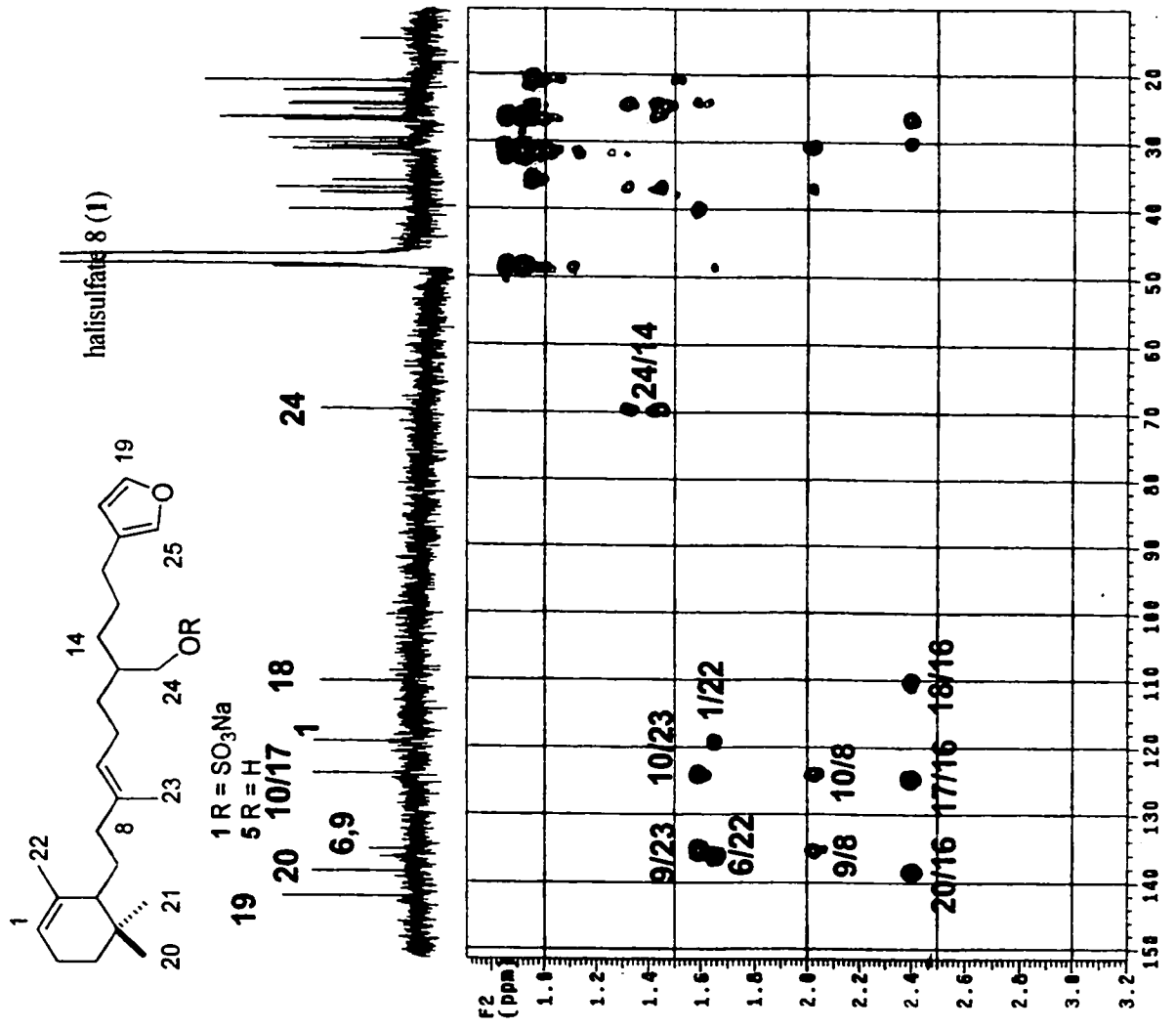
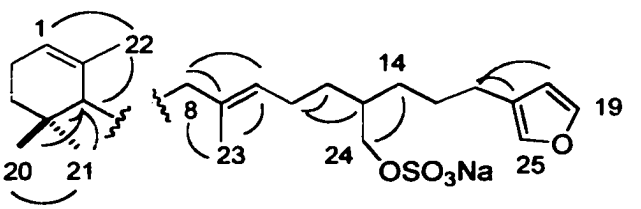


**Figure 8** gHMBC spectrum for halisulfate 8 (**1**) ($CDCl_3$, 500MHz).

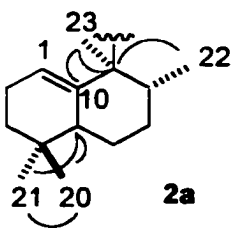


A DEPT experiment (Figure 4) reveled a carbon count of $4CH_3$'s, $10CH_2$'s, 7CH's and 4C, establishing a total of $C_{25}H_{39}$, and accounting for all carbons and hydrogens. Four isolated spin systems were revealed by the gCOSY experiment (Figure 6) between H10 (δ 5.06) / H11 (δ 1.99), H2 (δ 1.41) / H3 (δ 1.40) / H22 (δ 1.60), H24 (δ 3.88) / H13 (δ 1.64), and H16 (δ 2.34) / H15 (δ 1.59). Correlations appeared in the gHMBC spectrum (Figures 7 and 8), which linked C5 (δ 47.0) to H20 (δ 0.82), H21 (δ0.88) and H3 (δ 1.13); C8 (δ 40.3) with H23 (δ 1.60); and C11 (δ 25.4) with H12 (δ 1.40) and H13 (δ 1.64). The two-dimensional data led to substructures **1a** and **1b** in Figure 9. Difficulties arose in the final connectivity of these substructures due to a lack of information regarding the connectivity of C7 (δ 30.3) to the ring and side chain protons H8 and H6. The structure of 1 was finalized by hydrolyzing the sulfate to an alcohol, facilitating an ease of separation, which removed impurity peaks in the NMR spectrum. The hydrolysis of **1** will be discussed after a brief introduction to the spectra of compound **2**.

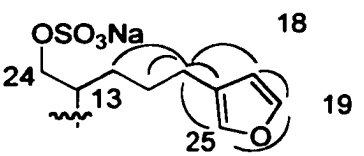
**Figure 9** Two Dimensional NMR correlations for halisulfate 8 substructures (**1a** and **1b**).



Correspondingly, compound **2**, halisulfate 7, was considered to likely be of identical molecular formula as **1** from negative ion LRESIMS data; *m/z*= 451 (Figure 10) corresponding to $C_{25}H_{39}SO_5$. The $^1H$ NMR data (Figure 11) indicated a sulfonated diastereotopic methylene (δ 3.82, 3.92), a furanoid structure (δ 6.25, 7.20, 7.32), one double bond (δ 5.30), four methyl singlets (0.82, 0.83 and 0.95) and one methyl doublet (δ 0.81). The $^{13}C$ NMR data (Figure 12) suggested a furan (δ 124.2, 110.9, 142.3 and 139.0) and a position for a sulfate group (δ 71.8). DEPT data (Figure 13) was inconclusive in the methyl region,[15] and also contained indistinguishable $CH_2$'s, and 7CH's. Summarized in Figure 16, the gCOSY (Figure 14) and the gHMBC data (Figure 15) was also inconclusive with a crowded $CH_2$ region, which made correlations from C11, C12, C2, C3, C4 and C5 in substructure **2a** difficult to identify. The furan portion of substuructure **2b** was firmly established by gHMBC correlations from H16 (δ 2.37) to C25, C17, C18, C14 and C16 (δ 139.2, 124.3, 111.1, 29.8, 24.7).




**2a**

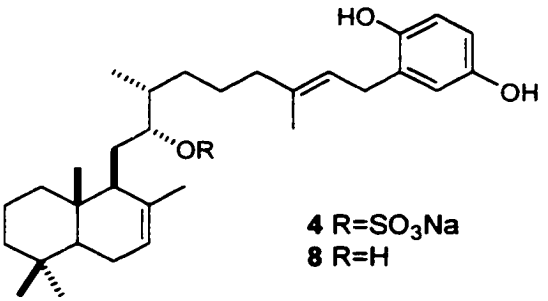



**2b**

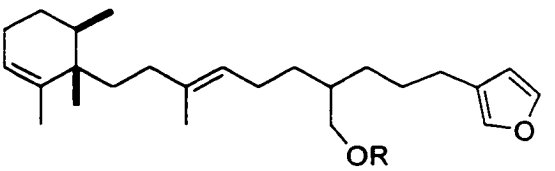
— gCOSY correlations

⌒ gHMBC correlations

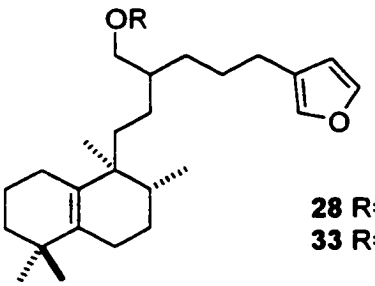
A MarinLit database search of substructures **1a**, **1b**, **2a** and **2b** led to the literature of the halisulfates 1-7 (**4**, **27-31**, **2**), six sulfated sesterterpene furans and one sulfated sesterterpene hydroquinone, which were discovered in a Californian sponge of the family Halichondridae, and from a *Cosinoderma* sp. from Yap, Micronesia.[13, 14] In both cases, the halisulfates were difficult to isolate as pure compounds. To facilitate their separation and characterization, the mixtures of halisulfates were hydrolyzed to produce the easily separable corresponding alcohols (**8**, **32-36**, **6**).



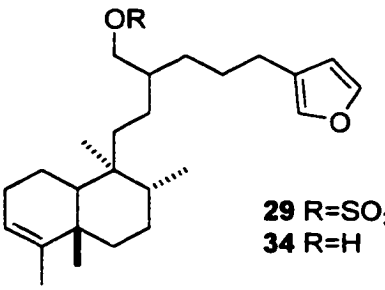

**4** R=$SO_3Na$
**8** R=H



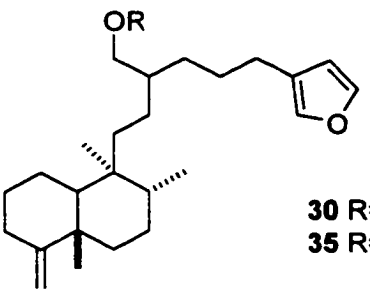

**27** R=$SO_3Na$
**32** R=H



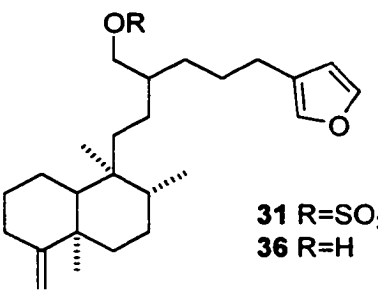

**28** R=$SO_3Na$
**33** R=H



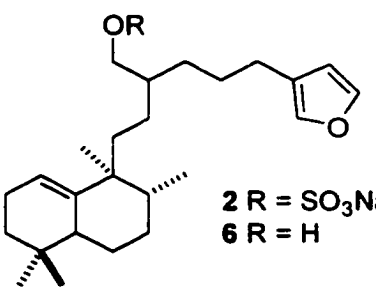

**29** R=$SO_3Na$
**34** R=H




**30** R=$SO_3Na$
**35** R=H




**31** R=$SO_3Na$
**36** R=H




**2** R = $SO_3Na$
**6** R = H

**Figure 10** Negative Ion ESI mass spectrum for halisulfate 7 (2).

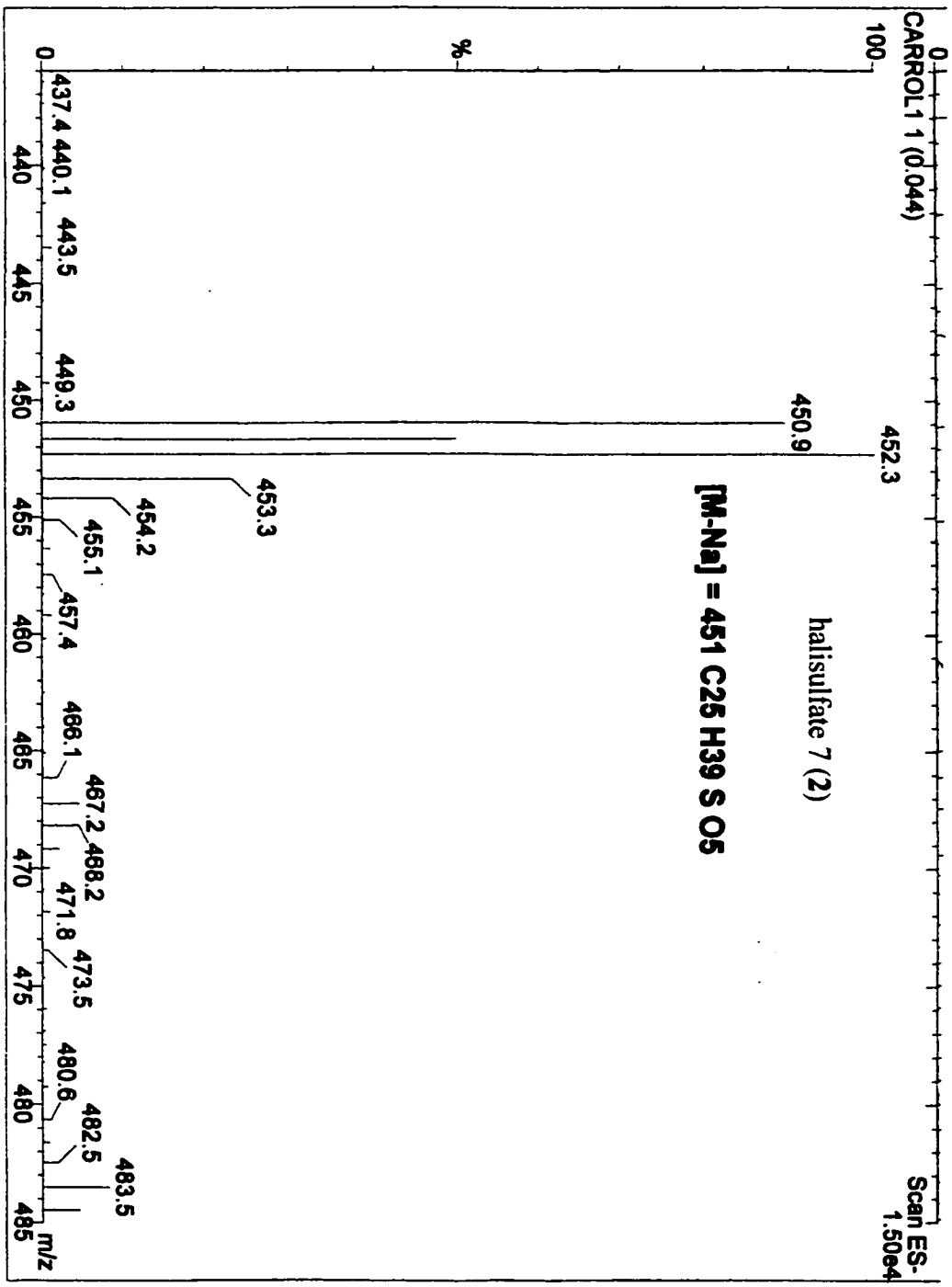


**Figure 11** Proton NMR spectrum for halisulfate 7 (**2**) ($CDCl_3$, 500MHz).

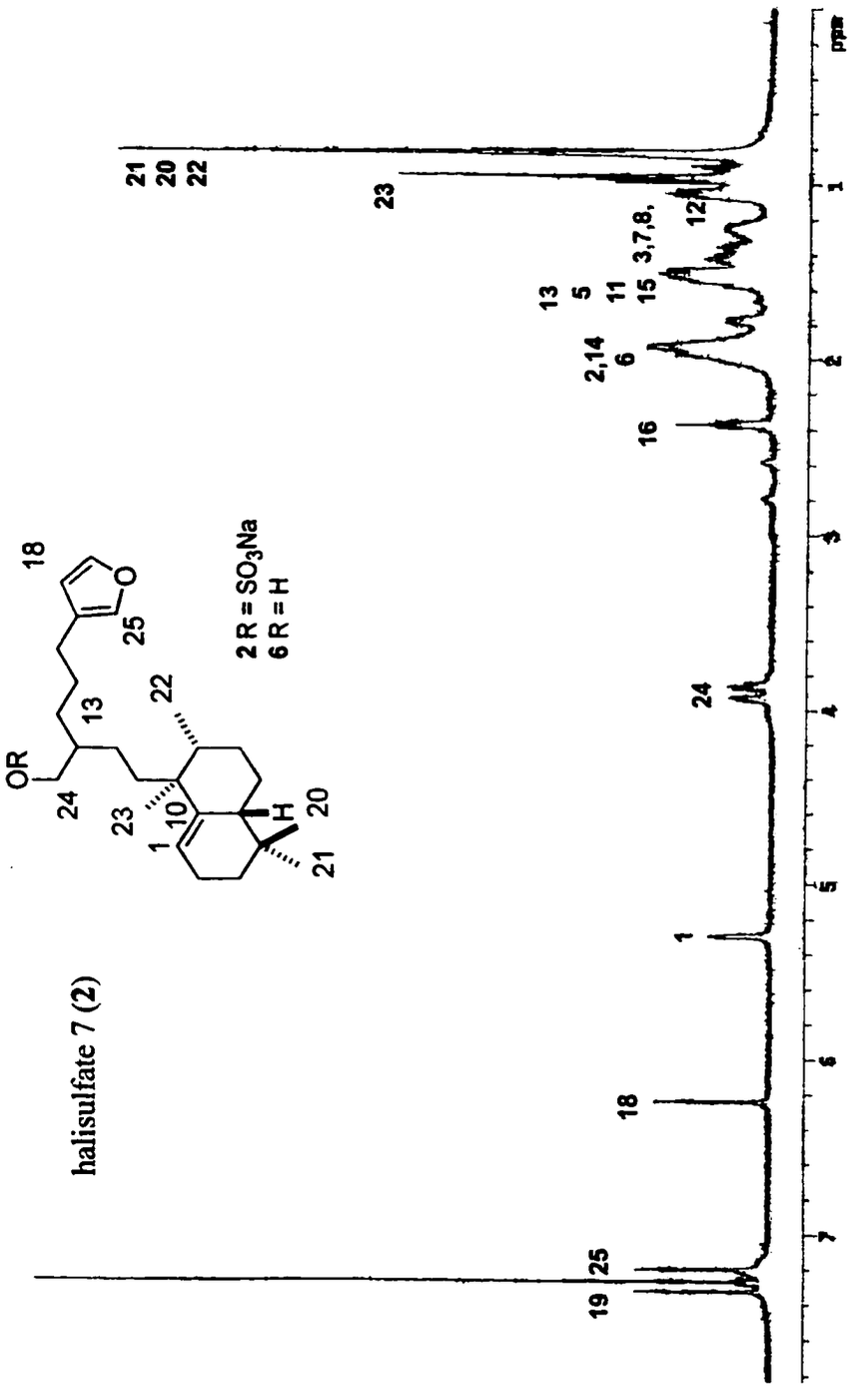


**Figure 12** Carbon NMR spectrum for halisulfate 7 (**2**) ($CDCl_3$, 500MHz).

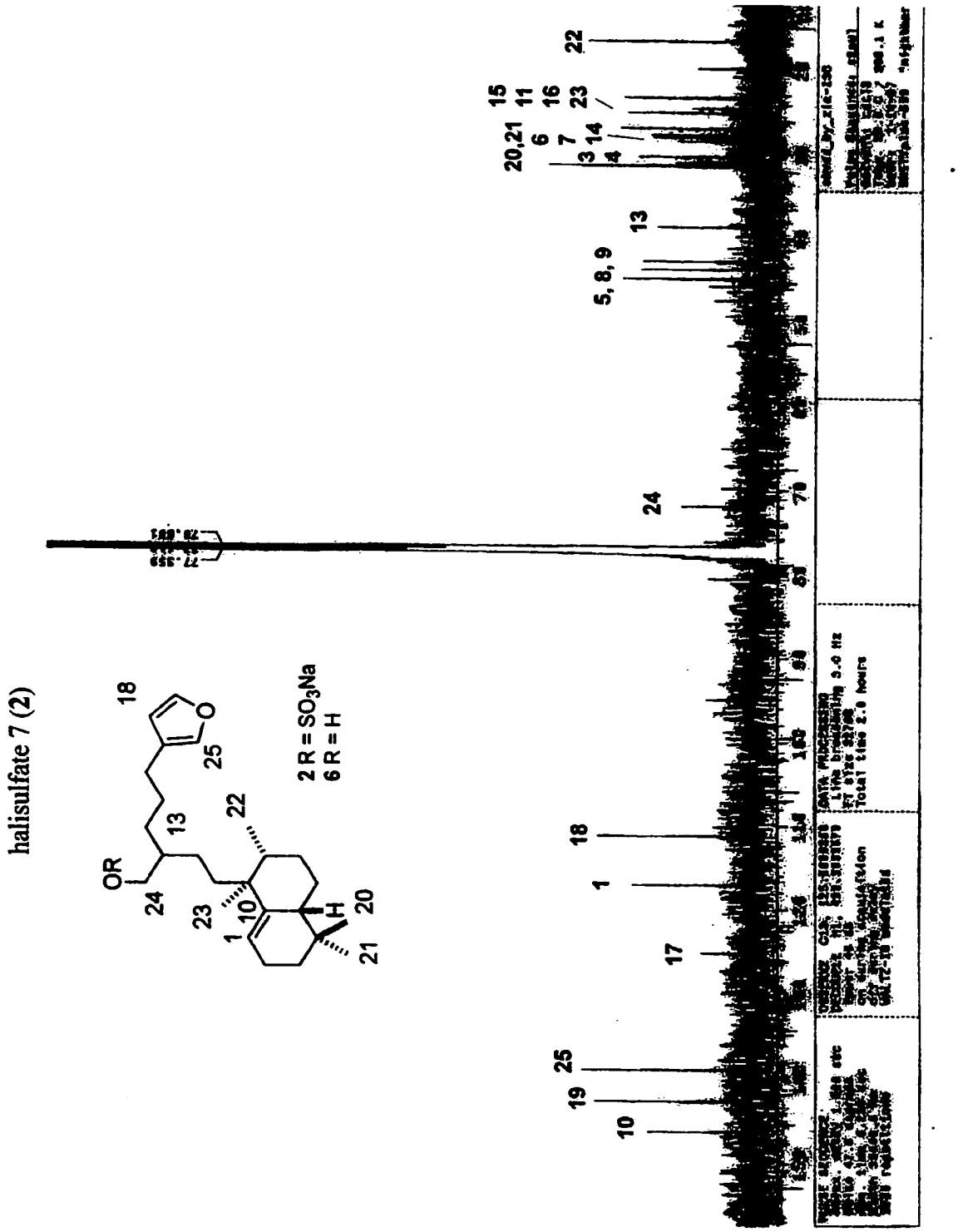


**Figure 13** DEPT NMR spectrum for halisulfate 7 (**2**) ($CDCl_3$, 500MHz).

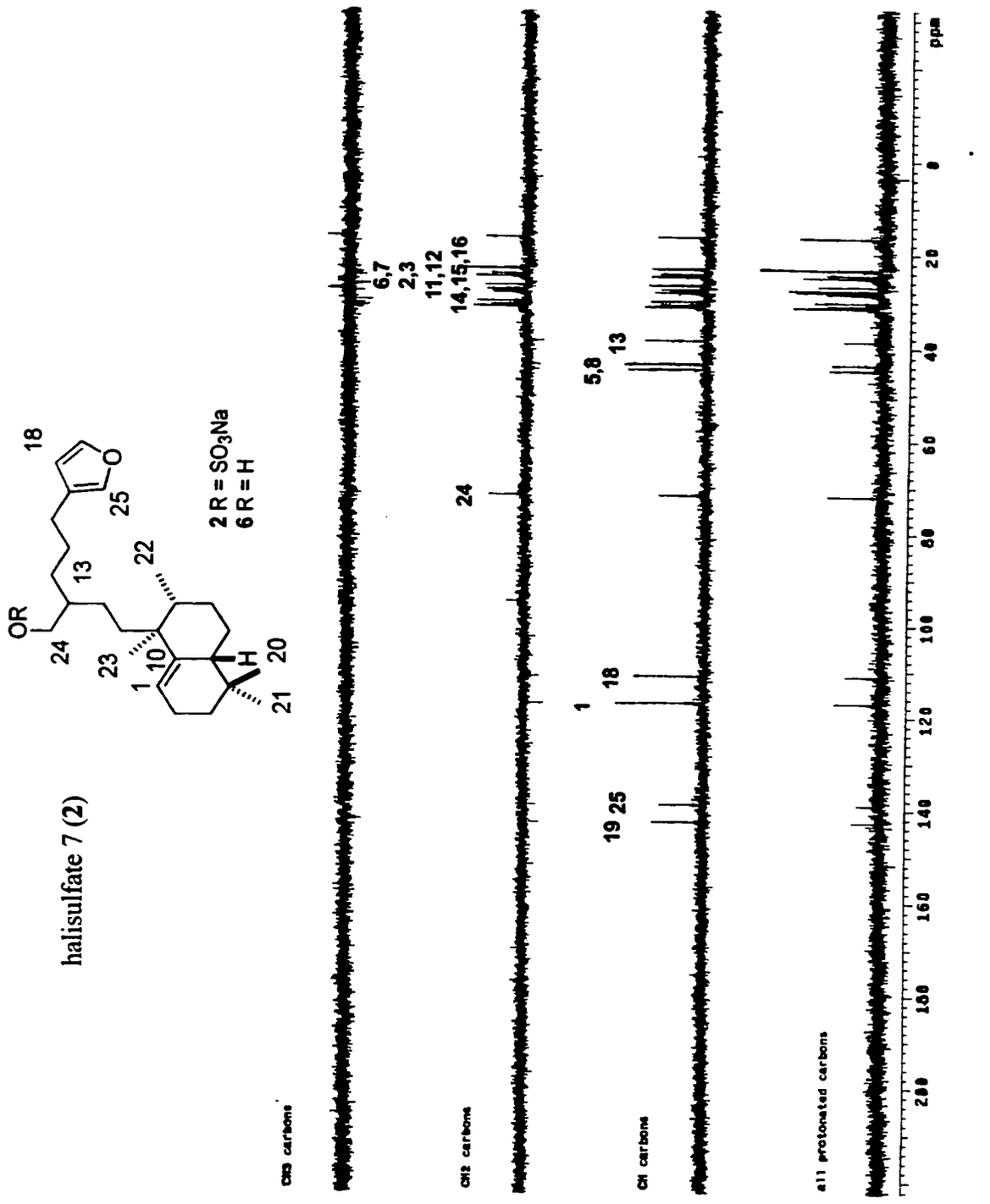


**Figure 14** gCOSY NMR spectrum for halisulfate 7 (**2**) ($CDCl_3$, 500MHz).

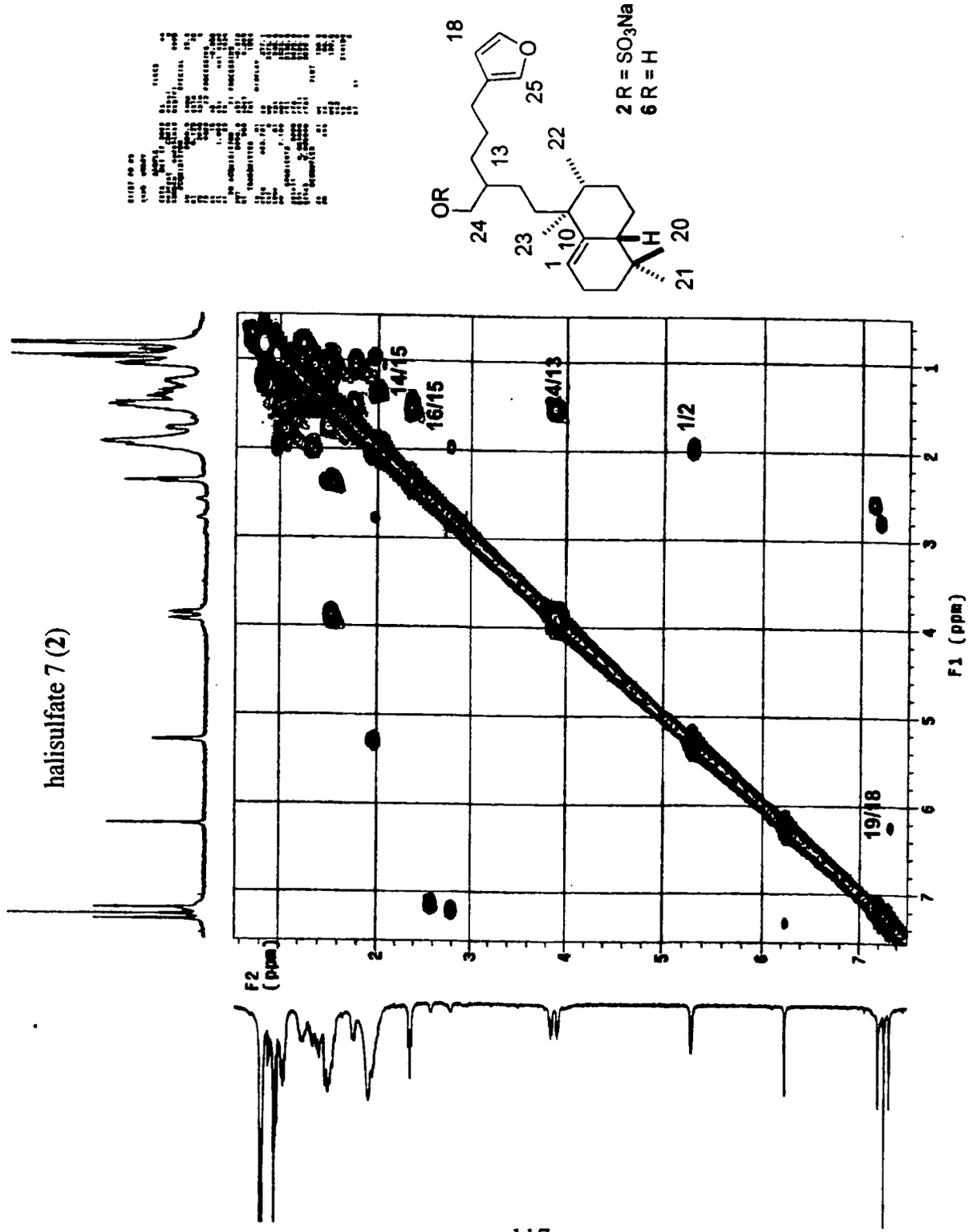
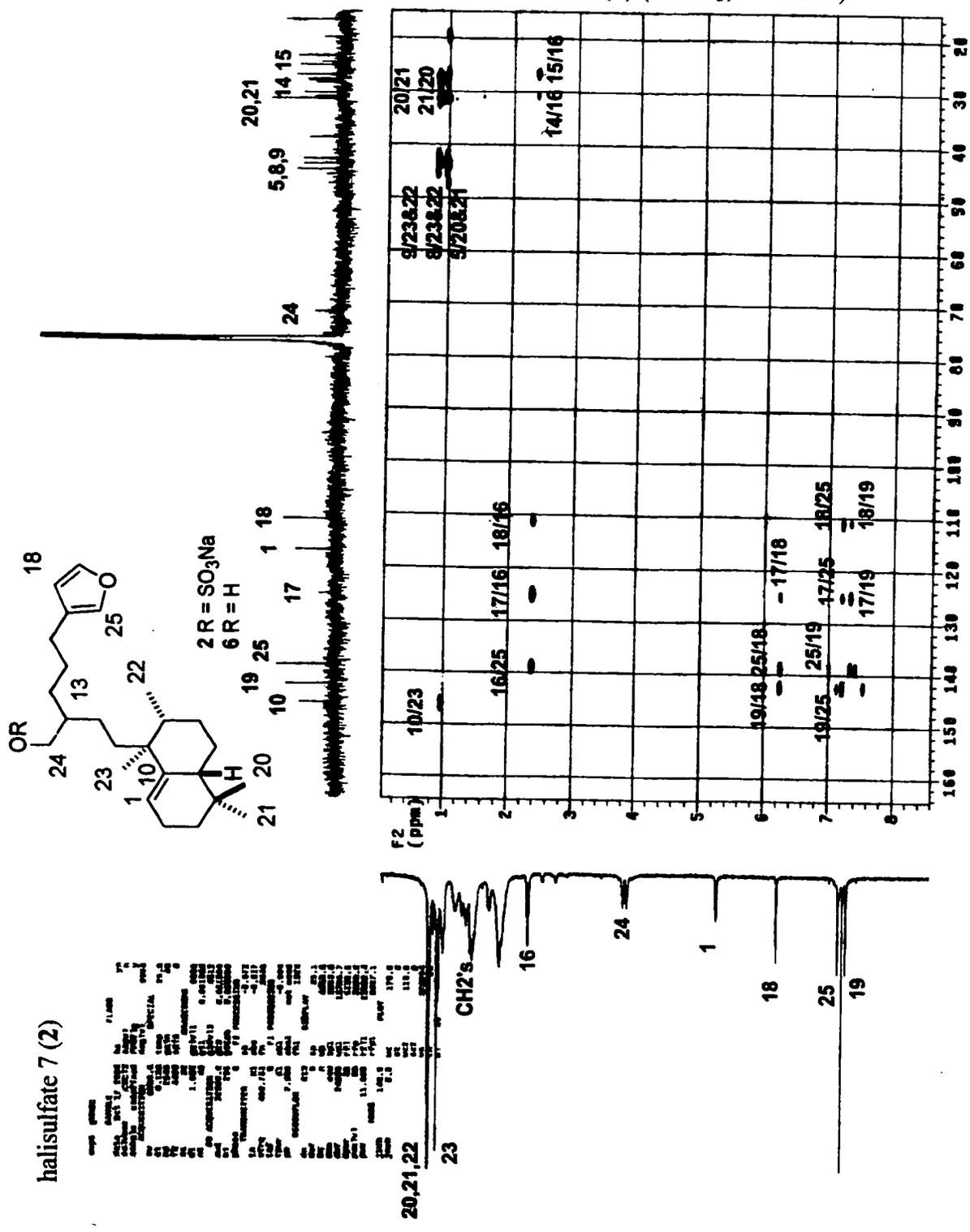
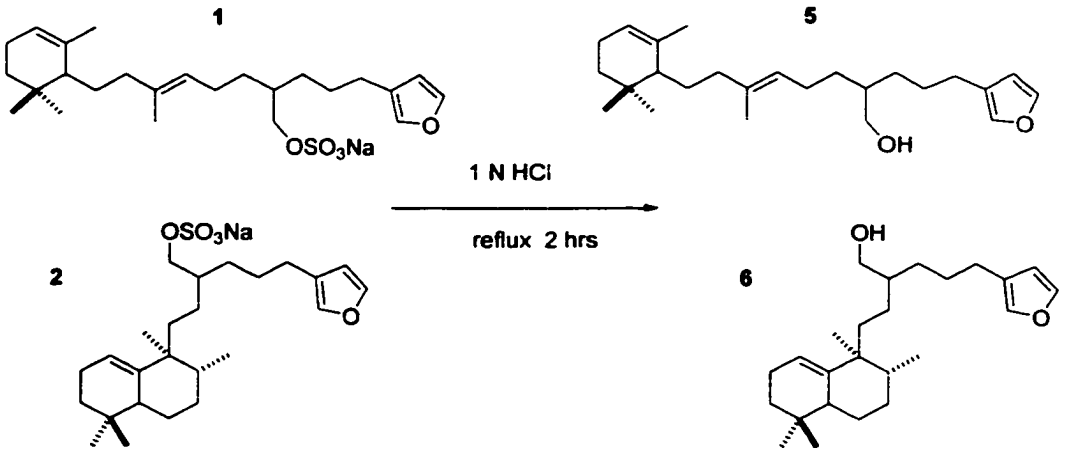


**Figure 15** gHMBC NMR spectrum for halisulfate 7 (**2**) ($CDCl_3$, 500MHz).






After an examination of the literature on sulfonated terpenes, it was decided to hydrolyze the sulfate moieties to alcohols within the crude extract in order to facilitate ease in separation and structural characterization. Treatment of the dichloromethane crude extract (97220FD) with 1N HCl[10] allowed the two corresponding primary alcohols, halisulfate 8 hydrolysis product (**5**) and halisulfate 7 hydrolysis product (**6**) to be easily separated under reverse phase HPLC conditions.

ESIMS data of **5** (Figure 16) showed a molecular ion of $[M+H]^+$ =373 amu indicating a difference of a $SO_3$ group ($\Delta$ 80 amu) from that of **1**. Evidence for the transformation also came from $^1H$ (Figure 17) and $^{13}C$ (Figure 18) NMR, which showed the diastereotopic methylene at position 24 shifted from $\delta$3.88/69.8 ppm in **1** to $\delta$3.52/65.8 ppm in **5**. Furthermore, the remaining connectivity for positions H24/H13 and H8/H7 became evident in the gCOSY experiment (Figures 19 and 20). ESIMS of halisulfate 7 hydrolysis product, **6** also indicated a loss of $SO_3$ with a molecular ion of $[M+H]^+$= 373 (Figure 21). The $^1H$ (Figure 22) and $^{13}C$ (Figure 23)

NMR spectra displayed position 24 at δ3.92/71.8 ppm in **2,** shifted upfield to δ3.55/66.2ppm in **6**, also pointing toward an alcohol moiety.[16]

Comparisons of the $^{13}C$ NMR data of compounds **5** and **6** with that of the literature indicated that these hydrolysis products were both previously known sponge derived compounds. Namely, alcohol **5** was the *Igernella* sp. metabolite igernellin, [17] (Table 1) and alcohol **6** was the hydrolysis product of halisulfate 7 (Table 2), which was isolated recently from a *Coscinoderma* sp.[18] Considering the variety in Spongiidae structural motifs to date, [12, 13] it is not surprising that structure types such as halisulfate 7 and 8 appear within this single sponge sample.

**Figure 16** Positive Mode ESI Mass spectrum of alcohol **5**.

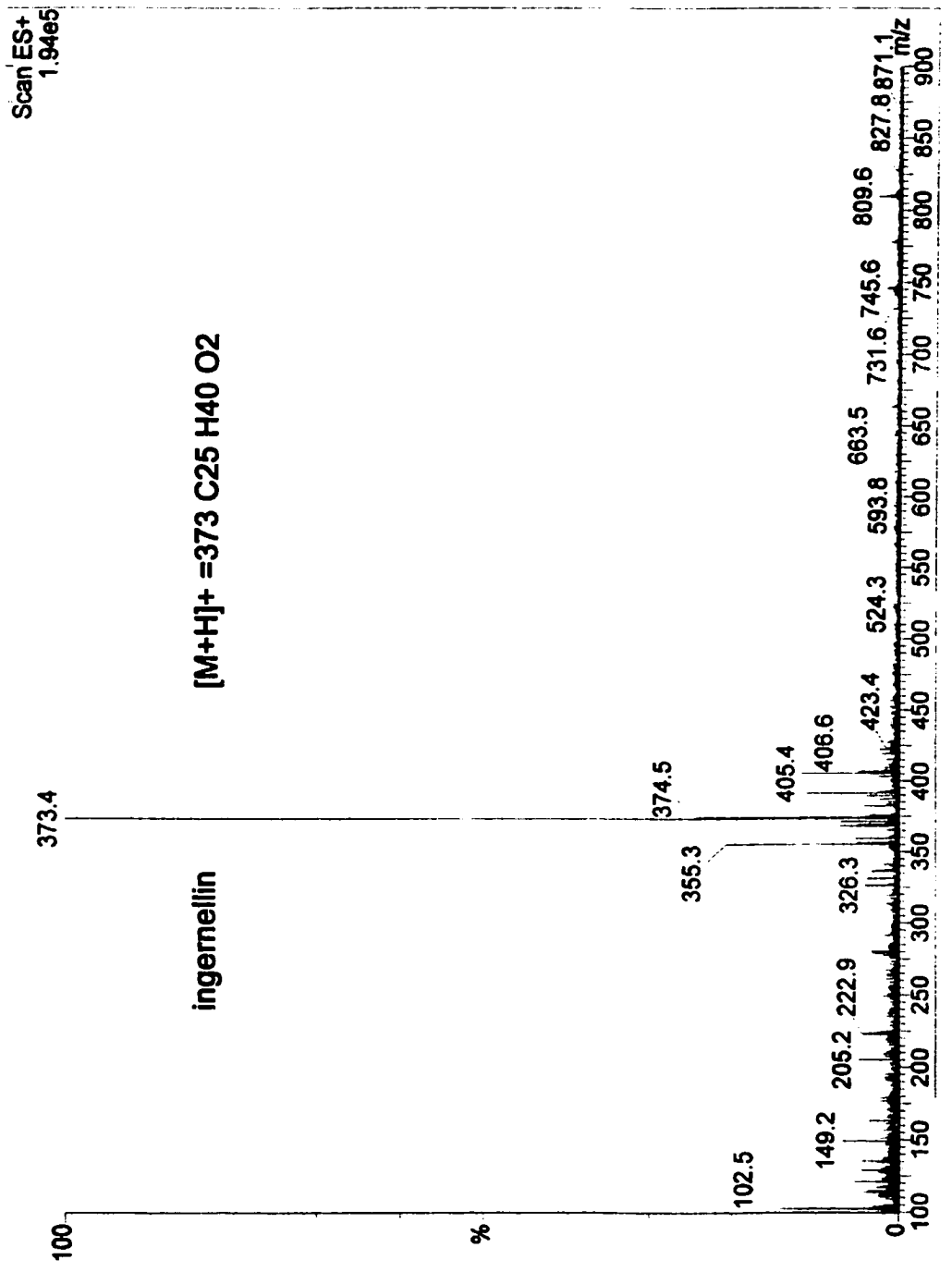


**Figure 17** Proton NMR spectrum of alcohol **5** ($CDCl_3$, 500MHz).

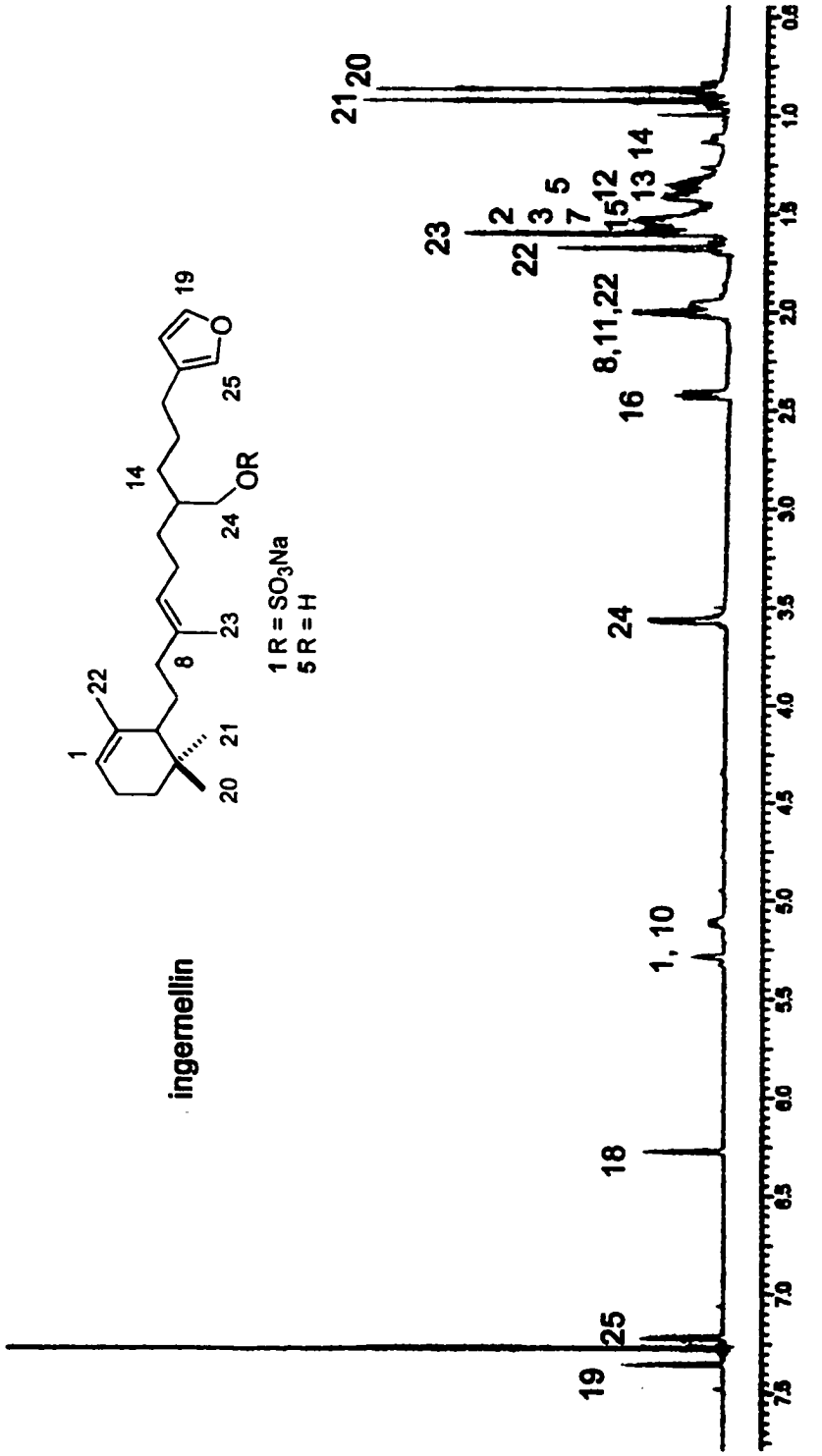


**Figure 18** Carbon NMR spectrum of alcohol **5** ($CDCl_3$, 500MHz).

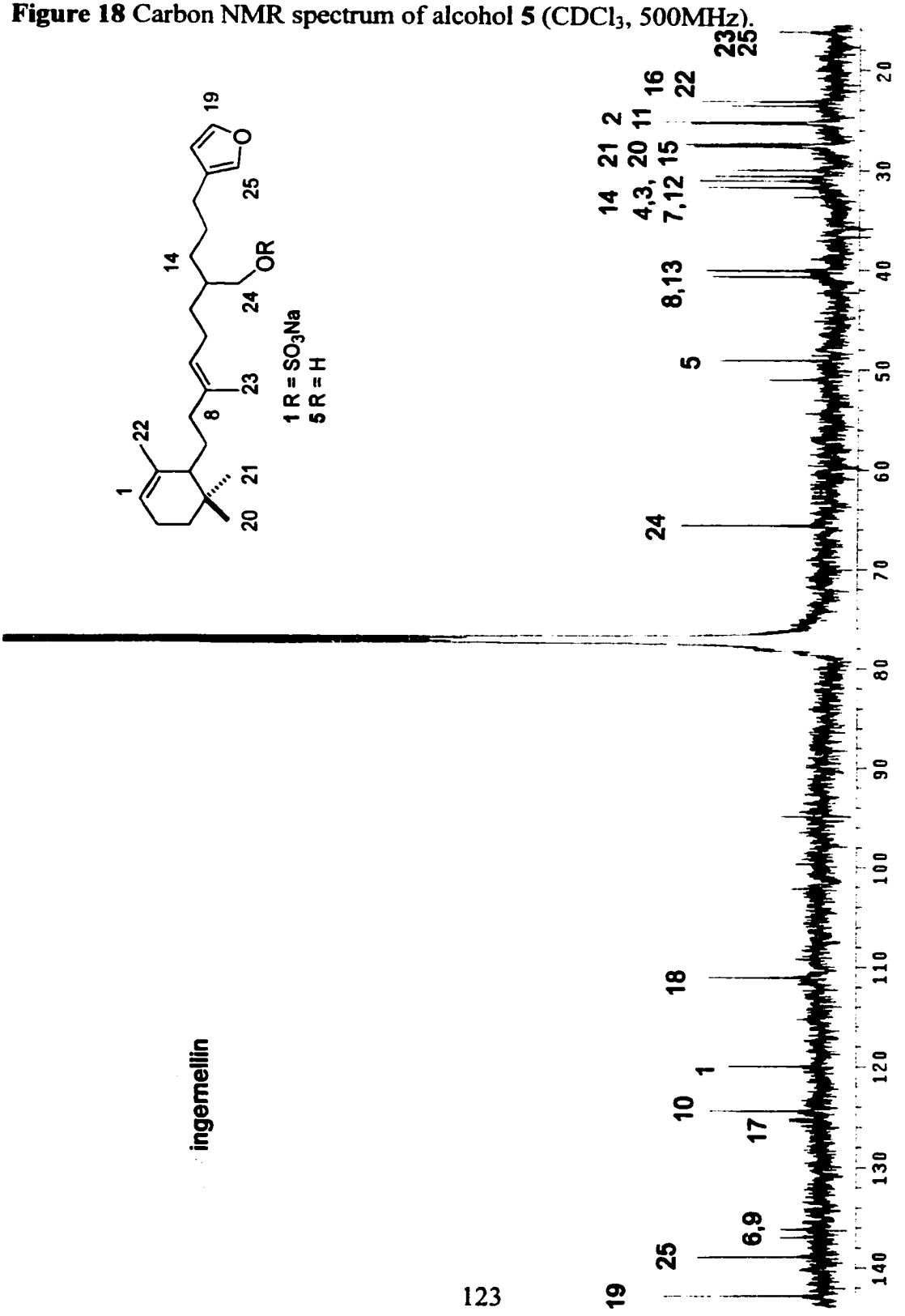


**Figure 19** gCOSY NMR spectrum of alcohol **5** ($CDCl_3$, 500MHz).

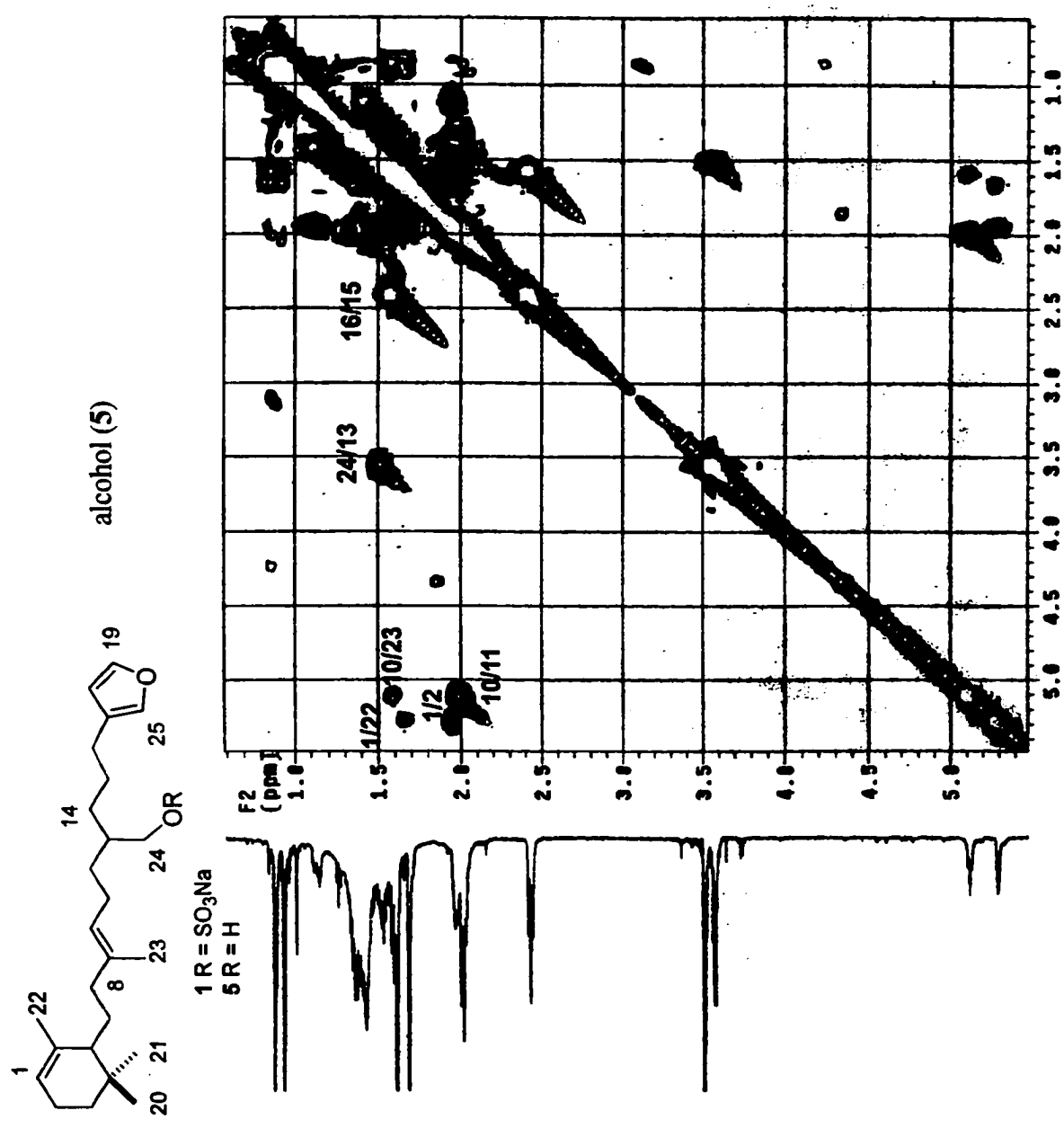


**Figure 20** gCOSY NMR spectrum of alcohol **5** ($CDCl_3$, 500MHz).

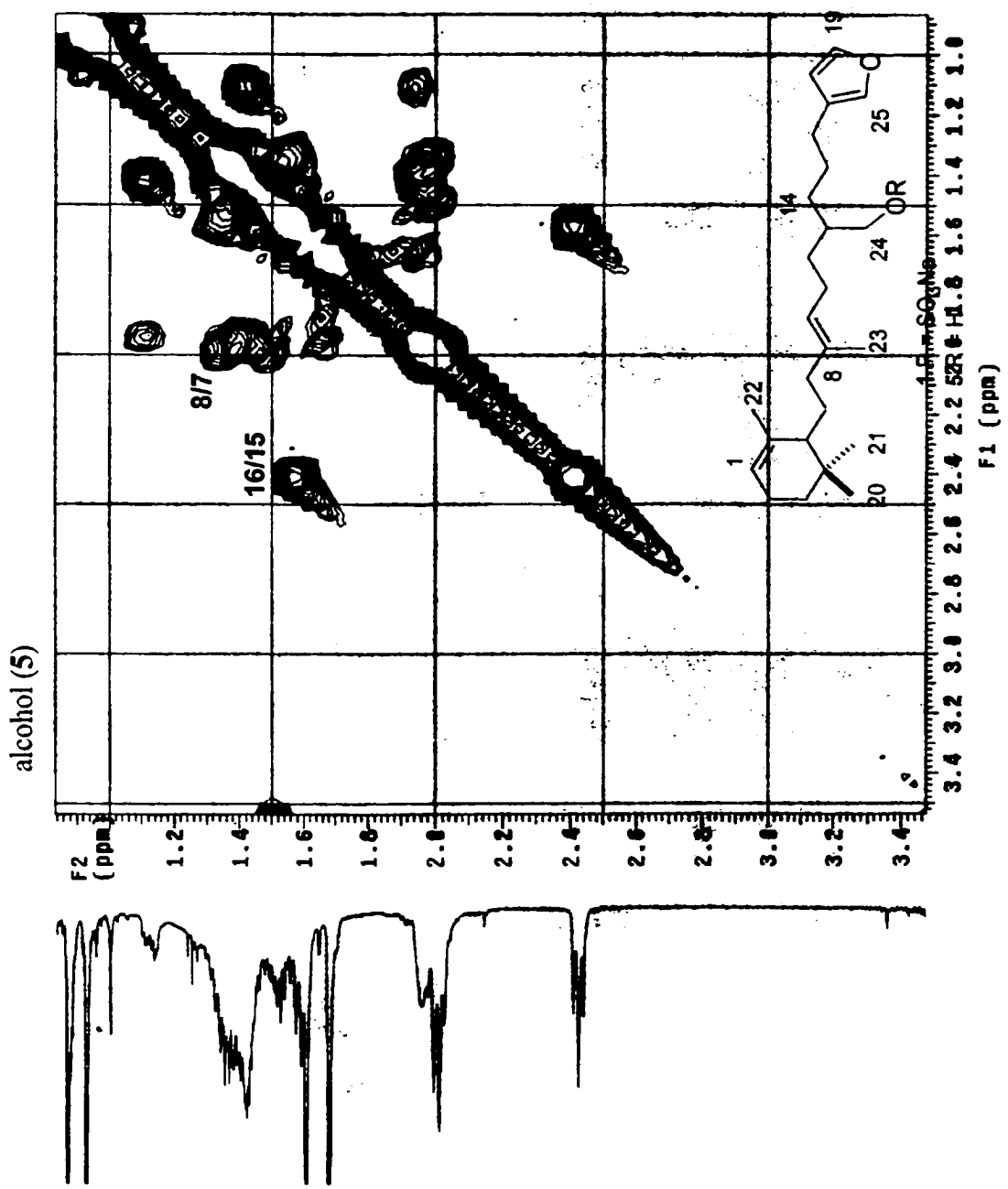


**Figure 21** Positive Mode ESI Mass spectrum of alcohol **6**.

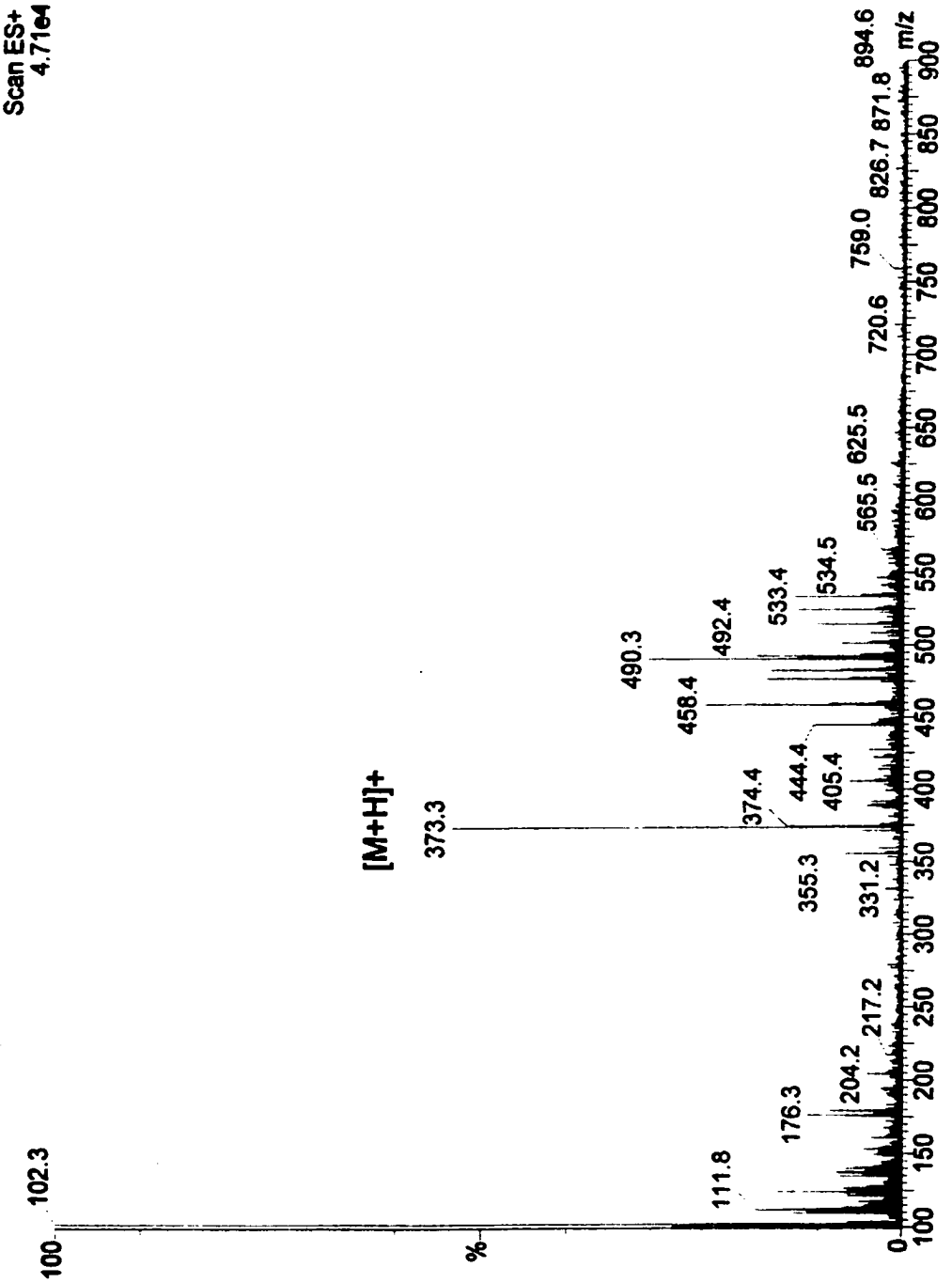


**Figure 22** Proton NMR spectrum of alcohol **6** ($CDCl_3$, 500MHz).

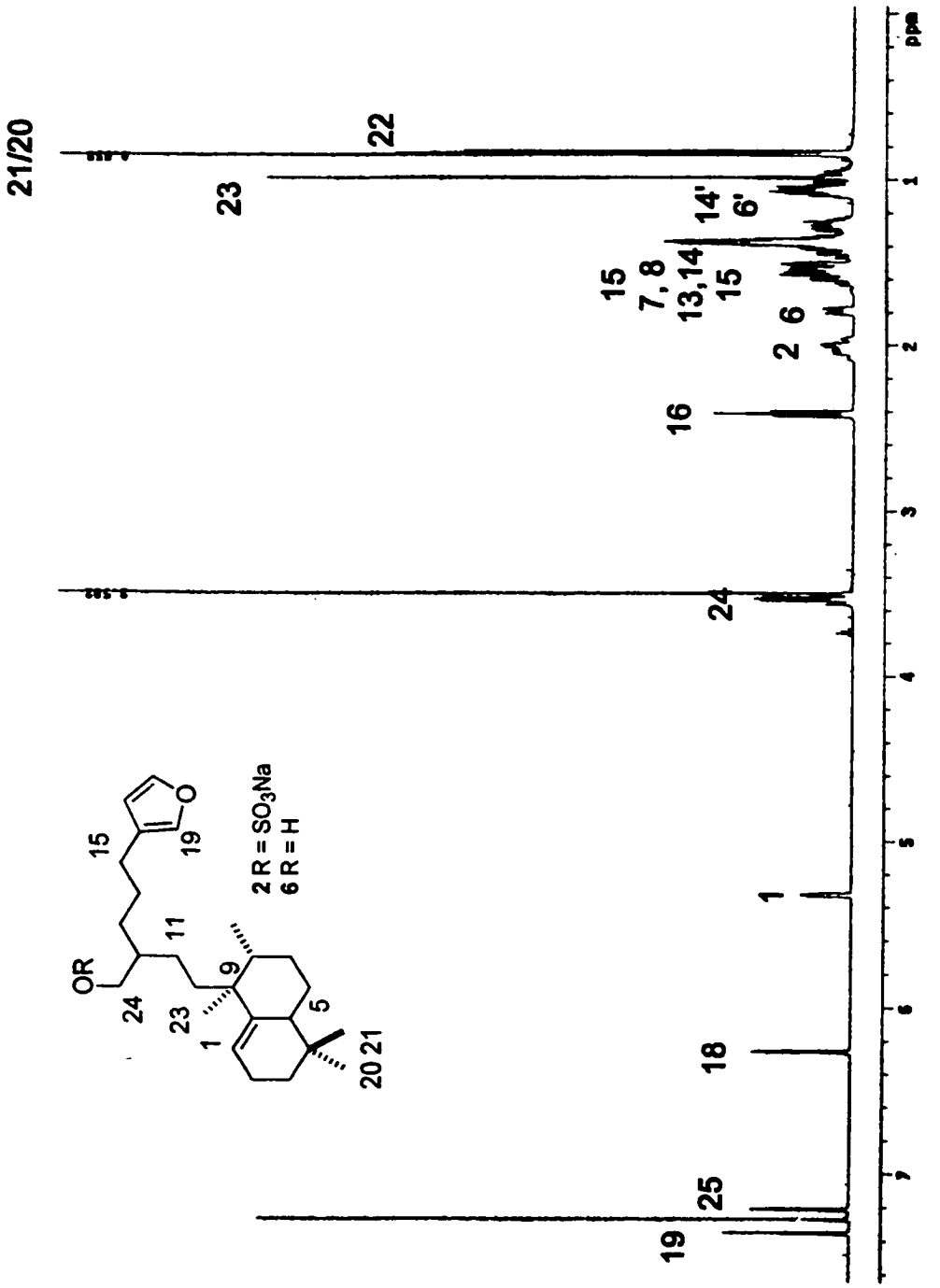


**Figure 23** Carbon NMR spectrum of alcohol **6** ($CDCl_3$, 500MHz).

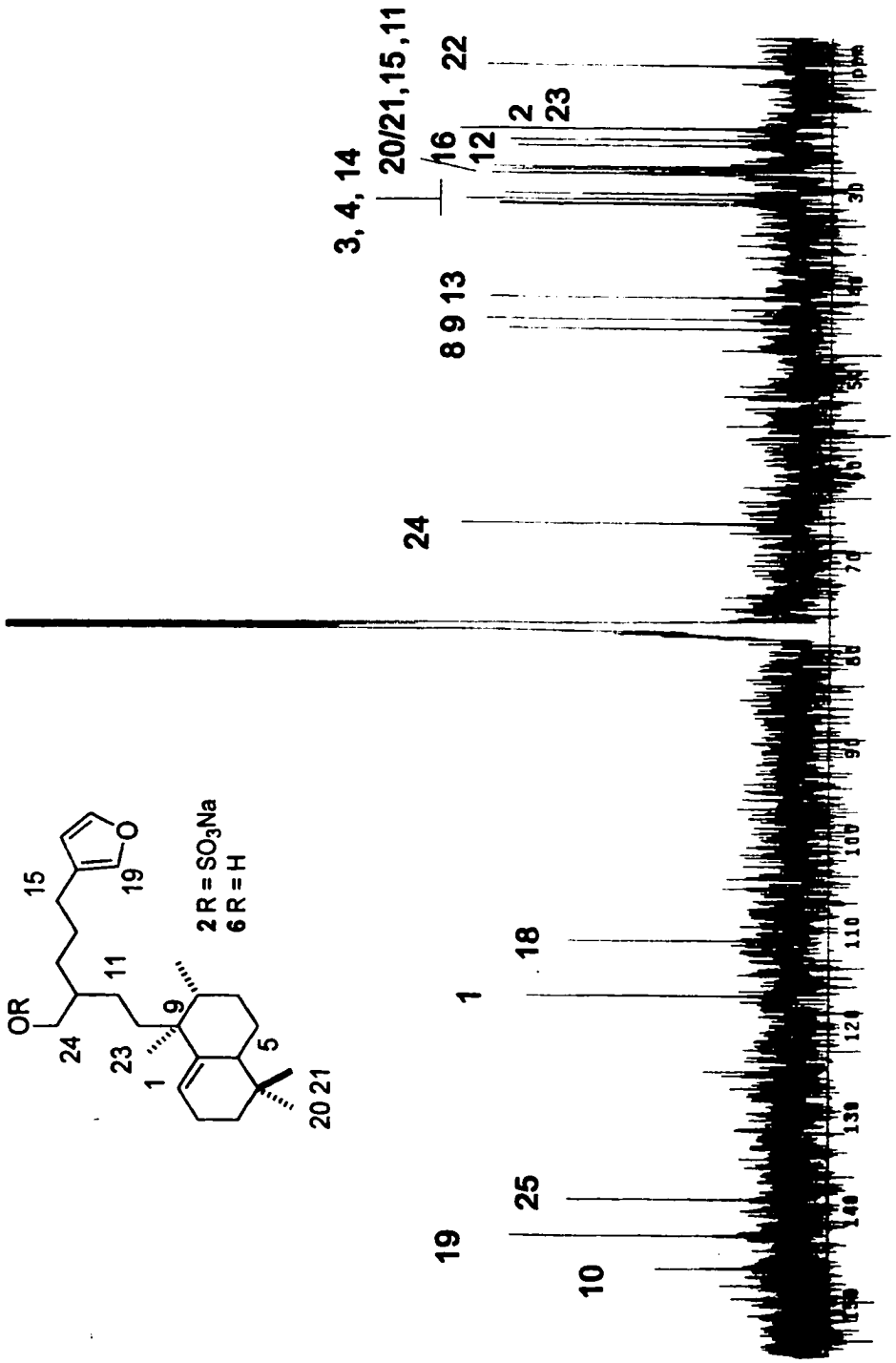


**Table 1.** NMR Data for Halisulfate 8 (**1**) and Its Corresponding Alcohol (**5**) and ingernellin. [a]

| Atom | halisulfate 8 (1) | | alcohol (5) | | ingernellin |
|---|---|---|---|---|---|
| | $\delta_C$ [b] (mult) | $\delta_H$ [c] (mult, *J* Hz) | $\delta_C$ [c] (mult) | $\delta_H$ [c] (mult, *J* Hz) | $\delta_C$ [c] (mult) |
| 1 | 119.4 (d) | 5.22 (bs) | 119.9 (d) | 5.29 (bs) | 119.8 (d) |
| 2 | 25.4 (t) | 1.41 (m) | 25.2 (t) | 1.71 (m) | 25.2 (t) |
| 3 | 31.5 (t) | 1.40 (m) | 31.7 (t) | 1.59 (m) | 31.6 (t) |
| 4 | 32.1 (s) | | 32.4 (s) | | 32.5 (s) |
| 5 | 47.0 (d) | 1.43 (m) | 48.1 (d) | 1.31 (m) | 48.9 (d) |
| 6 | 137.0 (s) | | 136.5 (s) | | 136.8 (s) |
| 7 | 30.3 (s) | 1.31 (m) | 30.0 (t) | 1.50 (m) | 29.8 (t) |
| 8 | 40.3 (t) | 1.94 (m) | 40.7 (t) | 2.01 (m) | 40.6 (t) |
| 9 | 135.4 (s) | | 136.0 (s) | | 136.0 (s) |
| 10 | 124.2 (d) | 5.06 (t,5) | 124.4 (d) | 5.12(t,10) | 124.2 (d) |
| 11 | 25.4 (t) | 1.99 (m) | 25.3 (t) | 2.00 (m) | 25.1 (t) |
| 12 | 31.2 (t) | 1.40 (m) | 31.1 (t) | 1.40 (m) | 30.9 (t) |
| 13 | 37.0 (d) | 1.64 (m) | 40.1 (d) | 1.45 (m) | 39.9 (d) |
| 14 | 30.8 (t) | 1.40 (m) | 30.6 (t) | 1.10 (m) | 30.4 (t) |
| 15 | 26.9 (t) | 1.59 (m) | 27.4 (t) | 1.56 (m) | 27.2 (t) |
| 16 | 24.4 (t) | 2.34 (t,10) | 23.6 (t) | 2.43 (t,7) | 23.8 (t) |
| 17 | 124.2 (s) | | 125.0 (s) | | 125.0 (s) |
| 18 | 110.9 (d) | 6.25 (s) | 111.0 (d) | 6.27 (s) | 110.9 (d) |
| 19 | 142.3 (d) | 7.32 (s) | 142.8 (d) | 7.36 (s) | 142.7 (d) |
| 20 | 26.5 (q) | 0.82 (s) | 27.5 (q) | 0.87 (s) | 27.4 (q) |
| 21 | 26.5 (q) | 0.88 (s) | 27.7 (q) | 0.96 (s) | 27.5 (q) |
| 22 | 22.3 (q) | 1.60 (s) | 23.2 (q) | 1.68 (s) | 23.5 (q) |
| 23 | 15.1 (q) | 1.55 (s) | 16.2 (q) | 1.55 (s) | 16.1 (q) |
| 24 | 69.8 (t) | 3.88 (p,8, 5) | 65.8 (t) | 3.52 (d,5) | 64.5 (t) |
| 25 | 139.0 (d) | 7.20 (s) | 138.9 (d) | 7.28 (s) | 138.8 (d) |

[a] Spectra were recorded at 125 MHz for $^{13}C$ and 500 MHz for $^{1}H$. [b] $CD_3OD$. [c] $CDCl_3$.

**Table 2.** NMR Data for Halisulfate 7 (**2**), Its Corresponding Alcohol (**6**) and the literature.[a]

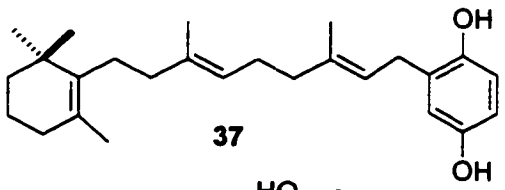
| | halisulfate 7 (**2**) | | | alcohol (**6**) | | |
|---|---|---|---|---|---|---|
| atom | $\delta_C$[b] (mult) | $\delta_H$[b] (mult) | literature | $\delta_C$[b] (mult) | $\delta_H$[b] (mult) | literature |
| 1 | 116.4 (d) | 5.28 (bs) | 116.8 (d) | 119.9 (d) | 5.30 (dd) | 116.7 (d) |
| 2 | 23.0 (t) | 1.98 (m) | 23.2 (t) | 25.2 (t) | 2.00 (m) | 23.2 (t) |
| 3 | 31.4 (t) | 1.07 (m) | 31.3 (t) | 31.7 (t) | 1.08 (m) | 31.3 (t) |
| | | 1.36 (m) | | | 1.36 (m) | |
| 4 | 31.3 (s) | | 31.2 (s) | 32.4 (s) | | 31.2 (s) |
| 5 | 44.1 (d) | 1.49 (m) | 43.6 (d) | 48.1 (d) | 1.50 (m) | 43.6 (d) |
| 6 | 30.1 (t) | 1.76 (m) | 30.1 (t) | 136.5 (s) | 1.78 (m) | 30.2 (t) |
| | | 1.05 (m) | | | 1.04 (m) | |
| 7 | 31.0 (t) | 1.50 (m) | 31.3 (t) | 30.0 (t) | 1.50 (m) | 31.1 (t) |
| | | 1.35 (m) | | | 1.35 (m) | |
| 8 | 44.5 (d) | 1.24 (m) | 44.6 (d) | 40.7 (t) | 1.25 (m) | 44.6 (d) |
| 9 | 42.8 (s) | | 42.5 (s) | 136.0 (s) | | 42.5 (s) |
| 10 | 146.3 (s) | | 146.0 (s) | 124.4 (d) | | 146.1 (s) |
| 11 | 26.4 (t) | 1.57 (m) | 26.7 (t) | 25.3 (t) | 1.00 (m) | 28.0 (t) |
| | | | | | 1.60 (m) | |
| 12 | 24.0 (t) | 1.05 (m) | 23.9 (t) | 31.1 (t) | 1.05 (m) | 24.5 (t) |
| 13 | 38.7 (d) | 1.64 (m) | 37.9 (d) | 40.1 (d) | 1.38 (m) | 41.2 (d) |
| 14 | 29.8 (t) | 1.77 (m) | 29.7 (t) | 30.6 (t) | 1.25 (m) | 30.6 (t) |
| | | | | | 1.45 (m) | |
| 15 | 26.7 (t) | 1.54 (m) | 26.8 (t) | 27.4 (t) | 1.56 (m) | 27.2 (t) |
| 16 | 24.7 (t) | 2.37 (t,8.0) | 24.9 (t) | 23.6 (t) | 2.41 (t,8.0) | 25.0 (t) |
| 17 | 124.2 (s) | | 125.0 (s) | 125.0 (s) | | 125.0 (s) |
| 18 | 111.1 (d) | 6.24 (bs) | 111.0 (d) | 111.0 (d) | 6.26 (bs) | 110.9 (d) |
| 19 | 142.3 (d) | 7.32 (bs) | 142.5 (d) | 142.8 (d) | 7.34 (bs) | 142.6 (d) |
| 20 | 28.8 (q) | 0.82 (s) | 27.8 (q) | 27.5 (q) | 0.84 (s) | 28.0 (q) |
| 21 | 27.5 (q) | 0.83 (s) | 27.5 (q) | 27.7 (q) | 0.85 (s) | 27.5 (q) |
| 22 | 16.1 (q) | 0.81 (d, 7.0) | 16.5 (q) | 23.2 (q) | 0.83 (d, 7.0) | 16.4 (q) |
| 23 | 21.1 (q) | 0.95 (s) | 23.1 (q) | 16.2 (q) | 0.98 (s) | 23.1 (q) |
| 24 | 71.8 (t) | 3.82 (t,8.0) | 71.9 (t) | 66.2 (t) | 3.55 (dd,5.0, | 65.7 (t) |
| | | 3.92 (dd,9.5, 5.0) | | | 8.0) | |
| 25 | 139.0 (d) | 7.21 (bs) | 138.9 (d) | 138.9 (d) | 7.20 (bs) | 138.7 (d) |

[a] Spectra were recorded at 125 MHz for $^{13}C$ and 500 MHz for $^{1}H$. [b] $CD_3OD$. [c] $CDCl_3$.

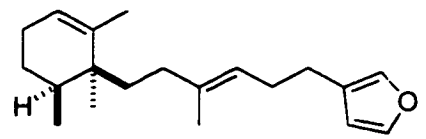
The four compounds mentioned thus far along with other sulfonated terpenes, hipposulfate (**3**), halisulfate 1(**4**), their respective corresponding alcohols **7** and **8**, and the quinone derivative of halisulfate 1 (**9**), obtained from the Crews Laboratory Pure

Compound repository, were screened at Galileo Laboratories. The primary aim of this assay is to prioritize compounds based on the percent of ischemic protection provided at varying concentrations for both cardiomyocyte and hippocampal cell lines. The data shown in Table 4 indicates that sulfate groups do not appear to affect the activity against cardiomyocyte cells. In the case of both **1** and its alcohol **5**, both were inactive at 75 μg/ml. Likewise, halisulfate 1 (**4**) and its alcohol **8** were just slightly active at 3.7 μg/ml and 11ug/ml (<30% protection). The quinone of halisulfate 1 (**4**) almost doubles the percent activity (51% protection at 11 μg/ml), indicating that the redox capability of hydroquinones, as discussed in Chapter Two, does not have an effect in the anti-ischemia assay. Halisulfate 9 (**2**) was moderately protective for cardiomyocyte cells (77 % protection at 25 μg/ml) but offered no protection at 10 μg/ml in the hippocampal cell line.

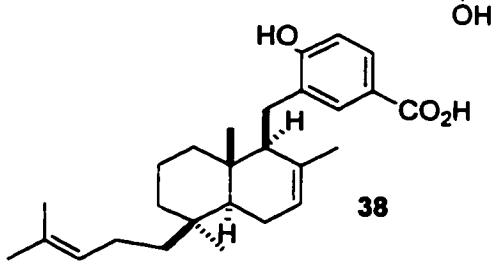
**Table 3** % Activity in Cardiomyocyte and Hippocampal Anoxia Assays



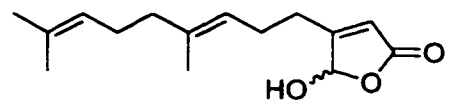

37



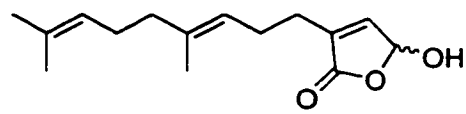

40



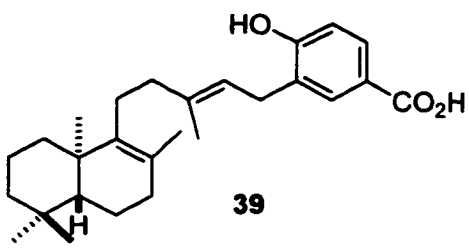

38



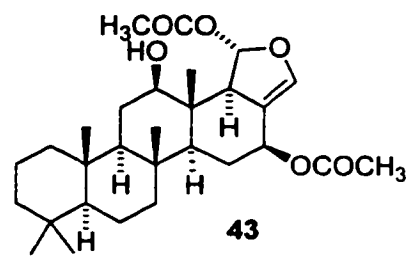

41




42




39




43

| Compound | Cardiomyocyte Activity (dose μg/mL) | Hippocampal Activity (dose μg/mL) |
|---|---|---|
| **1** | n/a (75) | 20 (10) |
| **2** | 77 (25) | n/a (10) |
| **3** | 70 (75) | n/a (10) |
| **4** | 22 (3.7) | n/t |
| **5** | n/a (75) | n/t |
| **6-7** | n/t | n/t |
| **8** | 28 (11) | n/t |
| **9** | 51 (11) | n/t |
| **37** | n/a (75) | 20 (0.1) |
| **38** | 59 (3) | 63 (1.0) |
| **39** | 61 (25) | n/a (10) |
| **40** | 78 (8) | 30 (1.0) |
| **41** | 46 (100) | n/t |
| **42** | n/a (100) | 56 (0.1) |
| **43** | n/a (100) | 21 (0.1) |

n/a = not active, n/t = not enough compound available for testing.

In the course of our studies on bioactive constituents from marine sponges, we have already reported that the terpenoids **37-40** from marine sponges have inhibitory effects against human 15-lipoxygenase enzymes (15-HLO).[19] In order to further examine the mechanism of action of these compounds, terpenoids **37-40** were also tested in the Galileo Assays. In addition, terpenoids **41** and **42**, and heteronemin (**43**) from the Crews lab compound repository were assayed. Interestingly, the most active anti-ischemic compound, jaspic acid (**38**) was an inhibitor of 15-HLO, whereas another highly anti-ischemic compound, subersin (**40**) was previously seen as an activator of 15-HLO enzymes.

This work has shown that the breadth of sulfonated terpenes from *Coscinoderma* sp. sponges includes a number of structures with activity in this novel assay. This includes new natural product halisulfates 8 (**1**), the known compound halisulfate 7 (**2**), synthetically modified ingernelin (**5**), alcohols **6**, **7**, and **8**, and quinone **9**. This information provided by the Galileo screen may lead to a better understanding of ischemic diseases such as heart attack and stroke. Further work in this area would include examination of the UCSC marine natural products repository for further sponges, the primary aim of which would be finding additional sulfonated or even non-sulfonated terpenoids with highly protective affects against oxygen depletion in cells. In addition, over 100 milligrams of subersin (**40**) material for *in vivo* experiments was obtained recently from this sponge. Hopefully these experiments will deepen our understanding of how these terpenoid compounds produce these anti-ischemic effects.

## Experimental

### General Methods

Biotage Si gel or Sephadex LH-20 columns were used for the initial chromatography, Alltima Silica (5μM) for normal phase HPLC, and a Phenomenex 5μ LUNA column was used for RPHPLC. HRMS were measured on a PE Biosystems Mariner and JEOL JMS-AX505HA Mass Spectrometers, and optical rotation data was obtained on a JASCO DIP-370 digital polarimeter. NMR experiments were conducted with a Varian VXR-500 instrument equipped with a 5mm switchable gradient probe. NMR spectra were recorded in $CDCl_3$ and $CD_3OD$ solutions at 500 MHz for $^1H$ and 125.7 MHz for $^{13}C$.

### Animal Material

*Coscinoderma* sp. (order Dictyoceratida, family Spongiidae, collection number 97220: photo Figure 28) was collected at a depth of 20 meters near Madang, Papua New Guinea (S 05°01.528' / E 145°48.131'). It was described as a ramose grayish sponge, yellow internally, with a regularly conulose surface (conules 1 mm high, 2-3 mm apart) with a rubbery consistency. The skeleton consisted of a very homogeneous network of clear fibers (20-25μm in diameter), where only secondaries were distinguished. The surface was packed with sand and foreign material. This specimen had typical Spongiidae fibers. Although primaries cored with sediments were not observed, the predominance of a secondary clear reticulation, the sand cortex and overall sponge morphology pointed to *Coscinoderma*.[20] Bergquist

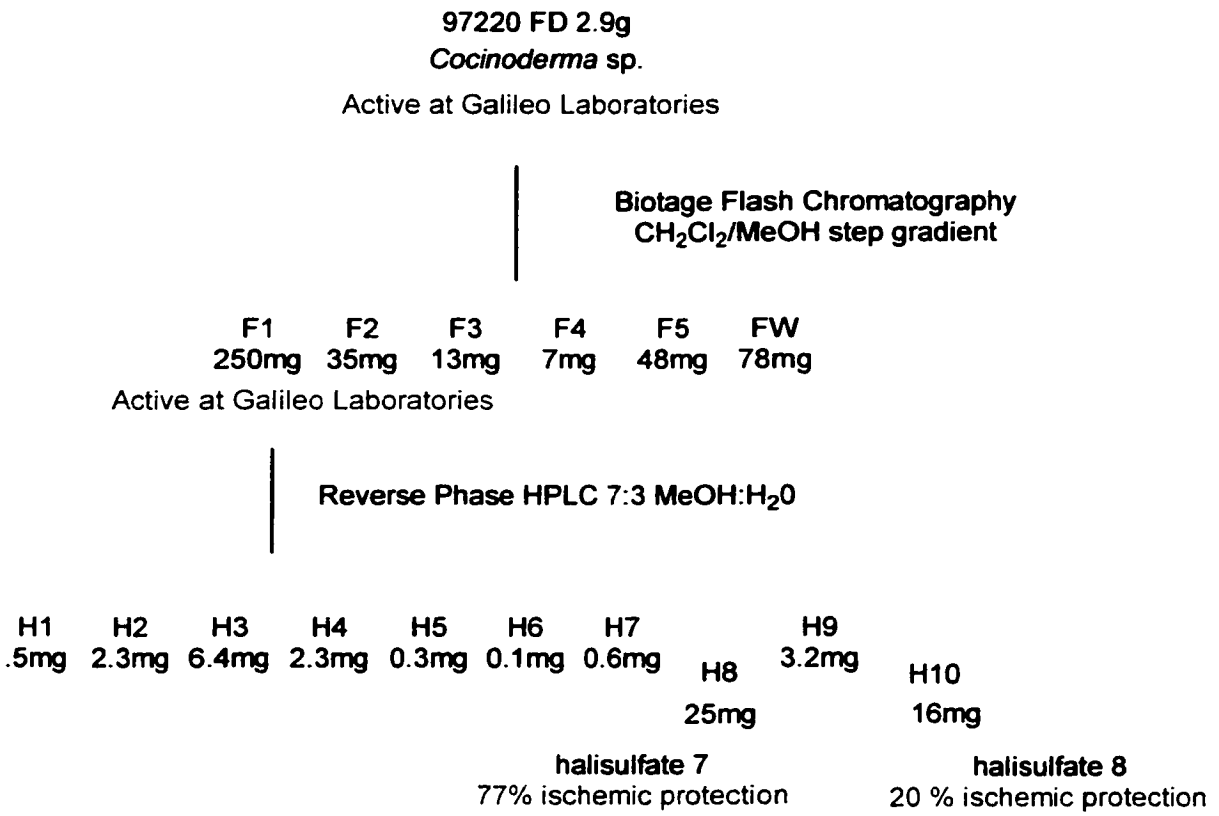
describes how the secondaries usually make up the bulk of the skeleton in this genus,[21] so here it is considered that primaries might be so low in abundance that they were not observed in the fragments studied.

**Figure 24** Underwater Photo of *Coscinoderma* sp.

**Extraction and Isolation**

The sponge specimen was returned to UCSC where it underwent extraction. As shown in Scheme 1, the bioassay-guided fractionation indicated that the active components were concentrated in the methylene chloride extract of 97220. Step gradient column chromatography (methylene chloride/methanol) of 97220 gave four fractions, the first of which showed increased activity in the Galileo assay. This was further purified by RPHPLC using a methanol/water gradient to give 16mg halisulfate 8 (**1**) and 25mg halisulfate 7 (**2**).

**Scheme 1** Isolation of halisulfate 8 (**1**) and halisulfate 8 (**2**).



**(-) Halisulfate 8 (1)**. Colorless oil $[\alpha]_D$ = -68° (*c* 2.3 MeOH) ; $^1H$ NMR and $^{13}C$ NMR see Table 1; HRFABMS *m/z* $[M-Na]^-$ = 451.2508 (calcd for $C_{25}H_{39}O_5S$, 451.2518).

**Halisulfate 7 (2)**. Colorless oil $[\alpha]_D$ = -68° (*c* 2.3 MeOH) ; $^1H$ NMR see Figure 11, $^{13}C$ NMR see Figure 12 and Table 2; LRESIMS *m/z* $[M-Na]^-$ = 450.9 see Figure 10.

**Hydrolysis of (1) and (2)**. 0.2mL of 3N HCl was added to 200 mg of crude *Cocinoderma* sp. extract (coll # 97220) in 20 mL methanol. Mixture was refluxed for 2 hours. After cooling, the mixture was extracted with methylene chloride, then washed (3x) with DI water, dried with anhydrous sodium sulfate and purified on a flash column with 100% $CH_2Cl_2$, then by RPHPLC using methanol-water gradient.

**Alcohol 5**. Clear oil, $[\alpha]_D$ = +60° (*c* 0.27 MeOH) ; $^1H$ NMR and $^{13}C$ NMR see Table 1. LRESIMS see Figure 16.

**Alcohol 6**. Clear oil, $[\alpha]_D$ = -109° (*c* 0.27 MeOH) ; $^1H$ NMR and $^{13}C$ NMR see Figures 22 and 23 and Table 2. LRESIMS see Figure 21.

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## Chapter 4. A Reinvestigation of the Microfilament and Microtubule Active Constituents of *Cacospongia mycofijiensis*.

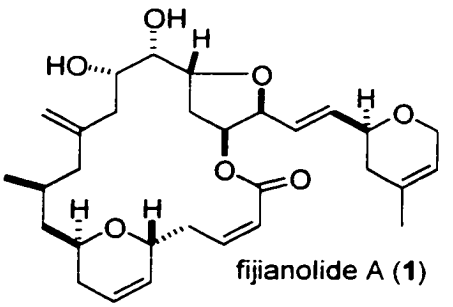
### Background

During the last decade the search for small molecule microtubule and microfilament inhibitors has intensified. The realization that such properties are associated with paclitaxel, introduced in 1982 as a potent anticancer agent, was major stimulus for this interest. There is also much interest concerning the chemistry and biosynthesis of the highly active marine microtubule inhibitors, fijianolide A[1] (also known as isolaulimalide[2]) (**1**) and fijianolide B (also known as laulimalide) (**2**) The $IC_{50}$ values reported for these paclitaxel-like microtubule inhibitors are in the low micromolar and nanomolar range respectively.[3] Moreover, their polyketide structural framework would be ideal for further manipulation of the biosynthetic polyketide synthase (PKS) gene code that was described in Chapter 1.

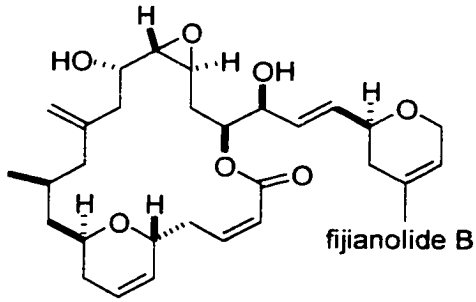
The goal of this work was to utilize the microtubule assay screening system developed by Dr. Susan Mooberry at the Southwest Foundation for Biomedical Research in San Antonio, Texas to search out new microtubule inhibitors from a recent Vanuatu collection of the *Cacospongia mycofijiensis* available in our repository. Mooberry's assay has been key in the discovery of a number of novel compounds that inhibit actin and tubulin.[4] Moreover, we wanted to verify that the Vanuatu collections of this sponge continued to produce fijianoldies so that DNA isolation work could lead to future biosynthetic PKS manipulations of these

structures. During the course of this research, a new fijianolide was discovered which inhibited microtubule formation in cells. We also obtained assay data on a number of crude extracts of this sponge which showed microfilament and microtubule inhibition (Scheme 1, Experimental Section) and will be the focus of a future study.

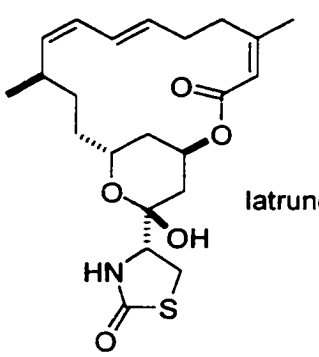
The sponge *Cacospongia mycofijiensis*, common throughout the Indo Pacific, was given the common name "mushroom sponge" because of its often button-like appearance. Already known from the literature are a number of compounds from the mushroom sponge that have interesting biological activity. In addition to fijianolide A (isolaulimalide **1**), and fijianolide B (laulimalide **2**), there are latrunculin A (**3**), mycothiazole (**4**), and dendrolasin (**5**). The incidence of these compounds varies depending upon the location of the sponge collection. Previous work in our laboratory has seen the occurrence of laturnculin A from sponges collected in Fiji, the Solomon Islands and Papua New Guinea, whereas sponges collected in Vanuatu and Indonesia contain latrunculin A, micothiazole and fijianolides.[5] Moreover, dendrolasin has only been isolated from species collected in Fiji.



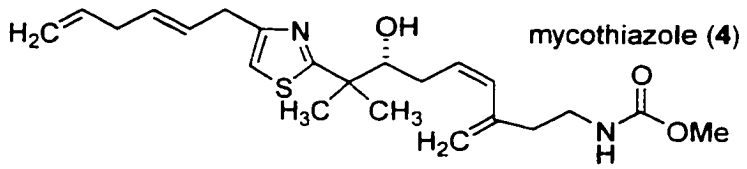
fijianolide A (**1**)



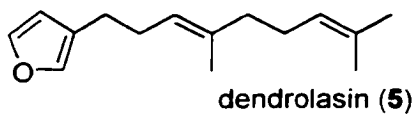
fijianolide B (**2**)



latrunculin A (**3**)



mycothiazole (**4**)

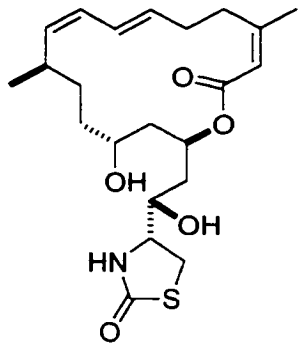


dendrolasin (**5**)

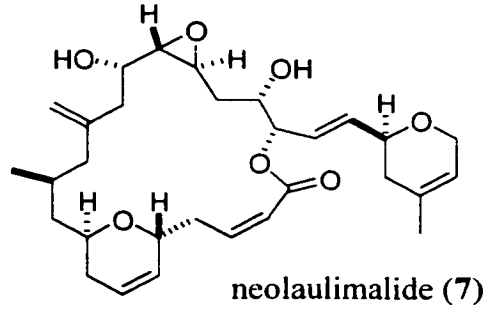
Additional sources for latrunculin A and dendrolasin have been seen in widely differing species. The original source of **3** and its 14-membered ring analogue, latrunculin B, was from a taxonomically distant sponge *Negombata magnifica* (formally *Latrunculia magnifica*) in the Red Sea.[6] The production of latrunculin A as an unusual toxin by two such distinct taxonomically and geographically remote species is unusual and not understood at this point. One rational could be the involvement of a common microbial fauna living within these sponges. Another source of **3** is from the nudibranchs *Chromadoris lochi*, *C. elizabethina*,[1] *C. williani*, and *Glossidoris quadricolor*[7] found in association with and grazing on the mushroom sponge.[8] Interestingly, dendrolasin has been previously found in the sponge

*Oligoceras hemorrhages*,[9] the wood oil of *Torreya nucifera*,[10] and the ant *Lasius (Dendrolasius) fulginosus*.

Other sources of latrunculin A and the fijianolides are the sponges characterized as *Fasciospongia rimosa* and *Dactylospongia* sp. In addition to the known compounds **1-3**, three new compounds have arisen from the former sponge collected off Okinawa; latrunculin S (**6**), neolaulimalide (**7**)[11] and zampanolide (**8**).[12] The *Dactylospongia* sp. collected in Vanuatu gave the new cyctotoxic macrolide dactylolide (**9**) and the known compounds **1-4**.[13] It is likely, because of the continuing occurrence of latrunculins and fijianolides within these samples, that they are the same species.[14]

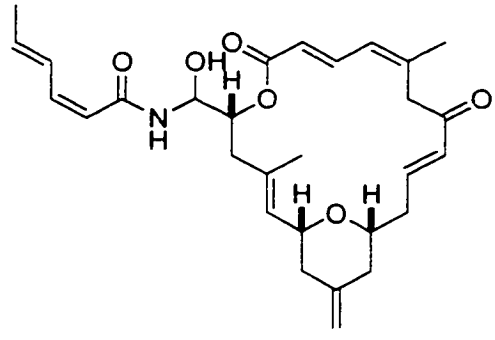


latrunculin S (**6**)

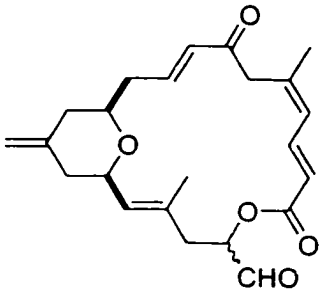


neolaulimalide (**7**)

zampanolide (**8**)



dactyloide (**9**)




The total synthesis of mycothiazole (**4**) was achieved by Shioiri's group in Nagoya, Japan.[15] The absolute stereochemistry of the alcohol moiety was determined to be (*R*) by analysis of the MTPA esters of a synthetic intermediate and by a comparison of the optical rotation data. The MTPA esters that I reported on in early 1996[16] indicated (*S*) stereochemistry. However, on further analysis it appears that the (*S*)-MTPA ester was not characterized adequately. An analysis of the MTPA esters of the natural product needs to be produced to support Shioiri's results.

The cellular activity of *Cacospongia mycofijiensis* compounds continues to be of interest. For example, latrunculin A (**3**) is used extensively as an agent to sequester monomeric acitn in living cells.[17] The unique, monomeric, actin-binding properties of **3** and its effects on the cytoskeletal matrix have been studied extensively.[18,19] Since latrunculin A's discovery in 1983, 166 references have been published on its unusual impacts on cellular structure.[20] The isolation of additional derivatives of latrunculin would serve to expand the knowledge of this important actin disruptor.

Also important is the microtubule-stabilizing effect of marine agents found from the mushroom sponge and their role in the discovery of new anti-cancer drugs.

Microtubules are proteins consisting of α and β-tubulin subunits. These tubules provide the structural framework for cell division. Most of the known microtubule inhibitors (vinca alkaloids or colchicines)[21] inhibit the tubulin polymerization into microtubules. Paclitaxel was the first compound found to stabilize preformed microtubules, and at higher concentrations, promote tublin polymerization.[22] The discovery of this effect has prompted the search for a new generation of paclitaxel-like anticancer agents.

The aquatic environment is the primary source of novel microtubule inhibitors. Highly active compounds include the epothilones, eleutherobin, the fijianoldies, and the milnamides. At low, nanomolar concentrations ($IC_{50}$), these stabilize microtubules, and at higher, micromolar concentrations, promote tubulin polymerization ($EC_{50}$). Microtubule stabilization at low concentrations is followed by abnormal mitotic spindle formation, which leads to G2/M mitotic arrest and eventually apoptosis.[22] Shown in Table 1 are the $IC_{50}$ and $EC_{50}$'s of these agents relative to paclitaxel (PTX). Epothilone B, isolated from a myxobacteria, shows the greatest microtubule stabilization effects ($IC_{50}$ = 0.2nM), whereas discodermolide from a marine sponge, at micromolar concentration is the strongest inhibitor of tubulin depolymerization ($EC_{50}$ = 1.3μM). Epothilone B, discodermolide and elutherobin also show inhibition of binding of paclitaxel to microtubules ($K_i$(PTX) > 1), which is suggestive of a competitive binding site for these compounds.[23] Fijianolide B (**2**) is somewhat less potent, with $IC_{50}$ and $EC_{50}$'s of 5.7 nM and 4.3 μM

respectively.[16] Interestingly, in the multidrug resistant cell line SKVLB, **2** is 800-fold more potent than paclitaxel.[16] Other sponge derived compounds, adociasulfate-2 and milnamide B are moderately good inhibitors of microtubule bundling ($IC_{50}$'s = 2.7nM and 2.5nM), but their tubulin polymerization effects ($EC_{50}$) and $K_i$(PTX) have yet to be determined.[17,18]

**Table 1** Activity of Microtubule-stabilizing agents.[24]

| Compound | Source | $IC_{50}$ (nM)† | $EC_{50}$ (μM)‡ | $K_i$(PTX) (μM)# |
|---|---|---|---|---|
| paclitaxel | *Taxis brevifolia* | 1.0 | 9.4 | NA |
| epothilone B | *Sorangium cellulosum* | 0.2 | 2.7 | 1.7 |
| discodermolide | *Discoderma dissolute* | 2.8 | 1.3 | 1.7 |
| elutherobin | *Elutherobia* sp. | 14 | 11 | 2.1 |
| fijianolide B (**2**)[25] | *Cacospongia mycofijiensis* | 5.7 | 4.3 | ND |
| adociasulfate-2* [26] | *Haliclona* sp. | 2.7 | ND | ND |
| milnamide B[27] | *Auletta* cf. *constricta* | 2.5 | ND | ND |

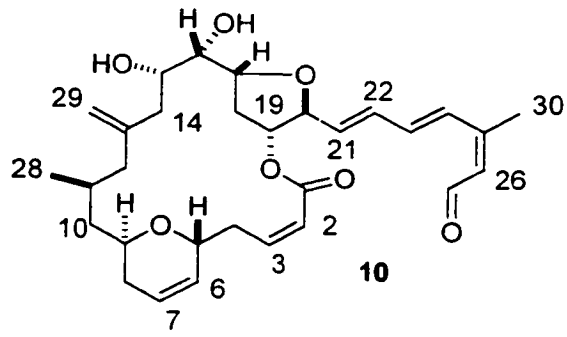
*Kinesin motor inhibitor. † Growth inhibition of human cancer cell lines. ‡Concentration required to induce 50% of maximum tublin polymerization. #Inhibition of binding of radiolabeled paclitaxel to microtubules (competitive inhibition). ND = not determined.

The high activity of **2** towards multidrug resistant cell lines and the determination of its absolute stereochemistry[28] have spurred interest in its synthesis. Although many approaches to fragments have been accomplished,[29a-j] to date only three total syntheses have been achieved, one by Ghosh and Wang,[30] and two by Mulzer.[31,32] This extensive amount of interest in the biological activity of the

fijianolides led to an investigation of a recent collection of the mushroom sponge from Vanuatu (coll. # 00600).

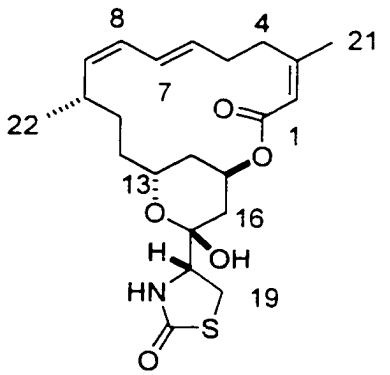
**Results and Discussion**

The methylene chloride (FD) extract of sponge 00600, collected in Vanuatu, showed microtubule bundling at 5 μg/ml, and mitotic spindle changes characteristic of the fijianolides. Bioassay guided Silica gel column chromatography of the FD led to increased microtubule bundling in the sixth fraction (Scheme 1, Experimental Section). The next step was to acquire coupled liquid chromatographic and low-resolution mass spectral data of this fraction. The positive mode ESITOF-LCMS data (Figure 1) indicated compounds with the known molecular weight of fijianolides (MW=514) and a compound with a molecular weight (MW=512), which had never before been seen in this sponge. Reversed phase high performance liquid chromatography yielded the known macrolides latrunculin A (**3**), fijianolide A (**1**), fijianolide B (**2**), and a new compound, fijianolide C (**10**).




**10**

Characterization of (+)-latrunculin A (**3**) $[\alpha]_D$ +130° (*c* 0.16 $CHCl_3$), began with low resolution ESIMS data (Figure 2) that revealed ions corresponding to $[M+H]^+$ *m/z* = 422 and $[M-OH]^+$ *m/z* = 404 in the positive mode, and in the negative mode $[M+Cl]^-$ *m/z* = 435/437. Characteristic $^1H$ NMR peaks of **3** (Figure 3) were two *Z* double bonds at positions 2/3 (δ 5.68 d, *J*=1.2 Hz) and 8/9 (δ 5.99 t, *J*=11.0; δ 5.01 t, *J*=11.0) and one *E* double bond at position 6/7 (δ 5.74 dt, *J*=15.0, 4.5; δ 6.42 dd, *J*=15.0, 11.0). Also visible were one doublet methyl $H_3C22$ (δ 0.98 d, *J*=7.0) and one singlet methyl $H_3C21$ (δ 1.95 s), and five hydrogens adjacent to heteroatoms:13, 15, 18, 19 and 19' (δ 4.29 m; 5.43 bt, 3.87 dd; 3.50 t; 3.48 dd). Table 2 shows the $^1H$ NMR data of reisolated **3** is nearly identical to that of the literature.[33]




3

**Table 2** NMR data comparison for latrunculin A (**3**) (500 MHZ, $CDCl_3$).

| Atom | literature<br>δ $^1$H (mult, *J* (Hz)) | **3**<br>δ $^1$H (mult, *J* (Hz)) |
|---|---|---|
| 1 | -- | -- |
| 2 | 5.69 (d, 1.3) | 5.68 (d, 1.2) |
| 3 | -- | -- |
| 4 | 3.00 (m) | 2.98 (m) |
| | 2.60 (m) | 2.63 (m) |
| 5 | 2.26 (m) | 2.26 (m) |
| 6 | 5.74 (dt, 15, 4.5) | 5.74 (dt, 15.0, 4.5) |
| 7 | 6.41 (dd, 15, 10.5) | 6.42 (dd, 15.0, 11.0) |
| 8 | 5.98 (t, 10.5) | 5.99 (t, 11.0) |
| 9 | 5.02 (t, 10.5) | 5.01 (t, 11.0) |
| 10 | 2.83 (m) | 2.76 (m) |
| 11 | NR | 1.4-1.7 |
| 12 | NR | 1.4-1.7 |
| 13 | 4.29 (m) | 4.29 (m) |
| 14 | NR | 1.4-1.7 |
| 15 | 5.43 (bt, 3) | 5.43 (bt, 2.0) |
| 16 | NR | 2.12 (d, 12) |
| | | 1.4-1.7 |
| 17 | -- | -- |
| 18 | 3.87 (dd, 8, 7) | 3.87 (dd, 7, 8) |
| 19 | 3.51 (dd, 11.5, 7) | 3.50 (t, 12.0) |
| | 3.48 (dd, 11.5, 8) | 3.48(dd, 12.0, 7) |
| 20 | -- | -- |
| 21 | 1.92 (d, 1.3) | 1.95 s |
| 22 | 0.98 (d, 6.7) | 0.98 (d, 7.0) |

**Figure 1** 00600FDF6 LCTOFMS Extracted Ion Chromatograms from (positive mode, Mariner).

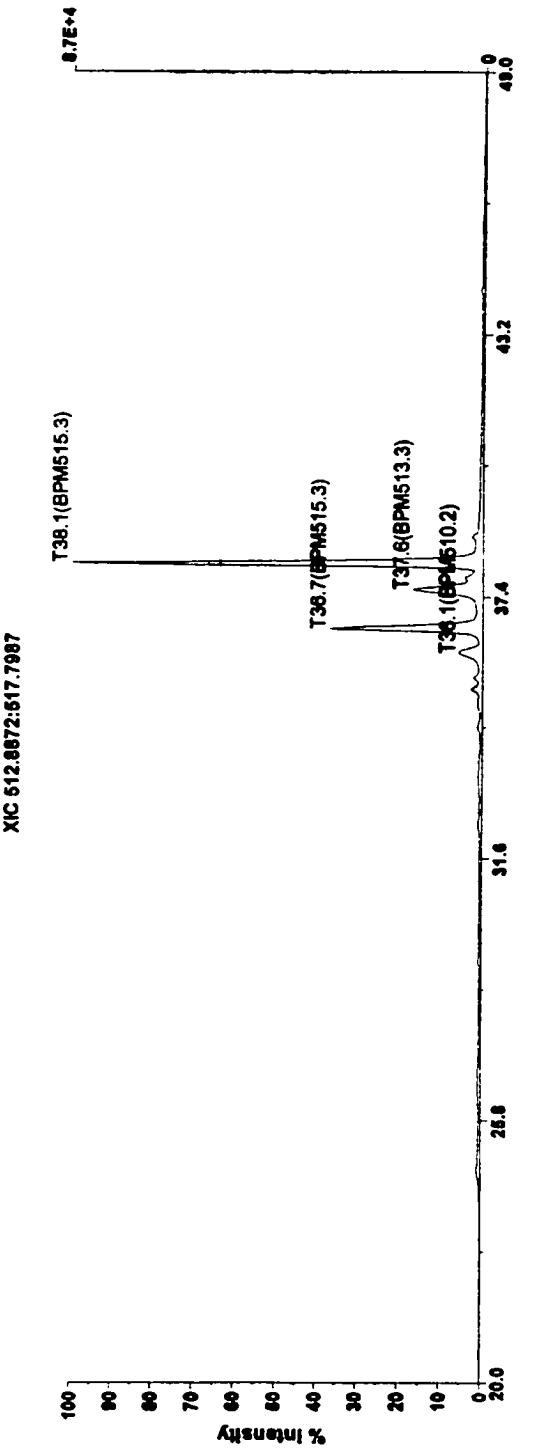


**Figure 2** Low Resolution ESI Mass Spectrum of **3** (negative mode above and positive mode below).

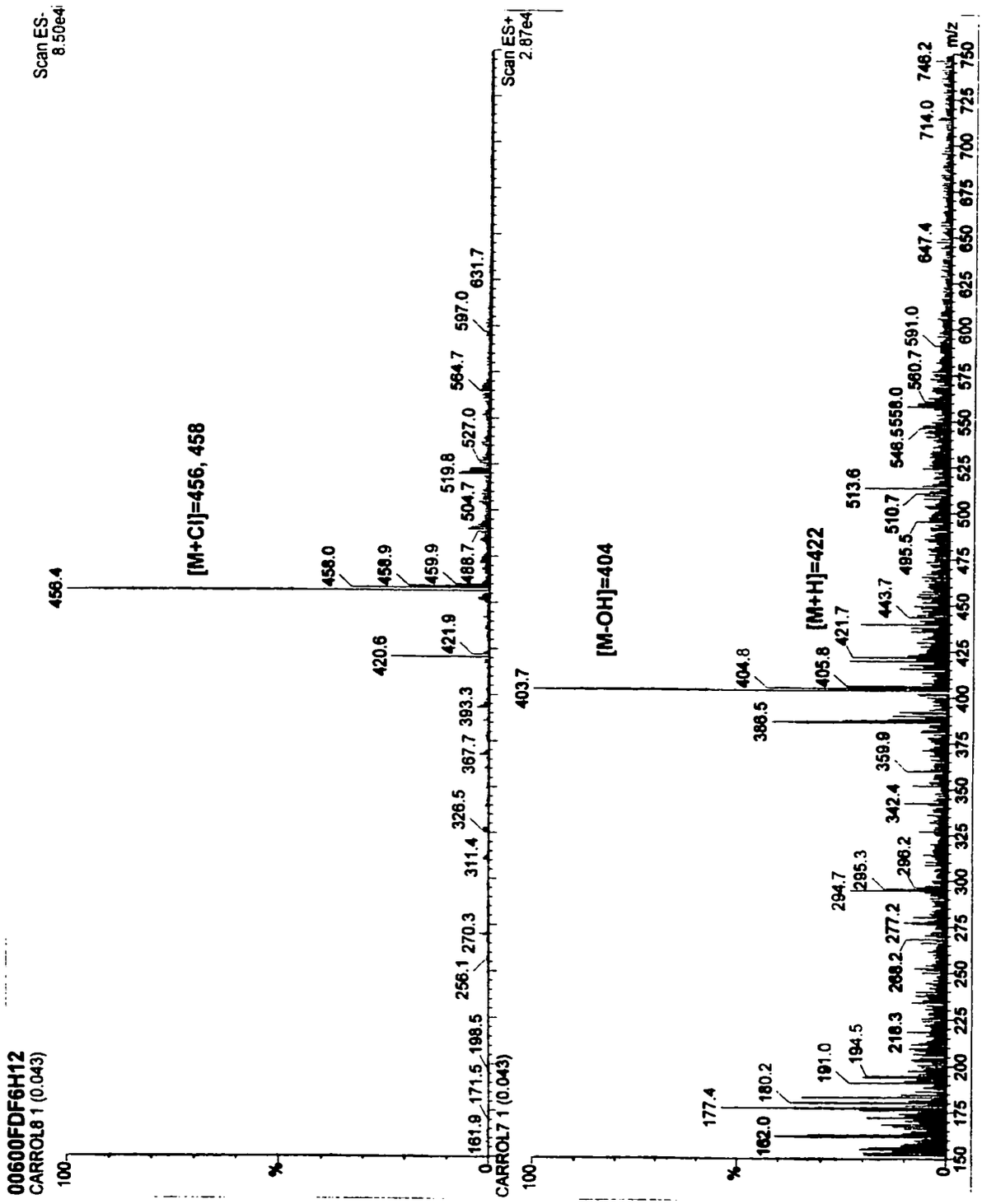
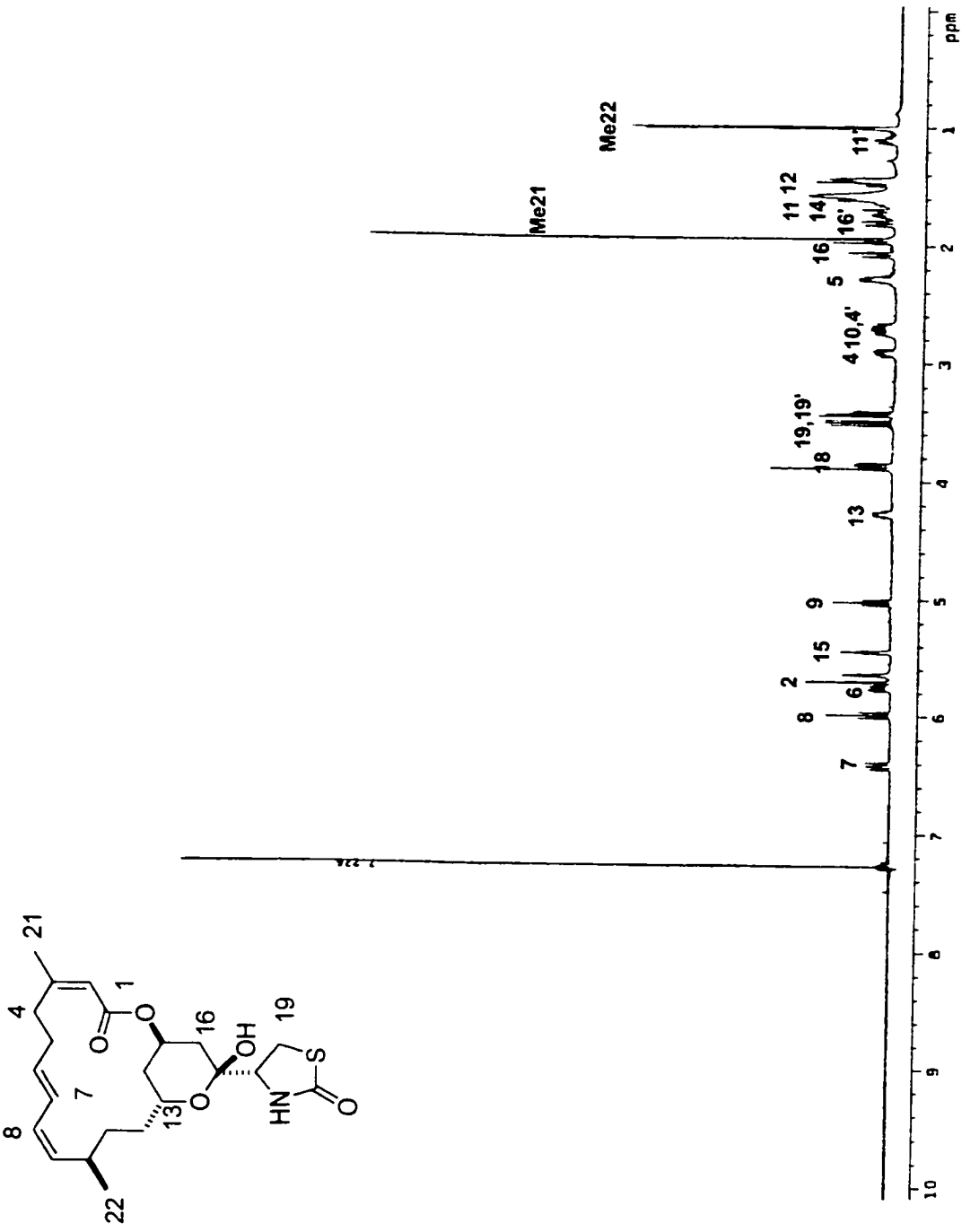
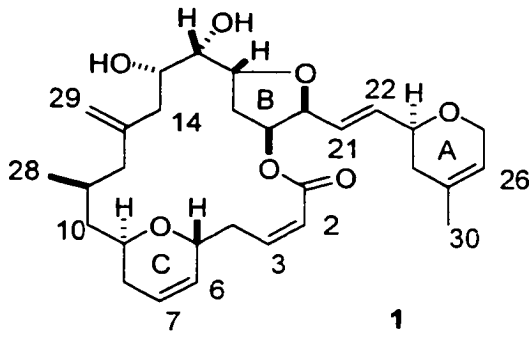


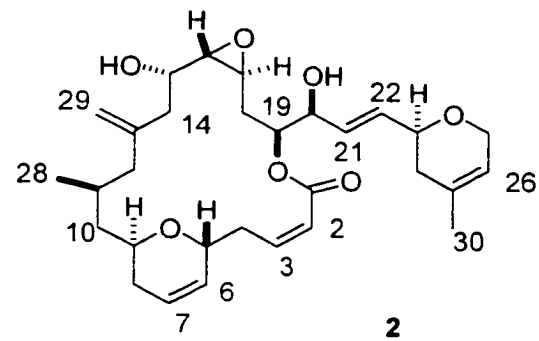
**Figure 3** $^{1}$H NMR Spectrum of latrunculin A (**3**) (500MHz, $CDCl_3$).



The low resolution ESIMS of (-)-fijianolide A (**1**), $[\alpha]_D$ -10° (*c* 0.5, $CHCl_3$) (Figure 4) showed characteristic *m/z* = 549/551 $[M+Cl]^-$, 515 $[M+H]^+$ and 532 $[M+H_20]^+$. The $^1H$ NMR spectrum of **1** (Figure 5) indicated the dihydropyran A ring (δ 3.91 m, H23; 1.90 m, $H_224$; 5.19 bs, H26; 4.11 bs H27; 4.04 bs H27') was intact with its vinylic methyl (δ1.45 s, $H_330$) and (*E*)-ethenyl groups (δ 5.85 dd *J*=15.3, 5.4, H21; 6.04 dd *J*=15.3, 5.1). The second dihydropyran C ring, with its (*Z*)-disubstituted double bond (δ 4.34 m, H5; 5.58 bd *J*=10.5, H6; 5.69 bd, *J*=10.3 H7; 1.65 m $H_28$; 3.50 m, H9) was apparent from the $^3J_{6-7}$ of 10Hz. The furan B ring was indicated by the following shifts; δ 4.46 m, H17; 2.30 m, H18; 2.20 m, H18'; 5.57 t, *J*=4, H19; 4.36 dd *J*= 4.2, 4.0, H20. Moreover, the $^{13}C$ NMR shifts agreed with that of the literature (Table 3).




**1**




**2**

The low-resolution mass spectrum of fijianolide B (**2**) $[\alpha]_D$ -10° (*c* 0.5, $CHCl_3$) (Figure 6), displayed peaks corresponding to $[M+Cl]^-$ *m/z* = 549, 551 in the negative mode, and in positive mode, $[M+H]^+$, $[M+H_20]^+$ and $[M+Na]^+$ at *m/z* = 515, 532 and 537 respectively, indicating a molecular weight identical to the fijianolides (MW=514). The key difference in the $^1H$ NMR spectra (Figure 7) indicated the

epoxide (δ 2.84 dd, *J*=10.8, 1.5, H16; 2.90 dd, *J*=10.5, 3.9, H17) rather than the furan B ring.

**Table 3** NMR data of fijianolides A (**1**) and B (**2**) (500MHZ, $C_6D_6$).

| atom | fijianolide A | | | fijianolide B |
|---|---|---|---|---|
| | $\delta$ $^1$H (mult), *J*(Hz) | $\delta^{13}$C | $\delta^{13}$C(lit.) | $\delta$ $^1$H (mult), *J*(Hz) |
| 1 | -- | 165.3 | 165.2 | -- |
| 2 | 5.80 (m) | 123.0 | 123.2 | 5.80 (d=12.3) |
| 3 | 5.80 (m) | 143.1 | 142.2 | 6.12 (ddd=12.0, 10.8, 4.5) |
| 4 | 3.09 (m) | 35.5 | 35.6 | 3.90 (m) |
| | 2.20 (m) | | | 2.08 (m) |
| 5 | 4.34 (m) | 72.8 | 72.9 | 4.12 (m) |
| 6 | 5.58 (bd=10.5) | 128.3 | 128.5 | 5.48 (bd=10.2) |
| 7 | 5.69 (bd=10.3) | 125.6 | 125.3 | 5.67 (bd=10.2) |
| 8 | 1.65 (m) | 31.9 | 31.8 | 1.75 (m) |
| 9 | 3.50 (m) | 66.9 | 66.5 | 3.71 (m) |
| 10 | 1.20 (m) | 43.0 | 42.9 | 1.50 (m) |
| | 0.95 (m) | | | 1.20 (bd=12.0) |
| 11 | 1.90 (m) | 27.5 | 27.0 | 1.75 (m) |
| 12 | 2.25 (m) | 45.6 | 45.4 | 2.55 (dd=10.8, 2.4) |
| | 2.20 (m) | | | 1.85 (dd=10.8, 5.4) |
| 13 | -- | 146.4 | 145.6 | -- |
| 14 | 2.25 (m) | 35.7 | 35.6 | 2.14 (bs) |
| | | | | 2.13 (bs) |
| 15 | 4.18 (m) | 70.5 | 71.3 | 4.04 (m) |
| 16 | 4.18 (m) | 74.6 | 75.7 | 2.84 (dd=10.8, 2.0) |
| 17 | 4.46 (m) | 78.3 | 78.3 | 2.90 (dd=11.1, 3.9) |
| 18 | 2.30 (m) | 34.7 | 34.9 | 2.36 (dd=10.8, 1.5) |
| | 2.20 (m) | | | 1.55 (m) |
| 19 | 5.57 (m) | 76.8 | 76.8 | 5.25 (dd=10.5, 3.9) |
| 20 | 4.56 (dd=4.2, 4.0) | 81.6 | 81.5 | 4.18 (dd=9.3, 4.5) |
| 21 | 5.85 (dd=15.3, 5.4) | 125.8 | 125.9 | 5.81 (dd=16.2, 5.4) |
| 22 | 6.04 (dd=15.3, 5.1) | 134.0 | 133.5 | 5.94 (dd=16.2, 5.4) |
| 23 | 3.91 (m) | 73.4 | 73.4 | 3.90 (m) |
| 24 | 1.90 (m) | 35.9 | 35.9 | 2.03 (m) |
| 25 | -- | 131.1 | 131.1 | -- |
| 26 | 5.19 (bs) | 119.8 | 120.0 | 5.16 (bs) |
| 27 | 4.11(bs) | 65.6 | 65.4 | 4.09 (bs) |
| | 4.04 (bs) | | | 3.95 (bs) |
| Me28 | 0.87 (d=5.7) | 19.9 | 19.7 | 0.86 (d=6.0) |
| 29 | 5.00 (s) | 113.7 | 113.4 | 4.94 (bs) |
| | 4.92 (s) | | | 4.89 (bs) |
| Me30 | 1.45 (s) | 23.0 | 22.7 | 1.50 (s) |

**Figure 4** Low Resolution Mass Spectrum of **1** (negative mode above and positive mode below).

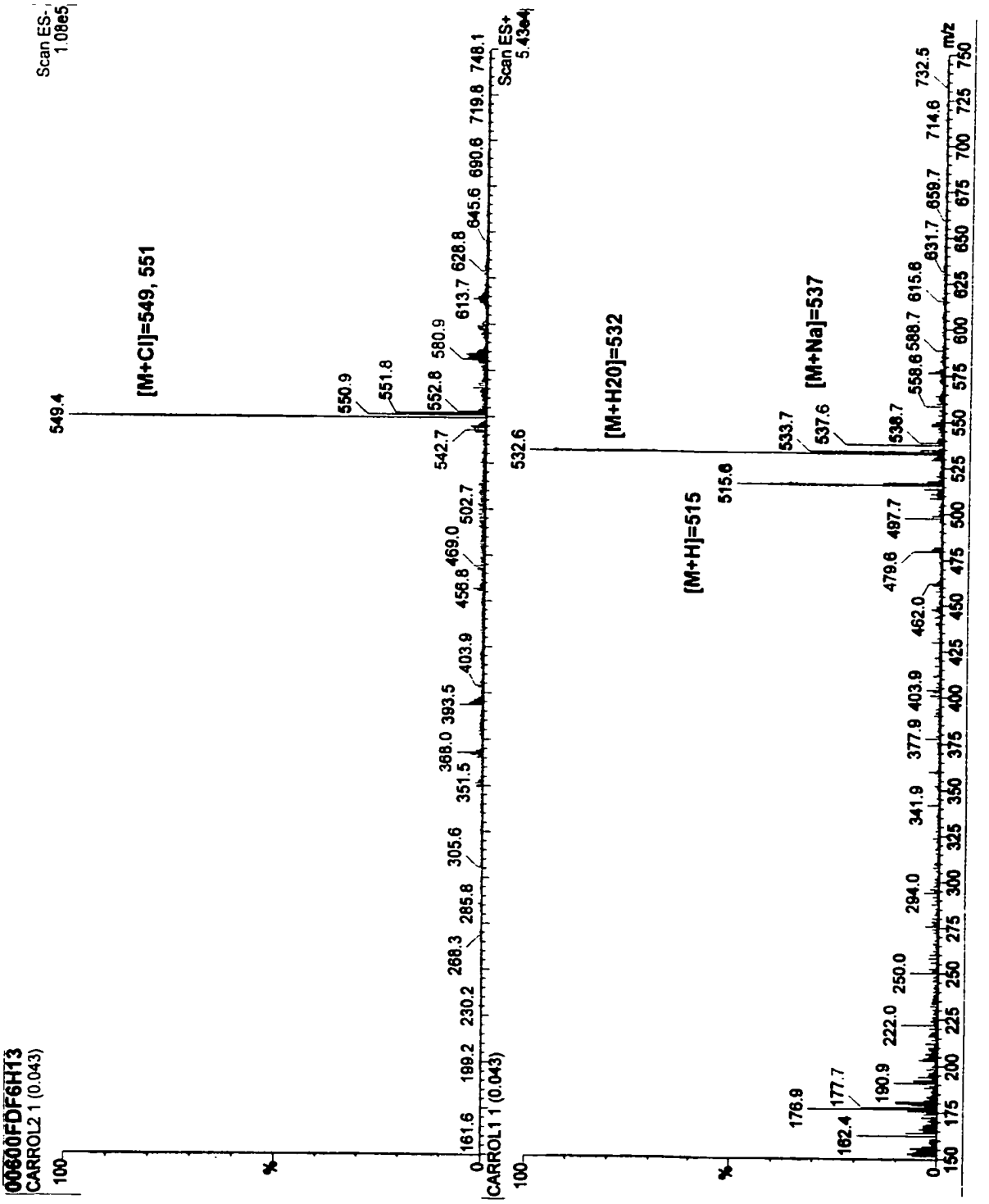


**Figure 5** $^{1}$H NMR Spectrum of fijianolide A (**1**) (500MHz, $C_6D_6$).

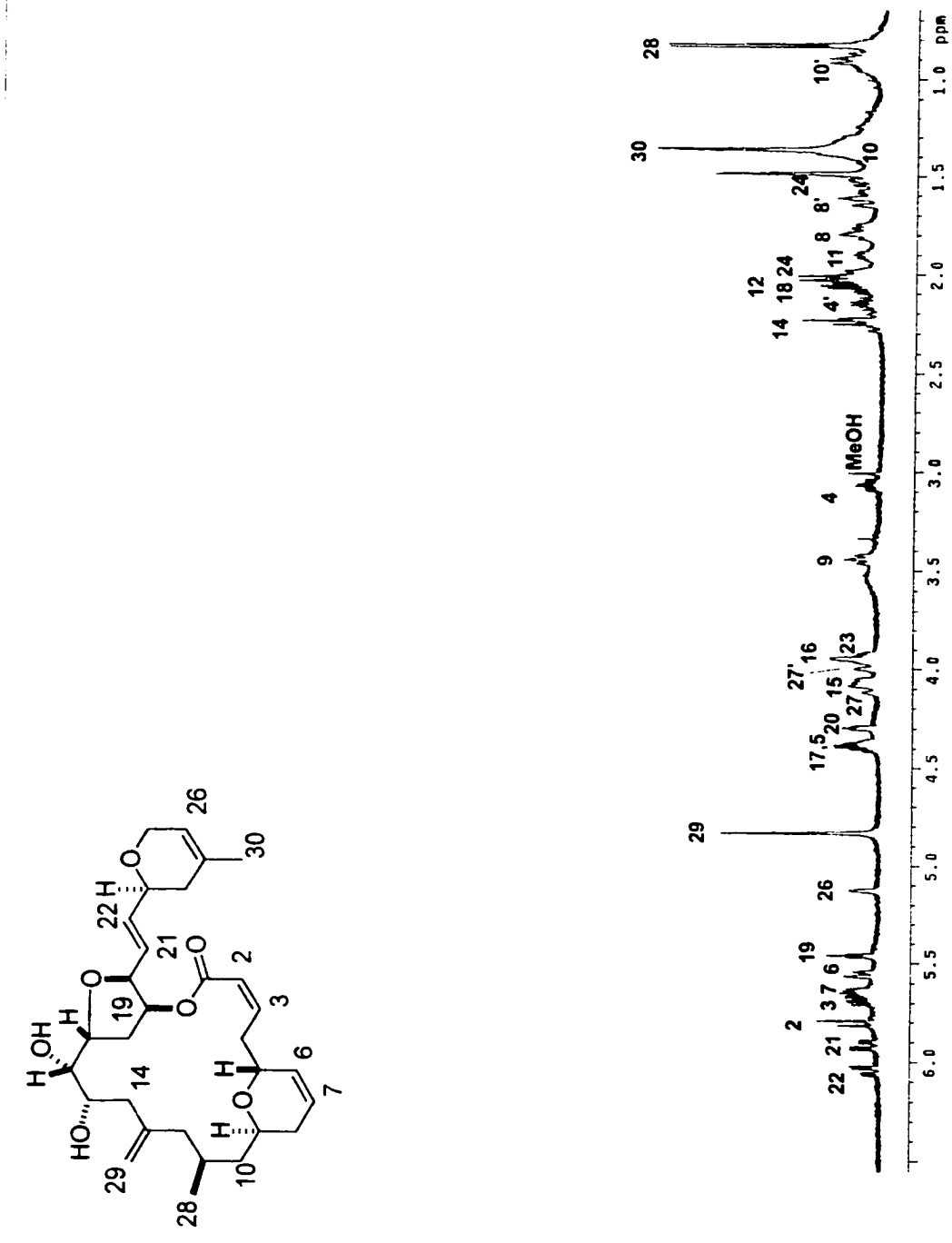


**Figure 6** Low Resolution Mass Spectrum of **2** (negative mode above and positive mode below).

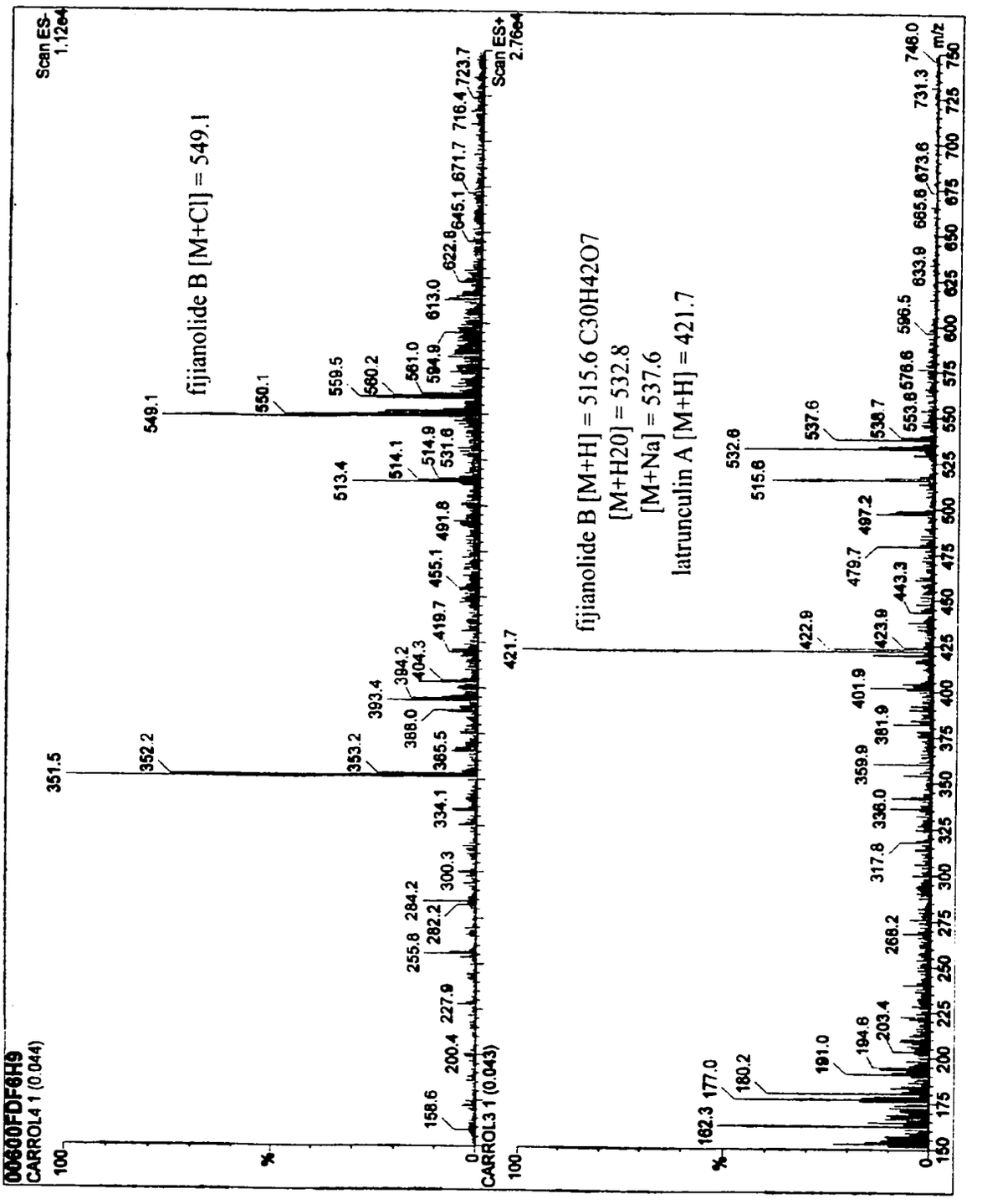
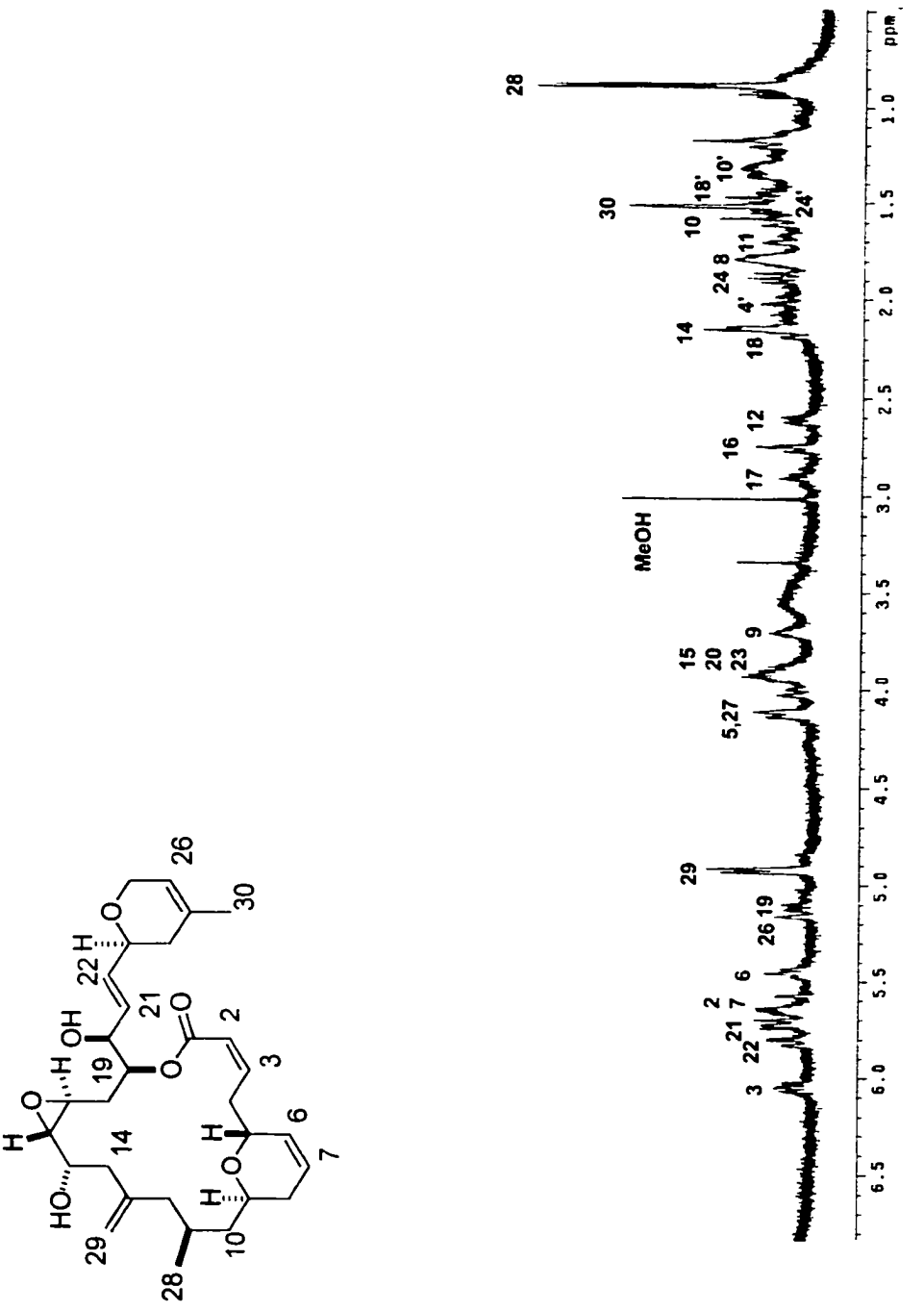
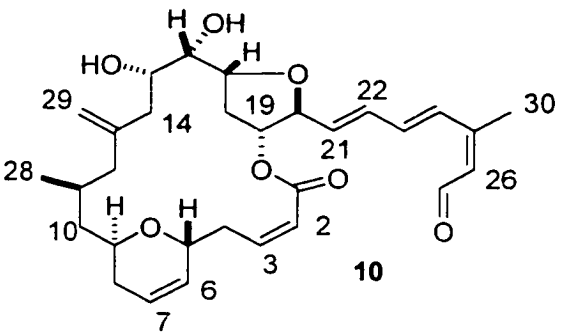


**Figure 7** $^{1}$H NMR Spectrum of fijianolide B (**2**) (500MHz, $C_6D_6$).



The next step was to establish the structure of the new (-)-fijianolide C $[\alpha]_D$ -16° (*c* 0.4, $CHCl_3$). This process was begun with the determination of the molecular formula $C_{30}H_{40}O_7$ from TOF high-resolution mass spectrum of **10** (Figure 8), which showed $[M+H]^+$ m/z = 513.2852 (Δ 1.7mmu calc. for $C_{30}H_{41}O_7$). The 2 amu difference between the known fijianoldies A and B vs. **10** indicated that an additional degree of unsaturation was present. Similarities in the $^1H$ NMR between **10** and **1** (Figures 9 and 10) indicated that this pair differs only in the A ring. For example H26, which in **1** appears as a broad singlet at δ 5.19, appears in **10** as a doublet (*J*=8.5) at δ 5.92. Also missing from **10** are the diastereotopic H27/H27'and H24/H24' which can clearly be seen in the $^1H$ NMR of **1**.




**10**

The gCOSY correlations of **10** differed only in the A ring from that of **1** (Figure 11 12 and 13). Namely, two new correlations were evident from H26-H27 and H23-H24. Further spin systems were identical to that of **1** and included H3-H4-H4'-H5, H6-H7-H8-H9-H10, H28-H11-H12, H13-H14 and H17-H18-H19-H20-H21-

H22. Numerous attempts have been made to obtain the $^{13}C$ of **10**, but due to its very low concentration this has not been achieved. Further attempts to obtain $^{13}C$ data from small scale, nanoprobe experiments are currently underway.

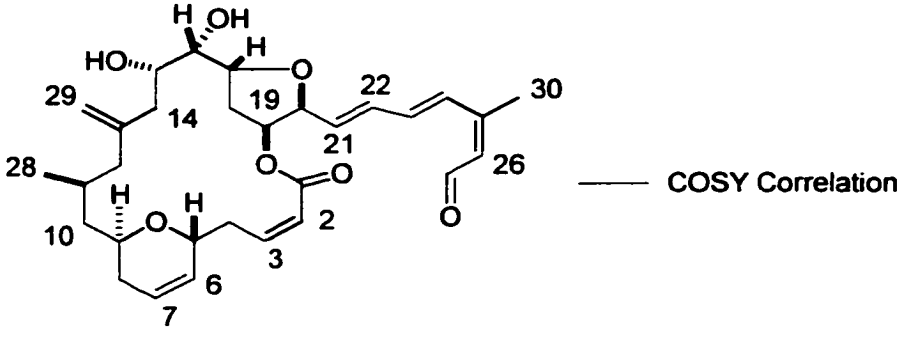


**Table 4** NMR Data of Fijianolide C (**10**) (500MHz, $C_6D_6$)

| atom | $\delta$ $^1$H (mult, $J$(Hz)) | gCOSY |
|---|---|---|
| 1 | -- | |
| 2 | 5.80 (d, 13.0) | |
| 3 | 5.70 (m) | 4, 4' |
| 4 | 3.06 (m) | 4' |
| | 2.16 (m) | |
| 5 | 4.29 (bd 16.0) | 4,4' |
| 6 | 5.50 (m) | 8' |
| 7 | 5.65 (m) | |
| 8 | 1.65 (m) | 9 |
| | 1.52 (s) | |
| 9 | 3.44 (dt, 8.0, 1.0) | 10 |
| 10 | 1.53 (t, 12.0) | 10' |
| | 0.93 (m) | |
| 11 | 2.05 (m) | |
| 12 | 2.13 (dd, 8.5, 6.5) | |
| | 2.14 (m) | |
| 13 | -- | |
| 14 | 2.36 (dd, 14.5, 3.5) | |
| | 2.32 (d, 9.5) | |
| 15 | 4.15 (dd, 10.0, 2.0) | 14 |
| 16 | 4.01 (t, 2.0) | |
| 17 | 4.47 (dt, 2.0, 6) | 18 |
| 18 | 2.16 (m) | 19 |
| 19 | 5.50 (m) | 20 |
| 20 | 4.34 (bs, $w_{1/2}$=5.0) | 21 |
| 21 | 5.84 (m) | 22 |
| 22 | 6.60 (dd, 15.5, 5.1) | |
| 23 | 6.38 (dd, 15.5, 5.1) | 24 |
| 24 | 6.08 (d, 15.5) | |
| 25 | -- | |
| 26 | 5.92 (d, 8.5) | 27 |
| 27 | 9.90 (d, 8.5) | |
| Me28 | 0.88 (d, 7.0) | 11 |
| 29 | 4.90 (bs) | |
| Me30 | 1.60 (s) | |

**Figure 8** Positive Mode High Resolution ESI-TOFMS of **10**.

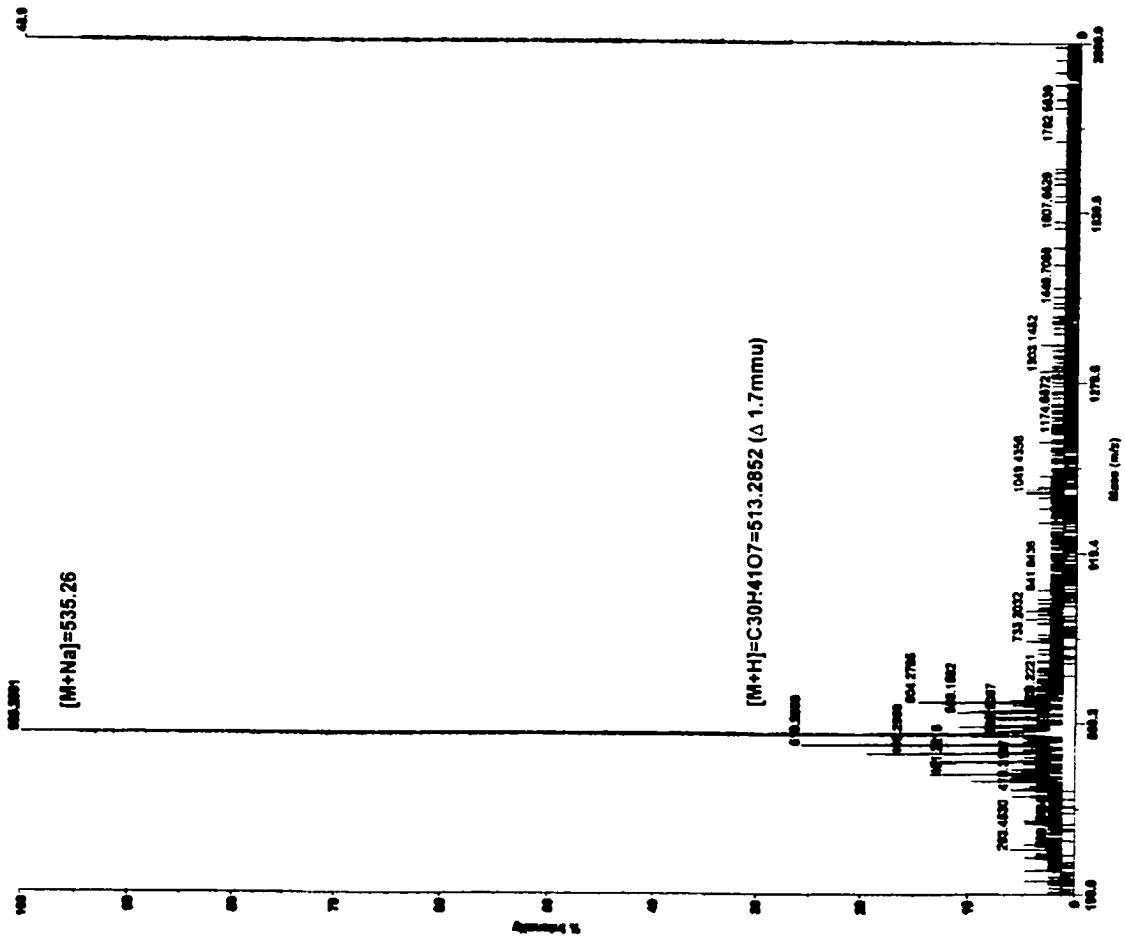


**Figure 9** $^{1}$H NMR Spectrum of fijianolide C (**10**) (500MHz, $C_6D_6$).

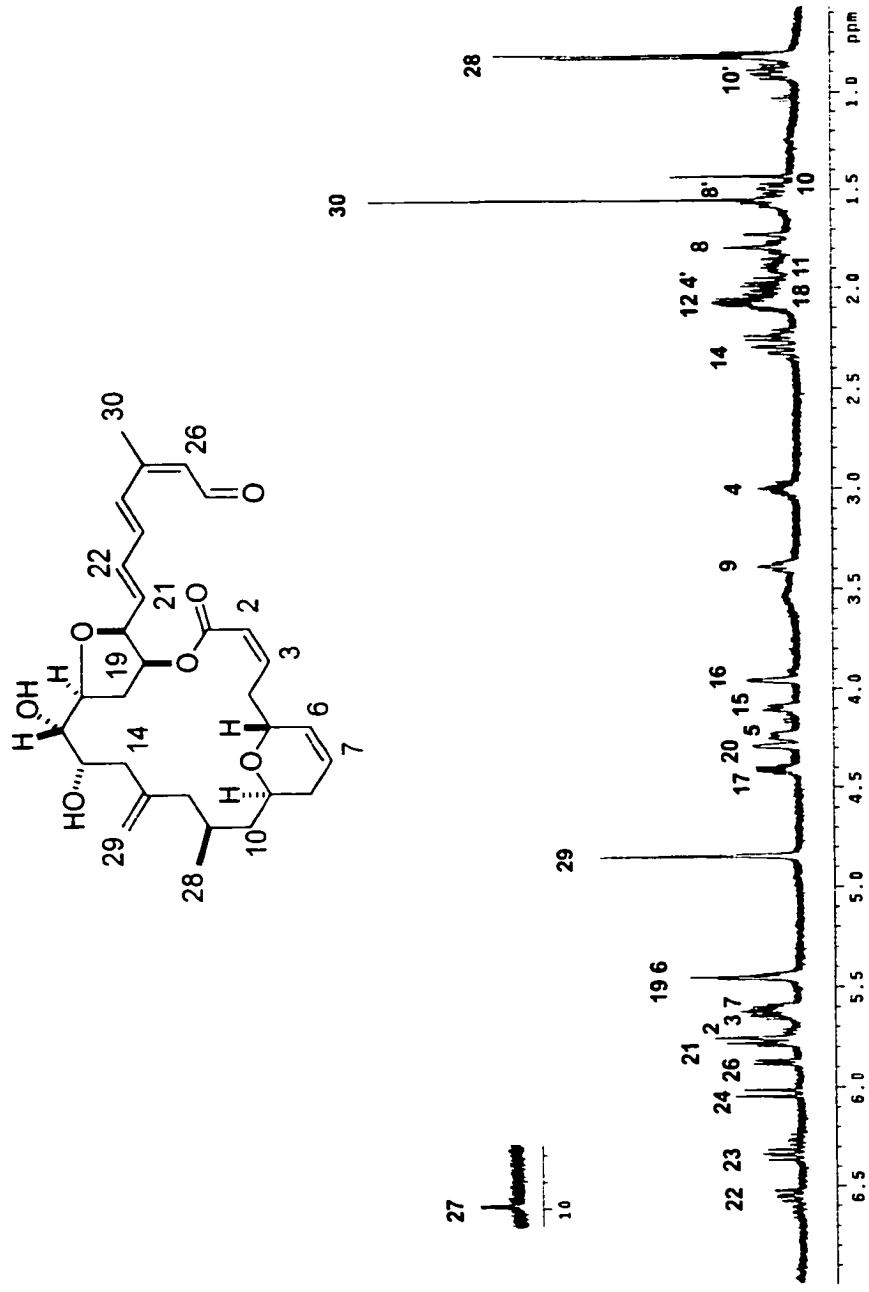


**Figure 10** $^{1}$H NMR Spectrum of fijianolide A (**1**) and fijianolide C (**10**) (500MHz, $C_6D_6$).

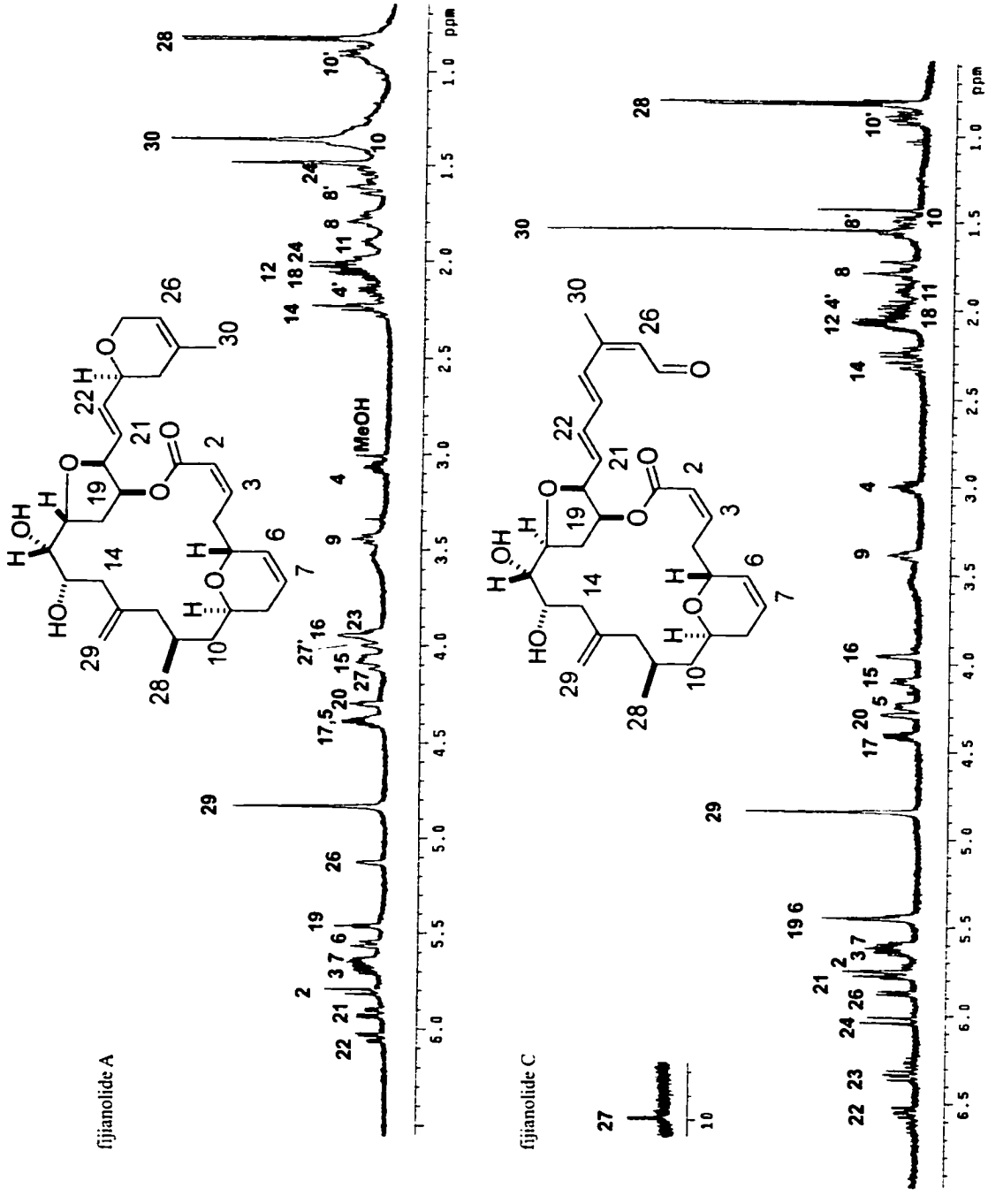


**Figure 12** gCOSY NMR Spectrum of fijianolide C (**10**) (500MHz, $C_6D_6$).

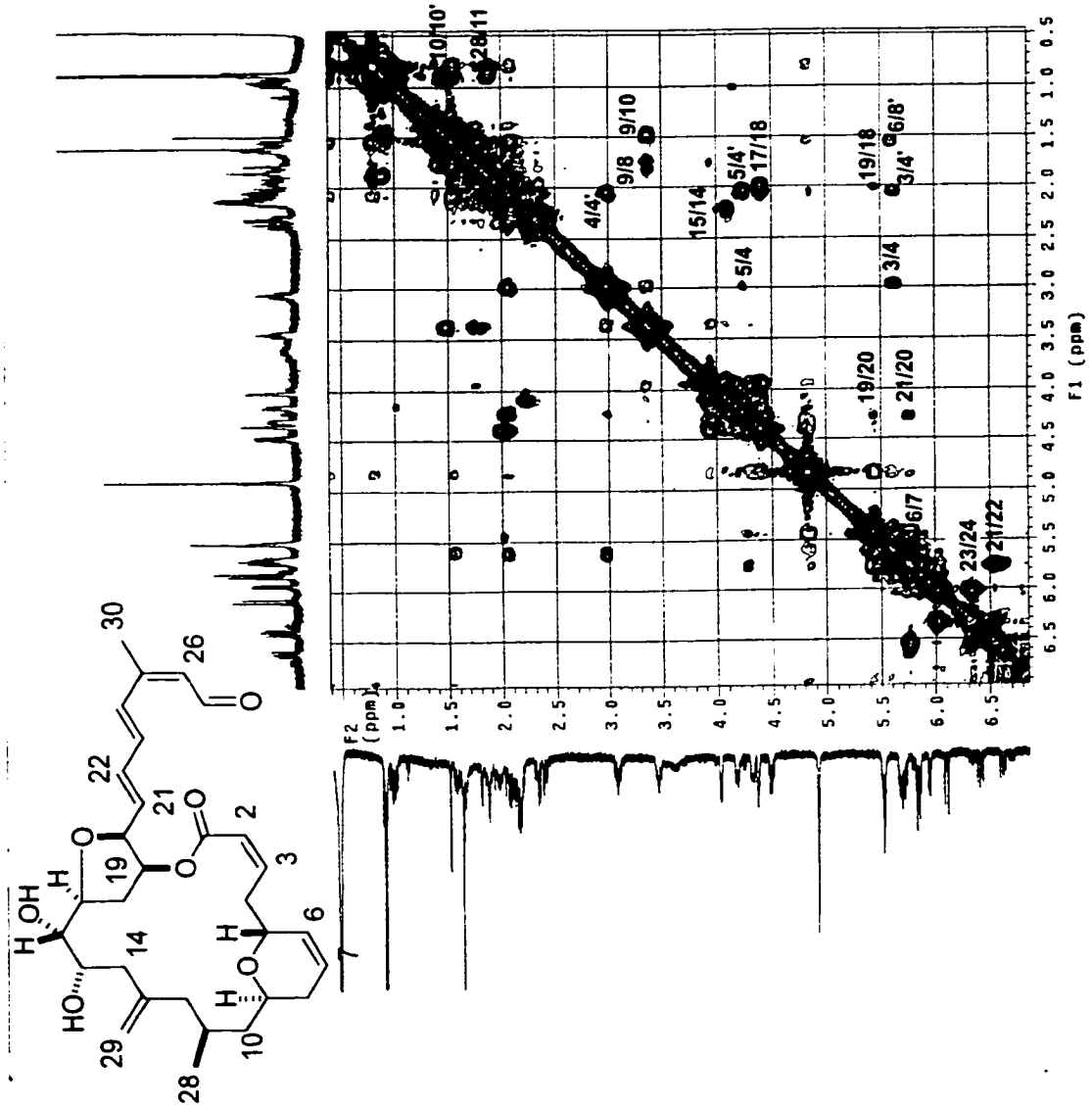


**Figure 13** gCOSY NMR Spectrum of fijianolide C (**10**) (500MHz, $C_6D_6$).

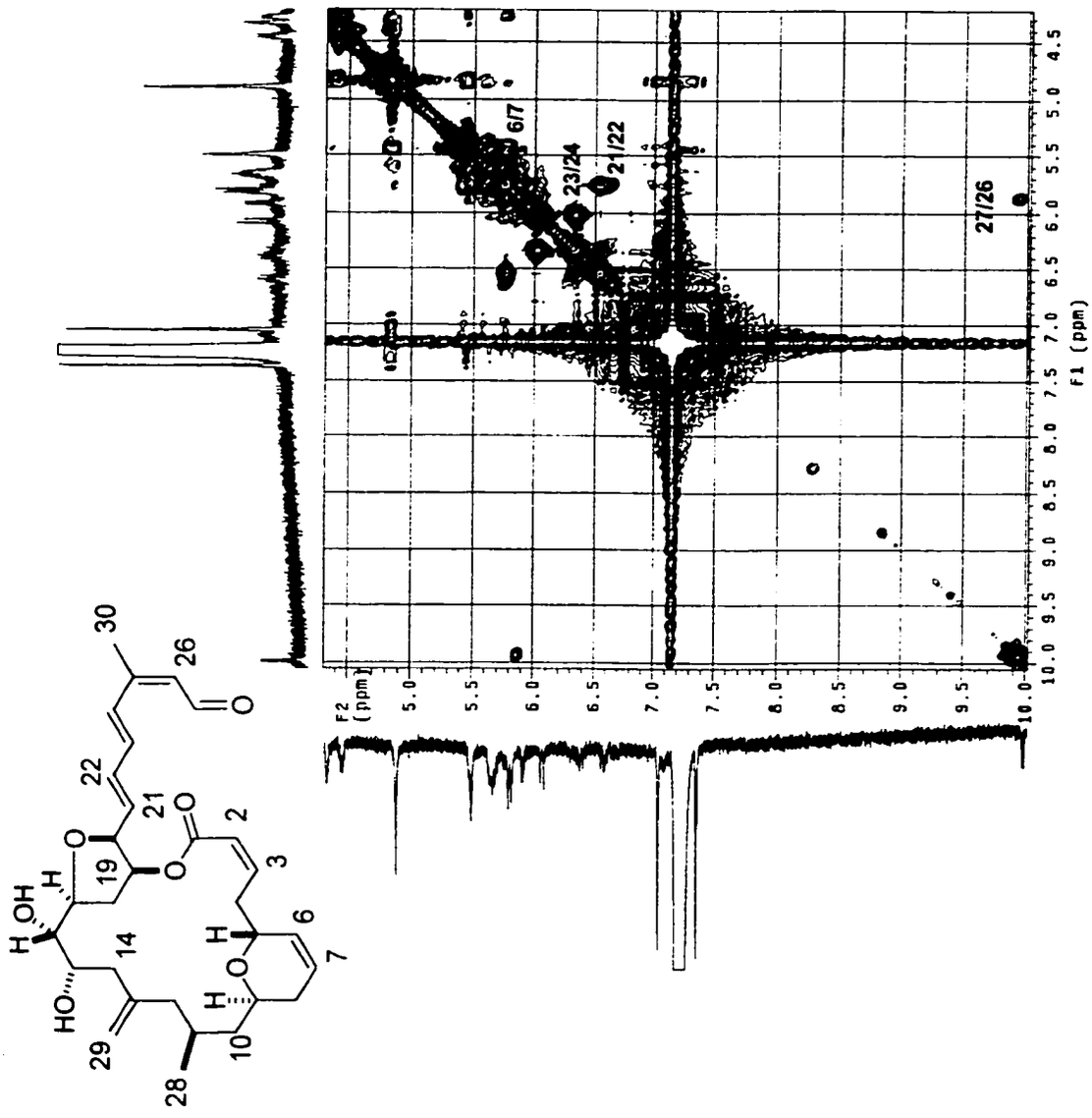


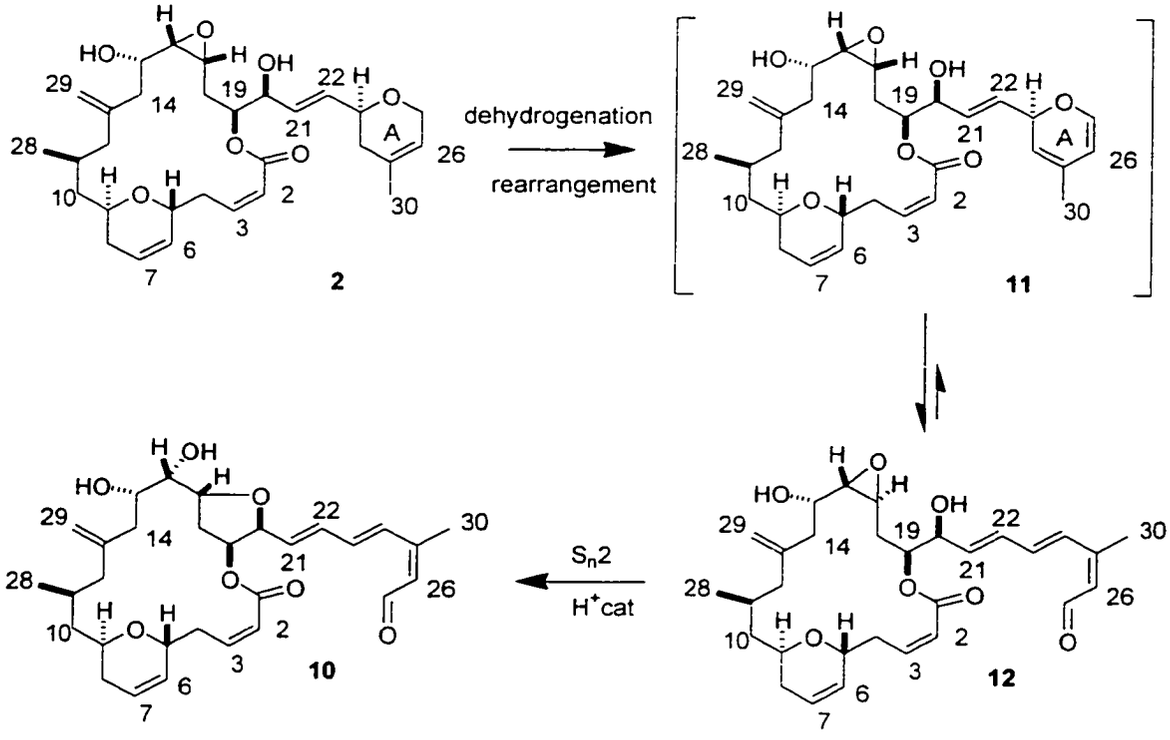
**Figure 14** $^{13}C$ NMR Spectrum of fijianolide C (**10**) (500MHz, $C_6D_6$).

**Conclusions**

Assay reports from Dr. Susan Mooberry on one of the early fractions containing compound **10** have yielded intriguing results. At low concentrations of this sample there is evidence of microtubule stabilization, but beginning at 5 μg/ml and peaking at 7.5 μg/ml there is unusual microtubule loss. At a higher concentration, 10 μg/ml, a microtubule stabilizing effect seems to be winning out. This is particularly interesting because in the cytotoxicity experiments there is a biphasic dose response curve in one cell line, a linear curve in another and in a multi-drug resistant line, there is evidence of resistance.[34] The existence of small amounts of Latrunculin A (**3**), a known actin inhibitor, within this sample might be responsible for these results. Further examination of a more pure sample of **10** by Dr. Mooberry will hopefully lead to a determination of the $IC_{50}$ and $EC_{50}$ of this compound.

The fijianolides A and B are biogenetically related by an obvious $S_n2$ attack, which involves an inversion of stereochemistry at C-17 in going from **2** to **1**.[1] This rearrangement has been seen to occur within the acidity levels of chromatographic conditions (0.01 N HCl in Acetone).[2] The appearance of **10** with a furan-type B ring similar to **1** is not surprising considering the HPLC conditions used (0.1% formic acid in methanol) in this work. The reported activity of **1** in the MDA-MB-435 cell line ($IC_{50}$ = 1.02 μM) is three orders of magnitude less than that of **2** ($IC_{50}$ = 5 nM),[3] thus, the preliminary activity of the impure **10**, at 5 μg/ml (10 μM) is typical for its structural motif. Future mushroom sponge workups should focus on normal phase

(i.e. nonacidic conditions) to preserve the epoxide form of the fijianolides throughout the isolation.




Another intriguing point is that of the possible biosynthesis of **10**. Dehydrogenation of **2**, followed by a rearrangement of double bonds could be envisioned which forms the highly unstable intermediate **11**. These 2*H*-pyrans such as **11** are known to be in equilibrium with the *Z* dienals represented by **12**.[35] Next, an acid catalyzed $S_n2$ reaction would lead to the formation of the B furan ring of **10**. A 13C NMR of fijianolide C would verify the proposed *Z* configuration of the C25/C26 double bond.

This work has shown the utility of Dr. Susan Mooberry's cellular protein assay, when linked to marine natural product isolations and structural

characterization, in the discovery of new biologically active agents. There is continued evidence for the production of fijanolide-like compounds from the mushroom sponge, which will hopefully be used in the future DNA isolation work geared toward the manipulation of the PKS pathway. Moreover, an examination of other bioactive extracts from this sponge sample might yield additional interesting chemistry.

## Experimental Section

**General Experimental Procedures.** NMR spectra were recorded in $CDCl_3$ and $C_6D_6$ solutions at 500 or 125.7 MHz for $^1H$ and $^{13}C$ respectively. Optical rotation was measured on a JASCO DIP-370 digital polarimeter, ESIMS spectra on a VG Quattro II, LC and HRMS on a PE Biosystems Mariner Mass Spectrometer.

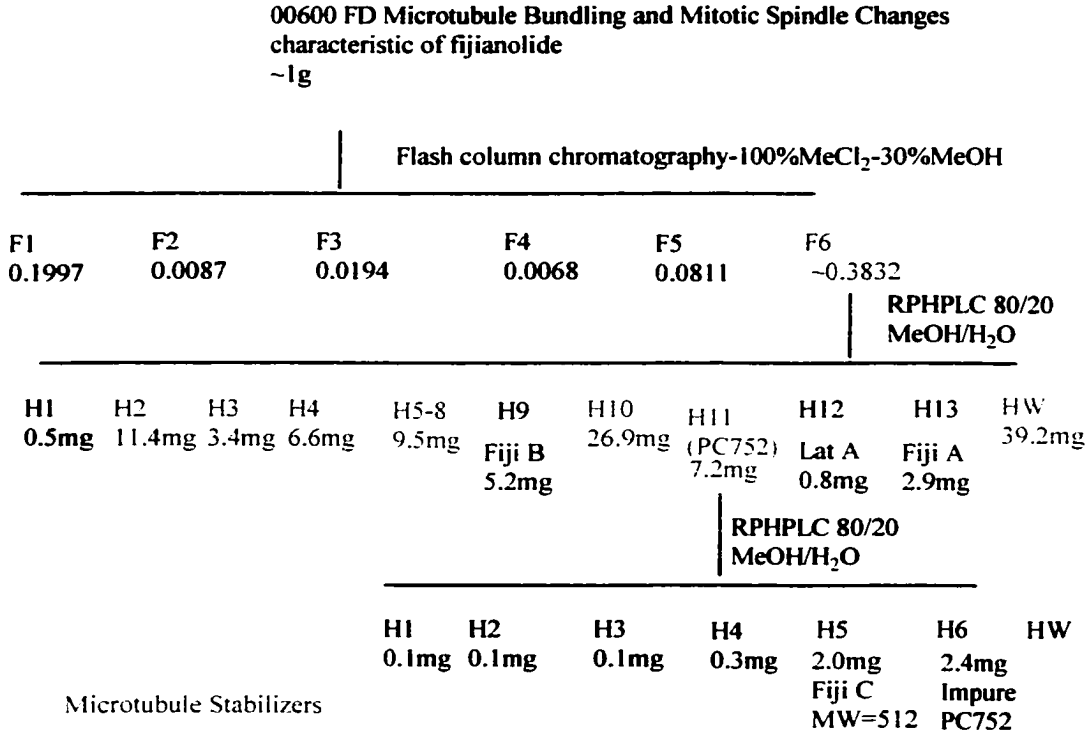
**Biological Material, Collection, and Identification.** The sponge, *Cacospongia mycofijiensis*has (Underwater photo Figure 15) has been collected on numerous occasions by our group, and has been given the following taxonomic description.[36] There are three distinct morphological forms: massive, lobate, or tubular. The size varies from 3 to 20 cm in height, and 2-10 cm in diameter. The surface is microconulose, and the colors are dark brown/black externally and tan internally. The sponge is also recognizable by a sweet, pungent odor. It is generally

found in sheltered reef habitats, under ledges or in caves, and is fairly rare despite its broad range of distribution in the South and Indo Pacific. Skeletal features are characterized by the presence of prominent, cored primary fibres, regularly spaced and oriented perpendicular to the surface. *Cacospongia* emerges as the most suitable genus for this species due to the similarity in fibre lamination and coring, rectangular reticulum, and the regularity and character of the overall networks. This sponge has also been known as *Spongia mycofijiensis*, *Hyattella* sp., a "new" genus of Thorectidae, *Dactylospongia* sp. and as *Fasciospongia rimosa*. This sample (UCSC collection number: 00600) was collected off Vanuatu in November 2000.

**Figure 15** Underwater photo of *Cacospongia mycofijiensis.*

**Extraction and Isolation.** The sample was preserved by immersion in a 1:1 alcohol/seawater solution. After 24h this solution was decanted and discarded. The damp organism was then transported in a Nalgene bottle back to the home lab at ambient temperature, was next extracted with 100% MeOH to give a crude oil which was then partitioned between various solvents. The methylene chloride fraction was further purified on Biotage Si gel column (100% $CH_2Cl_2$ to 70/30 $CH_2Cl_2$/MeOH) to give six fractions. The sixth fraction was further purified by reverse phase gradient HPLC to give **1** (2.9mg), **2** (5.2mg), **3** (0.8mg) and impure **10** (Scheme 1). Repeated reverse phase HPLC let to the isolation of **10** (2.0mg).

**Scheme 1** Isolation of fijianoldies A (**1**), B (**2**), C (**10**) and latrunculin A (**3**).




Testing not completed as of 11/28/01

**Other Fractions:**

**F5=Microtubule Bundling and Mitotic Spindle Changes characteristic of fijianolide (latrunculin A & mycothiazole by ESIMS and NMR)**

**FM=Microtubule bundling.**
**FMM11Microtubule Stabilizer. FMM12 minor stabilizing changes**

**DMH=Slight microtubule depolymerization**
**DMM=short broken pieces of very thick microtubules**
**WB,FH=No effect**

**Fijianolide A** (**1**): white powder; 2.9mg; $[\alpha]_D$ -10° (*c* 0.5, $CHCl_3$), Lit$[\alpha]_D$ = -8°(c 0.04 $CHCl_3$); *m/z* = 549/551, $[M+Cl]^-$ negative mode, *m/z* = 515 $[M+H]^+$, 532 $[M+H_20]^+$, positive mode (Figure 4). $^1H$ NMR (500MHz, $C_6D_6$), see Table 3 and Figure 5. $^{13}C$ NMR (125 MHz, $C_6D_6$), see Table 3.

**Fijianolide B** (**2**): white powder; 5.2mg; $[\alpha]_D$ -10° (*c* 1.04, $CHCl_3$), Lit$[\alpha]_D$ = -8°(c 0.04 $CHCl_3$); *m/z* = 549/551, $[M+Cl]^-$ negative mode, *m/z* = 515 $[M+H]^+$, 532 $[M+H_20]^+$, 537 $[M+Na]^+$ positive mode (Figure 6). $^1H$ NMR (500MHz, $C_6D_6$), see Table 3 and Figure 7.

**Latrunculin A (3)**: clear oil 0.8mg; $[\alpha]_D$ +130° (*c* 0.16 $CHCl_3$), Lit$[\alpha]_D$ = + 152 (c 1.2 $CHCl_3$) *m/z* = 435/437, $[M+Cl]^-$ negative mode, *m/z* = 422 $[M+H]^+$, 404 $[M-OH]^+$, positive mode (Figure 6). $^1H$ NMR (500MHz, $C_6D_6$), see Table 3 and Figure 7.

**Fijianolide C (10)**: white powder 2.0mg $[\alpha]_D$ -16° (*c* 0.4, $CHCl_3$), *m/z* = 513.2852, $[M+H]^+$ ($\Delta$ 1.7 mmu calc. for $C_{30}H_{41}O_7$) (Figure 8). $^1H$ NMR (500MHz, $C_6D_6$), see Table 4 and Figure 9. COSY NMR spectrum see Figure 10.

**References**


[1] Quiñoà, E.; Kakou, Y.; Crews, P. *J. Org. Chem.* **1988**, *53*, 3642-3644.

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[3] Mooberry, S.; Davidson, B. S. Laulimalide Compounds as Microtubule Stabilizing Agents. U.S. Patent 09/493,897, World Patent WO 01/54689, August 2, 2001.

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