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DISSERTATION

Flavonolignan and Flavone Inhibitors of a *Staphylococcus aureus* Multidrug Resistance (MDR) Efflux Pump: Synthesis and Structure Activity Relationships

Submitted by
Nathan R. Guz
Department of Chemistry

In partial fulfillment of the requirements
for the degree of Doctor of Philosophy
Colorado State University
Fort Collins, Colorado
Summer, 2001

Colorado State University

June 27th, 2001

We hereby recommend that the dissertation prepared under our supervision by Nathan R. Guz entitled "Flavonolignan and Flavone Inhibitors of a *Staphylococcus aureus* Multidrug Resistance (MDR) Efflux Pump: Synthesis and Structure Activity Relationships" be accepted as fulfilling in part the requirements for the degree of Doctor of Philosophy.

Committee on Graduate Work

Advisor

Department Head

Abstract of Dissertation

Flavonolignan and Flavone Inhibitors of a *Staphylococcus aureus* Multidrug Resistance (MDR) Efflux Pump: Synthesis and Structure Activity Relationships

Multidrug resistance (MDR) represents an increasing problem in the treatment of bacterial infections and cancer. It often appears after prolonged exposure of cells to a single drug and is often characterized by resistance to a series of structurally unrelated compounds. One important resistance mechanism involves drug depletion in cells by transmembrane efflux proteins, for example P-glycoprotein (P-gp) in mammalian cells, Bmr in *Bacillus subtilis*, and NorA in *Staphylococcus aureus*. There has been extensive work in the field of mammalian tumor research and a multitude of structure-activity relationship (SAR) studies of P-gp inhibitors have been reported. The identification of bacterial MDR efflux inhibitors is in its infancy and only a limited number have been identified.

A potent inhibitor of the NorA efflux pump in the gram-positive bacteria *S. aureus* was previously isolated from *Berberis fremontii* and identified as the flavonolignan 5'-methoxyhydnocarpin. This flavonolignan was also reported in the mid 1970's as a minor constituent of *Hydnocarpus wightiana*, but the authors were unable to report a definitive regiochemical assignment of the 1,4-benzodioxane substituents due to the low resolution and lack of two-dimensional NMR techniques available at that time. A regiocontrolled total synthesis of the flavonolignan was performed and it was shown that the original regiochemical assignments were incorrect. The flavonolignan was renamed 5'-methoxyhydnocarpin-D to denote the correct regiochemistry.

Because of the high activity of 5'-methoxyhydoncarpin-D, a SAR study of structurally reminiscent synthetic flavonolignans was performed to see if a more potent inhibitor of the NorA efflux pump could be found. Numerous inhibitors were synthesized using a Ag_2CO_3 mediated biomimetic coupling of various flavonoids and hydroxycinnamyl alcohols. Hydnocarpin-D, 5-deoxyhydnocarpin-D, 5,7-deoxyhydnocarpin-D, and 5-deoxyscutellaprostin-A proved to be more potent inhibitors of the NorA efflux pump than the natural product. It was also shown that some small lipophilic ether derivatives of 4'-hydroxyflavone were also more potent than 5'-methoxyhydoncarpin-D.

Preliminary results from bioassays investigating MDR efflux pump inhibitors of the gram-negative bacteria *Escherichia coli* and *Pseudomonus aeruginosa* showed that coumarins may potentiate anthraquinones. *Harbouria trachypleura* belongs to the *Apiaceae*, a family of plants known to often contain coumarins. Bioassay results showed that *H. trachypleura* did not contain the desired inhibitors, but the plant belongs to a monotypic genus and this distinction warranted its further investigation. Two new coumarins, (+)-trachypleuranin-A and (±)-trachypleuranin-B were isolated and identified by spectroscopic and synthetic means.

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Chapter One

Synthesis and Structures of Regioisomeric Hydnocarpin-Type Flavonolignans

Introduction

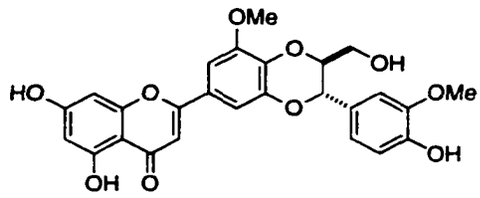
A flavonolignan is the phytochemical oxidation product between a flavonoid and a hydroxycinnamyl alcohol (Figure 1.1). It is well documented that flavonoids are excellent radical scavengers.[1] To date the biomechanistics of flavonolignan formation have not been investigated but there are two principal explanations for flavonolignan production. The first is that flavonolignans are formed via enzyme catalysis. A few flavonolignans have been isolated with optical activity and this is good evidence of enzyme mediated formation.[2] However, the optical purity of these isolates has not been

Figure 1.1. Flavonoid (left) and hydroxycinnamyl alcohol (right) general structures.

discussed in any literature reports, so it is unclear whether these isolates are optically pure or scalemic material. Probably the more accepted view on flavonolignan formation is that they are formed as a result of a random two component coupling induced by

superoxide, *hv*, or other oxidative process. The fact that most flavonolignans have been isolated devoid of optical activity is good evidence for this mechanism. A random coupling mechanism for flavonolignan formation is supported by extensive research in lignan biosynthesis. Lignans are dimers, trimers, or polymers of hydroxycinnamyl alcohols and are the main constituent of plant cell walls. It has been speculated since the 1960's that lignans are formed by a plant regulated random coupling mechanism where the plant initially oxidizes the hydroxycinnamyl alcohol phenol but does not bind or orientate it. This biomechanism most likely parallels that of flavonolignan formation. Until the late 1990's, the random coupling mechanism of lignan biosynthesis had not been challenged. Lewis, however, reports that "dirigent" proteins are responsible for lignan biosynthesis.[3,4,5] Lewis explains that dirigent proteins are also responsible for the initial phenolic oxidation of a hydroxycinnamyl alcohol, but these proteins also orientate, but do not bind, the lignan for dimerization or polymerization. This hypothesis was suggested as to account for optical activity of lignins. Evidence of dirigent proteins is questionable at best and has not been accepted by the mainstream of lignin biosynthetic researchers.

The majority of flavonolignans contain a 1,4-benzodioxane moiety, for example **1**, but structural isomers that result from other phenolic oxidative couplings, for example silychristin (**3**) and isohydnocarpin (**8**), have also been placed in the flavonolignan category. Flavonolignans are best known for their antihepatoxic properties,[6] but they also possess a wide variety of biological activity. These compounds possess anti-inflammatory properties,[7] hypolipidemic activities, and activity against six human cancer cell wall lines and the murine cell line L-1210.[2] Flavonolignans also inhibit superoxide



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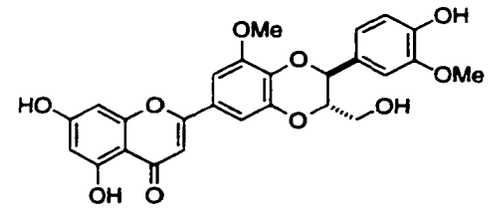

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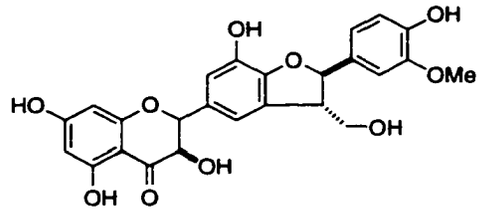

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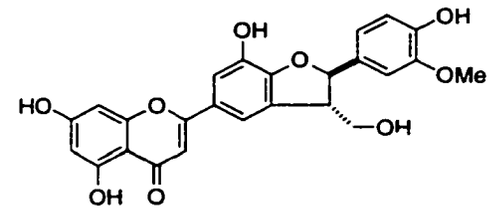

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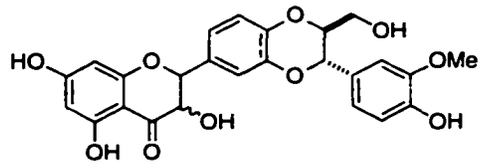
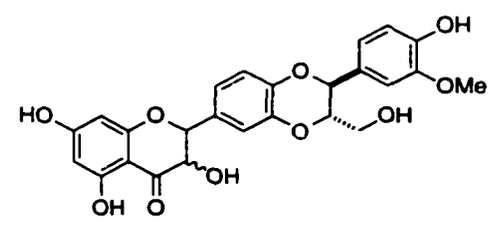
release by human polymorphonuclear leukocytes.[8] A major issue of flavonolignan identification and synthesis is the correct regiochemical assignment of the substituents on the 1,4-benzodioxane ring. Many flavonolignans were isolated in the early 1970's when ^{1}H and ^{13}C NMR spectra did not have the resolution to determine regiochemical identities and two-dimensional NMR techniques were non-existent. Literature reports as recent as 1999 have given incorrect regiochemical assignments[7] or failed to give any definitive proof of the regiochemical structure.[9]

The first flavonolignan, silybin, was isolated in 1968 from a fruit extract of the medicinal plant *Silybum marianum* and identified on the basis of spectroscopic analysis as either **4a** or **5a**.[10] It was stated that the data did not distinguish between these structures and that the structure **4a** was used only "for convenience" when the data were discussed.



4a: α-OH
4b: β-OH



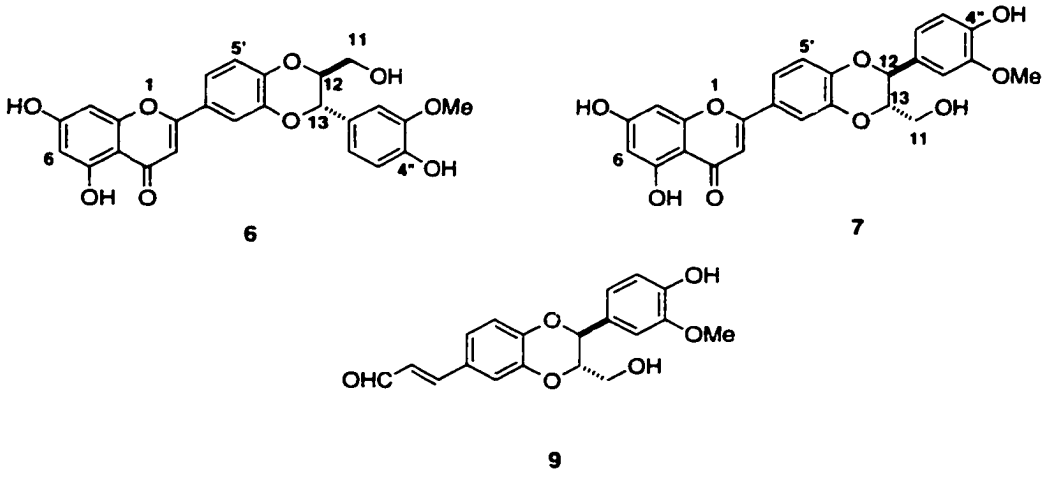
5a: α-OH
5b: β-OH

It was later shown in the mid-1970's through degradation experiments that silybin had the structure **4b** and that isosilybin, isolated at the same time as silybin, had the structure **5b**.[11,12,13] Moreover, recent synthetic studies of dehydrosilybin have also confirmed the regiochemical assignments of silybin and isosilybin.[14]

The second flavonolignan reported, identified in 1973 as an isolate from *Hydnocarpus wightiana*, was given the name hydnocarpin and on the basis of spectroscopy and chemical reactions was assigned structure **6**,"based on analogy with silymarin and also on reactivity considerations."[15] Silymarin was an alternate name for silybin.[16] The spectral data for hydnocarpin could not distinguish between **6** and the alternate structure **7** (Figure 1.2), and there was no discussion of what the "reactivity considerations" were for the assignment. In 1974, the same research group reported the isolation of two flavonolignans from *H. wightiana*, isohydnocarpin **8**[17] (a structural, not regioisomeric structure of hydnocarpin) and what was termed 5'-methoxyhydocarpin (**2**).[18] NMR and mass spectral data for **2** as well as for derivatives of **2** were presented and the structure was assigned **2** based on these data and "the similarity in the spectra [of the isolate and that of hydnocarpin]." Hydnocarpin was also reported in 1977 from the seeds of *Cassia absus* and compared to the *H. wightiana* isolate by spectral data, mixed melting point, co-TLC and co-IR.[19]

Shortly thereafter, americanin-A was isolated from *Phytolacca americana* and, on the basis of spectroscopic evidence, given the structure **9** (Figure 1.2).[20] It was recognized that spectroscopy alone could not distinguish **9** from its regioisomer, and hence definitive proof for the structure **9** was established by correlation of degradation products of **9** derivatives with the same degradation derivatives available from silybin (**4b**) and

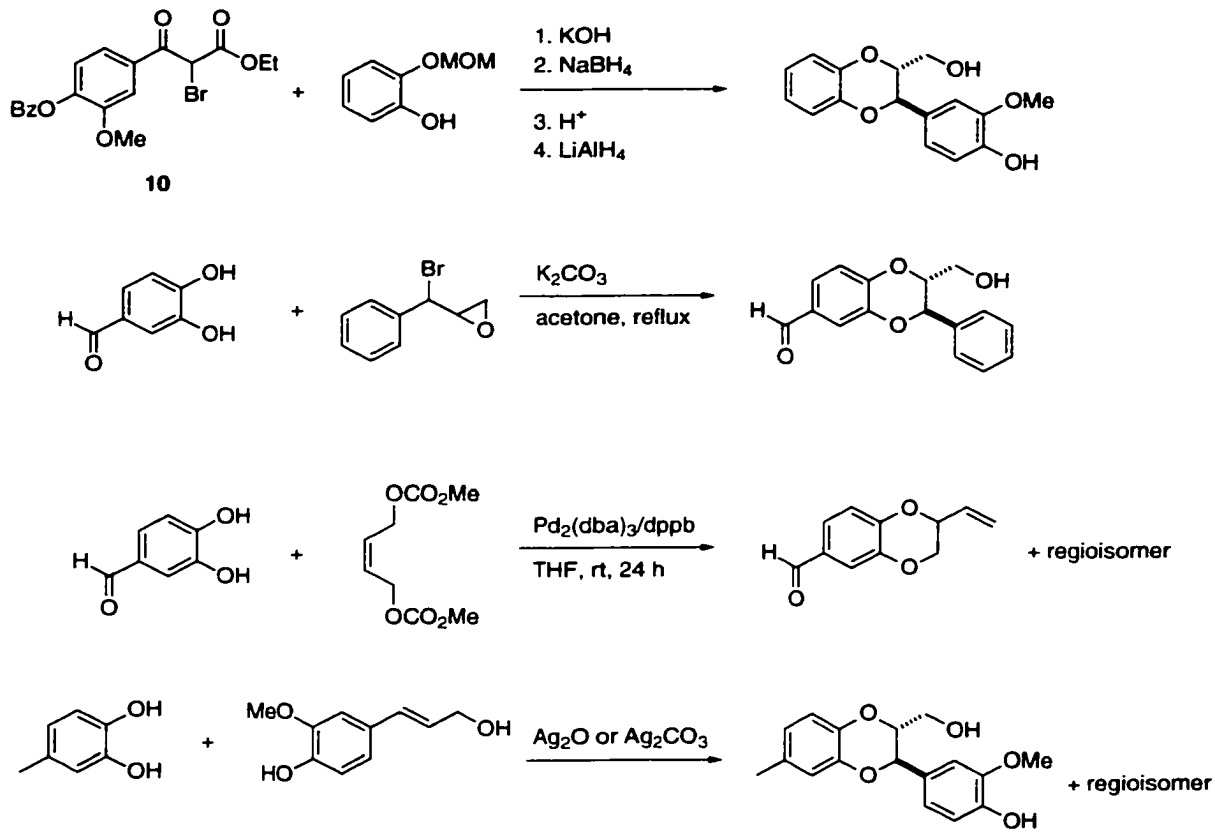
isosilybin (**5b**). One of the americanin-A degradation products was used to synthesize a trimethyl ether of isolated hydnocarpin and this resulted in the regiochemical verification of hydnocarpin as **7**.[21]



6 7 9

Figure 1.2. Structures of hydnocarpin (**6**), hydnocarpin-D (**7**), americanin-A (**9**), and a suggested numbering system for flavonolignans.

Since the initial isolation of **4b**, a number of other flavonolignans have been isolated. These compounds include silandrin,[22] 5"-methoxyhydnocarpin,[23] silychristin,[24] the scutellaprostins,[25] the xanthocercins,[26] a flavonolignan from *Distemonanthus benthamianus*,[27] and sinaiticin.[2] There have also been a multitude of flavonolignan syntheses reported with the majority of these targeting silybin[11,13,14,28]. Silybin (**4b**) has attracted much attention because of its ready availability from the common milk thistle *S. marianum* and its diverse biological activity. The reported syntheses of **4b** in the literature are exemplary of most flavonolignan syntheses with the key step being the formation of the 1,4-benzodioxane ring. The formation of this moiety can be achieved in numerous ways (Scheme 1.1). This ring can be formed by a reaction of brominated

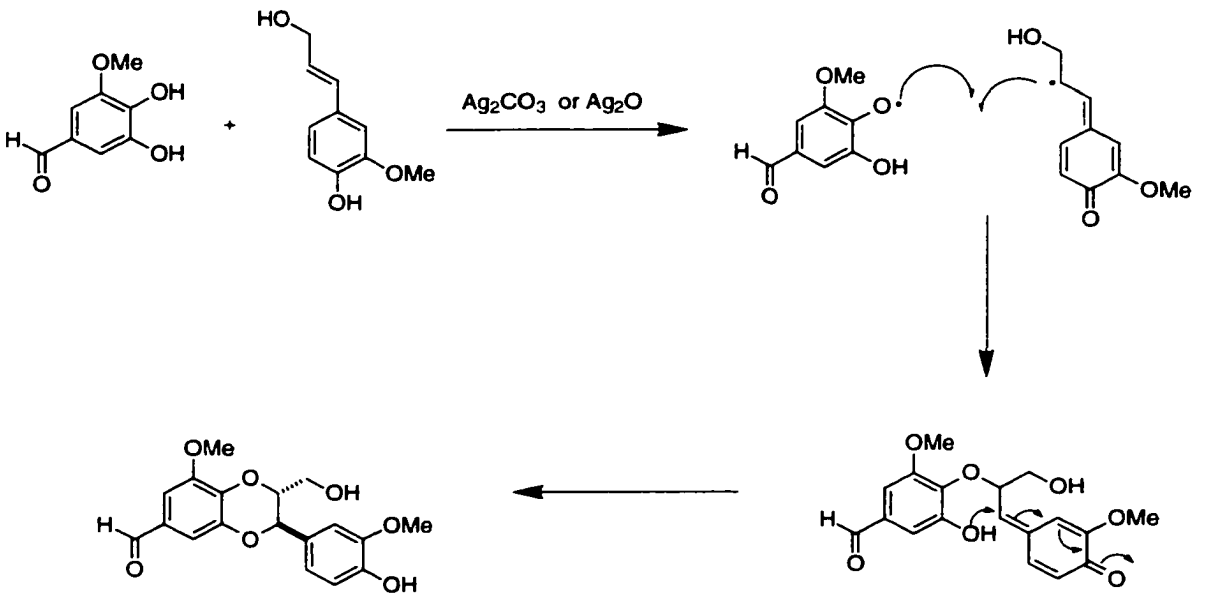


10

Scheme 1.1. Four synthetic techniques for 1,4-benzodioxane formation.

substrates such as **10** in basic media to give, after a reduction, hydrolysis and another reduction, 1,4-benzodioxane flavonolignan precursors.[12] Moreover, it was recently reported that the coupling of 3-bromo-2-epoxyphenyl derivatives with catechols in a basic medium provides regiopure flavonolignan precursors.[29] The use of palladium mediated reactions to create 1,4-benzodioxanes has also been reported, but poor regioselectivity is of concern with this class of reaction.[30] Perhaps the most common method to prepare 1,4-benzodioxane flavonolignan precursors is by a one step Ag_2CO_3, Ag_2O, or horseradish peroxidase mediated oxidative radical coupling of a catechol with a hydroxycinnamyl alcohol (Scheme 1.1, 1.2). This class of reaction is often described as a

biomimetic coupling. The flavonolignans silybin[31] (**4b**), hydnocarpin[32] (**7**), and sinaiticin[9] (**11**) were synthesized using this oxidative radical coupling strategy.



Scheme 1.2. Mechanism of 1,4-benzodioxane formation initiated by Ag_2CO_3.

The syntheses of enantiopure 1,4-benzodioxanes appeared in the literature in the 1990's. Li *et al.* obtained an enantiopure sample of silybin by preparing the flavonoid (+)-taxifolin using a Sharpless asymmetric epoxidation. An Ag_2CO_3/Celite mediated coupling was used to create a 1,4-benzodioxane ring and a chiral HPLC resolution was used to provide enantiopure (+)-**4b**.[28] The synthesis of unnatural silybin-type flavonolignans was reported by Czompa and coworkers in 2000.[8] They employed (+)-glycidyl tosylate and a $CaCO_3$ mediated coupling to create chiral 1,4-benzodioxane rings. Gu *et al.* created chiral 1,4-benzodioxane rings using a Sharpless asymmetric dihydroxylation.[33]

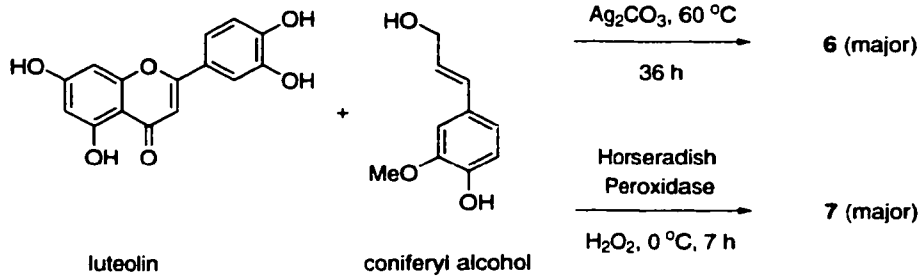
Recently, a flavonolignan was isolated from *Berberis fremontii* whose ^1H and ^{13}C NMR, mass and UV spectra were essentially identical with those reported for 5'-

methoxyhydnocarpin (**2**).[34] It was also shown that this isolate is a potent inhibitor of the NorA MDR efflux pump in *Staphylococcus aureus*.[35] A definitive regiochemical assignment had not been reported for 5'-methoxyhydnocarpin, so it was elected to perform a regiocontrolled total synthesis of the natural product to prove its structure. To ensure that the regiochemical assignment of the isolate was correct, both regioisomers of the hydnocarpin series were synthesized to help shed light into ^{1}H and ^{13}C NMR resonance relationships between flavonolignan regioisomers. These syntheses would also provide a starting point for a small flavonolignan structure activity relationship study with *S. aureus*. There were no literature reports on the synthesis of 5'-methoxyhydnocarpin and only a single report on that of hydnocarpin, via a coupling of the flavone luteolin and the hydroxycinnamyl alcohol coniferyl alcohol in a cell-free suspension culture from the fruit of *S. marianum*.[36]

Results

Hydnocarpin and Hydnocarpin-D

Biomimetic oxidative couplings were used as the primary method to access both regioisomers of the hydnocarpin series. Schrall and Becker used horseradish peroxidase (HRP) to synthesize isosilybin (**5b**), a flavonolignan whose pendant aromatic ring is attached at the 12 position in our suggested numbering scheme (or in an informal sense,



Scheme 1.3. Biomimetic synthesis of hydnocarpin series flavonolignans.

the "up" position, Figure 1.2).[36] A coupling of luteolin and coniferyl alcohol with HRP should thus give hydnocarpin (**7**), a flavonolignan with analogous regiochemistry to isosilybin (Scheme 1.3). The literature suggested that access to hydnocarpin-D (**6**) could be achieved by a Ag_2O or Ag_2CO_3 mediated coupling to give a previously unknown flavonolignan with the pendant aromatic ring attached at the 13 position (or in the "down" position, Figure 1.2).[37] These couplings were indeed achieved (Scheme 1.3) although the resulting regioselectivities were less than expected based upon literature results for somewhat analogous systems.[37,38] In our hands, **6** and **7** were not separable by C-8 or C-18 HPLC methods using a wide variety of solvents. This separation difficulty parallels that of similar separations with silybin/isosilybin.[13,39] Normal phase separations were not attempted. The **6/7** regiochemical product ratios in the crude preparations were easily determinable, however, from the ratios of the two ^{1}H NMR doublets for H-13 (in **6**) and H-12 (in **7**), which appeared at δ 5.02 and δ 5.06, respectively, in acetone-d_6 (Figure 1.3). These ratios showed the crude products to be 9:1 **6/7** for the Ag_2CO_3-mediated reaction and 3:2 **7/6** for the HRP mediated reaction. It was found that in the case of **6** and **7** and in other syntheses of flavonolignans that will be described later, the H-12 benzyl proton resonance in "up" isomers was always 0.1-0.3 ppm downfield of that of the H-13 benzyl proton on "down" regioisomers (Figure 1.3). ^{13}C NMR resonances were for **6** and **7** were essentially identical (all within 0.2 ppm), with the exception of those for C-12 and C-13. For **6**, C-12 was at δ 78.0 and C-13 at δ 76.0, while for **7** C-13 was at δ 78.5 and C-12 at δ 75.9. Although small, these resonance differences were normally indistinguishable in a mixture of the two. It is highly unlikely that ^{1}H or ^{13}C

NMR spectra of a regiopure flavonolignan would differentiate between exactly which regioisomer was on hand unless both regioisomers were available.

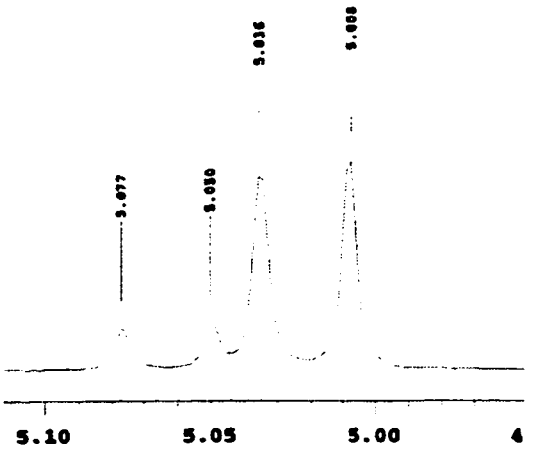
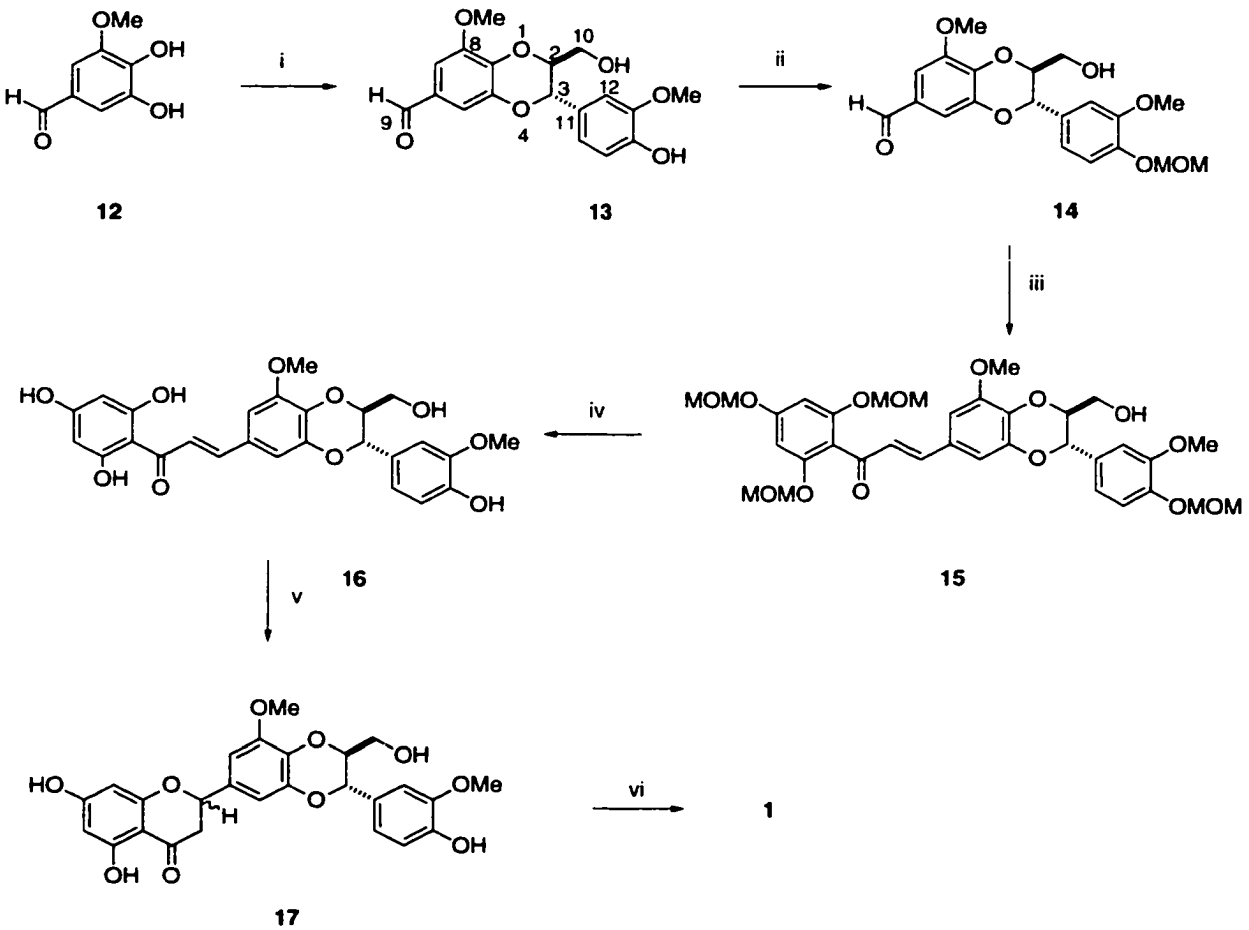


Figure 1.3. Doublets representing the two regioisomers of the hydnocarpin series. The left doublet corresponds to the "up" regioisomer, and the right doublet to the "down" regioisomer.

It was necessary to use both normal phase chromatography and reverse phase C-18 vacuum-liquid chromatography to purify **6** and **7**. These purification techniques yielded pure regiochemical mixtures of the flavonolignans in the ratios described above. To obtain a regiopure sample of **7**, the chromatographed material was recrystallized from 9:1 MeOH/H_2O. Recrystallization of **6** or its peracetate with a variety of systems was unsuccessful in yielding regiopure samples. It was found that washing the peracetate of **6** with 1:1 hexanes/ethyl acetate or technical grade acetone provided regiopure material. A standard deacetylation with K_2CO_3/MeOH provided a regiopure sample of **6**. The chromatographic systems as well as the recrystallization solvents and washing techniques for **6** and **7** proved invaluable for the purification of synthetic flavonolignans described in Chapter 2.

5'-Methoxyhydnocarpin-D

Because regiochemical mixtures were obtained with the hydnocarpin series syntheses, it was elected to attempt a regiospecific synthesis of 5'-methoxyhydnocarpin. The literature provided a reactivity profile of substituted catechols and hydroxycinnamyl alcohols with Ag_2O or Ag_2CO_3 mediated couplings. It was shown that catechols with para-electron donating groups, such as 3,4-dihydroxytoluene, would yield a 1,4-benzodioxane product with the major regioisomer having the pendant aromatic ring in the "down" position. Conversely, a similar reaction with a catechol containing a para electron



i: coniferyl alcohol, Ag_2CO_3, 5/1 PhH/acetone, 60 °C, 7 h, 72%; ii: MOMCl, THF, NaH, rt,7 h, 85%, iii: KOH, EtOH, 2,4,6-tris(methoxymethoxy)acetophenone, 48 h, 68%; iv: conc. HCl, MeOH, rt, 12 h, 72%; v: NaOAc, MeOH, reflux, 3 h, 92%; vi: DDQ, dry dioxane, reflux, 36 h, 74%.

Scheme 1.4. Regiocontrolled synthesis of 5'-methoxyhydnocarpin-D.

withdrawing group, such as 3,4-dihydroxybenzaldehyde, would yield a 1,4-benzodioxane product with the major isomer having the pendant aromatic ring in the "up" position.[37] Reactions with ortho substituted catechols like 3,4-dihydroxy-5-methoxybenzaldehyde (**12**) were not discussed and it was unclear what the product mixture would be for such a reaction. The Ag_2CO_3 mediated coupling of coniferyl alcohol with **12** in 2:1 benzene/acetone at 60 °C gave a single regioisomeric product in high yield (74%). This product was assumed to have the "up" regiochemistry based upon literature precedence. Surprisingly, an X-ray diffraction crystal structure of the 1,4-benzodioxane product (**13**) showed that the product was the unexpected "down" regioisomer (see X-ray crystal structure, Figure 1.4 and Scheme 1.2).

Substituent effects of substituted benzenes are well documented and this case is no exception. The product seen in this biomimetic coupling is most likely the result of the strong stabilizing effect by the ortho-methoxy on the phenoxide radical. This stabilization effect transcends the destabilization of the para-carbaldehyde on reactivity considerations. It was hypothesized that if the plant produced flavonolignan was in fact a result of a random coupling of **12** and coniferyl alcohol, then the phytochemical would also have "down" regiochemistry.

With the regiochemistry of the 1,4-benzodioxane carbaldehyde **13** established, the synthesis of the flavonolignan was continued (Scheme 1.4) by protecting the free phenol as the MOM ether using NaH and MOMCl in THF at room temperature to yield **14**. Protection of the primary alcohol as a MOM ether was sluggish even under refluxing THF conditions (<30% protected after 6 hours) and the traditional MOM protection sequence of MOMCl in Hünig's base provided no product because of the insolubility of

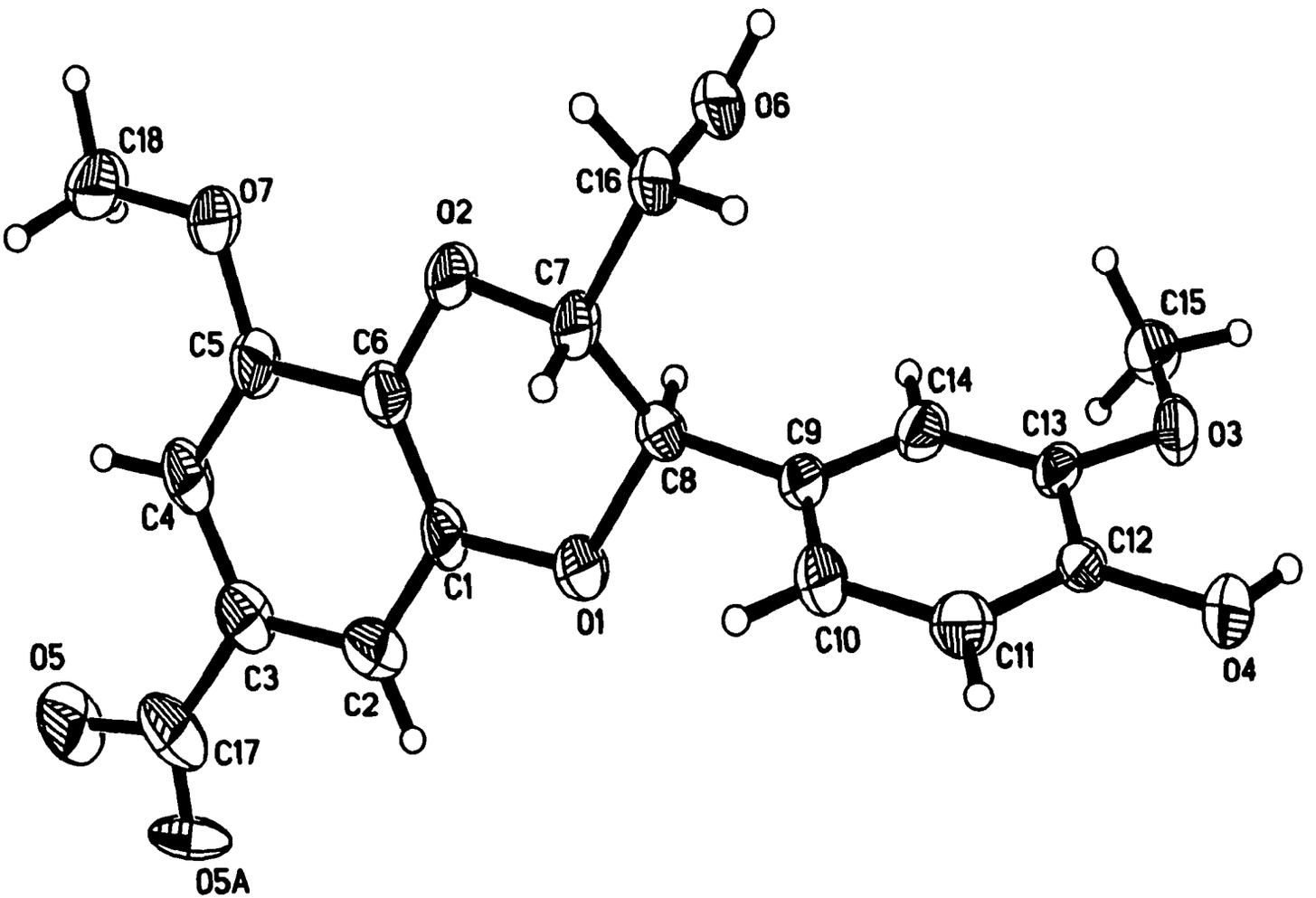


Figure 1.4. ORTEP diagram representing X-ray crystal structure of synthetic intermediate **13**. O5 and O5A represent an average position of the aryl aldehyde functional group.

13. It was found that protection of the primary alcohol was not necessary because the subsequent aldol reaction with 2,4,6-tris(methoxymethoxy)acetophenone proceeded smoothly to give **15** in 68% yield. Deprotection of the MOM groups using conc. HCl in MeOH at room temperature provided the free chalcone (**16**) and cyclization to the chromone (**17**) was achieved using NaOAc in MeOH with both reactions proceeding in good yield. Formation of the final flavonolignan was achieved using DDQ in refluxing dry 1,4-dioxane for 48 h. to yield a product (**1**) that had a ^{1}H and ^{13}C NMR spectrum identical to that of the natural product.

Although the NMR spectra of synthesized **1** and an authentic sample from *B. fremontii* were apparently identical,[34] the experience with hydnocarpin and hydnocarpin-D suggested that one could not be certain which whether **1** or **2** was on hand if only one regioisomer was available. Attempts to grow X-ray diffraction quality crystals of the *B. fremontii* isolate proved unsuccessful; thus, independent evidence for the structure of the isolated flavonolignan was necessary. HMBC NMR achieved this confirmation with the

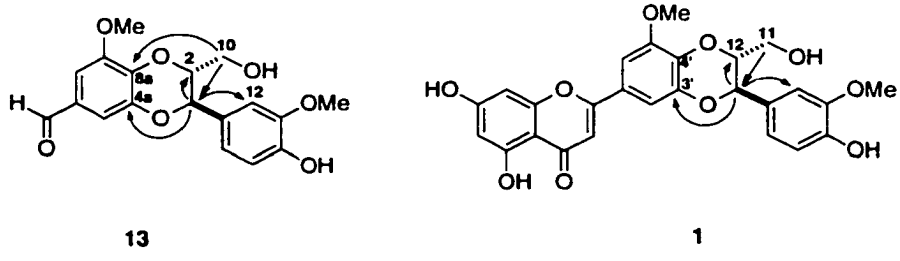
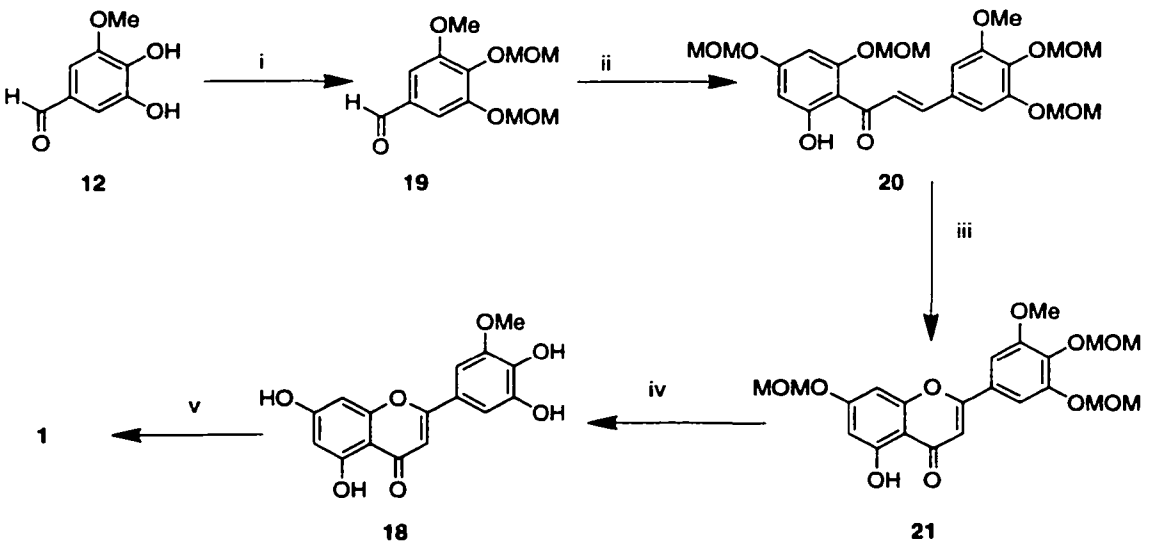


Figure 1.5. HMBC correlations of an intermediate with known regiochemistry (**13**) and the natural product (**1**).

coupling constant optimized to 1.6 Hz, a technique similar to the "selective heteronuclear decoupling" experiment used to prove the regiochemistry of some coumarinolignoids.[40] As a model, compound **13** (whose regiochemical structure was known from X-ray crystal data, Figure 1.4) showed the following diagnostic HMBC correlations (Figures 1.5 and

1.10): H-3 (δ 5.0 resonance) to C-2 (79.1), C-12 (109.7) and C-4a (144.9); H-10 (δ 3.6 resonance) to C-3 (76.2) and C8a (138.7). The experiment was the repeated on the phytochemical. Although the same array of correlations were not seen, the key H-13 to C-3' correction was indeed seen (Figure 1.5, 1.16 and 1.17). Thus, the natural 5'-methoxy compound does not have the same regiochemistry as hydnocarpin (**7**) and hence was designated 5'-methoxyhydnocarpin-D (**1**, the suffix "D" designating a "down" regioisomer).

Because 5'-methoxyhydocarpin-D proved to be a potent inhibitor of the *Staphylococcus aureus* NorA MDR efflux pump,[35] a sample of its regioisomer **2** was desired for a future structure-activity relationship between flavonolignans. By analogy with the syntheses of **6** and **7**, radical-initiated coupling of coniferyl alcohol to the rare flavone selgin should yield a mixture of **1** and **2**. Selgin (**18**), also known as selagine and selagin, was first isolated from *Huperzia selago* and its structure proven by spectroscopic analysis and synthesis.[41] The flavonoid **18** was prepared by a much more efficient method



i: MOMCl, K_2CO_3, dry acetone, 0.25 h, 85%; ii: KOH, EtOH, 2-hydroxy-4,6-bis(methoxymethoxy)acetophenone, 17 h; 75%; iii: DDQ, dry dioxane, reflux, 48 h, 65%; iv: HCl, MeOH, reflux, 20 min., 72%; v: coniferyl alcohol, Ag_2CO_3, 5/1 PhH/acetone, 60 °C, 7 h, 33%; coniferyl alcohol, HRP, H_2O_2, 0 °C,7h, 7%

Scheme 1.5. Synthesis of selgin (**18**) and attempted synthesis of 5'-methoxyhydnocarpin.

(Scheme 1.5) using standard chemical transformations and was coupled with coniferyl alcohol using Ag_2CO_3 as well as horseradish peroxidase. Unlike the reactions in the preparation of **6** and **7**, only a single flavonolignan, again **1**, resulted in low (33% with Ag_2CO_3) and poor (7% with HRP) yield. The formation of only one regioisomer gives further evidence that a 5'-methoxy group has a pronounced stabilizing effect on the 4'-phenoxy radical.

While disappointed with the fact that **2** could not be synthesized from **18**, it was soon found in a structure-activity relationship study that "up" regioisomers were not as potent *S. aureus* MDR efflux pump inhibitors as their regioisomeric cousins. Thus, it was decided not to pursue synthesis of **2** and focus synthetic efforts towards an array of "down" flavonolignans for further biological testing.

Experimental

General Experimental Procedures. ^1H and ^{13}C NMR spectra were recorded on a Varian Inova spectrometer at 400 and 100 MHz, respectively, using $CDCl_3$, acetone-d_6, or DMSO-d_6 as the solvent and internal reference. Melting points were determined on a Laboratory Device's Mel-Temp and are uncorrected. All solvents were distilled prior to use. THF and 1,4-dioxane were freshly distilled from benzophenone-ketyl and benzene was freshly distilled from CaH_2. ACS acetone was stored over 4 Å molecular sieves. All non-aqueous reactions were performed in dry glassware under an argon atmosphere. All column chromatography separations (CC) were performed with normal phase silica gel (Scientific Adsorbents Incorporated, 32-63 μm particle size, 60 Å pore size). All starting materials were used as received. Luteolin was purchased from Indofine Chemical Co. and

all other reagents purchased from Aldrich Chemical Co. ^{1}H and ^{13}C NMR spectra of final products and selected intermediates are presented after the experimental section.

Hydnocarpin-D (6). To a 250 mL three-neck round-bottom flask was added 0.360 g (1.26 mmol) of luteolin and 0.227 g (1.26 mmol) of coniferyl alcohol with 50 mL dry benzene and 25 mL dry acetone. The reaction vessel was placed in a 60 °C oil bath and let stir for 10 min. Next, 0.347 g (1.26 mmol) of Ag_2CO_3 was added and the reaction solution stirred vigorously for 36 h. The reaction was then allowed to cool, filtered through a Buchner funnel, and the solvent removed by rotary evaporation to yield a yellow powder. The yellow powder was subjected to CC using 95:5 $CHCl_3$/MeOH to yield 0.220 g of a 9:1 (by NMR) mixture of **6** and **7** plus other minor impurities. The sample was recrystallized from 9:1 MeOH/H_2O to yield 0.125 g (21%) of pure **6** and **7**. This mixture was acetylated using standard acetic anhydride/pyridine conditions to yield an off-white powder which was washed with acetone until bright white. A standard deprotection using K_2CO_3/MeOH was used to remove the acetyl groups and yielded 0.040 g (7%, yellow-white powder) of pure **6**. ^{1}H NMR (DMSO-d_6): δ 3.37 (dd, J=12.6, 4.8 Hz, H-11a), 3.57 (dd, J=12.6, 2.8 Hz, H-11b), 4.31 (m, H-12), 4.97 (d, J=8.0 Hz, H-13), 6.19 (d, J=2.0 Hz, H-6), 6.51 (d, J=2.0 Hz, H-8), 6.82 (dd, J=8.0, 1.6 Hz, H-5"), 6.87 (s, H-3), 6.89 (dd, J=8.0, 1.6 Hz, H-6"), 7.05 (d, J=1.6 Hz, H-2"), 7.12 (d, J=8.4 Hz, H-5'), 7.63 (dd, J=8.8, 2.0 Hz, H-6'), 7.67 (d, J=2.0 Hz, H-2'), 9.18 (bs), 10.85 (bs), 12.90 (bs). ^{13}C NMR (DMSO-d^6): δ 55.7 (OMe), 60.0 (C-11), 75.9 (C-13), 78.5 (C-12), 94.1 (C-8), 98.9 (C-6), 103.8 (C-3), 103.8 (C-10), 111.8 (C-2"), 115.0 (C-2'), 115.3 (C-5"), 117.4 (C-5'), 120.1 (C-6'), 120.6 (C-6"), 123.4 (C-1'), 127.0 (C-1"), 144.0 (C-4'), 146.9

(C-3'), 147.1 (C-4"), 147.6 (C-3"), 157.3 (C-9), 161.4 (C-5), 162.9 (C-7), 164.2 (C-2), 181.8 (C-4). Peracetate (**6a**): ^{1}H NMR ($CDCl_3$): δ 2.08 (s), 2.34 (s), 2.35 (s), 2.44 (s), 3.88 (s, OMe), 4.03 (dd, J=12.4, 4.0 Hz), 4.34 (m), 4.41 (dd, J=12.0, 3.0 Hz), 5.00 (d, J=8 Hz), 6.57 (s), 6.84 (d, J= 2.1 Hz), 6.98 (d, J=1.8 Hz), 7.02 (m), 7.11 (d, J=8.7 Hz), 7.12 (d, J=7.8 Hz), 7.32 (d, J=2.1), 7.45 (dd, J=8.4, 2.1), 7.53 (d, J=2.1). ^{13}C NMR ($CDCl_3$): δ 20.7, 21.1, 21.1, 21.2, 56.0, 62.4, 75.9, 76.3, 107.5, 108.9, 110.9, 113.5, 115.3, 117.8, 119.7, 120.2, 123.3, 124.4, 133.9, 140.6, 143.6, 146.0, 150.0, 151.6, 153.7, 157.4, 161.8, 167.9, 168.6, 169.3, 170.2, 176.2. mp 233-234 °C. *anal.* C 63.00%, H 4.11 %, calcd for $C_{33}H_{28}O_{13}$, C 62.66%, H 4.46%. See Figures 1.6 (hydnocarpin-D ^{1}H), 1.7 (hydnocarpin-D OAc ^{1}H), and 1.8 (hydnocarpin-D ^{13}C) for NMR spectra.

Hydnocarpin (7). To a 100 mL three-neck round-bottom flask was added 0.300 g (1.05 mmol) of luteolin and 0.189 g (1.05 mmol) of coniferyl alcohol. Next, 20 mL of dry ACS acetone and 5 mL of a 0.2 M citric acid/phosphate buffer were added to the reaction flask and the flask cooled to 0 °C. Two drops of 30% H_2O_2 and 1 mL of a horseradish peroxidase solution (1.5 mg HRP (1100 U/mg) / 3 mL water) were added. The HRP solution (1 mL) was added every 15 min. thereafter, and the reaction then allowed to stir at 0 °C for 7 h. The reaction solution was allowed to warm to room temperature, washed with brine, and extracted with EtOAc. The EtOAc was dried with anhyd $MgSO_4$, filtered, and removed by rotary evaporation to yield a brown-orange solid. This solid was subjected to CC using 95:5 $CHCl_3$/MeOH to yield 0.187 g (40%) of a white-yellow solid, 3:2 ratio of **7** to **6** by ^{1}H NMR. This solid was further purified by reverse-phase (C-18) vacuum-liquid chromatography (75:25 H_2O/MeOH to 20:80 H_2O/MeOH) to yield 0.087

g of a white-yellow solid that was recrystallized from 9:1 MeOH/H_2O to yield 0.025 g (5%, white-yellow powder, mp 258-259 °C) of pure **7**. ^1H and ^{13}C NMR spectra were essentially identical to the literature.[2]

3-(4-Hydroxy-3-methoxyphenyl)-2-hydroxymethyl-8-methoxy-2,3-dihydro-benzo[1,4]dioxine-6-carbaldehyde (13). To a 250 mL three-neck round-bottom flask was added 0.486 g (2.77 mmol) of **12** and 0.500 g (2.77 mmol) of coniferyl alcohol with 100 mL dry benzene and 20 mL dry acetone. This solution was allowed to stir 20 min in a 60 °C oil bath then 0.765 g (2.77 mmol) of Ag_2CO_3 was added and the reaction stirred vigorously for 7 h. The reaction was then allowed to cool, filtered through a Buchner funnel, and the solvent removed by rotary evaporation to yield a brown microcrystalline solid. This solid was subjected to CC using 4:6 hexanes/EtOAc to yield 0.687 g (72%) of a near pure sample of **7**. This solid was recrystallized from 7:3 EtOH/H_2O to yield 0.344 g (36%, white microcrystalline solid, mp 103-105 °C) of pure **13**. ^1H NMR ($CDCl_3$): δ 3.60 (dd, J=12.6, 4.0 Hz), 3.93 (s, OMe), 3.94 (m), 3.97 (s, OMe), 4.10 (m), 4.99 (d, J=8.0 Hz), 6.94 (br s, 1H), 6.97 (m, 2 H), 7.13 (d, J=2.0 Hz), 7.16 (d, J=2.0 Hz), 9.80 (s). ^{13}C NMR ($CDCl_3$): δ 56.0, 56.3, 61.2, 76.1, 79.1, 103.0, 109.6, 114.6, 114.8, 120.8, 127.3, 129.4, 138.7, 144.4, 146.5, 147.0, 149.5, 190.8. *anal.* C 62.55%, H 5.00%, calcd for $C_{18}H_{16}O_7$, C 62.61%, H 4.96%. See Figures 1.9 and 1.10 for ^1H and HMBC NMR spectra, respectively.

2-Hydroxymethyl-8-methoxy-3-(3-methoxy-4-methoxymethoxyphenyl)-2,3-dihydro-benzo[1,4]dioxine-6-carbaldehyde (14). To a 100 mL round-bottom was added 0.196 g

(0.569 mmol) of **13** and 35 mL dry THF. The flask was placed into an ice-water bath and 0.017 g (0.683 mmol) of NaH (washed prior with hexanes) added. The solution was stirred 1 h, then 0.055 g (0.683 mmol) of MOMCl added and the reaction allowed to warm to rt. The reaction was stirred for 6 h at rt, poured into a saturated solution of $NaHCO_3$, and extracted with EtOAc. The EtOAc was dried with anhyd Mg_2SO_4, filtered, and removed by rotary evaporation to yield an off-white microcrystalline solid. This solid was subjected to CC using 3:7 hexanes/EtOAc to yield 0.187 g (85%, white microcrystalline solid, mp 67-68 °C) of pure **14**. ^{1}H NMR ($CDCl_3$): δ 3.52 (s), 3.60 (dd, J=12.0, 3.3 Hz), 3.91 (s, OMe), 3.93 (m), 3.95 (s, OMe), 4.10 (m), 5.03 (d, J=8.1 Hz), 5.25 (s), 6.98 (d, J=1.8 Hz), 6.99 (dd, J= 8.7, 1.8 Hz), 7.12 (d, J=1.8Hz), 7.15 (d, J=1.8 Hz), 7.20 (d, J = 8.7 Hz), 9.79 (s). ^{13}C NMR ($CDCl_3$): δ 30.9, 56.0, 56.2, 61.2, 75.9, 78.9, 95.3, 103.0, 110.5, 114.5, 116.2, 120.0, 129.3, 129.5, 138.6, 144.2, 147.1, 149.4, 149.9, 190.6. See Figure 1.11 for ^{1}H NMR spectrum.

3-[2-Hydroxymethyl-8-methoxy-3-(3-methoxy-4-methoxymethoxyphenyl)-2,3-dihydrobenzo[1,4]dioxin-6-yl]-1-(2,4,6-tris-methoxymethoxyphenyl)-propenone (15). To a 25 mL round-bottom flask was added 0.098 g (0.252 mmol) of **14** and 0.076 g (0.252 mmol) of 2,4,6-tris(methoxymethoxy)acetophenone, 10 mL EtOH, and 0.339 g of crushed solid KOH. The blocked acetophenone was prepared from reaction of the bis-protected acetophenone[42] with NaH (1.3 eq), MOMCl (1.3 eq) in THF at 0 °C for 4 hrs; 86%. This was stirred at room temperature for 17 h, washed with brine, and extracted with EtOAc. The EtOAc was dried with anhyd. $MgSO_4$, filtered, and removed by rotary evaporation to yield a yellow oil that was subjected to CC 2:8 using hexanes/EtOAc to

yield 0.115 g (68%, yellow solid) of pure **15**. ^{1}H NMR (acetone-d_6): δ 3.36 (s), 3.46 (s), 3.52 (dd, J=12.8, 3.6 Hz), 3.83 (m), 3.85 (s, OMe), 3.89 (s, OMe), 4.14 (m), 5.06 (d, J=7.6 Hz), 5.14 (s), 5.19 (s), 5.22 (s), 6.88 (d, J=2.0 Hz), 6.88 (d, J=16.0 Hz), 6.98 (d, J=2.0 Hz), 7.03 (dd, J=8.4, 2.0 Hz), 7.15 (d, J=8.4 Hz), 7.17 (d, J=2.0 Hz), 7.24 (d, J=16.0 Hz). ^{13}C NMR (acetone-d_6): δ 56.3, 56.3, 56.3, 56.4, 56.4, 56.5, 61.6, 76.8, 79.6, 95.3, 95.3, 95.4, 95.4, 96.3, 97.9, 105.2, 111.4, 112.7, 118.1, 121.1, 128.1, 128.6, 132.2, 136.8, 145.5, 148.0, 150.5, 151.5, 156.6, 160.3, 193.6. See Figure 1.12 for ^{1}H NMR spectrum.

3-[2-Hydroxymethyl-8-methoxy-3-(4-hydroxy-3-methoxyphenyl)-2,3-dihydrobenzo[1,4]dioxin-6-yl]-1-(2,4,6-trihydroxyphenyl)-propenone (16). To a 25 mL round-bottom flask was added 0.042 g of **15**, 5 mL of MeOH, and 2 drops of concentrated HCl. This solution was stirred at room temperature for 17 h, poured into a saturated $NaHCO_3$ solution, and extracted with EtOAc. The EtOAc was dried with anhyd. $MgSO_4$, filtered, and removed by rotary evaporation to yield a brown-orange oil that was subjected to CC using 92:8 $CHCl_3$/MeOH to yield 0.022 g (72%, brown-orange microcrystalline solid, mp 175-177 °C) of pure **16**. ^{1}H NMR (acetone-d_6): δ 3.52 (dd, J=12.8, 3.6 Hz), 3.83 (m), 3.88 (s, OMe), 3.91 (s, OMe), 4.13 (m), 5.03 (d, J=8.0 Hz), 5.97 (s), 6.89 (d, J=8.0 Hz), 6.93 (d, J=2.0 Hz), 6.94 (d, J=2.0 Hz), 6.98 (dd, J=8.0, 2.0 Hz), 7.14 (d, J=2.0 Hz), 7.70 (d, J=15.6 Hz), 8.13 (d, J=15.6 Hz). ^{13}C NMR (acetone-d_6): δ 56.4, 56.5, 61.8, 77.1, 79.8, 96.2, 105.8, 106.1, 110.8, 112.0, 115.9, 121.7, 126.7, 128.8, 129.2, 136.9, 143.4, 145.8, 148.2, 148.6, 150.4, 165.6, 165.8, 193.2. *anal.* C 56.10%, H 5.42%, calcd for $C_{26}H_{24}O_{10} \cdot 3.5\ H_2O$ C 55.81%, H 5.58 %.

5,7-Dihydroxy-2-[3-(4-hydroxy-3-methoxyphenyl)-2-hydroxymethyl-8-methoxy-2,3-dihydrobenzo[1,4]dioxin-6-yl]-chroman-4-one (17): To a 50 mL round-bottom flask was added 0.021 g (0.043 mmol) of **16**, 15 mL of MeOH, and 0.035 g (0.43 mmol) of NaOAc. This solution was heated at reflux for 3 hours, poured into a saturated $NaHCO_3$ solution, and extracted with EtOAc. The EtOAc was dried with anhyd. $MgSO_4$, filtered, and removed by rotary evaporation to yield an off-white solid. This solid was subjected to CC using 1:9 hexanes/EtOAc to afford 0.019 g (white powder) of **17** as a 1:1 (^{1}H NMR) diastereomeric mixture (from integration of the singlets at δ 12.170 and δ 12.173). ^{1}H NMR ($CDCl_3$): δ 3.19 (d, J=12.4 Hz), 3.23 (d, J=12.8 Hz), 3.52 (m), 3.81 (m), 3.87 (s, OMe), 3.88 (s, OMe), 4.07 (m), 5.01 (d, J=7.6 Hz), 5.45 (dd, J=12.4, 2.4 Hz), 5.89 (m), 5.95 (m), 6.76 (m), 6.83 (m), 6.88 (d, J=8.0 Hz), 6.98 (dd, J=8.0, 2.0 Hz), 7.13 (m), 7.76 (bs), 9.62 (bs), 12.17 (bs). HRFAB$^+$ 497.1430 (calcd for $C_{26}H_{25}O_{10}$ 497.1447). See Figure 1.13 for ^{1}H NMR spectrum.

5'-Methoxyhydocarpin-D (1). To a 25 mL round-bottom flask was added 0.016 g (0.032 mmol) of **17**, 0.018 g of DDQ (0.081 mmol), and 7 mL of dry 1,4-dioxane. The solution was heated at reflux for 36 h, let cool, and solvent removed by rotary evaporation to yield a dark brown solid. The solid was chromatographed on 95:5 $CHCl_3$/MeOH to yield 0.012 g of pure **5** (72%, pale yellow powder). HRFAB$^+$ *m/z* 495.1310 (calcd for $C_{26}H_{23}O_{10}$ 495.1291). ^{1}H and ^{13}C NMR spectral data were identical with those of the isolate.[34] See Figures 1.14, 1.15, 1.16, 1.17 for ^{1}H, ^{13}C, and HMBC NMR spectra, respectively.

1-(2-Hydroxy-4,6-bis-methoxymethoxyphenyl)-3-(3-methoxy-4,5-bis-methoxymethoxyphenyl)-propenone (20). To a 100 mL three-neck round-bottom flask was added 0.202 g (1.20 mmol) of **12**, 1.75 g (16.22 mmol) of K_2CO_3 and 35 mL of dry acetone. This solution was stirred for 10 min, then 0.242 g (3.00 mmol) of MOMCl was added. The solution was heated at reflux for 15 min, poured into a saturated $NaHCO_3$ solution, and extracted with EtOAc. The EtOAc was dried with anhyd. $MgSO_4$, filtered, and removed by rotary evaporation to yield a brown, viscous oil. The oil was subjected to CC using 1:1 hexanes/EtOAc to yield a near pure sample of **19** (0.262g, 85%, clear oil that partially solidified upon standing) which was carried directly to the next step. To a 50 mL round-bottom flask was added 0.129 g (0.505 mmol) of **19**, 0.129 g (0.505 mmol) of 2-hydroxy-4,6-bis(methoxymethoxy)acetophenone,[42] 20 mL EtOH, and 0.680 g (12.12 mmol) of crushed solid KOH. This was stirred at rt for 17 h, brought to pH 7 using 1 N HCl, and extracted with EtOAc. The EtOAc was dried with anhyd. Mg_2SO_4, filtered, and removed by rotary evaporation to yield a bright yellow oil. The oil was subjected to CC using 1:1 hexanes/EtOAc to yield 0.154 g (75%, yellow solid) of pure **20**. ^{1}H NMR ($CDCl_3$): δ 3.49 (s), 3.52 (s), 3.54 (s), 3.63 (s), 3.90 (s, OMe), 5.19 (s), 5.20 (s), 5.25 (s), 5.30 (s), 6.26 (d, J=2.4 Hz), 6.33 (d, J=2.4 Hz), 6.86 (d, J=2.0 Hz), 7.17 (d, J=2.0 Hz), 7.71 (d, J=15.6 Hz), 7.87 (d, J=15.6 Hz). ^{13}C NMR ($CDCl_3$): δ 56.0, 56.2, 56.5, 56.9, 57.2, 94.0, 94.6, 95.0, 95.3, 97.5, 98.4, 106.9, 107.4, 108.6, 127.0, 131.6, 137.3, 142.4, 151.4, 153.5, 159.9, 163.5, 167.4, 192.6. *anal.* C 58.11%, H 6.16% , calcd for $C_{24}H_{30}O_{11}$, C 58.29%, H 6.12%. See Figure 1.18 for ^{1}H NMR spectrum.

5-Hydroxy-2-(3-methoxy-4,5-bis-methoxymethoxyphenyl)-7-methoxymethoxychromen-4-one (21). To a 25 mL round-bottom flask was added 0.095 g (0.192 mmol) of **20**, 0.109 g (0.480 mmol) of DDQ, and 10 mL dry 1,4-dioxane. This solution was heated at reflux for 48 h, let cool, and solvent removed by rotary evaporation to yield a dark brown solid. This solid was subjected to CC using 97:3 CH_2Cl_2/Me_2CO to yield 0.056 g (65%, pale yellow powder, mp 122-123 °C) of pure **21**. ^1H NMR ($CDCl_3$): δ 3.52 (s), 3.55 (s), 3.63 (s), 3.97 (s, OMe), 5.23 (s), 5.27 (s), 5.29 (s), 6.50 (d, J=2.4 Hz), 6.63 (s), 6.69 (d, J=2.4 Hz), 7.14 (d, J=2.4 Hz), 7.34 (d, J=2.4 Hz). ^{13}C NMR ($CDCl_3$): δ 56.3, 56.4, 56.4, 57.3, 94.1, 94.3, 95.4, 98.4, 100.2, 104.4, 105.8, 106.3, 107.8, 127.0, 138.8, 151.4, 153.8, 157.6, 162.0, 163.0, 163.8, 182.4. *anal.* C 58.83 %, H 5.27%, calcd for $C_{22}H_{24}O_{10}$ C 58.93%, H 5.39%. See Figure 1.19 for ^1H NMR spectrum.

Selgin (18). To a 25 mL round-bottom flask was added 0.092 g (0.291 mmol) of **21**, 3 mL 3 N HCl, and 10 mL of MeOH. This solution was heated to reflux for 20 min., allowed to cool, washed with a saturated $NaHCO_3$ solution, and extracted with EtOAc. The EtOAc was dried with anhyd. $MgSO_4$, filtered, and removed by rotary evaporation to yield 0.047 g (72%, pale yellow powder) of pure **18**. The ^1H NMR spectra was essentially the same as in the literature,[41] but the ^{13}C NMR spectra was not previously reported. ^1H NMR (DMSO-d_6): δ 3.87 (s, OMe), 6.20 (d, J=2.0 Hz), 6.48 (d, J=2.0 Hz), 6.83 (s), 7.15 (d, J=2.0 Hz), 7.17 (d, J=2.0 Hz), 9.25 (bs), 9.41 (bs), 10.85 (bs), 12.98 (bs). ^{13}C NMR (DMSO-d_6): δ 56.3, 93.2, 98.8, 102.4, 103.3, 103.7, 107.5, 120.4, 138.6, 146.0, 148.6,

157.3, 161.5, 163.9, 164.2, 181.8. See Figures 1.20 and 1.21 for ^{1}H and ^{13}C NMR spectra, respectively.

5'-Methoxyhydnocarpin-D (1). To a 50 mL three-neck round-bottom flask was added 0.011 g (0.035 mmol) of **18**, 0.007 g (0.035 mmol) of coniferyl alcohol, 15 mL dry benzene and 7.5 mL of dry acetone. The flask was placed in a 60 °C oil bath and stirred for 20 min. Next, 0.010 g of Ag_2CO_3 was added and the reaction solution stirred vigorously for 7 h. The reaction was then allowed to cool, filtered through a Buchner funnel, and the solvent removed by rotary evaporation to yield a yellow powder. The powder was subjected to CC using 8:2 CH_2Cl_2/Me_2CO to yield 0.006 g (33%, pale yellow powder) of pure **1**. ^{1}H and ^{13}C spectral data were identical with those of **1** prepared regiospecifically (see above) and with those of the isolate.[34]

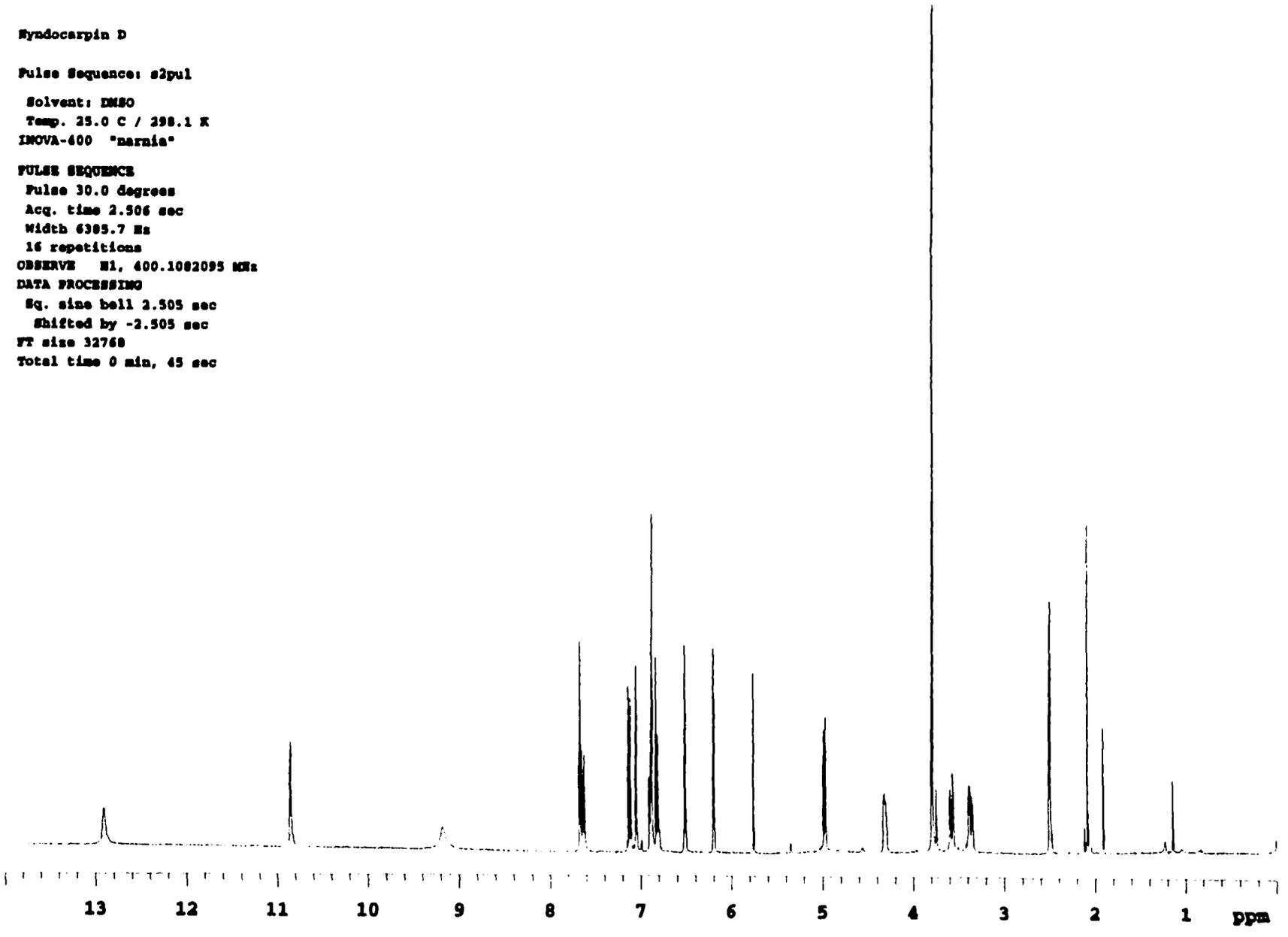


Figure 1.6. ^{1}H NMR spectrum of hydnocarpin-D (**6**).

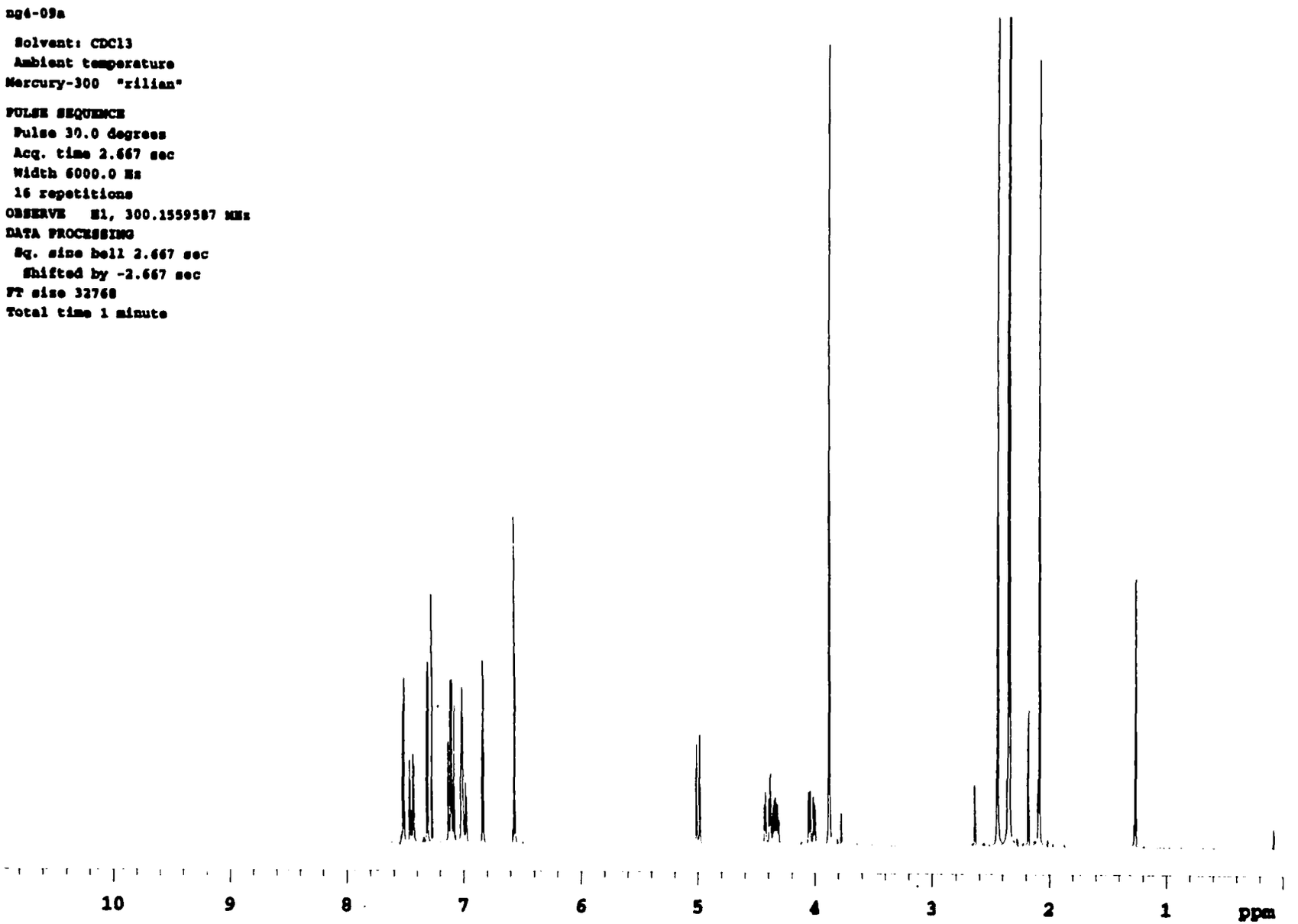


Figure 1.7. ^{1}H NMR spectrum of hydnocarpin-D peracetate (**6a**).

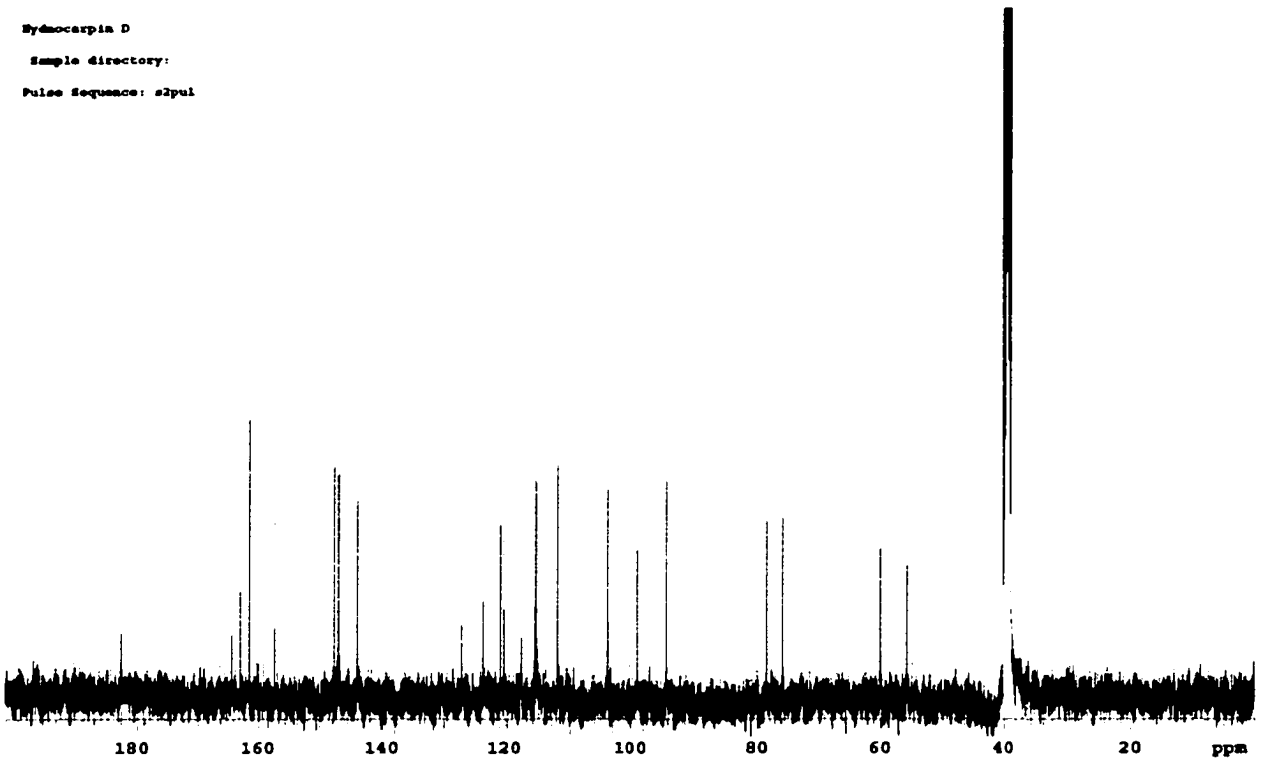


Figure 1.8 ^{13}C spectrum of hydnocarpin-D (**6**).

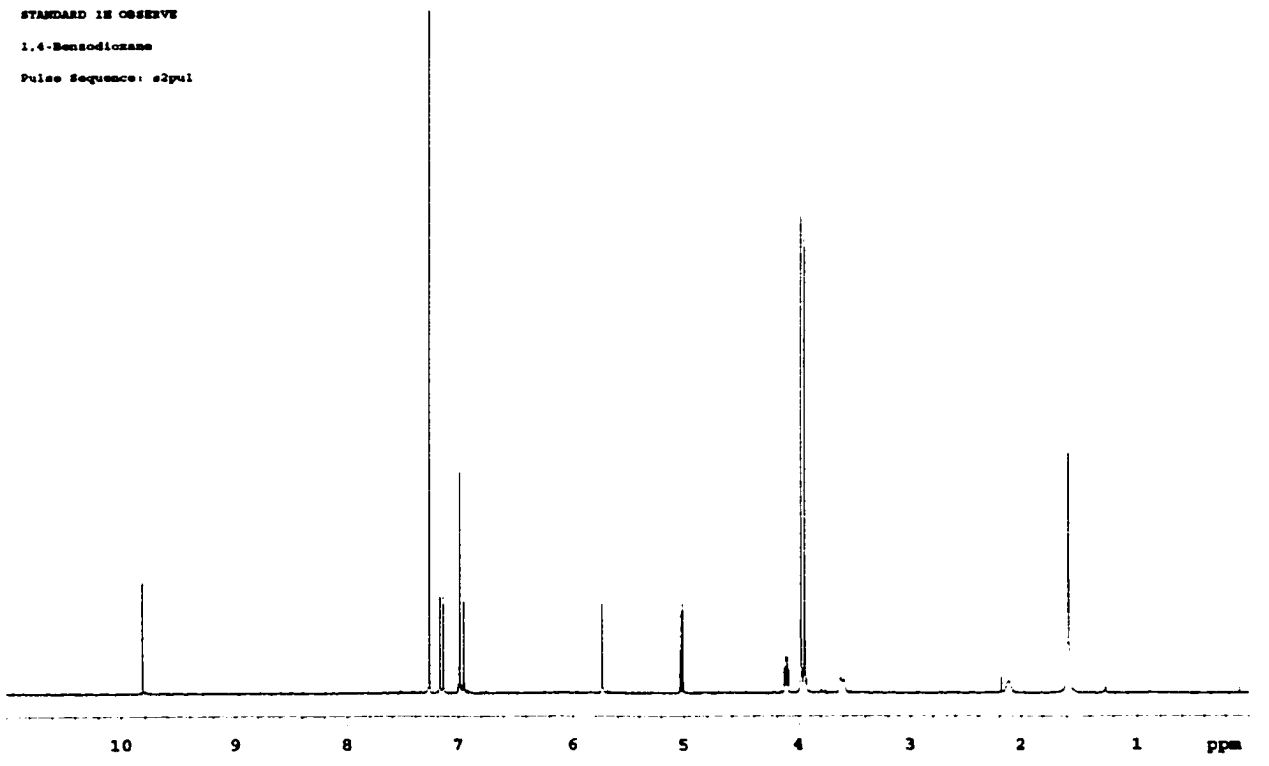


Figure 1.9. ^{1}H spectrum of 1,4-benzodioxane product synthetic intermediate (**13**).

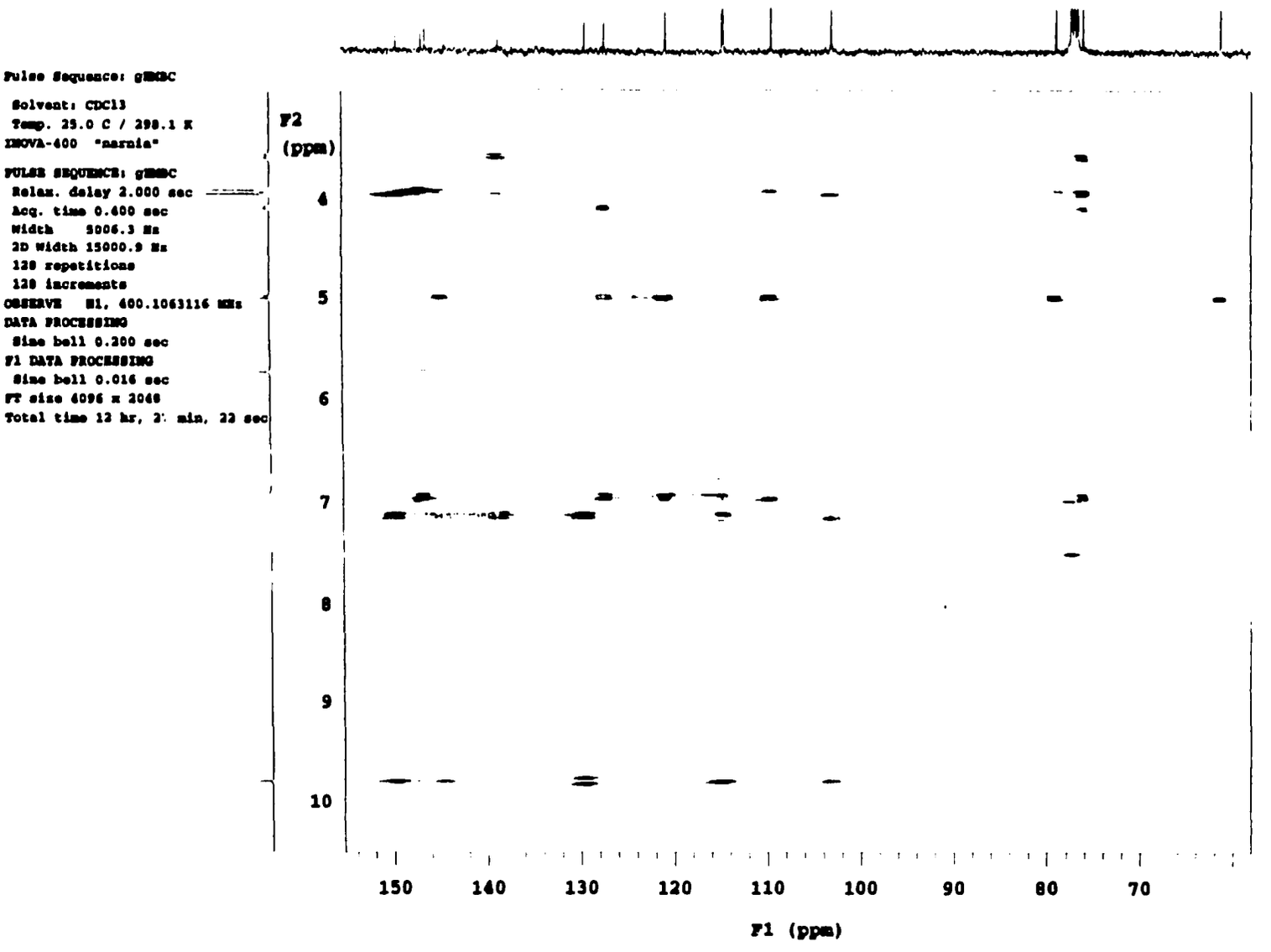


Figure 1.10. HMBC NMR (J=1.6 Hz) spectrum of synthetic intermediate **13**.

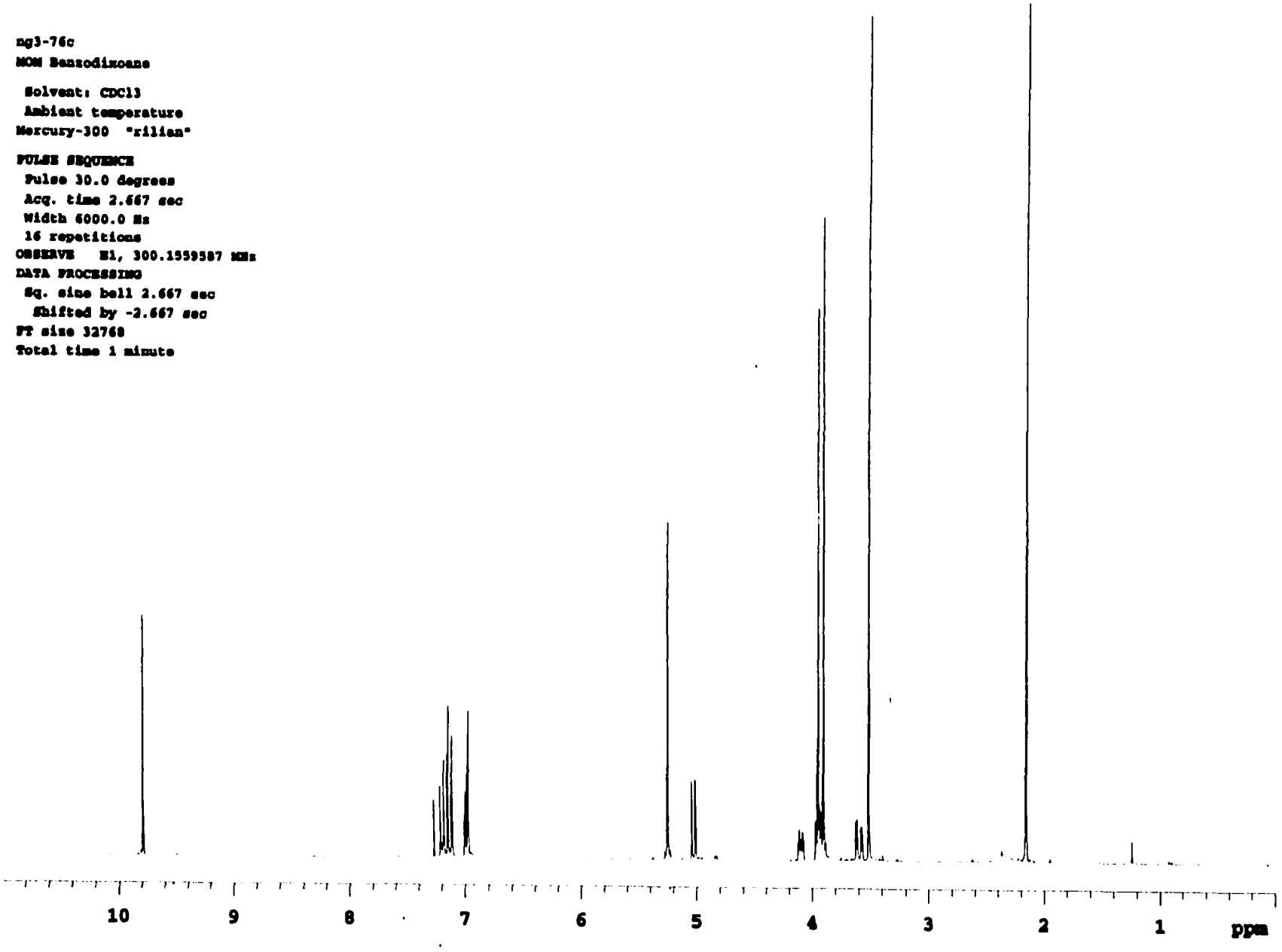


Figure 1.11. ^{1}H NMR spectrum of a MOM protected 1,4-benzodioxane (**14**).

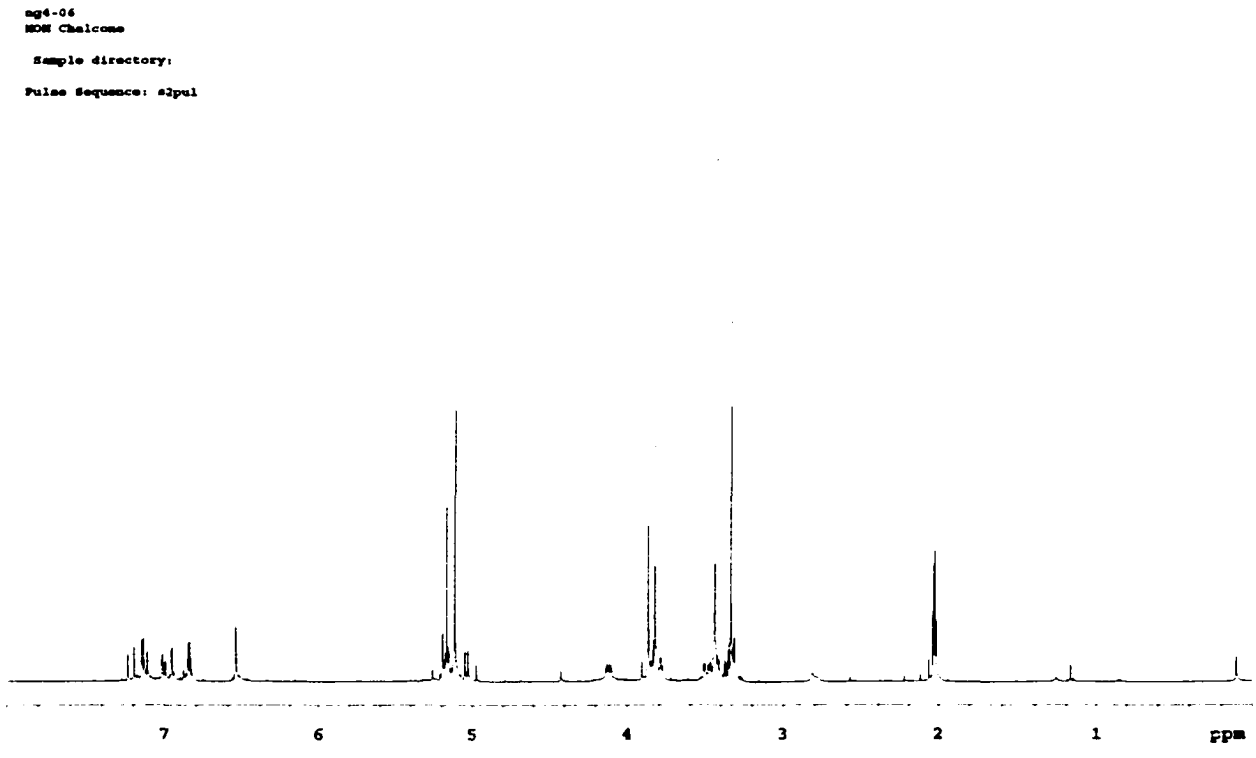


Figure 1.12. ^{1}H spectrum of a MOM protected chalcone (**16**).

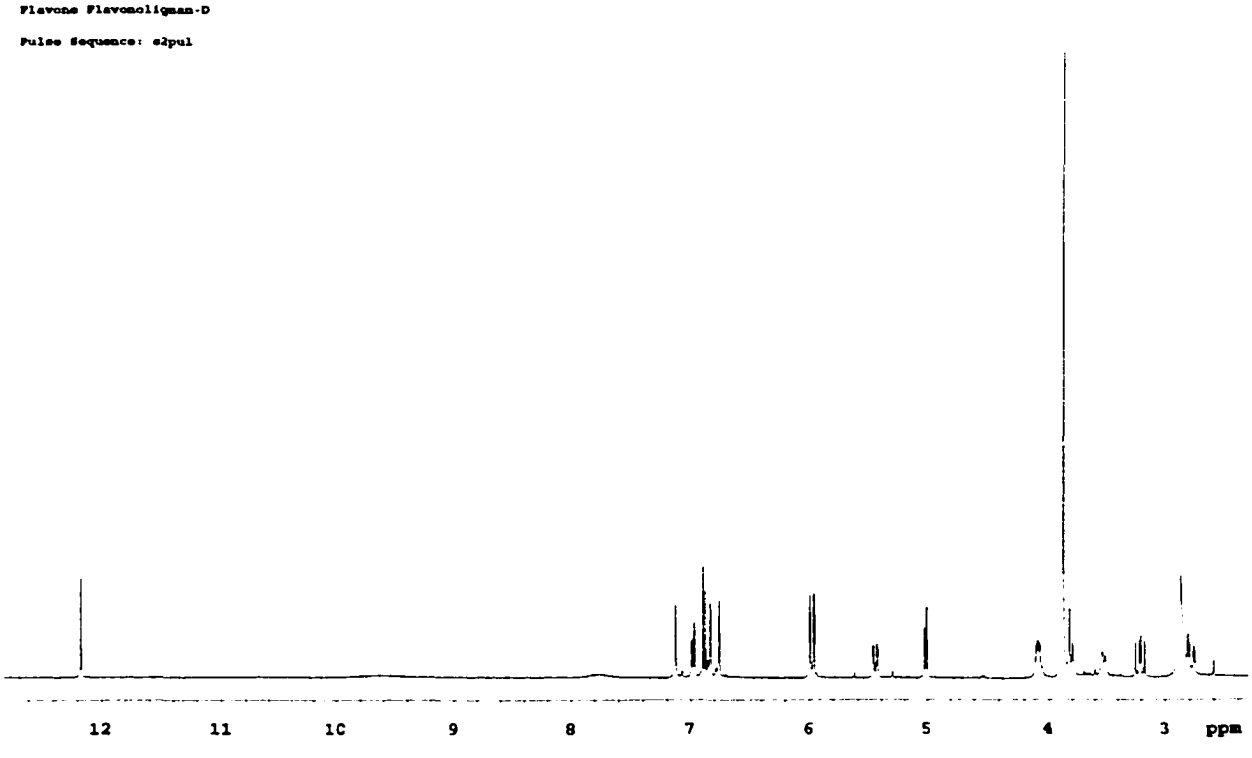


Figure 1.13. ^{1}H spectrum of cyclized flavanolignan diastereomers (**17**).

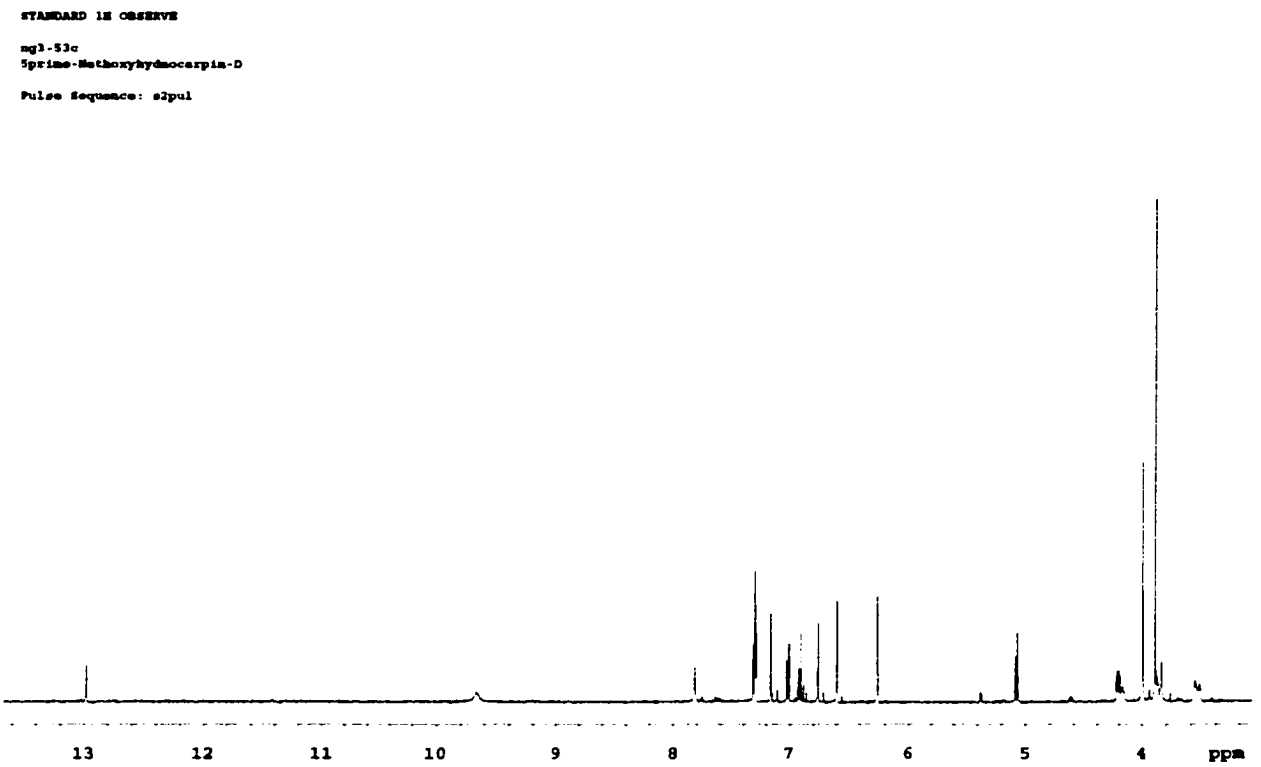


Figure 1.14. ^{1}H spectrum of synthetic 5'-methoxyhydnocarpin-D (**1**).

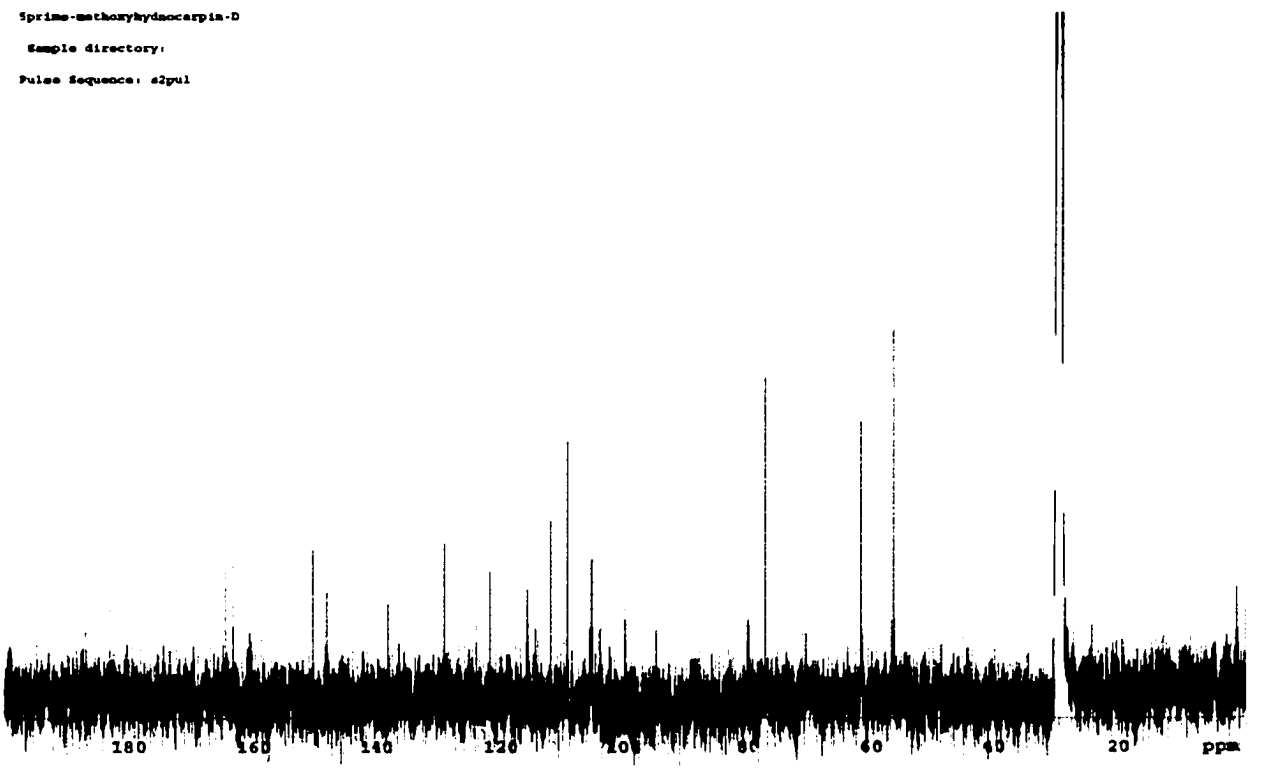


Figure 1.15. ^{13}C spectrum of synthetic 5'-methoxyhydnocarpin-D (**1**).

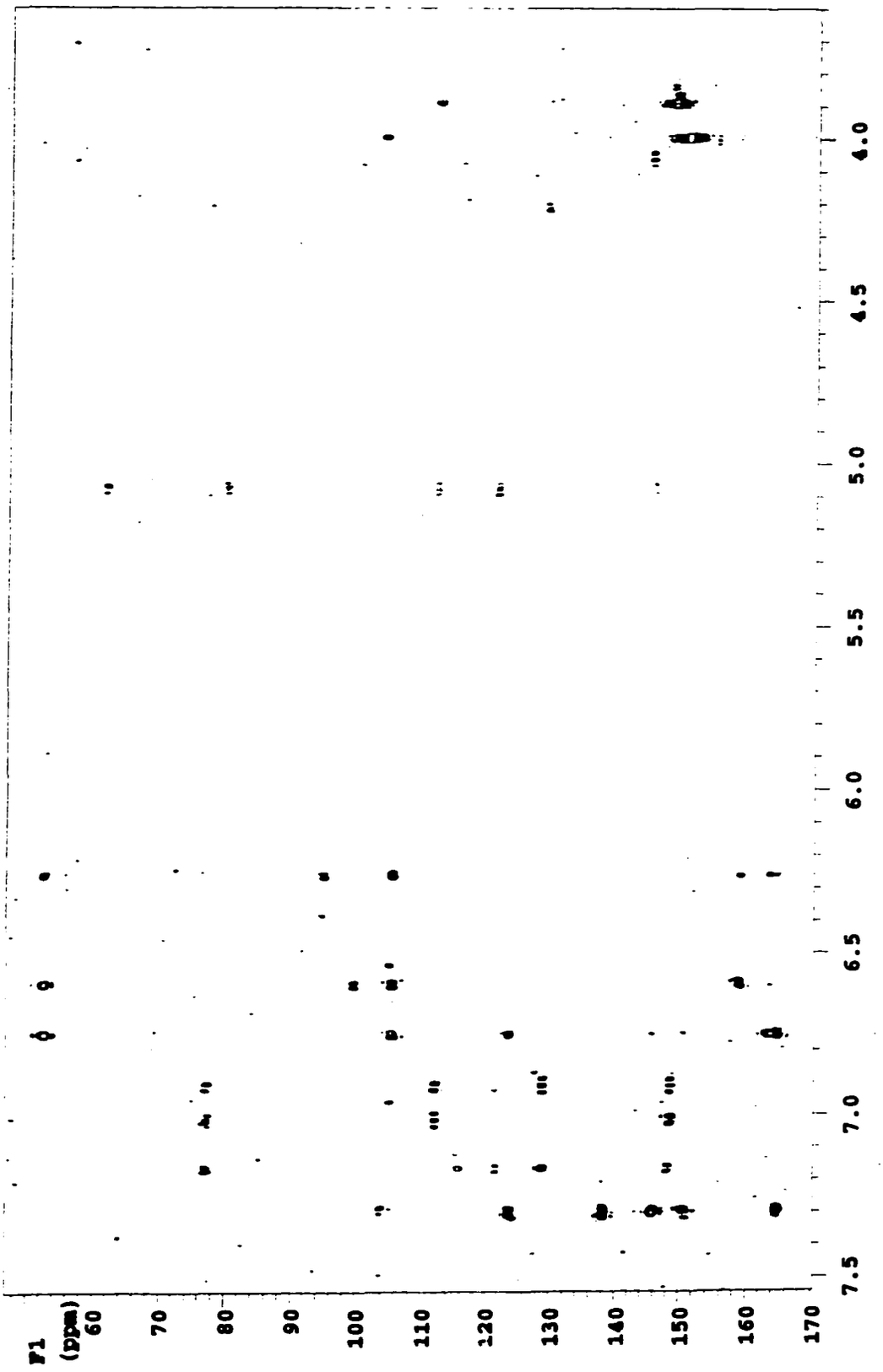


Figure 1.16. HMBC NMR (J=1.6 Hz) spectrum of 5'-methoxyhydnocarpin-D (1)

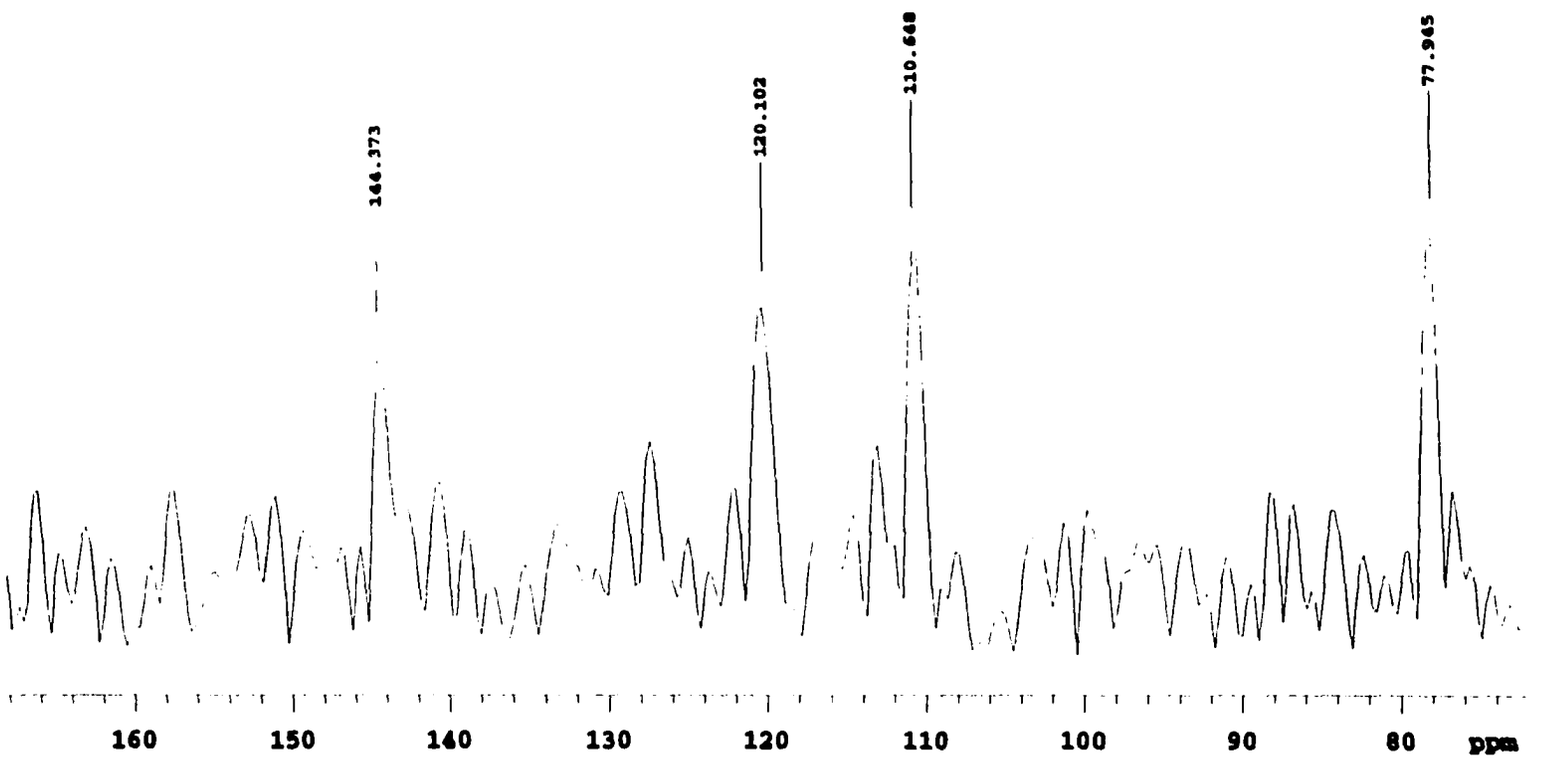


Figure 1.17. HMBC NMR (J=1.6 Hz) spectrum of 5'-methoxyhydnocarpin-D (**1**). Blow up of carbon correlations with benzyl proton (δ 5.0 resonance). They key δ 144.4 ^{13}C resonance is seen.

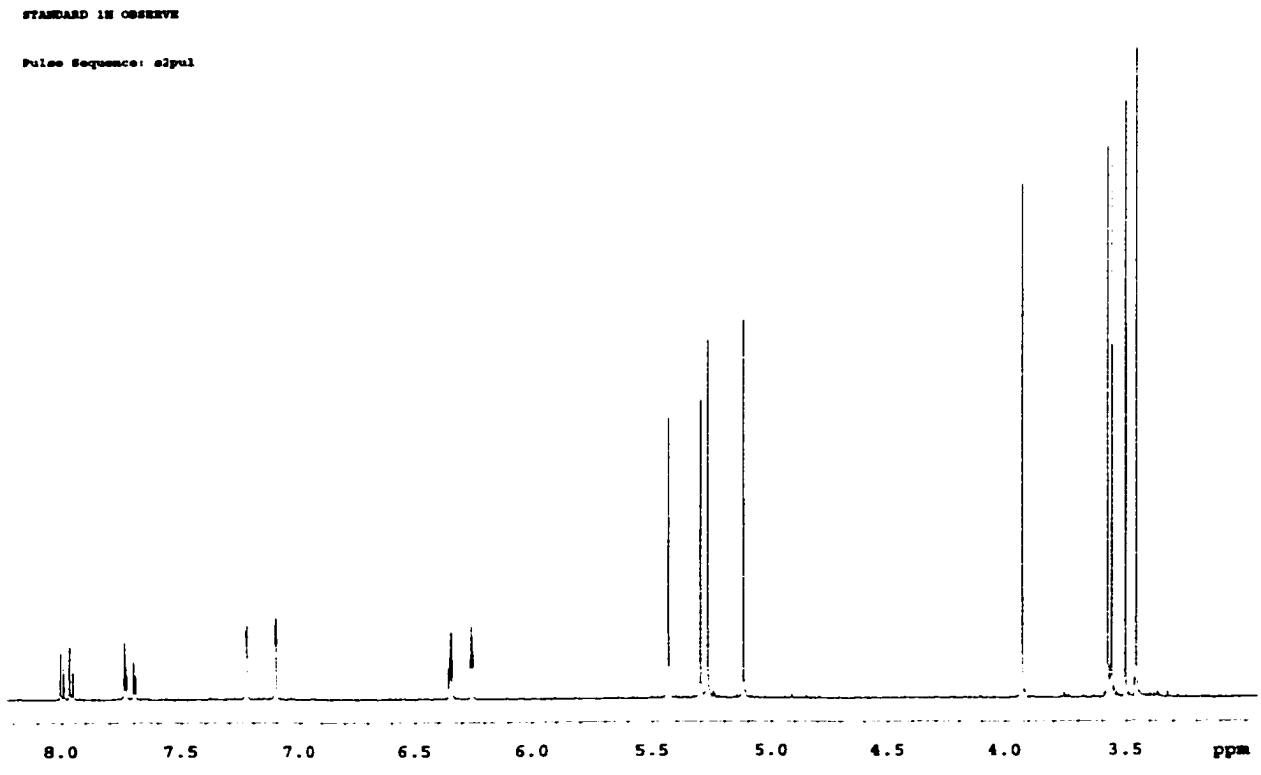


Figure 1.18. ^{1}H spectrum of a MOM protected chalcone (**20**).

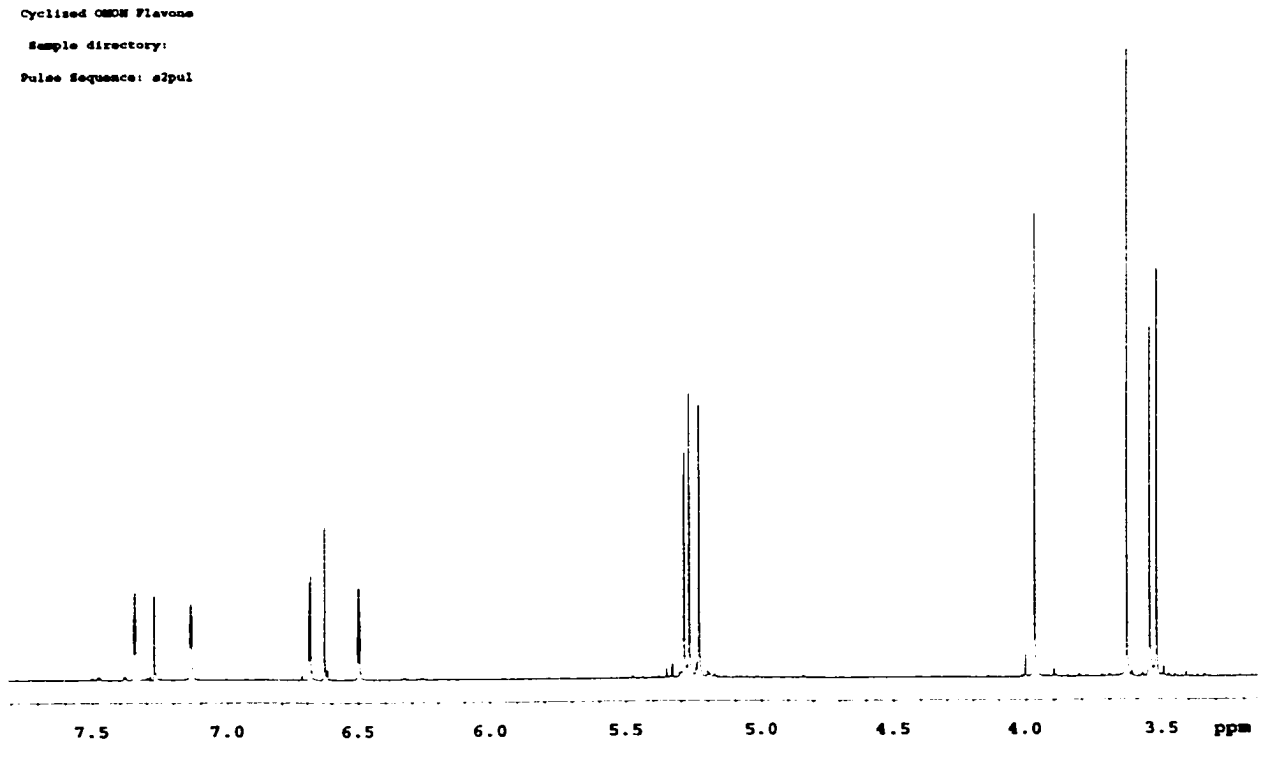


Figure 1.19. ^{1}H spectrum of a MOM protected flavone (**21**).

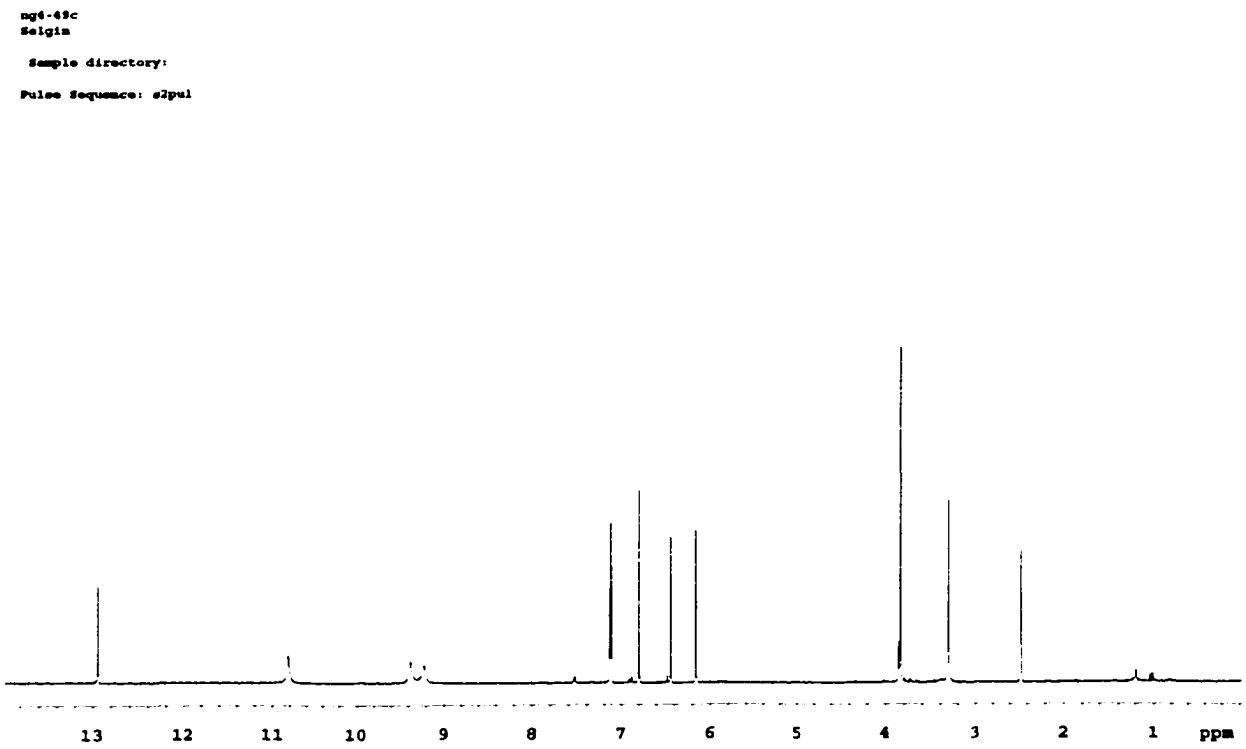


Figure 1.20. ^{1}H spectrum of the flavone selgin (**18**).

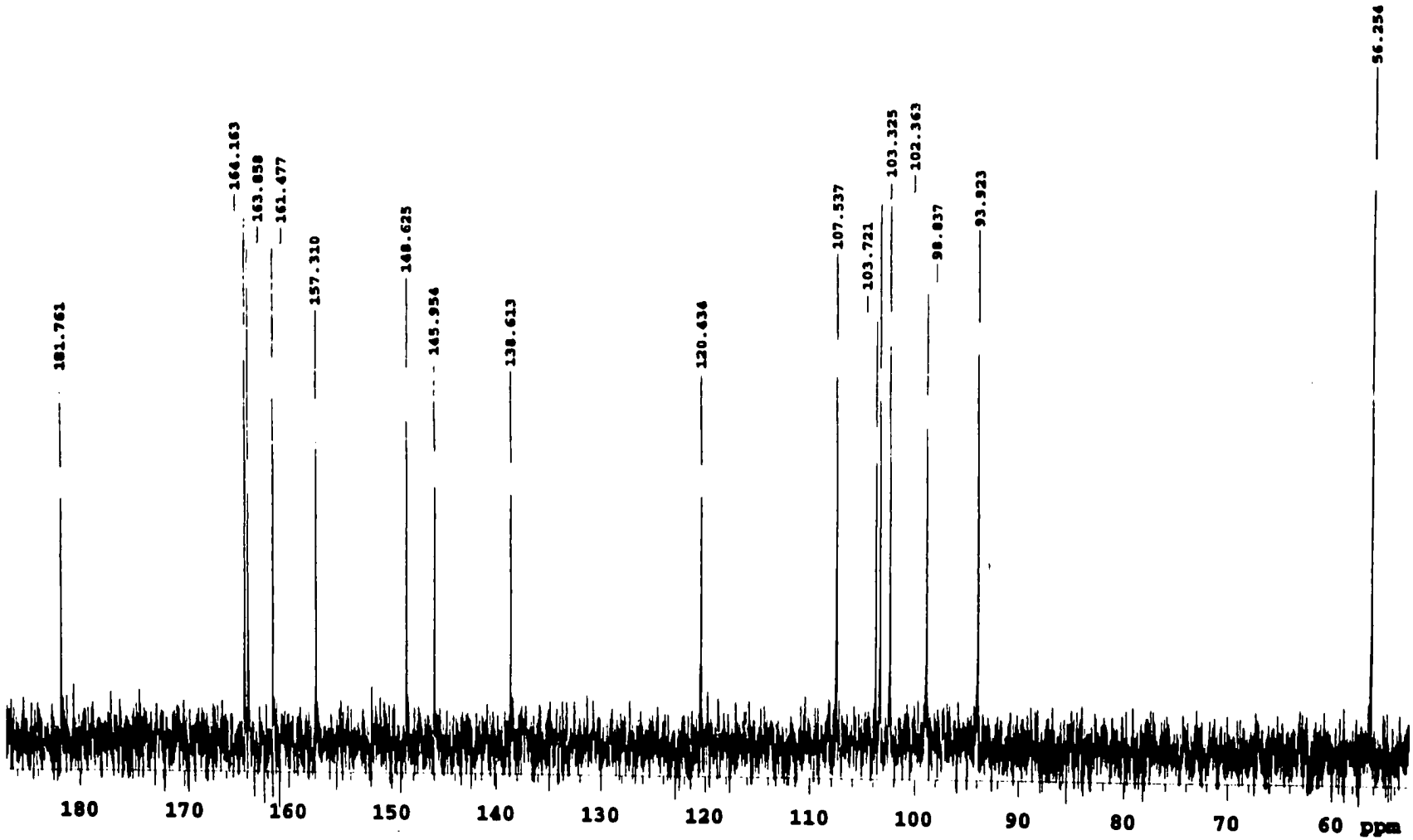


Figure 1.21. ^{13}C NMR spectrum of the flavone selgin (**18**).

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Chapter 2

Synthesis and Structure Activity Relationship Study of Flavonolignans and Flavones

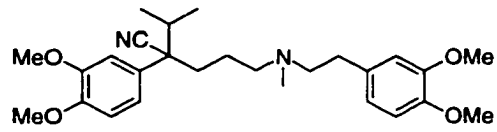
Introduction

The introduction of antibiotics for the treatment of bacterial infections in the 1940's was heralded as one of the greatest advances of the modern world. Previously untreatable diseases quickly succumbed to the powerful effects of antibiotics. It is unlikely that the problem of antibiotic resistance was conceivable in the early days of these medications.[1] Multidrug resistance, however, is now becoming of increasing importance in the treatment of bacterial infections and cancer.[2,3,4] Bacterial multidrug resistance evolved from hospitals and chronic care facilities where bacteria were exposed to large quantities and varieties of antibiotics. This resulted in the rapid selection of bacteria that were resistant to numerous structurally unrelated antibiotics. These resistant bacteria include *Pseudomonas aeruginosa* and *Enterococcus* species, often called "opportunistic pathogens" for their efficient infection of debilitated hospital patients.[5] Recently, multidrug resistant pathogens have been seen outside of hospital situations and have shown resistance to some of the "last resort" medications such as vancomycin.

Resistance to chemotherapeutic cancer drugs parallels that of antibiotic resistance. After an initial period of successful treatment, mammalian tumor cells became resistant to not only the chemotherapeutic drug, but also a wide variety of structurally unrelated compounds.[6,7] Until the mid 1980's, there was not a concrete explanation of how or why bacterial or cancer multidrug resistance occurred. In mammalian tumors, it was found that the overexpression of a transmembrane efflux pump protein named P-glycoprotein (P-gp) was responsible for drug depletion.[8,9] Several mechanisms on how P-gp effects drug depletion have appeared since P-gp's initial discovery. One model suggests that efflux pumps simply use membrane potential and no binding mechanism to promote the efflux of a diverse structure of positively charged compounds.[10,11] This method, however, does not explain how neutral molecules are removed from the cell. The "flippase" model suggests that transmembrane efflux pumps extrude drugs not from the cytoplasm but directly from the cell wall membrane.[11,12] This model assumes that as drugs pass through the lipid membrane bilayer, they are recognized by the transport membrane and "flipped" out of the bilayer to the external medium.[13] These nonspecific pumping mechanisms have generally given way to experimental evidence that efflux pumps directly bind their substrates from the cytoplasm and then extrude them from the cell to keep cellular concentrations of the drug at a minimum.[11,14] It has been shown that P-gp contains four identified binding domains, two hydrophobic transmembrane domains involved in drug efflux and the cytosolic nucleotide-binding domains (NBD) responsible for ATP hydrolysis.[15] Other domains most likely exist but have yet to be identified.

The inhibition of P-gp is a major focus in anti-cancer research at the present time. The first generation of inhibitors included verapamil (**1**) and cyclosporin (**2**), two

pharmaceutical reagents that had already been in use in the late 1970's as calcium channel blockers.[16] While these structurally unrelated compounds were found to inhibit P-gp's efflux pump, the high concentrations of the drug necessary proved to be toxic to healthy cells.[17] Since this first generation of research, flavonoids,[18] flavonolignan derivatives,[15] chalcones,[19] cyclosporin analogues[20] and ardeemin derivatives[21] have shown great promise as less toxic alternatives.



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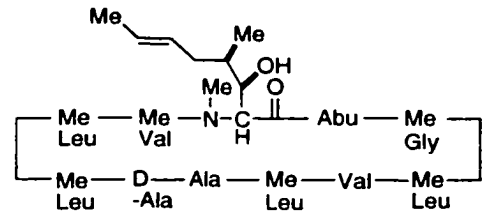

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Many multidrug resistance efflux pumps, to the order of over 100, have been identified since the discovery of P-gp. Multidrug efflux pumps are found in nearly every cell but all are not necessarily overexpressed to promote multidrug resistance (Figure 2.1).[2,11] These pumps belong to one of four families of transporters: ATP binding cassette (ABC), major facilitator (MF), small-multidrug-resistance (SMR), and resistance-nodulation (RN) classes with the latter three being proton-dependent transporters.[4] Multidrug efflux pumps have been identified in bacteria, yeasts, and protozoa. Of the organisms examined to date, only *Mycoplasma* appears to lack MDR efflux pumps.[11]

ABC efflux proteins similar to P-gp have recently been discovered in the protozoan parasites *Leishmania* and *Plasmodium* species. The classical P-gp inhibitors such as verapamil (**1**) and cyclosporine (**2**) and their modern derivatives, however, showed poor inhibition in this MDR phenotype.[22] This poor inhibition indicates that the

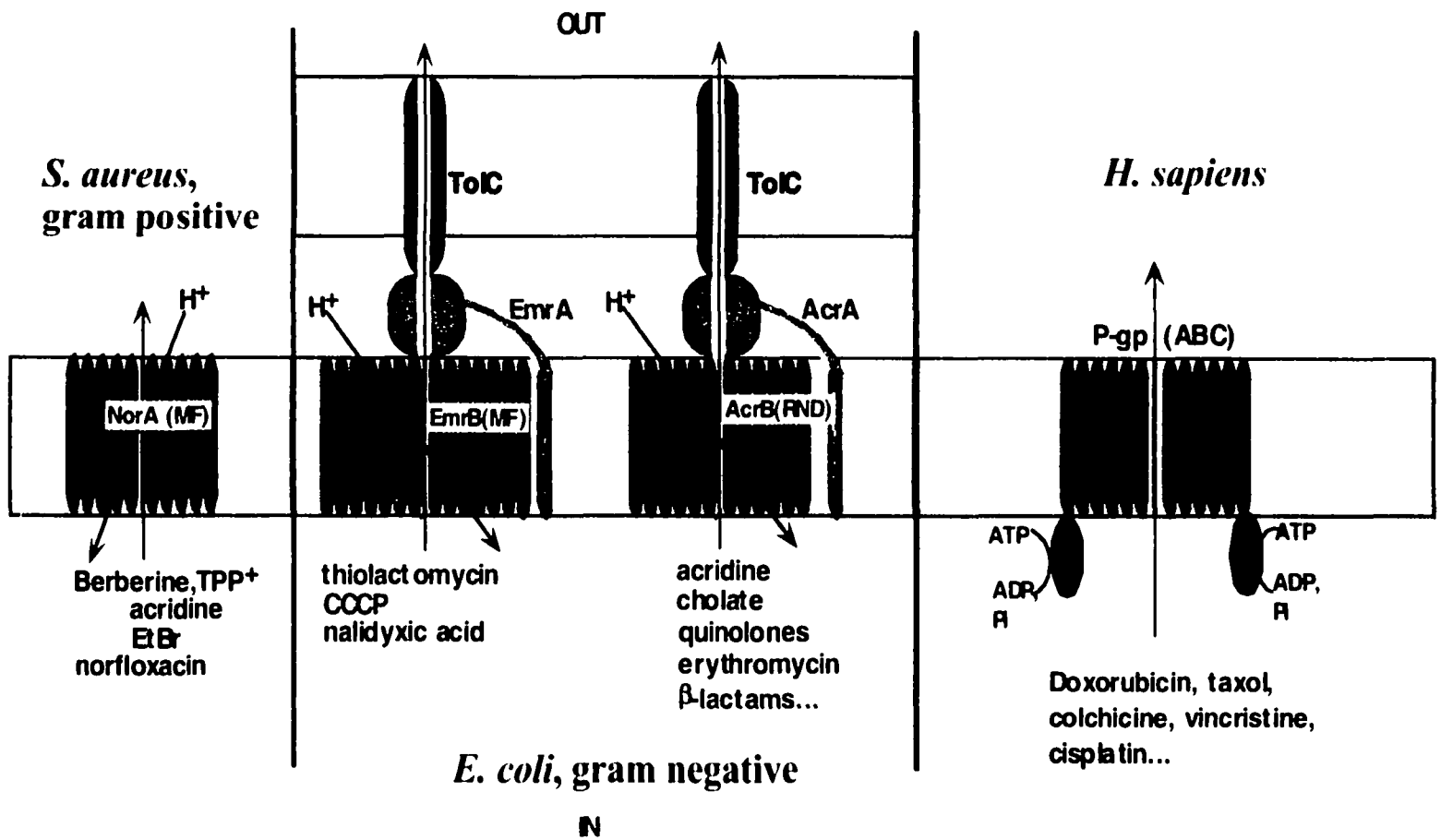
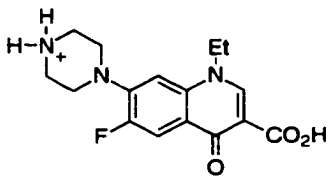


Figure 2.1. A general depiction of a gram-postive, two gram negative, and a mammalian transmembrane efflux protein.

specific structure of the MDR efflux protein, together with the properties of the substrates themselves, dictate what compounds will be extruded from the cell or inhibit the pump.

Numerous MDR efflux pumps such as EmrAB, AcrAB, TetA, and TetB have been identified in the gram-negative bacteria *Escherichia coli*.[3] These transporters belong to the major facilitator family of transport proteins and like P-gp, are capable of pumping a wide variety of structurally unrelated compounds out of the cell. EmrB was found to be responsible for the extrusion of carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), phenylmercuric acid acetate, the weak acid nalidixic acid, and the neutral compound thiolactomycin, all structurally unrelated compounds. The AcrAE system is also broad in it substrate range and includes the macrolide erthromycin, mitomycin C and tetracycline.[5] The TetA and TetB MDR pumps are responsible for extrusion of tetracycline based compounds.[23]

Pseudomonas aeruginosa also has numerous MF transporter MDR efflux pumps including CmlA and MexB. *P. aeruginosa* also protects itself from antimicrobials with a low-permeability outer membrane and is thus often called an intrinsically resistant bacterium. CmlA and MexB efflux proteins both extrude choramphenicol. MexB is less substrate specific and pumps out tetracycline analogs, fluoroquinolones, β-lactams and pyoverdine.[4,5]



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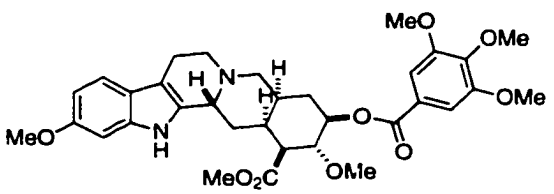

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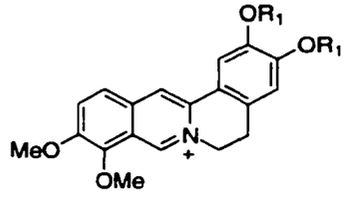
The NorA membrane efflux pump in *Staphylococcus aureus* is another MF transporter and recognizes basic dyes, fluoroquinones such as norfloxacin (**3**), tetra-phenylphosphonium salts and benzalkonium salts as well as quaternary amines like reserpine (**4**), berberine (**5**) and palmatine (**6**).[4,24] Other pumps in *S. aureus*, such as QacA and QacC pumps, have also been identified. The Bmr efflux pump in *Bacillus subtilis* extrudes compounds similar to NorA and is also a MF transporter. *B. subtilis* also contains the Tel(L) efflux pump that extrudes tetracycline analogs.[5]



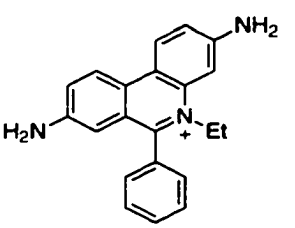
5: R_1 = -CH_2-
6: R_1 = OMe

It of interest to note that a comparison of the structures of the Bmr MDR pump and the TetA and -B proteins show that they are structurally similar with a 24 to 25% sequence identity. This sequence identity is also true with between the NorA and Tet MDR pumps. The similarities between the NorA and Bmr MDR pumps are more striking, with the homology of the proteins being about 44%. Neyfakh notes further that at the DNA level, the identity of the *bmr* and *norA* coding sequences is about 51%. It is thought that homologs of NorA and Bmr are widespread among bacteria.[25]

Although a multitude of small molecule inhibitors of P-gp have been identified, there are relatively few known inhibitors of bacterial MDR efflux pumps. Some tetracycline analogues were found to inhibit the TetB protein in *E. coli*.[26] Diamide amines were found to inhibit *P. aeruginosa* efflux pumps.[27] MDR inhibitors of *S. aureus* efflux pumps have also been identified.

S. aureus is one of the more important human pathogens and chemicals that can inhibit its main efflux pump, the NorA pump, would be of great value. The alkaloid reserpine (**4**) was found to inhibit the NorA pump in *S. aureus* in the early 1990's.[28] Unfortunately, **4** cannot be used as an inhibitor in human subjects infected with resistant strains of *S. aureus* because it is a potent neurotoxin.[29] It has been shown, however, that non-toxic concentrations of **4** can be used to inhibit the structurally related Bmr efflux pump in *B. subtilis*.[30] Recently, a structure-activity relationship study of resperine analogs was reported using a commercially available drug-screening library to search for new inhibitors of NorA. Approximately 4% of the library (of 9600 substrates tested) showed some sort of NorA inhibition. There were eleven compounds that showed inhibition better than reserpine and of these, five were selected for further investigation because of their pharmacologically compatible chemical structures with humans. These effective inhibitors were structurally diverse nitrogen-containing polyphenolics that showed a two-fold increase of inhibition over reserpine. There was no report of human toxicity studies with these new NorA inhibitors.[29]

Another approach to finding effective inhibitors of *S. aureus* was reported by Lewis *et al.*[31] This approach had its beginnings in the use of mutant *S. aureus* strains. Lewis and coworkers were able to disrupt the *norA* gene that encodes for the NorA efflux pump. The removal of the NorA efflux pump increased the sensitivity of *S. aureus* to amphicationic substrates, such as ethidium bromide (**7**) and fluoroquinolones, 5 to 30 fold. Strong amphicationic antibiotic substrates, because of their positive charge, are expected to naturally accumulate in cells because of



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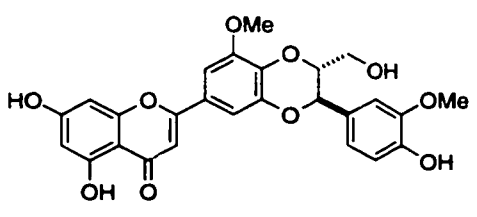
the pH potential of the cell membrane; however, these substrates are rarely seen in cells even after cells are dosed with such antibiotics. This experiment provided further evidence that NorA was the major efflux pump responsible for the removal of amphicationic substrates from *S. aureus*. Some of the preferred substrates of NorA were the alkaloids berberine (**5**) and palmatine (**6**), two compounds known to be prevalent in many plant species. It was hypothesized that plant species producing berberine or berberine like alkaloids might also evolve natural MDR pump inhibitors.

As was discussed above, there are striking structural similarities among efflux pumps.[25] While *S. aureus* is not a plant pathogen, berberine or palmatine containing plants may have evolved an inhibitor to a plant pathogen with efflux pumps structurally reminiscent of Bmr or NorA.[32] To test this hypothesis, a method was developed using plants containing amphicationic substrates such as berberine. Using bioassay guided fractionation, two structurally unrelated inhibitors were isolated from *Berberis fremontii*. These inhibitors did not possess antibiotic activity themselves but worked in synergy with berberine to effectively inhibit growth of resistant strains of *S. aureus*.[33] The minimum inhibitory (MIC) concentrations necessary to inhibit growth of *S. aureus* in the presence of a 30 μg/ml dose of berberine (subinhibitory dose, 200 μg/ml alone will inhibit *S. aureus*) was 2 μg/mL for the flavonolignan 5'-methoxyhydonocarpin-D (**8**). The other



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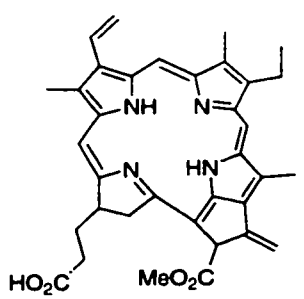

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inhibitor, a porphyrin identified as pheophorbide *a* (**9**), had a MIC of 0.9 μg/mL in the same assay. As a comparison of antibiotic efficacy, the MIC's of the potent antibiotics vancomycin and norfloxacin alone were 1.5 μg/mL and 1.25 μg/mL, respectively.

Although *in vivo* toxicity studies have not been performed, it is speculated that the two isolated inhibitors will not have the deleterious pharmacological effects associated with the NorA inhibitor reserpine (**4**). Pheophorbide *a* (**9**) is a natural intermediate in the breakdown of chlorophyll and 5'-methoxyhydocarpin-D (**8**) is a product of plant oxidation of a flavonoid and a hydroxycinnamyl alcohol, two common components in fruits and vegetables.

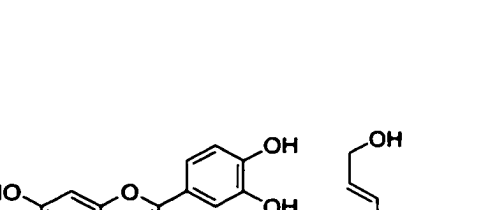
Results and Discussion

Synthesis and Structure Verification Potential MDR Inhibitors

A preliminary study of 5'-methoxyhydnocarpin-D (**8**), hydnocarpin-D (**10**) and hydnocarpin (**11**) showed that they were potent inhibitors of the NorA efflux pump in *Staphylococcus aureus.* This study suggested that the synthesis of a series of structurally reminiscent flavonolignan analogs would be a good starting point for a structure-activity relationship study. To access a large number of similar flavonolignans, the previous syntheses of **8**, **10** and **11** proved invaluable as synthetic models.[34] It was decided to focus the structure activity relationship on flavonolignans containing 1,4-benzodioxane rings and leave other flavonolignans, such as the benzofuran-type flavonolignans, for a future study. Many of the proposed synthetic flavonolignans were new while some were isolated previously. Regiochemical assignment problems with previously isolated

flavonolignans were anticipated and all efforts were to be made to correct structures when necessary.

A wide array of synthetic flavonolignans were prepared by reaction of a commercially available flavonoid catechol with coniferyl (**12a**), sinapyl (**12b**), or *p*-coumaryl alcohol (**12c**). All but one coupling was performed with Ag_2CO_3 in 2:1 benzene/acetone at 60 °C with the exception being a horseradish peroxidase initiated coupling in an acetone/water buffer/H_2O_2 solution at 0 °C (Scheme 2.1). These methods gave different regioselectivities as described for preparation of **10** and **11**.[34] The Ag_2CO_3 reactions yielded a mixture having the major product with the pendant aromatic ring (D ring) in the "down" position (e.g. **10**) and the HRP reaction with the pendant aromatic

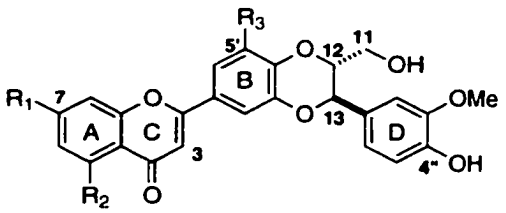


12a: R_1 = OMe, R_2 = H
12b: R_1 = R_2 = OMe
12c: R_1 = R_2 = H

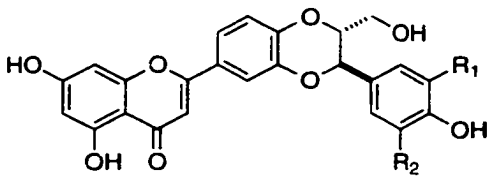
Scheme 2.1 Examples of favonolignan synthesis.

ring in the "up" position (e.g. **11**). As was discussed in the last chapter with the synthesis of the hydnocarpin series, extensive chromatography using normal and reverse phase silica gel and recrystallizations were necessary to obtain regiopure samples of the desired flavonolignans. Yields were not optimized in any of the reactions.

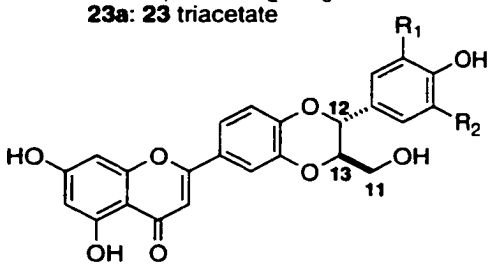
Compounds **13**, **14** and **15** are exemplary of the syntheses and structure verification of the prepared flavonolignans. The compounds **13** and **14** were synthesized using luteolin (**30**) and sinapyl alcohol (**12b**) in a manner discussed above (Scheme 2.1).



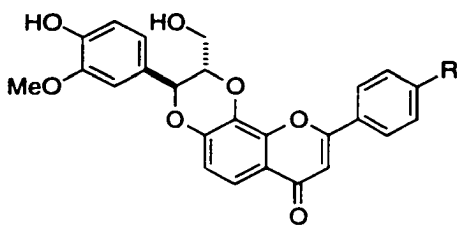
8 : $R_1 = R_2 = OH$, $R_3 = OMe$
10 : $R_1 = R_2 = OH$, $R_3 = H$
10a: **10** tetraacetate
22 : $R_1 = R_2 = R_3 = H$
23 : $R_1 = OH$, $R_2 = R_3 = H$
23a: **23** triacetate



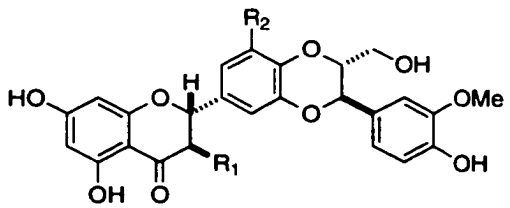
13: $R_1 = R_2 = OMe$
17: $R_1 = R_2 = H$



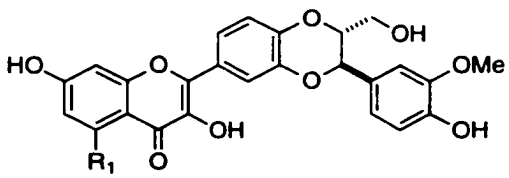
11: $R_1 = OMe$, $R_2 = H$
14: $R_1 = R_2 = OMe$
18: $R_1 = R_2 = H$



15: R = H
16: R = OH



19: $R_1 = OH$, $R_2 = H$
20: $R_1 = R_2 = H$
20a: **20** tetraacetate
21: $R_1 = H$, $R_2 = OMe$



24: $R_1 = H$
24a: **24** tetraacetate
25: $R_1 = OH$
25a: **25** pentaacetate

Figure 2.2. Flavonolignans tested as inhibitors of an *S. aureus* NorA MDR efflux pump

In 1990, a new flavonolignan was isolated from the plant *Onopordon corymbosum* and was assigned structure **14**.[35] The authors did not present data that distinguished **14** from its regioisomer **13**. Samples of **13** and **14** were prepared synthetically and they both had very similar ^{1}H and ^{13}C NMR resonances to the reported isolate. A definitive structure assignment for the isolate was thus not possible. This ambiguity parallels the cases of silybin and 5'-methoxyhydocarpin-D discussed in Chapter 1. The authors of the isolation report did not have an authentic sample available,[35] so which strucuture represents the actual plant isolate is unknown at this time. The structures for synthetic **13** and **14** were verified by HMBC NMR.[34,36]

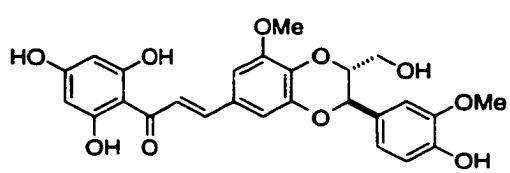
The structure activity relationship study was not limited to flavonolignans derived from 3',4'-catecholic flavonoids (Scheme 2.1). Coniferyl alcohol (**12a**) was coupled with A ring catecholic flavonoids to yield regiopure flavonolignans, unlike those exemplified in Scheme 2.1. The 5-OH analogs of **15** and **16** are known as scutellaprostin A and B, respectively, and were isolated from *Scutellaria prostrata*.[37] The syntheses of numerous scutellaprostins has been documented and the authors report similar regiospecific couplings mediated by Ag_2O.[37,38]

The flavonolignan sinaiticin (**18**) was isolated from *Verbascum sinaiticum* and its structure confirmed by the authors using INEPT NMR.[39] A HRP initiated coupling of luteolin and *p*-coumaryl alcohol (**12c**) provided a 3:2 mixture of **18/17**, but unlike the case of **11/10**, extensive attempts to obtain a regiopure sample of **18** by recrystallization were met with failure. Regiopure **17** was obtained via an Ag_2CO_3 coupling and its regiochemistry verified by HMBC NMR. A synthesis of sinaiticin was recently reported[40] but no evidence was given which would distinguish the proposed final product from

isomer **18**. A request for a sample of the synthetic flavonolignan was not answered, so it is unclear at this time which regioisomer was actually synthesized.[40]

An extract of *Silyibum marianum*, known as silymarin, is commercially available and contains a number of flavonolignans along with the major constituent flavonolignan, silybin (**19**). A sample of a **19** was obtained by a methanol recrystallization as a 50:50 mixture of diastereomers (the other diastereomer has the alternate trans stereochemistry of substituents on the benzodioxane ring). Silandrin-D (**20**) is the 3-deoxy analog of silybin and one of the minor constituent flavonolignans in *S. marianum*. A multistep synthesis of **20** was reported[41] and regiochemistry proven through degradation experiments.[42] A sample of silandrin could not be obtained by a HRP mediated coupling of the flavanone (±)-eriodictyol and **12a**. Its regioisomer, however, was available through a Ag_2CO_3 based coupling. A 1:1 diastereomeric ratio of regiopure products resulted from this coupling and the mixture, as well as the individual diastereomers, were tested in the *S. aureus* assay. The diastereomers were separated using preparative C-18 HPLC chromatography. The corresponding 5'-methoxy derivative (**22**) of silandrin-D, an intermediate in the synthesis of **8**, was also available for biological testing.[34]

The remainder of the synthesized flavonolignans were new compounds and their structures verified by spectroscopic means. A number of the flavonolignans were derivatized as peracetates to help chromatographic purification or recrystallization. These



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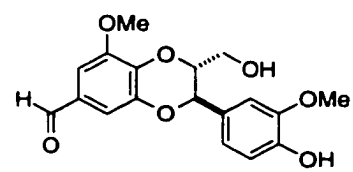

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Figure 2.3. Flavonolignan synthetic precursors tested as inhibitors of the *S. aureus* NorA MDR efflux pump.

peracetates were also submitted to the biological assay. A chalcone (**45**) and its precursor (**46**), intermediates in the synthesis of **8** (Figure 2.3), and all flavonoid and hydroxycinnamyl alcohol starting materials were added to the testing pool. By testing detached functional groups of flavonolignans, it was hoped that the functional group(s) responsible for inhibition could be isolated and their structures further utilized to create more potent inhibitors.

Biological Assays. Cell culturing and susceptibility testing.

Growth of bacteria and susceptibility measurements were performed at Tufts University, Medford, Massachusetts according to National Center for Clinical Laboratory Standards recommendations and the results communicated to us. *S. aureus* RN4222 were cultured in Mueller-Hinton (MH) broth overnight with aeration at 37 °C. Cells were then inoculated into fresh MH medium at a 1:10 dilution and were allowed to grow for 1 hour. This suspension was diluted 1:2000 into MH broth and 0.05 mL was dispensed per well of microtiter plates. For measurements of direct antimicrobial activity, test substances were serially diluted two-fold in the wells. The final volume of a well was 0.2 mL, and the cells concentration was 10^5/mL. MIC was defined as a concentration of an antimicrobial that completely prevented cell growth during an 18 hour incubation at 37 °C. Growth was assayed with a microtiter plate reader (Biorad) by absorption at 600 nm. Tests for MDR inhibitory activity were done similarly, but with berberine present at a subinhibitory concentration (30 μg/mL, 1/8 MIC) throughout. The test substance was then serially diluted 2-fold, and MIC for test substances was then defined as their

minimal concentration that completely inhibited cell growth in the presence of 30 μg/ml berberine. MIC values (Tables 2.1 and 2.2) could vary within a factor of two.

Results and Discussion

Bioassay results are summarized for flavonolignans (Table 2.1) and for flavones (Table 2.2). None of compounds showed independent activity (with no berberine present) against *S. aureus*, except flavonolignan **15** and flavone **38**, which were only very weakly active (MIC = 125 μg/ml). Many compounds were, however, synergistic (some very potently so) with a subinhibitory concentration of berberine, **5**. A number of the tested flavonolignans (**10**, **10a**, **22**, **23**, **23a**, **15**, and **16**) were as potent as or more potent (<2 μg/mL) than the natural product **8**. Flavonolignans with and without free phenolic groups at the 5 and 7 position were comparably active (**10**, **22**, **23**, **15**, and **16**), although the most potent, **22**, completely lacks A ring OH groups. The potency of peracetate derivatives (**10a**, **23a**, **20a**) was approximately the same as their parent compounds. These peracetates could be inherently active or, perhaps less likely, deacetylated *by S. aureus* and hence considered "pro-drugs." The presence of a 3-hydroxy group resulted in markedly decreased activity (**24**) or no activity (**25**, **25a**) in flavone-derived systems. In the case of the one 3-hydroxyflavanolignan that was tested, silybin (**19**), potency was, however, still fairly high. The diastereomers of **20**, a 2,3-dihydro derived flavonolignan, as well as the individual diastereomers, tested with equal potency and **21**, the 5'-methoxy derivative of **20**, also showed good inhibition.

Table 2.1. Minimum Inhibitory Concentrations (MIC) of flavonolignans that inhibit growth of *S. aureus* in the presence of subinhibitory (30 μg/mL) **5**.

Compound	MIC (μg/ml)	Compound	MIC (μg/mL)	Compound	MIC (μg/mL)
8	1-2	**17**	Inactive	**23**	0.8
10	0.1	**18**	Inactive	**23a**	1.6
11	3.1	**19**	12.5	**24**	163
13	4-8	**20***	3.1	**25, 25a**	Inactive
14	7.8	**20a**	1.6	**45, 46**	Inactive
15	1.9	**21**	1.9		
16	0.6	**22**	0.08		

* Individual diastereomers of **20** were active at about the same potency as the mixture.

In contrast to the case of A ring substitution, some changes on the D ring played an important role. The majority of flavonolignans had 3-methoxy-4-hydroxy substitution and were quite active, while 3,5-dimethoxy-4-hydroxy D ring substituted compounds (**13**, **14**) were slightly less active. On the other hand, **17**, with only 4-hydroxy substitution on ring D was, perhaps remarkably, completely inactive. The regiochemistry of the D ring benzodioxane ring fusion in the flavonolignans was important in one case where we were able to compare isomers (**10** vs **11**), but not in another case (**13** vs **14**). The two scutelleprostin analogs **15** and **16** were both quite active so in this system H vs. OH in ring B is not critical. Two synthetic intermediates, chalcone **45** and its precursor **46** were inactive.

Most of the commercially available simple flavones (Figure 2.4 and Table 2.2), especially those with free phenolic groups, or as peracetates, had little or no activity (**30**, **30a**, **31**, **32**, **32a**, **33**, **36**). Luteolin, **30**, which was the flavone most often used for preparation of flavonolignans (Scheme 2.1), was not active, nor was coniferyl alcohol, often used as the coupling partner with the catecholic flavones. Flavones devoid of B ring substitution (chrysin, **33**, and its dimethyl ether **34**) as well as two devoid of substitution on the A ring (**26** and **27**) had moderate activity. Diosmetin, **28**, which is the 4' methyl

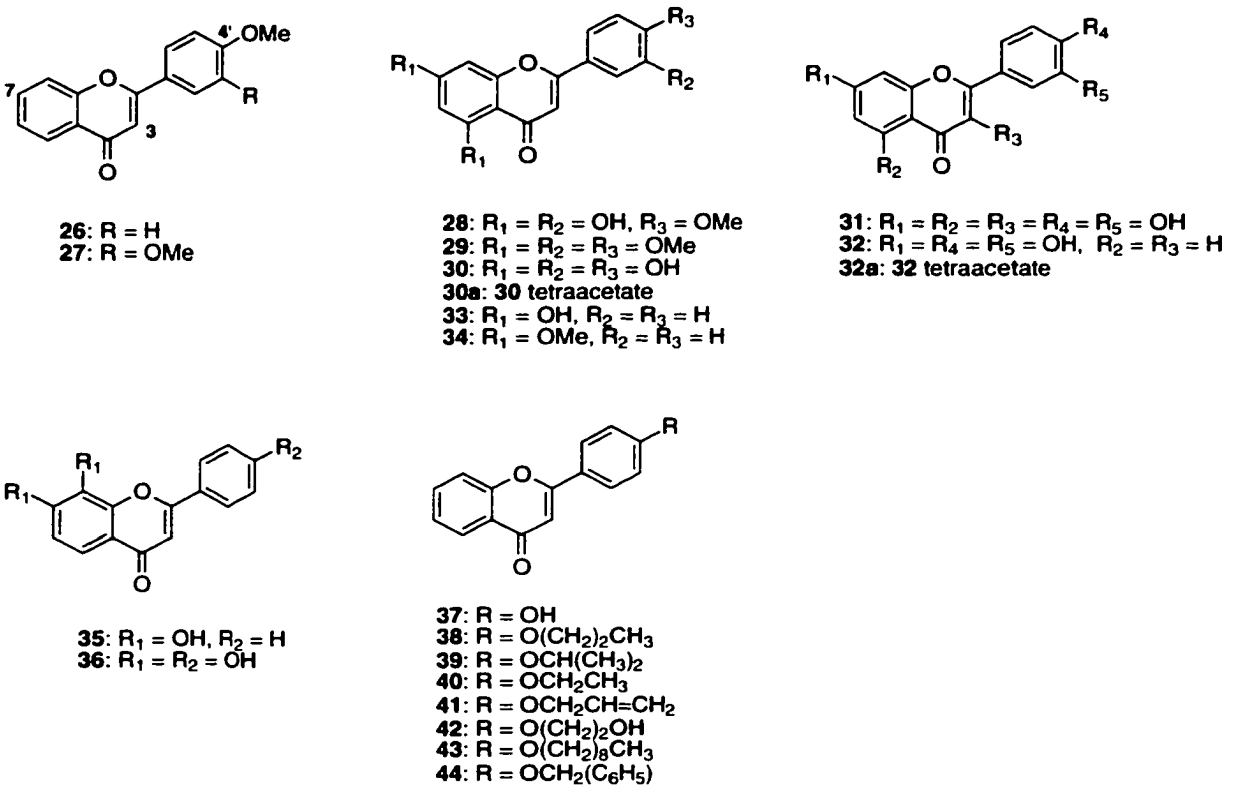

Figure 2.4. Flavones tested as inhibitors of the *S. aureus* NorA MDR efflux pump.

ether of the inactive luteolin, was surprisingly active (15 μg/ml). The relatively high activity of **26**, **27**, and **28** suggested that 4'-alkylation might be of importance and led to the synthesis of various ethers of 4'-hydroxyflavone (**37**). While **37** was inactive, derivatives with small lipophilic alkylated sidechains (**38-41**) were quite active. As lipophilicity was increased, however, activity was completely lost (**43** and **44**). The difference between **37** and **38** is particularly striking. The importance of maintaining lipophilicity at the 4'-carbon was also demonstrated by the decreased activity for **43** as compared to **38**. Because of the potency of **38-40** and their simplified structures as compared to the flavonolignans, they were also tested against a mutant *S. aureus* strain lacking NorA. They did not potentiate the activity of norfloxacin against the mutant, as

was similarly the case for the flavonolignan **8**, thus indicating that the activities for **38-40** against the wild type *S. aureus* were indeed due to NorA inhibition.

Table 2.2. Minimum Inhibitory Concentrations (MIC) of flavones that inhibit growth of *S. aureus* in the presence of subinhibitory (30 μg/mL) **5**.

Compound	MIC (μg/ml)	Compound	MIC (μg/mL)	Compound	MIC (μg/mL)
26	6.3	**34**	25	**41**	6.3
27	6.3	**35-37**	Inactive	**42**	25
28	15	**38**	0.4	**43, 44**	Inactive
29-32a	Inactive	**39**	1.9		
33	25	**40**	1.6		

The flavonolignan **8** was also tested as an inhibitor against the gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, but was ineffective. An activity comparison of the above inhibitors versus structurally similar inhibitors for P-gp has been reviewed.[43] In summary, many of the functional groups that were necessary for P-gp inhibition were deleterious for NorA inhibition.[18,43] Acidic functionalities, such as free phenols or free carboxylic acids, were often detrimental to inhibition in both systems.[43,44] The above *S. aureus* SAR study is important because it will complement the multitude of P-gp SAR studies in the future design of NorA specific or P-gp specific inhibitors.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded at 25 °C on a Varian Inova spectrometer at 400 and 100 MHz, respectively, using $CDCl_3$, acetone-d_6, methanol-d_4, or DMSO-d_6 as the solvent and internal reference. Melting points were determined on a Laboratory Device's Mel-Temp and are uncorrected. All solvents were distilled prior to use. THF was freshly distilled from benzophenone-ketyl and benzene was freshly distilled from CaH_2. ACS acetone was stored over 4 Å

molecular sieves. All non-aqueous reactions were performed in dry glassware under an argon atmosphere. All starting materials were used as received. Acetylations were performed using standard acetic anhydride/pyridine conditions. All flavonoids except quercetin dihydrate were purchased from Indofine Chemical Co. and all other reagents purchased from Aldrich Chemical Co. All column chromatography separations (CC) were performed with normal phase silica gel (Scientific Adsorbents Incorporated, 32-63 μm particle size, 60 Å pore size). Coniferyl, sinapyl, and *p*-coumaryl alcohols were prepared by a DIBAL reduction (THF, 0 °C, 12 h. >75% yield) of the corresponding aldehyde. ^{1}H and ^{13}C NMR spectra of final products and selected intermediates are presented after the experimental section.

Flavonolignans

5"-Methoxyhydnocarpin-D (13). To a 100 mL three-neck round-bottom flask was added 0.160 g (0.559 mmol) of luteolin, 0.118 g (0.559 mmol) of sinapyl alcohol, 40 mL of benzene and 20 mL of acetone. The reaction vessel was placed in a 60 °C oil bath and let stir for 10 min. Next, 0.154 g (0.559 mmol) of Ag_2CO_3 was added and the reaction solution stirred vigorously for 10 h. The reaction was then allowed to cool, filtered through a Buchner funnel, and the solvent removed by rotary evaporation to yield a pale yellow powder. The powder was subjected to CC using 4:1 $CH_2Cl_2/(CH_3)_2CO$ to yield 0.052 g of pure **13** (19%, pale yellow powder, 6:1 **13/14**). The purified material was recrystallized from 1:1 H_2O/MeOH to give 0.033 g of regiopure **13** (pale yellow white crystals, mp=213-214 °C). ^{1}H NMR (DMSO-d_6): δ 3.38 (dd, J=12.6, 4.4 Hz), 3.57 (dd, J=12.6, 2.8 Hz), 3.78 (s, OMe), 4.33 (m), 4.95 (d, J=8.0 Hz), 6.19 (d, J=2.0 Hz), 6.50 (d,

J=2.0 Hz), 6.77 (s), 6.87 (s), 7.12 (d, J=8.4 Hz), 7.63 (dd, J=8.4, 2.0 Hz), 7.67 (d, *J*=2.0 Hz) 12.91 (s). ^{13}C NMR (DMSO-d_6): δ 56.1, 60.0, 76.2, 78.5, 94.1, 98.9, 103.8, 103.9, 105.4, 115.1, 117.4, 120.1, 123.4, 126.1, 136.1, 144.0, 146.9, 148.0, 157.3, 161.4, 162.9, 164.3, 181.8. *anal.* C 57.87%, H 5.09%, calcd for $C_{26}H_{22}O_{10}$ ·2.5 H_2O, C 57.88%, H 5.04%. See Figures 2.5 and 2.6 for ^{1}H and ^{13}C NMR spectra, respectively.

5"-Methoxyhydnocarpin (14). To a 100 mL three-neck round-bottom flask was added 0.300 g (1.05 mmol) of luteolin and 0.220 g (1.05 mmol) of sinapyl alcohol. Next, 20 mL of acetone and 5 mL of a 0.2 **M** citric acid/phosphate buffer were added to the reaction flask and the flask cooled to 0 °C. Two drops of 30% H_2O_2 and 1 mL of a horseradish peroxidase solution (1.5 mg HRP (1100 U/mg) / 3 mL water) were added. The HRP solution (1 mL) was added every 15 min. thereafter, and the reaction then allowed to stir at 0 °C for 7 h. The reaction solution was allowed to warm to room temperature, washed with brine, and extracted with EtOAc. The EtOAc was dried with anhyd. $MgSO_4$, filtered, and removed by rotary evaporation to yield a brown-orange solid. The solid was subjected to CC using 92:8 $CHCl_3$/MeOH to yield 0.192 g (37%) of a yellow-orange solid, 3:2 ratio of **14/13** (by ^{1}H NMR) with minor impurities present. This solid was recrystallized from 9:1 MeOH/H_2O to give 0.025 g of regiopure **14** (yellow crystals, mp=260 °C (dec)). ^{1}H NMR (75 °C, DMSO-d_6): δ 3.46 (m), 3.63 (dd, J=12.6, 2.8 Hz), 4.29 (m), 4.77 (t, CH_2-O*H*), 5.03 (d, J=7.8 Hz), 6.22 (d, J=2.0 Hz), 6.51 (d, J=2.0 Hz), 6.76 (s), 6.79 (s), 7.09 (d, J=8.4 Hz), 7.58 (d, J=8.6,2.0 Hz), 7.63 (d, J=2.0 Hz). ^{13}C NMR (25 °C, DMSO-d_6): δ 56.1, 60.1, 76.7, 77.9, 94.1, 98.9, 103.8, 103.9, 105.4, 114.8, 117.6, 119.9, 123.7, 126.0, 136.1, 143.7, 147.1, 148.0, 157.3, 161.4, 162.9, 164.3, 181.8. *anal.* C

56.58%, H 5.09%; calc d for $C_{26}H_{22}O_{10} \cdot 3\ H_2O$, C 56.93%, H 5.15%. See Figures 2.7 and 2.8 for ^1H and ^{13}C NMR spectra, respectively.

5-Deoxyscutellaprostin-A (15). To a 250 mL three-neck round-bottom flask was added 0.400 g (0.157 mmol) of 7,8-dihydroxyflavone, 0.284 g (0.157 mmol) of coniferyl alcohol, 130 mL benzene, and 65 mL acetone. The reaction vessel was placed in a 60 °C oil bath and let stir for 10 min. Next, 0.758 g (2.75 mmol) of Ag_2CO_3 was added and the reaction vigorously stirred for 24 h. The reaction was then allowed to cool, filtered through a Buchner funnel, and the solvent removed by rotary evaporation to yield an orange solid. The solid was subjected to CC using 95:5 $CHCl_3$/MeOH to yield 0.270 g of near pure sample of **15**. This sample was subjected to VLC using C-18 silica gel (1:1 H_2O/MeOH to 3:7 H_2O/MeOH) to yield 0.142 g of pure **15** (21%, yellow microcrystals, mp=233-234 °C). ^1H NMR (35 °C, DMSO-d_6): δ 3.48 (br d, J=12.4 Hz), 3.74 (br d, J=12.4 Hz), 3.80 (s, OMe), 4.40 (m), 5.08 (t, CH_2-OH), 5.14 (d, J=7.6 Hz), 6.83 (d, J=8.0 Hz), 6.92 (dd, J=8.0, 2.0 Hz), 7.01 (s), 7.07 (d, J=2.0 Hz), 7.09 (d, J=8.8 Hz), 7.54 (d, J=8.8 Hz), 7.61 (m), 8.13 (m), 9.17 (s). ^{13}C NMR (35 °C, DMSO-d_6): δ 55.7, 60.0, 76.3. 78.0, 106.6, 111.9, 114.8, 115.4, 115.9, 117.9, 120.6, 126.2, 126.7, 129.1, 131.2, 131.7, 132.2, 145.8, 147.2, 147.6, 161.8, 176.4. *anal.* C 69.31%, H 4.84%, calcd for $C_{25}H_{20}O_7$, C 69.44%, H 4.66%. See Figures 2.9 and 2.10 for ^1H and ^{13}C NMR spectra, respectively.

5-Deoxyscutellaprostin-B (16). The title compound was synthesized in the same manner as **15** using 0.350 g (1.30 mmol) of 7,8,4'-trihydroxyflavone, 0.233 g (1.30 mmol) of coniferyl alcohol, 110 mL benzene, and 55 mL acetone and 0.625 g (2.28 mmol) of

Ag_2CO_3 to yield an orange oil. The oil was subjected to column chromatography (CC) using 95:5 $CHCl_3$/MeOH to yield 0.145 g of a regiopure sample of **16** and dehydrodiconiferyl alcohol. VLC using C-18 silica gel (1:1 H_2O/MeOH to 1:4 H_2O/MeOH) yielded 0.050 g of pure **16** (12%, white powder, mp=254 °C). ^{1}H NMR (DMSO-d_6): δ 3.45 (br d, J=12.8 Hz), 3.71 (br d, J=12.8 Hz), 3.79 (s, OMe), 4.39 (m), 5.10 (t, CH_2-OH), 5.11 (d, J=7.6 Hz), 6.82 (d, J=8.0 Hz), 6.83 (s), 6.91 (d, J=8.0, 2.0 Hz), 6.94 (d, J=8.8 Hz), 7.06 (d, J=8.8 Hz), 7.07 (d, J=2.0 Hz), 7.50 (d, A of A_2B_2), 8.13 (d, B of A_2B_2), 9.22 (bs), 10.32 (bs). ^{13}C NMR (DMSO-d_6): δ 55.7, 60.0, 76.4, 78.1, 104.4, 111.8, 114.5, 115.4, 115.8, 115.9, 117.9, 120.6, 121.6, 126.8, 128.2, 132.2, 145.7, 147.2, 147.5, 147.7, 160.9, 162.3, 176.3. *anal.* HRFAB$^+$ 449.1239, calcd for $C_{25}H_{20}O_8$ 449.1236.

Sinaiticin-D (17). The title compound was synthesized in the same manner as **13** using 0.200 g (0.699 mmol) of luteolin, 0.105 g (0.699 mmol) of *p*-coumaryl alcohol, 25 mL benzene, 12.5 mL acetone, and 0.193 g (0.699 mmol) of Ag_2CO_3 to yield a brown-orange solid. The solid was subjected to CC using 91:9 $CHCl_3$/MeOH to yield 0.146 g (48%) of a 1:2 mixture of **18/17** and minor impurities. The solid was recrystallized from MeOH to yield 0.038 g of regiopure **17** (13%, yellow crystals, mp=269 °C). ^{1}H NMR (DMSO-d_6): δ 3.34 (dd, J=12.0, 4.0 Hz), 3.58 (dd, J=12.0, 2.4 Hz), 4.26 (m), 4.98 (d, J=8.0 Hz), 6.19 (d, J=1.8 Hz), 6.50 (d, J=1.8 Hz), 6.81 (d, J=8.8 Hz), 6.87 (s), 7.11 (d, J=8.8 Hz), 7.29 (d, J=8.8 Hz), 7.62 (d, J=8.8, 2.0 Hz), 7.65 (d, J=2.0 Hz), 12.90 (s). ^{13}C NMR (DMSO-d_6): δ 60.0, 75.7, 78.6, 94.1, 98.9, 103.7, 103.9, 115.0, 115.3, 117.4, 120.1, 123.5, 126.5, 129.2, 144.0, 146.9, 157.3, 157.9,161.4, 162.9, 164.5, 181.8. *anal.* C 66.33%, H 3.99%, calcd

for $C_{24}H_{18}O_8$, C 66.36%, H 4.18%. See Figures 2.11 and 2.12 for ^1H and ^{13}C NMR spectra, respectively.

***rac*-Silandrin-D (20).** The title compound was synthesized in the same manner as **13** using 0.400 g (1.39 mmol) of (±)-eriodictyol, 0.250 g (1.39 mmol) of coniferyl alcohol, 60 mL benzene, 30 mL acetone, and 0.383 g (1.39 mmol) of Ag_2CO_3 to yield a brown-white solid. This solid was subjected to CC 95:5 $CHCl_3$/MeOH to yield 0.262 g of a diastereomeric mixture of near pure **20** with only the down regioisomer detectible by ^1H NMR. This sample was further purfied using CC (7:3 hexanes/EtOAc) to yield 0.208 g of a regiopure diastereomeric mixture of **20** (24%, white microcrystalline solid). The two diastereomers were separated via preparative C-18 HPLC (gradient 1:9 H_2O/MeOH to 2:3 H_2O/MeOH.) Diastereomer #1 (**20b**) ^1H NMR (acetone-d_6): δ 2.78 (dt, J=17.0, 2.8 Hz), 3.17 (dd, J=17.0, 12.8 Hz), 3.51 (dd, J=12.0, 4.0 Hz), 3.75 (dd, J=12.0, 2.4 Hz), 3.89 (s, OMe), 4.15 (m), 5.00 (d, J=8.0 Hz), 5.47 (dd, J= 12.8, 2.8 Hz), 5.94 (d, J=2.0 Hz), 5.98 (d, J=2.0 Hz), 6.89 (d, J=8.0 Hz), 6.97 (d, J=8.0 Hz), 6.98 (dd, J=8.0, 2.0 Hz), 7.05 (dd, J=8.0, 2.0 Hz), 7.13 (d, J=2.0 Hz), 7.14 (d, J=2.0 Hz) 12.17 (s). ^{13}C NMR (acetone-d_6): δ 43.6, 56.4, 61.9, 77.3, 77.4, 79.7, 96.1, 97.0, 112.0, 115.8, 116.3, 116.3, 117.8, 120.6, 121.7, 129.2, 133.0, 145.0, 145.0, 148.1, 148.6, 164.3, 165.1, 168.0, 197.0. Diastereomer #2 (**20c**) ^1H NMR (acetone-d_6): δ 2.80 (dt, J=17.0, 2.8 Hz), 3.18 (ddd, J=17.0, 12.8, 2.8 Hz), 3.51 (ddd, J=12.4, 4.0, 2.0 Hz), 3.75 (dd, J=12.4, 1.2 Hz), 3.88 (s, OMe), 4.15 (m), 5.00 (d, J=8.0 Hz), 5.50 (dd, J=12.8, 2.8 Hz), 5.96 (d, J=2.0 Hz), 5.99 (d, J=2.0 Hz), 6.89 (d, J=8.0 Hz), 6.95 (d, J=8.0 Hz), 6.98 (dd, J=8.0, 2.0 Hz), 7.04 (dd, J=8.0, 2.0 Hz), 7.12 (m, 2H), 12.18 (s). ^{13}C NMR (acetone-d_6): δ 43.6, 56.4, 61.9, 77.3,

77.4, 79.7, 96.0, 97.0, 112.0, 115.8, 116.1, 116.1, 117.9, 120.3, 121.7,129.2, 133.2, 144.7, 145.3, 148.1, 148.6, 164.3, 165.4, 167.6, 197.1. *anal.* (pure diastereomers) C 62.19%, H 4.76 %, calcd for $C_{33}H_{30}O_{13}$, 62.46%, 4.77%. See Figures 2.13 and 2.14 for ^{1}H NMR spectra of each diastereomer.

5,7-Deoxyhydnocarpin-D (22). The title compound was synthesized in the same manner as **13** using 0.291 g (1.15 mmol) of 3,4-dihydroxyflavone, 0.207 g (1.15 mmol) of coniferyl alcohol, 25 mL benzene, 12.5 mL acetone, and 0.317 g (1.15 mmol) of Ag_2CO_3 to yield a dark yellow solid. This solid was subjected to CC using 93:7 $CHCl_3$/MeOH to yield 0.129 g of a 7:1 down/up mixture of regioisomers and minor impurities. A portion (0.016 g) of the purified product (**22**) was subjected to acetylation to give 0.014 g of acetylated product that was further purified by CC (1:1 hexanes/EtOAc) to give 12 frs. of pure **22a**, in which 3 frs. (0.006 g) contained a 15:1 down/up mixture (by ^{1}H NMR) of regioisomers (white microcrystalline solid, mp=172-174 °C). Peracetate: ^{1}H NMR ($CDCl_3$): δ 2.09 (s), 2.35 (s), 3.89 (s, OMe), 4.04 (dd, J=12.4, 4.4 Hz), 4.36 (m), 4.42 (dd, J=12.4, 3.2 Hz), 5.01 (d, J=8.0 Hz), 6.76 (s), 7.00 (d, J=2.0 Hz), 7.02 (m), 7.12 (J=8.0 Hz), 7.12 (d, J=8.0 Hz), 7.42 (ddd, J=8.2, 6.8, 0.8 Hz), 7.52 (d, J=2.0 Hz), 7.54 (d, J=2.0 Hz), 7.54 (dd, J=8.4, 1.6 Hz), 7.61 (d, J=2.0 Hz), 7.63 (dd, J=8.0, 2.0 Hz), 7.70 (ddd, J=8.6, 7.0, 1.6 Hz), 8.23 (dd, J=8.4, 1.6 Hz). ^{13}C NMR ($CDCl_3$): δ 20.7, 20.7, 56.0, 62.5, 75.9, 76.4, 106.7, 111.0, 115.5, 117.8, 118.0, 119.8, 120.4, 123.4, 123.9, 125.2, 125.4, 125.7, 133.7, 134.0, 140.7, 143.7, 145.9, 151.7, 156.2, 163.0, 168.7, 170.4, 178.3. *anal* C 68.33%, H 4.87%, calcd for $C_{25}H_{20}O_7 \cdot 0.5\ H_2O$, C 68.02%, H 4.80 %.

5-Deoxyhydnocarpin-D (23). To a 250 mL three-neck round-bottom flask was added 0.400 g (1.48 mmol) of 7,3',4'-trihydroxyflavone, 0.267 g (1.48 mmol) of coniferyl alcohol, 70 mL of benzene, and 35 mL of acetone. The reaction vessel was placed in a 60 °C oil bath and let stir for 10 min. Next, 0.408 g (1.48 mmol) of Ag_2CO_3 was added and the reaction solution stirred vigorously for 36 h. The reaction was then allowed to cool, filtered through a Buchner funnel, and the solvent removed by rotary evaporation to yield a yellow powder. The yellow powder was subjected to CC using 95:5 $CHCl_3$/MeOH to yield 0.172 g of a pale yellow powder that contained a 6:1 down/up (by ^1H NMR) mixture of regioisomers and dehydrodiconiferyl alcohol. VLC (vacuum liquid chromatography) using C-18 silica gel (4:1 H_2O/MeOH to 100% MeOH) yielded 0.073 g of pure **23** (11%, pale yellow powder, 6:1 down/up regioisomers). A portion (0.035 g) of the purified flavonolignan was acetylated (0.039 g, 89%) and recrystallized from 1:1 H_2O/MeOH to yield 0.025 g (64% from acetylated product) of regiopure **23a**. Peracetate (white microcrystalline sold, mp=212 °C): ^1H NMR ($CDCl_3$): δ 2.09 (s), 2.34 (s), 2.37(s), 3.88 (s, OMe), 4.05 (dd, J=12.3, 4.0 Hz), 4.35 (m), 4.42 (dd, J=12.3, 3.2 Hz), 6.75 (s), 7.01 (m, 2H), 7.11 (d, J=8.8 Hz), 7.12 (d, J=8.0 Hz), 7.16 (dd, J=8.0, 2.2 Hz), 7.37 (d, J=2.2 Hz), 7.50 (dd, J=8.4, 2.2 Hz), 7.57 (d, J=2.2 Hz), 8.24 (d, J=8.8 Hz). ^{13}C NMR ($CDCl_3$): δ 20.6, 20.7, 21.2, 56.0, 62.4, 75.9, 76.3, 106.6, 110.9, 111.0, 115.4, 117.9, 119.3, 119.8, 120.4, 121.6, 123.3, 125.0, 127.1, 134.0, 140.7, 143.7, 146.1, 151.7, 154.5, 156.6, 163.2, 168.5, 168.7, 170.3, 177.6. anal. 64.70%, 4.34%, calcd for $C_{31}H_{26}O_{11}$, C 64.81%, H 4.56%.

5-Deoxy-3-hydroxyhydocarpin-D (24). The title compound was synthesized in the same manner as **23** using 0.200 g (0.699 mmol) of fisetin, 0.126 g (0.699 mmol) of coniferyl alcohol, 50 mL of benzene, 25 mL of acetone and 0.193 g (0.699 mmol) of Ag_2CO_3 for 36 h to yield a yellow powder. The yellow powder was subjected to CC using 95:5 $CHCl_3$/MeOH to yield 0.172 g of a pale yellow powder that contained a 14:1 down/up (by ^{1}H NMR) mixture of regioisomers and dehydrodiconiferyl alcohol. VLC using C-18 silica gel (4:1 H_2O/MeOH to 100% MeOH) yielded 0.086 of regiopure **24** (27%, pale yellow powder). ^{1}H NMR (acetone-d_6): δ 3.55 (dd, J=12.3, 4.0 Hz), 3.79 (dd, J=12.3, 2.5 Hz), 3.89 (s, OMe), 4.24 (m), 5.06 (d, J=8.4 Hz), 6.90 (d, J=8.1 Hz), 6.99 (dd, J=8.7, 2.1 Hz), 7.01 (dd, J=8.1, 1.8 Hz), 7.08 (d, J=9.0 Hz), 7.09, (d, J=2.1 Hz), 7.18 (d, J=1.8 Hz), 7.86 (m, 2H), 8.02 (d, J=8.7 Hz). Peracetate (**24a**, white microcrystalline solid, mp=199-200 °C) : ^{1}H NMR ($CDCl_3$): δ 2.09 (s), 2.34 (s), 2.37 (s), 2.38 (s), 3.88 (s, OMe), 4.03 (dd, J=12.3, 4.0 Hz), 4.34 (m), 4.42 (dd, J=12.3, 3.2 Hz), 5.01 (d, 8.0 Hz), 7.00 (dd, J=8.4, 1.6 Hz), 7.01 (d, J=1.6 Hz), 7.11 (d, J=8.4 Hz), 7.12 (d, J=8.4 Hz), 7.17 (dd, J=8.8, 2.4 Hz), 7.39 (d, J=2.4 Hz), 7.51 (J=8.4, 2.4 Hz), 7.57 (d, J=2.4 Hz), 8.25 (d, J=8.8 Hz). ^{13}C NMR ($CDCl_3$): δ 20.6, 20.6, 20.7, 21.2, 56.0, 62.5, 75.9, 76.3, 110.9, 111.0, 117.5, 119.4, 119.8, 121.3, 122.4, 123.2, 123.3, 127.4, 133.4, 134.0, 140.6, 143.5, 145.7, 151.7, 154.7, 155.7, 155.9, 168.0, 168.4, 168.7, 170.4, 171.5. *anal.* C 63.13%, H 4.00%, calcd for $C_{33}H_{28}O_{13}$, C 62.86%, 4.16%. See Figure 2.15 for ^{1}H NMR spectrum.

3-Hydroxyhydnocarpin-D (25). The title compound was synthesized in the same manner as **23** using 0.600 g (1.77 mmol) of quercetin dihydrate, 0.320 g (1.77 mmol) of coniferyl alcohol, 90 mL of benzene and 45 mL of acetone, and 0.489 g (1.77 mmol) of

Ag_2CO_3 to yield a yellow powder. The yellow powder was subjected to CC using 95:5 $CHCl_3$/MeOH to yield 0.341 g of a yellow powder that contained >30:1 down/up ratio of regioisomers (by ^1H NMR) plus minor impurities. In the more concentrated CC fractions, the product crystallized out to give regiopure samples of pure **25** (0.055 g). The remaining frs. were subjected to VLC using C-18 silica gel (3:2 H_2O/MeOH to 1:4 H_2O/MeOH) to give 0.142 g of a regiopure sample of pure **25** (23% combined, yellow powder, mp=279-280 °C). ^1H NMR (acetone-d_6): δ 3.54 (dd, J=12.4, 4.0 Hz), 3.78 (dd, J=12.4, 2.4 Hz), 3.89 (s, OMe), 4.25 (m), 5.06 (d, J=8.4 Hz), 6.27 (d, J=2.0 Hz), 6.59 (d, J=2.0 Hz), 6.90 (d, J=8.0 Hz), 7.01 (dd, J=8.4, 2.0 Hz), 7.10 (d, J=8.4 Hz), 7.17 (d, J=2.0 Hz), 7.85 (dd, J=8.4, 2.0 Hz), 7.86 (d, J=2.0 Hz), 12.14 (s). ^{13}C NMR (acetone-d_6): δ 56.3, 61.7, 77.2, 80.0, 94.6,, 99.2, 104.2, 111.9, 115.8, 117.2, 117.7, 121.7, 122.2, 124.9, 128.9, 137.1, 144.8, 146.1, 146.5, 148.1, 148.5, 157.8, 162.3, 165.1, 172.8, 176.6. Peracetate (**25a**, pale yellow microcrystals, mp=176-178 °C), ^1H NMR ($CDCl_3$): δ 2.09 (s), 2.34 (s), 2.35 (s), 2.35 (s), 2.44 (s), 3.88 (s, OMe), 4.02 (dd, J=12.0, 4.0 Hz), 4.34 (m), 4.41 (dd, J=12.0, 3.2 Hz), 5.00 (d, J=8.0 Hz), 6.99 (dd, J=8.4, 2.0 Hz), 7.01 (d, J=2.0 Hz), 7.09 (d, J=8.4 Hz), 7.12 (d, J=8.4 Hz), 7.32 (d, J=2.0 Hz), 7.47 (dd, J=8.4, 2.0 Hz), 7.53 (d, J=2.0 Hz). ^{13}C NMR ($CDCl_3$): δ 20.8, 20.9, 20.9, 21.3, 21.4, 56.2, 62.7, 76.1, 76.5, 109.1, 111.2, 113.9, 117.6, 117.7, 120.0, 122.6, 123.0, 123.5, 133.7, 134.2, 140.9, 143.7, 146.0, 150.5, 151.9, 154.3, 154.9, 157.0, 168.0, 168.1, 168.9, 169.5, 170.3, 170.6. *anal.* C 60.61 %, H 4.30%, calcd for $C_{35}H_{30}O_{15}$ C 61.05%, H 4.10%. See Figure 2.16 for ^1H NMR spectrum.

Flavones

4'-*n*-Propoxyflavone (38). To a 100 mL three-neck round-bottom flask was added 0.074 g (0.311 mmol) of **37**, 25 mL dry acetone, 0.076 g (0.622 mmol) of 1-bromopropane, and 0.215 g (1.56 mmol) of anhydrous K_2CO_3. The reaction solution was heated at reflux for 12 h, cooled to r.t., poured into a solution of brine, the acetone removed *in vacuo*, and the organics extracted with EtOAc. The EtOAc was dried with $MgSO_4$, filtered and removed *in vacuo* to yield a white powder. The white powder was subjected to CC using 7:3 hexanes/EtOAc to yield 0.071 g of pure **38** (82 %, white powder, mp=121-122 °C). ^{1}H NMR ($CDCl_3$): δ 1.08 (t), 1.86 (m), 4.01 (t), 6.76 (s), 7.02 (A of A_2B_2), 7.42 (ddd, J=8.4, 7.0, 0.8 Hz), 7.56 (dd, J=8.0, 1.2 Hz), 7.69 (ddd, J=8.4, 7.0, 1.2 Hz), 7.88 (B of A_2B_2), 8.23 (dd,J=8.0, 1.2 Hz). ^{13}C NMR ($CDCl_3$): δ 10.5, 22.4, 69.8, 106.1, 114.9, 117.9, 123.7, 123.9, 125.0, 125.6, 128.0, 133.5, 156.2, 162.0, 163.5, 178.4. *anal.* C 77.26% H 5.90%, calcd for $C_{18}H_{16}O_3$ C 77.12%, H 5.75%. See Figures 2.17 and 2.18 for ^{1}H and ^{13}C NMR spectra, respectively.

4'-*i*-Propoxyflavone (39). The title compound was synthesized in the same manner as **38** using 0.075 g (0.315 mmol) of **37**, 25 mL dry acetone, 0.078 g (0.630 mmol) of 2-bromopropane, and 0.218 g (1.58 mmol) of anhydrous K_2CO_3 heating at reflux for 12 h to give an off-white powder. The powder was subjected to CC using 7:3 hexanes/EtOAc to yield 0.041 g of pure **39** (47%, white powder, mp=102 °C). ^{1}H NMR ($CDCl_3$): δ 1.39 (s), 1.40 (s), 4.67 (m), 6.75 (s), 7.00 (A of A_2B_2), 7.41 (ddd, J=8.4, 7.0, 0.8 Hz), 7.55 (dd, J=8.4, 1.2 Hz), 7.69 (ddd, J=8.4, 7.0, 1.2 Hz), 7.87 (B of A_2B_2), 8.23 (dd, J=8.0, 1.2 Hz). ^{13}C NMR ($CDCl_3$): δ 21.9, 70.2, 106.0, 115.9, 117.9, 132.5, 123.9, 125.0, 125.6, 128.0,

133.5, 156.2, 160.9, 163.5, 178.4. *anal.* C. 77.22%, H 5.87%, calcd for $C_{18}H_{16}O_3$, C 77.12%, H 5.75%

4'-Ethoxyflavone (40). To a 100 mL three-neck round-bottom flask was added 0.078 g (0.327 mmol) of **37**, 25 mL dry acetone, 0.237 g (1.31 mmol) of bromoethane, and 0.226 g (1.63 mmol) of anhydrous K_2CO_3. The reaction flask was placed in a 37 °C oil bath and stirred for 17 h. The reaction was then allowed to cool to r.t., poured into a solution of brine, the acetone removed *in vacuo*, and the organics extracted with EtOAc. The EtOAc was dried with $MgSO_4$, filtered, and removed *in vacuo* to yield a white powder of near pure **40**. The powder was subjected to CC using 7:3 hexanes/EtOAc to yield 0.076 g of pure **40** (87%, white amorphous solid, mp=122-123 °C). ^{1}H NMR ($CDCl_3$): δ 1.47 (t), 4.13 (q), 6.76 (s), 7.02 (A of A_2B_2), 7.42 (ddd, J=8.0, 7.6, 1.2 Hz), 7.56 (dd, J=7.6, 1.2 Hz), 7.70 (ddd, J=8.6, 7.0, 1.4 Hz), 7.89 (B of A_2B_2), 8.24 (dd, J=8.0, 1.6 Hz). ^{13}C NMR ($CDCl_3$): δ 14.7, 63.8, 106.2, 114.9, 123.9, 124.0, 125.0, 125.7, 128.0, 133.5, 156.2, 161.8, 163.5, 178.4. *anal.* C 76.90%, H 5.10%, calcd for $C_{17}H_{14}O_3$ C 76.68%, H 5.30%.

4'-Allyloxyflavone (41). The title compound was synthesized in the same manner as **38** using 0.075 g (0.315 mmol) of **37**, 25 mL dry acetone, 0.076 g (0.630 mmol) of allyl bromide, and 0.218 g (1.58 mmol) of anhydrous K_2CO_3 heating at reflux for 18 h to yield an brown-white powder. The powder was subjected to CC using 7:3 hexanes/EtOAc to yield 0.065 g of near pure **41.** This sample was recrystallized from hot 99:1 hexanes/EtOAc to yield 0.050 g of pure **41** (57%, white fibrous crystals, mp=108-109 °C). ^{1}H NMR ($CDCl_3$): δ 4.64 (dd, J=5.2, 1.6 Hz), 4.65 (dd, J=3.2, 1.6 Hz), 5.35 (dd,

J=10.2, 1.6 Hz), 5.46 (dd, J=17.2, 1.6 Hz), 6.08 (dddd, J=17.2, 10.2, 5.2, 3.2 Hz), 6.76 (s), 7.05 (A of A_2B_2), 7.42 (ddd, J=8.4, 7.0, 0.8 Hz), 7.56 (dd, J=8.4, 0.8 Hz), 7.70, (ddd, J=8.4, 7.0, 2.0 Hz), 7.89 (B of A_2B_2), 8.24 (dd, J=7.6, 1.6 Hz). ^{13}C NMR ($CDCl_3$): δ 69.0, 106.3, 115.2, 118.0, 118.3, 124.0, 124.2, 125.1, 125.7, 128.0, 132.5, 133.6, 156.2, 161.4, 163.3, 178.4. *anal.* C 77.55%, H 5.27%, calcd for $C_{18}H_{14}O_3$ C 77.68%, H 5.07%.

4'-(2"-Hydroxyethyl)-flavone (42). To a 100 mL three-neck round-bottom flask was added 0.075 g (0.315 mmol) of **37**, 25 mL dry acetone, 0.075 g (0.315 mmol) of (2-bromoethoxy)-*tert*-butyldimethylsilane, and 0.218 g (1.58 mmol) of anhydrous K_2CO_3. The reaction solution was heated at reflux for a total of 9 h with 2 separate additions of 1 eq. of (2-bromoethoxy)-*tert*-butyldimethylsilane added at 2 h and 5 h. The reaction solution was then cooled to r.t., poured into a solution of brine, the acetone removed *in vacuo*, and the organics extracted with EtOAc. The EtOAc was dried with $MgSO_4$, filtered, and removed *in vacuo* to yield a white powder. The sample was subjected to CC using 1:1 hexanes/EtOAc to yield 0.030 g of the pure ether (24%, white microcrystalline powder). The ether and 15 mL of THF were added to a 50 mL three-neck round-bottom flask and the reaction solution cooled to 0 °C by an ice bath. After stirring 10 min, 0.2 mL of tetrabutylammonium fluoride (TBAF, 1.0 M in THF) was added and the reaction stirred for 30 min at 0 °C. The contents of the flask were then poured into water, THF removed *in vacuo*, and the organics extracted with EtOAc. The EtOAc was dried with $MgSO_4$, filtered, and removed *in vacuo* to yield a white powder. The powder was subjected to CC using 3:7 hexanes/EtOAc to yield 0.018 g of pure **42** (86%, white microcrystalline powder, mp = 142-143 °C). ^1H NMR (methanol-d_4): δ 3.93 (m, 2H), 4.15

(m, 2H), 6.84 (s), 7.15 (*A* of A_2B_2), 7.50 (ddd, J=8.4, 7.2, 0.8 Hz), 7.72 (dd, J=8.4, 0.8 Hz), 7.82 (ddd, J = 8.4, 7.0, Hz), 8.02 (*B* of A_2B_2), 8.14 (dd, J=8.4, 1.6 Hz). ^{13}C NMR (methanol-d_4): δ 61.7, 71.1, 106.3, 116.4, 119.5, 124.7, 125.0, 126.3, 126.8, 129.6, 135.7, 157.9, 163.9, 166.2, 180.7. anal. HRFAB$^+$ 283.0970, cald for $C_{17}H_{14}O_4$ 283.0973.

4'-Nonoxyflavone (43). The title compound was synthesized in the same manner as **38** using 0.075 g (0.315 mmol) of **37**, 25 mL dry acetone, 0.065 g (0.315 mmol) of 1-bromononane, and 0.218 g (1.58 mmol) of anhydrous K_2CO_3 heating at reflux for 12 h to yield an off-white powder. The powder was subjected to CC using 7:3 hexanes/EtOAc to yield 0.048 g of pure **43** (45%, white powder, mp=102 °C). ^{1}H NMR ($CDCl_3$): δ 0.90 (t), 1.34 (m, 10 H), 1.49 (m, 2H), 1.83 (m, 2H), 4.04 (t), 6.76 (s), 7.02 (A of A_2B_2), 7.42 (ddd, J=8.4, 7.0, 1.2 Hz), 7.56 (dd, J=8.4, 1.2 Hz), 7.69 (ddd, J=8.4, 7.0, 1.2 Hz), 7.88 (B of A_2B_2), 8.23 (dd, J=8.0, 1.2). ^{13}C NMR ($CDCl_3$): δ 14.1, 22.7, 26.0, 29.1, 29.2, 29.3, 29.5, 31.8, 68.3, 106.1, 114.9, 117.9, 123.7, 123.9, 125.0, 125.6, 127.9, 133.5, 156.2, 162.0, 163.5, 178.4. *anal.* C 79.17% H 7.98%, calcd $C_{24}H_{28}O_3$ C 79.09%, H 7.74%.

4'-Benzyloxyflavone (44). The title compound was synthesized in the same manner as **38** using 0.075 g (0.315 mmol) of **37**, 25 mL dry acetone, 0.060 g (0.351 mmol) of benzyl bromide, and 0.218 g (1.58 mmol) of anhydrous K_2CO_3 heating at reflux for 9 h to yield a white powder. The sample was recrystallized from MeOH to yield 0.086 g of pure **44** (84%, white powder, mp=192 °C). ^{1}H NMR ($CDCl_3$): δ 5.17 (s), 7.10 (A of A_2B_2), 7.42 (m, 6H), 7.55 (d, J=8.4, 0.8 Hz), 7.70 (ddd, J=8.4, 7.0, 2.0 Hz) 7.90 (B of A_2B_2), 8.24 (J=8.0, 2.0 Hz). ^{13}C NMR ($CDCl_3$): δ 70.2, 106.2, 115.3, 117.9, 123.9, 124.3, 125.1,

125.7, 127.5, 128.0, 128.3, 128.7, 133.6, 136.2, 156.2, 161.5, 163.3, 178.4. *anal.* C 80.57%, H 5.07%, calcd for $C_{22}H_{16}O_3$ C 80.47%, H 4.91%.

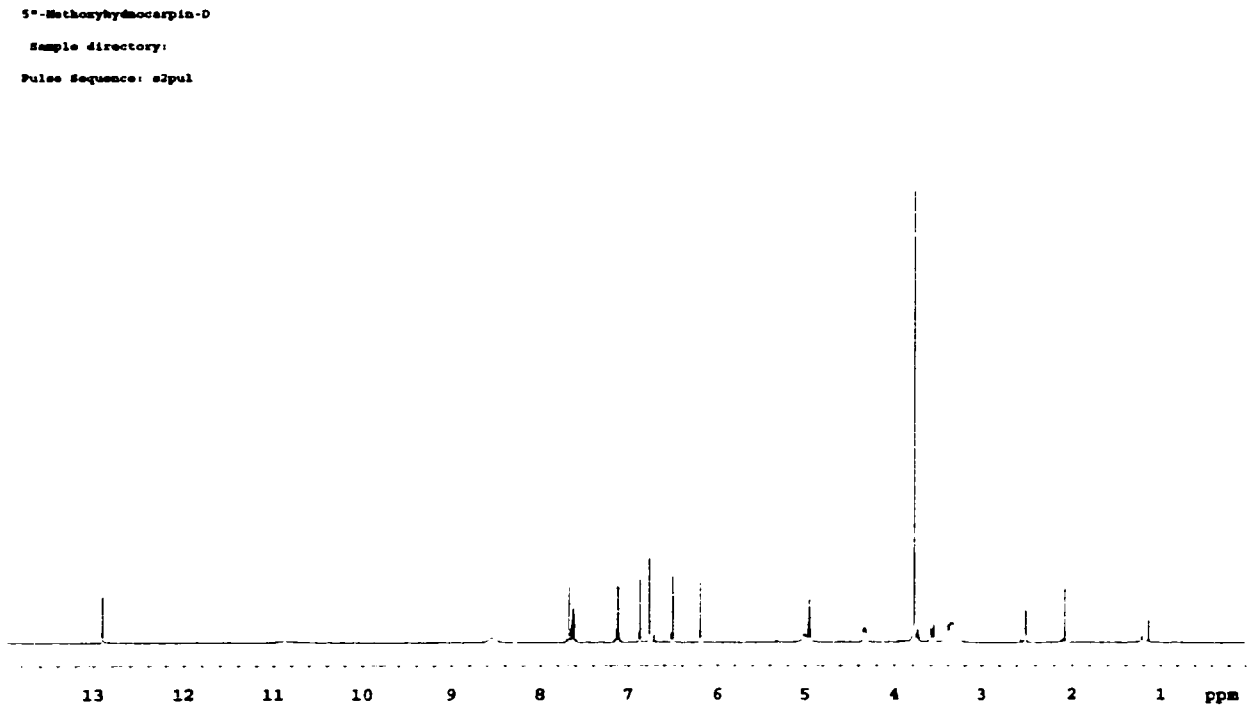

Figure 2.5. ^{1}H spectrum of 5"-methoxyhydnocarpin-D (**13**).

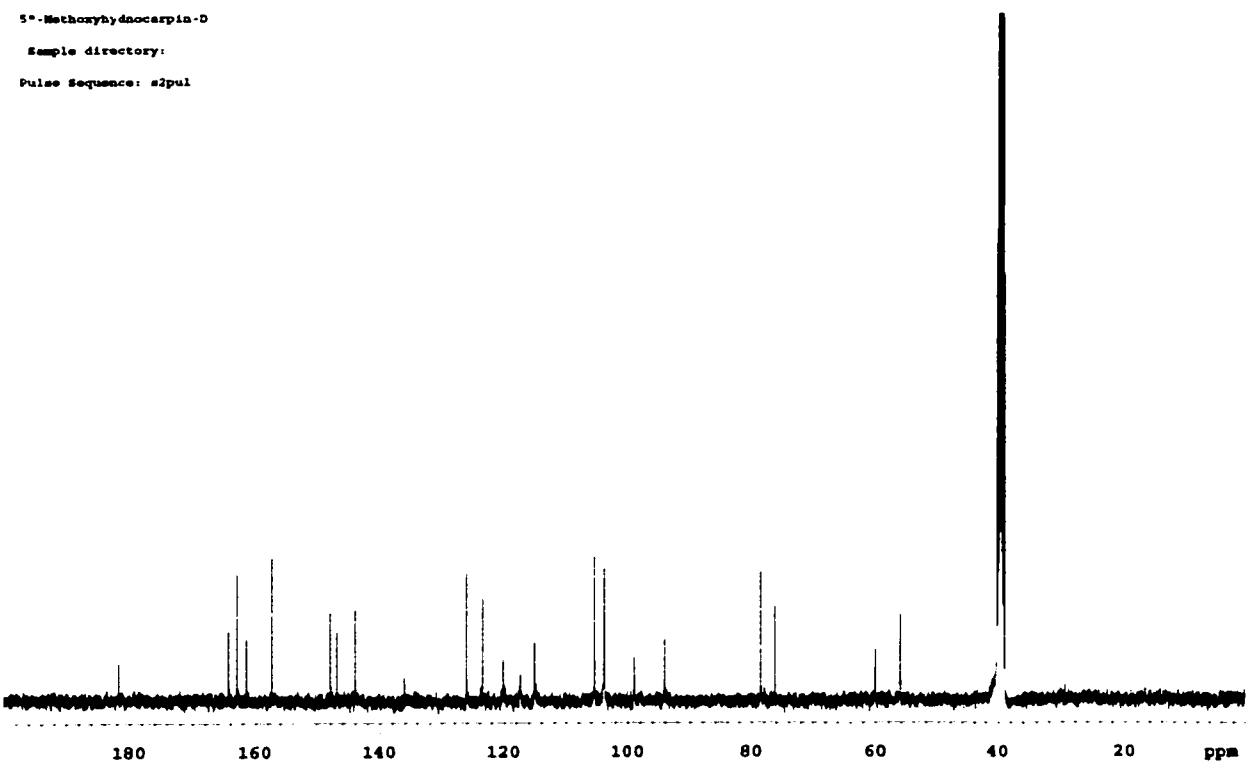

Figure 2.6. ^{13}C spectrum of 5"-methoxyhydnocarpin-D (**13**).

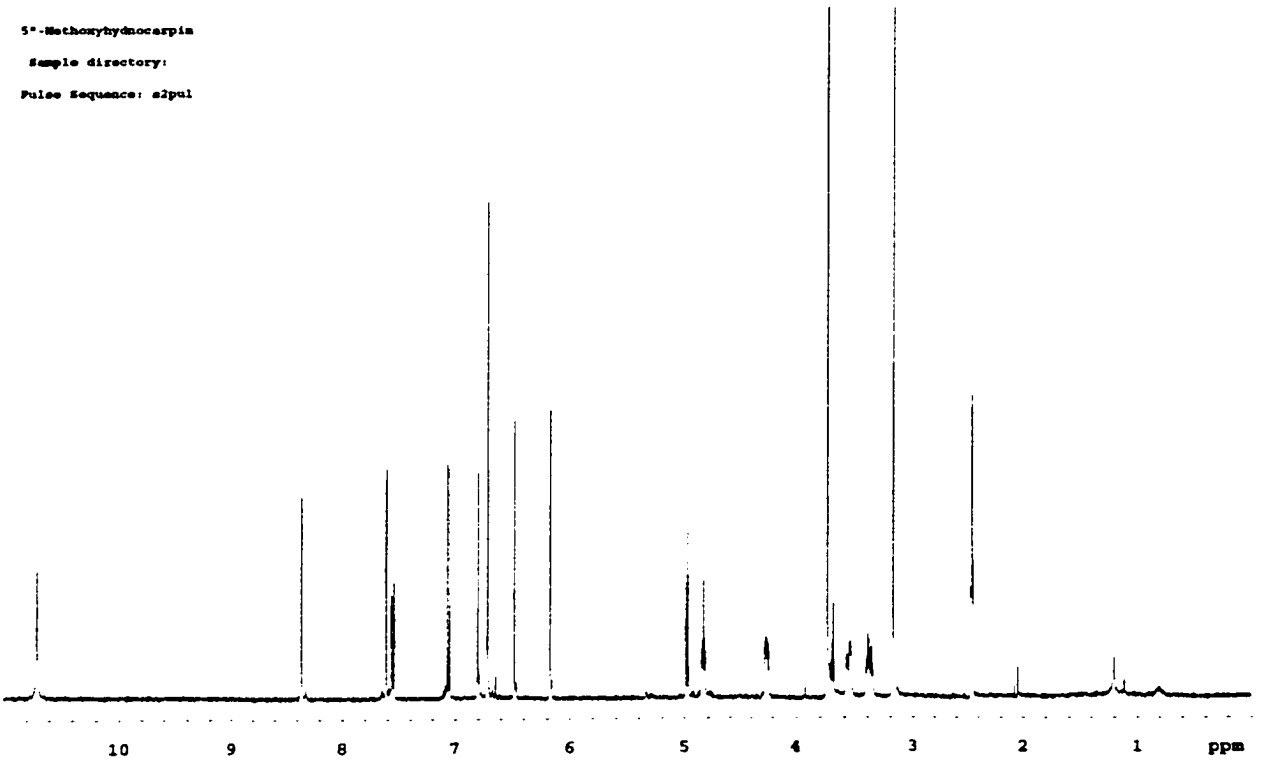

Figure 2.7. ^{1}H spectrum of 5"-methoxyhydnocarpin (**14**).

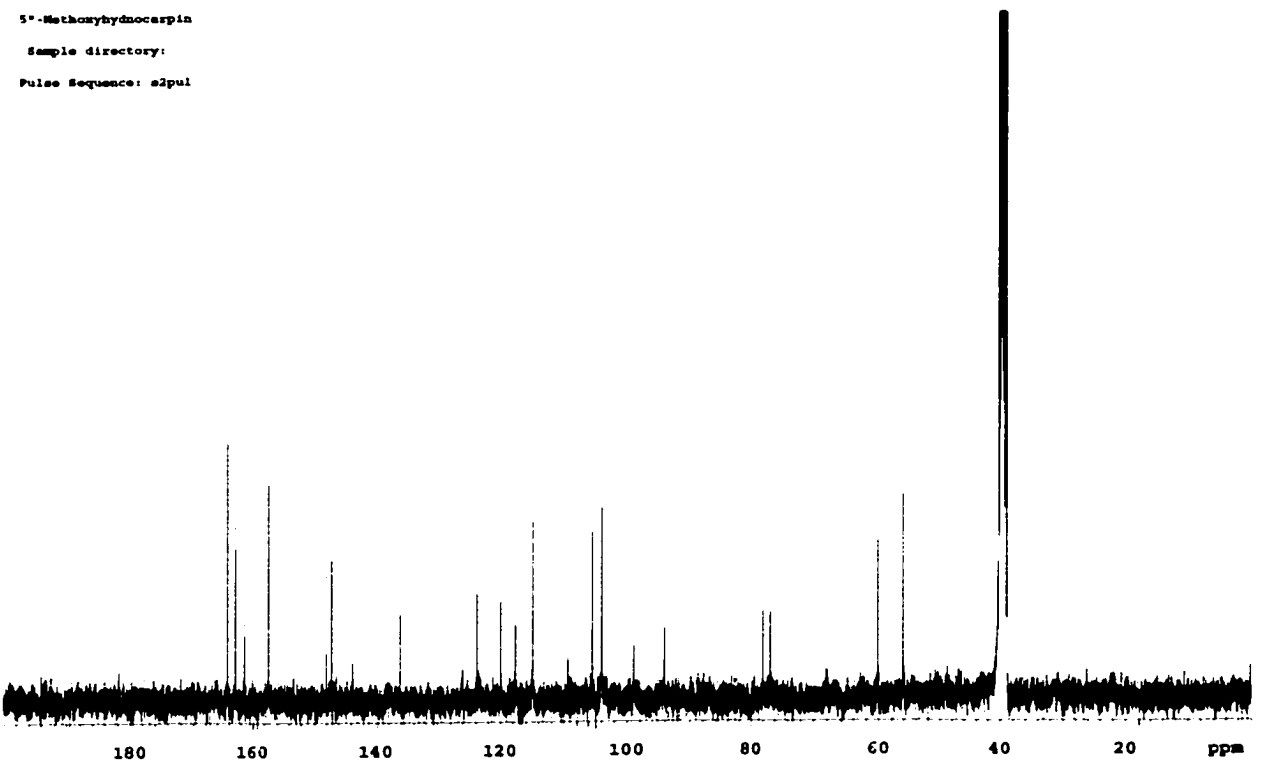

Figure 2.8. ^{13}C spectrum of 5"-methoxyhydnocarpin (**14**).

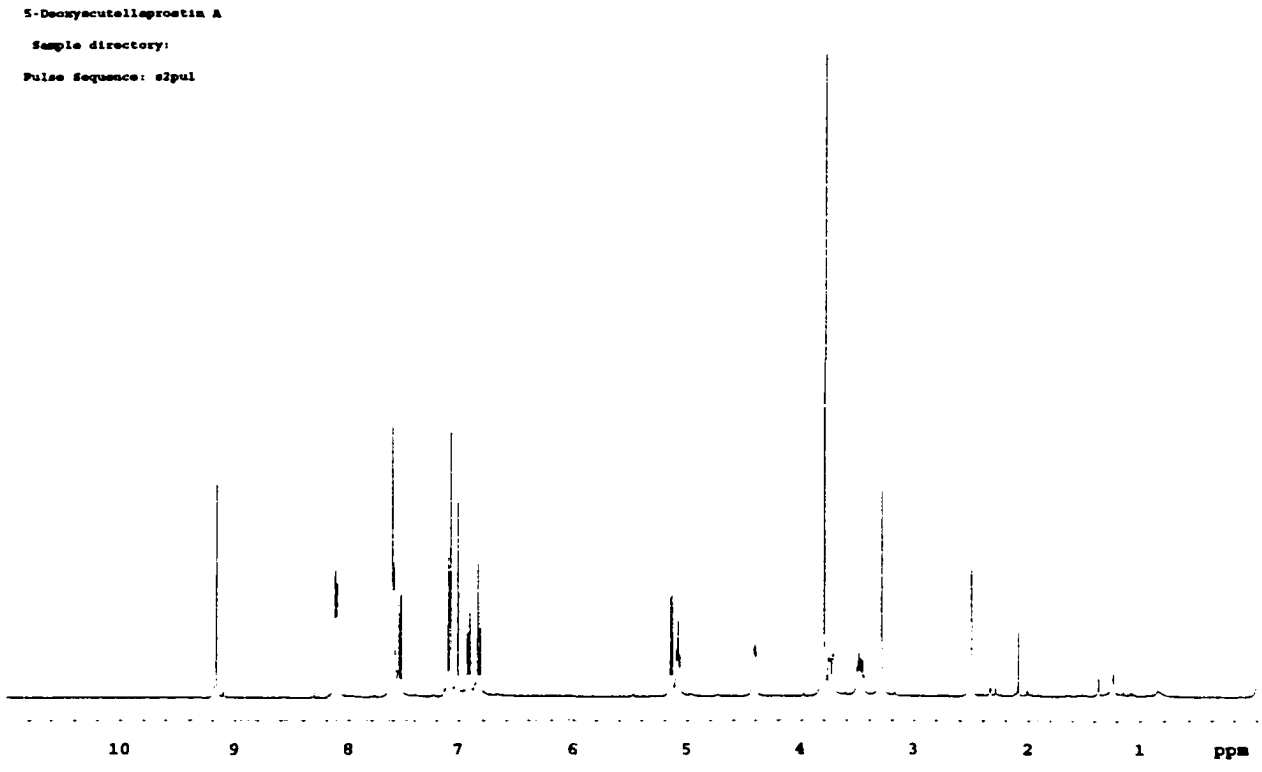

Figure 2.9. ^{1}H spectrum of 5-deoxyscutellaprostin-A (**15**).

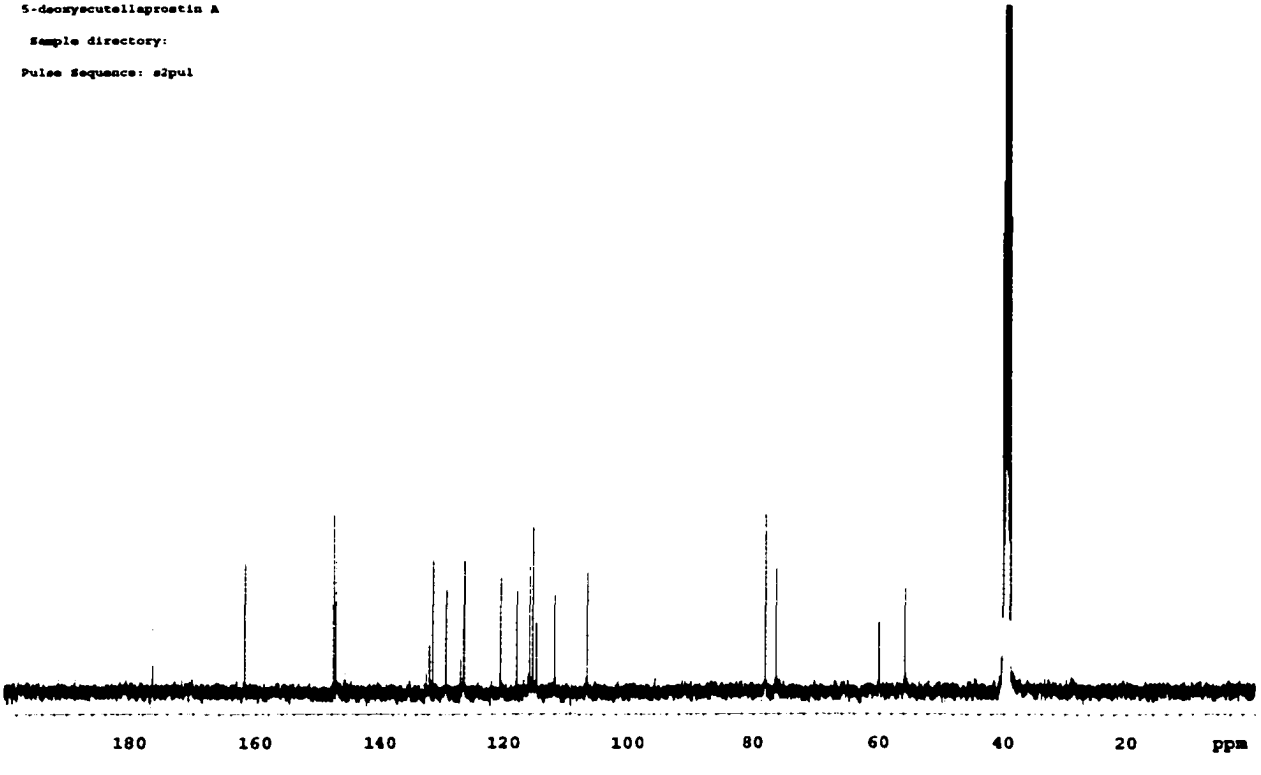

Figure 2.10. ^{13}C spectrum of 5-deoxyscutellaprostin-A (**15**).

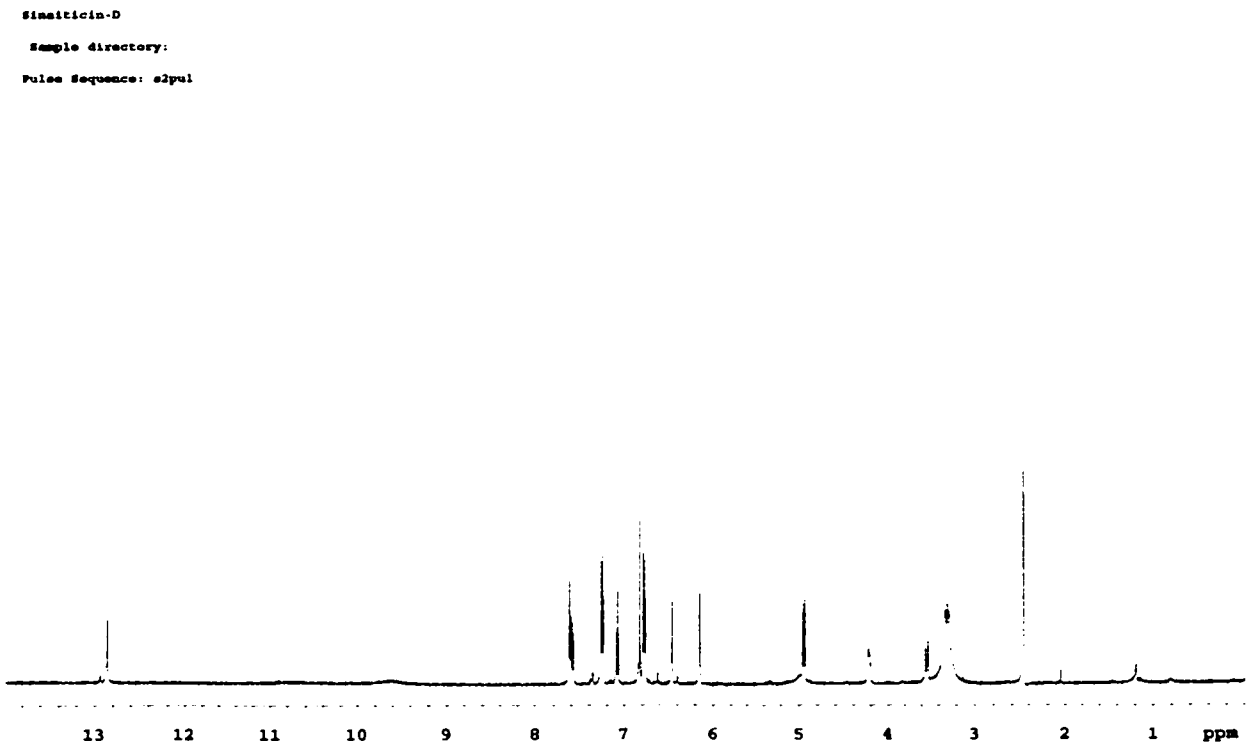

Figure 2.11. ^{1}H spectrum of sinaiticin-D (17).

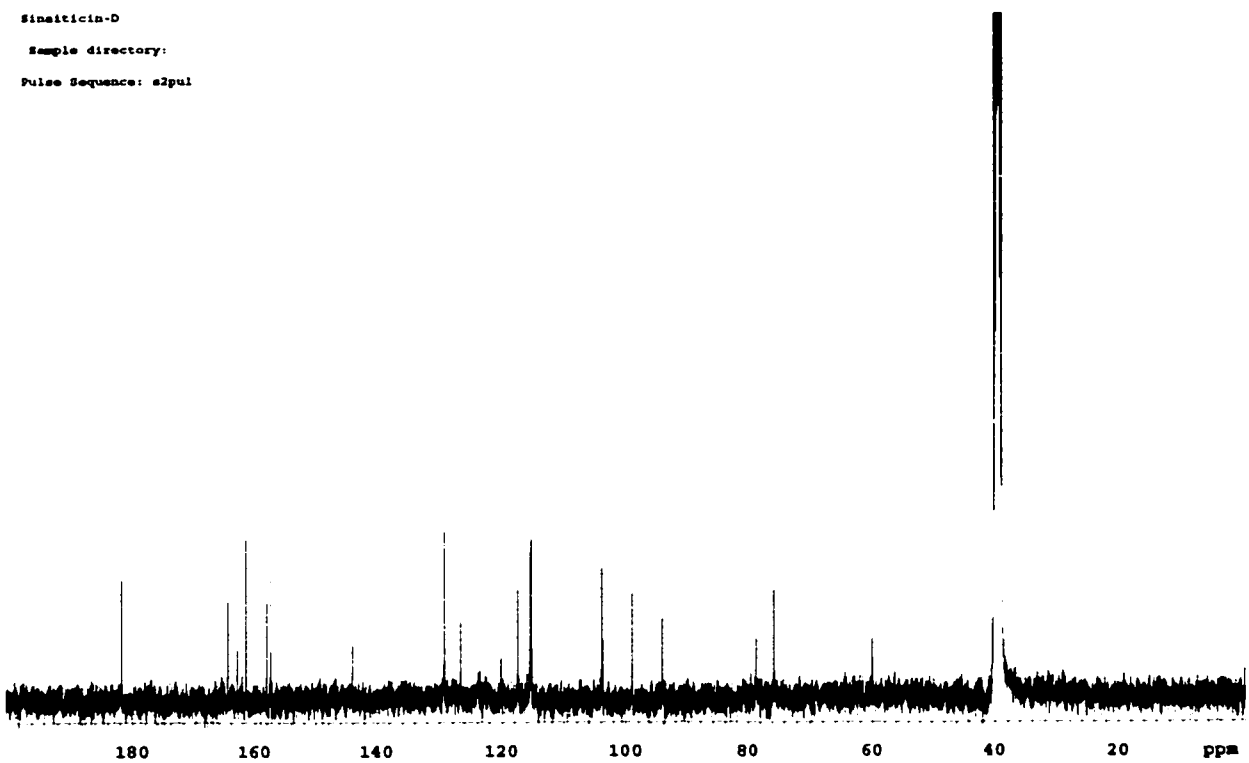

Figure 2.12. ^{13}C spectrum of sinaiticin-D (17).

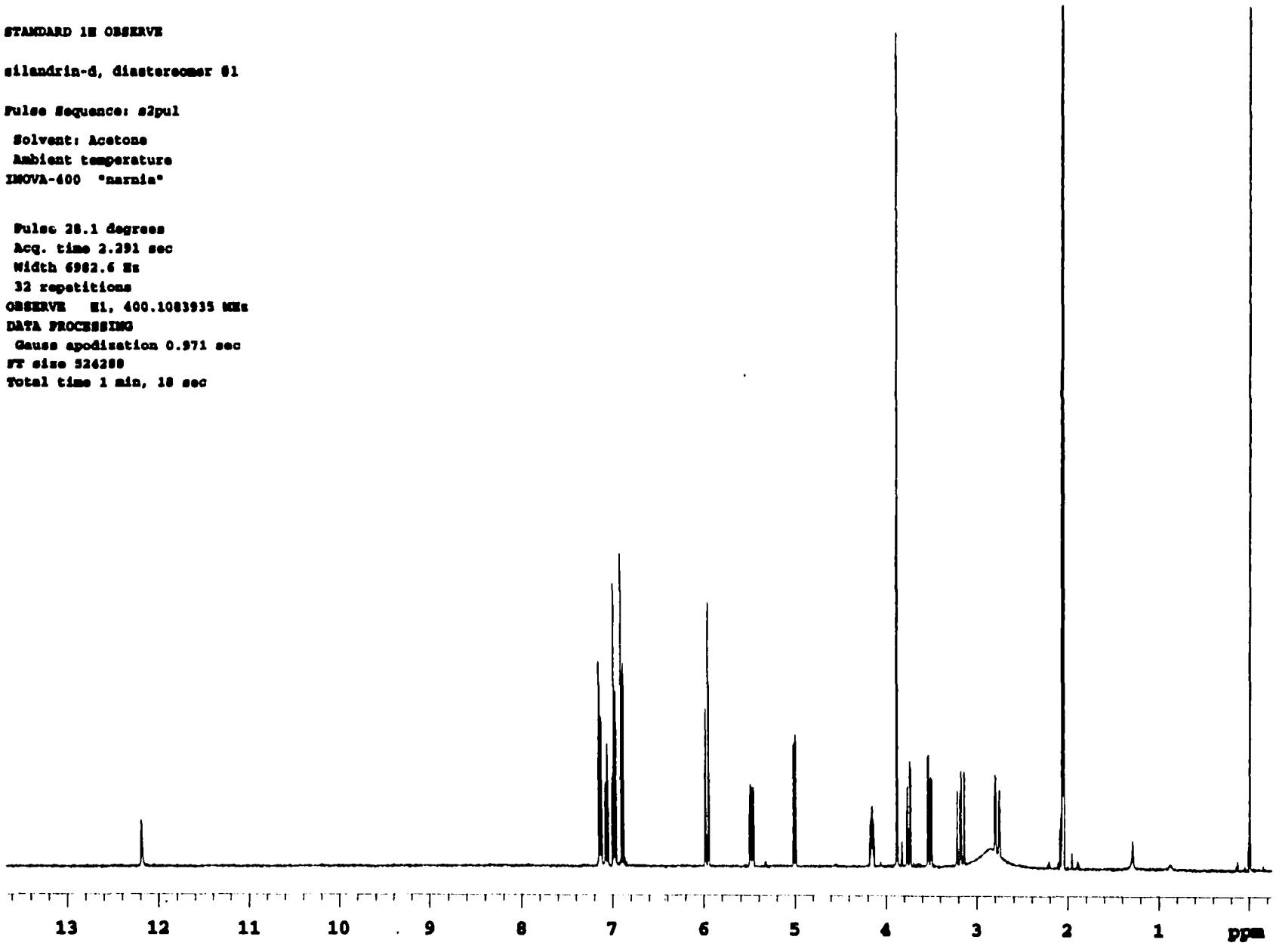

Figure 2.13. ^{1}H NMR spectrum of silandrin-D diastereomer #1 (**20b**).

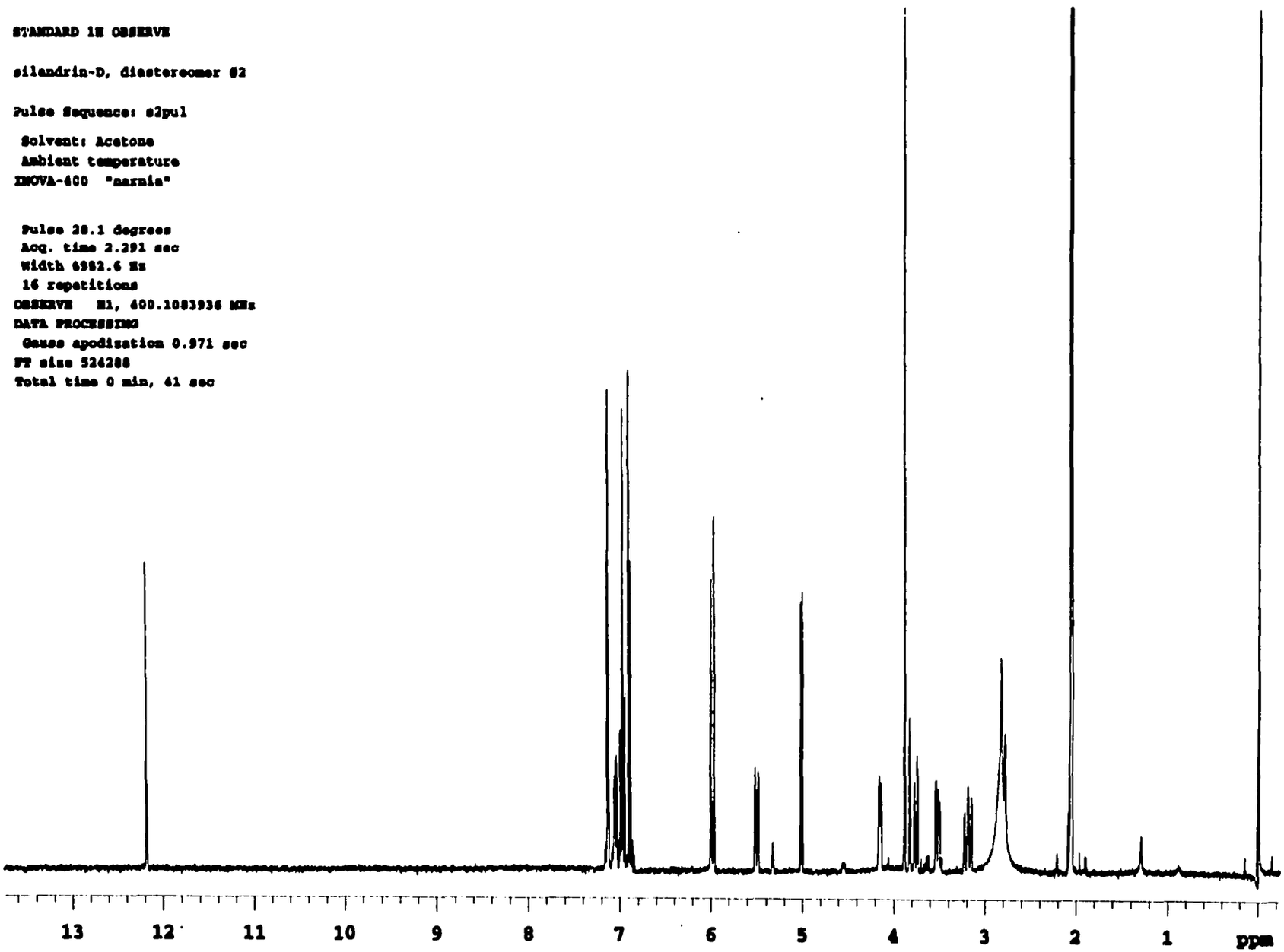

Figure 2.14. ^{1}H NMR spectrum of silandrin-D diastereomer #2 (20c).

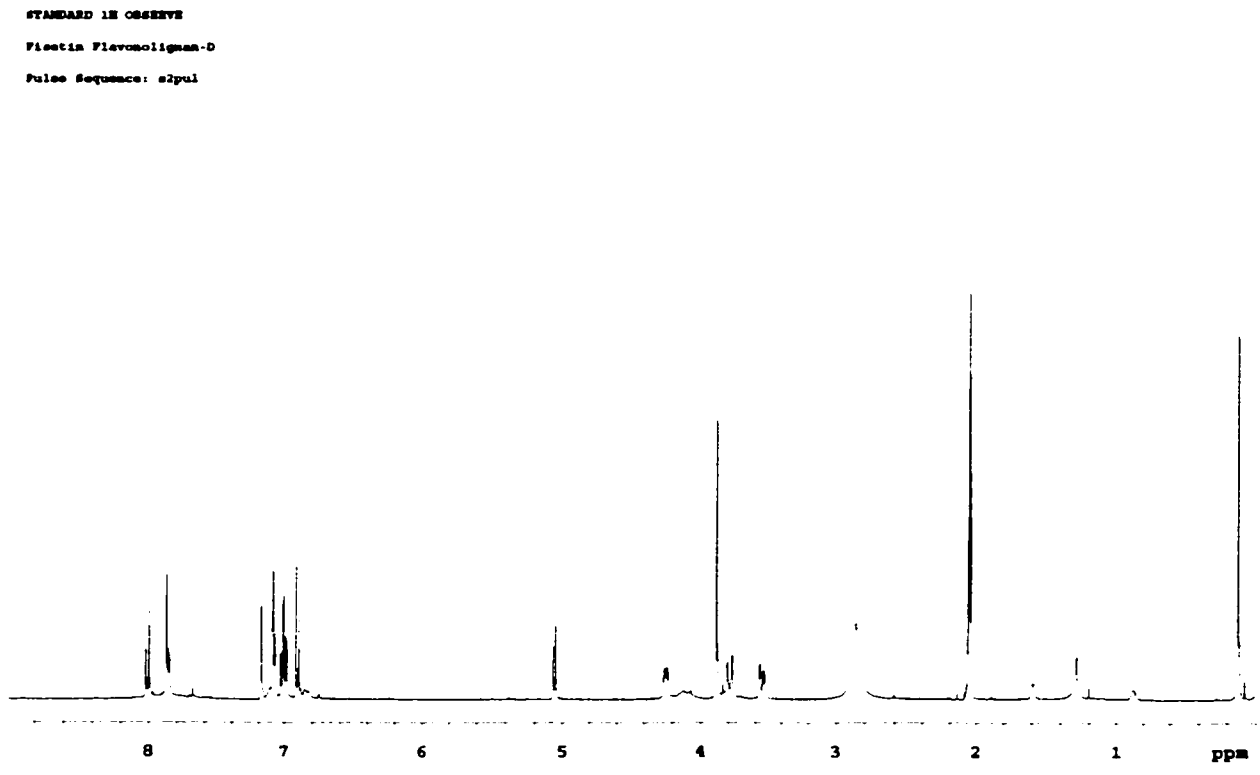

Figure 2.15. ^{1}H spectrum of 5-deoxy-3-hydroxyhydnocarpin-D (**24**).

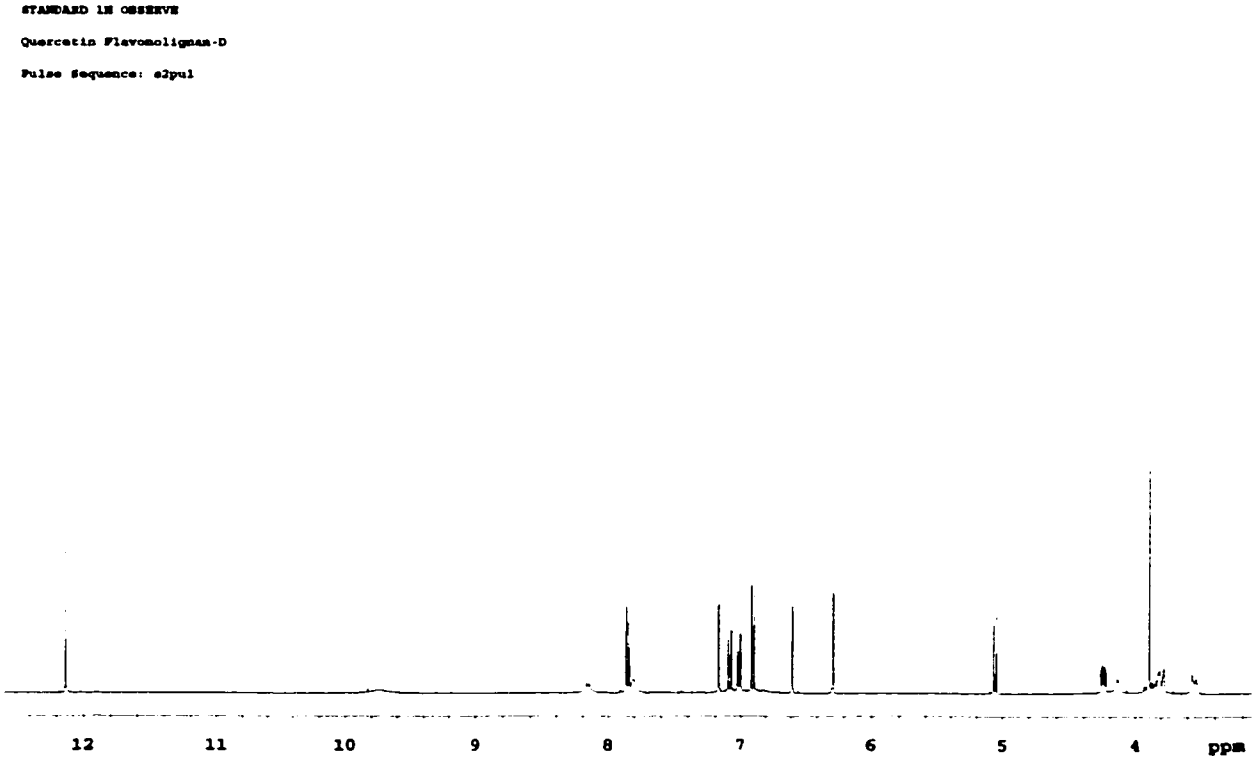

Figure 2.16. ^{1}H spectrum of 3-hydroxyhydnocarpin-D (**25**).

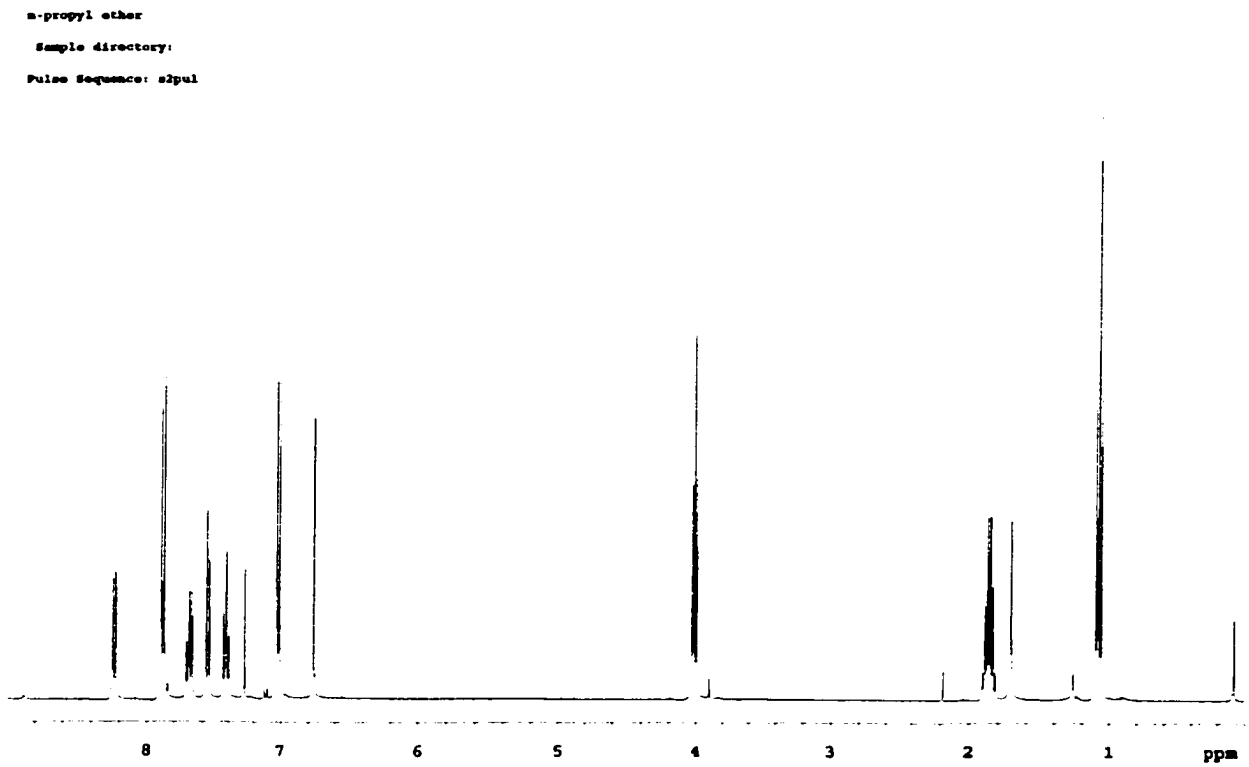

Figure 2.17. ^{1}H spectrum of 4'-*n*-propoxyflavone (38).

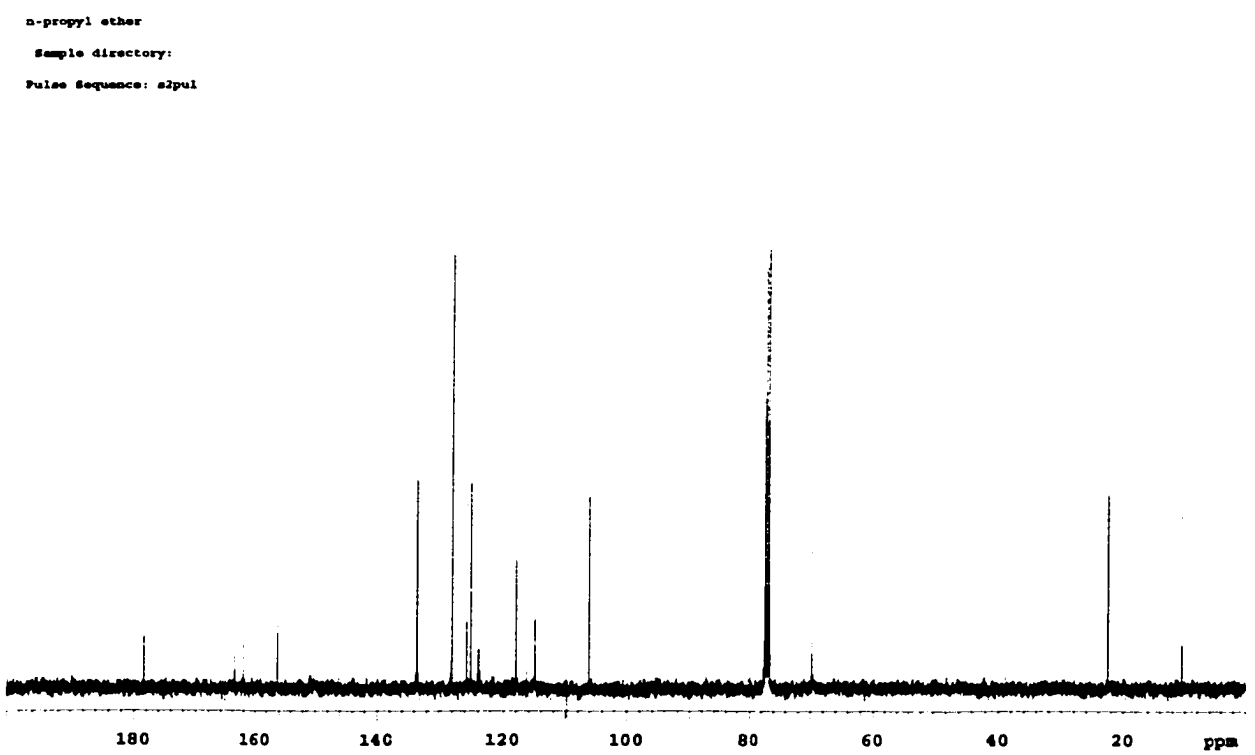

Figure 2.18. ^{13}C spectrum of 4'-*n*-propoxyflavone (38).

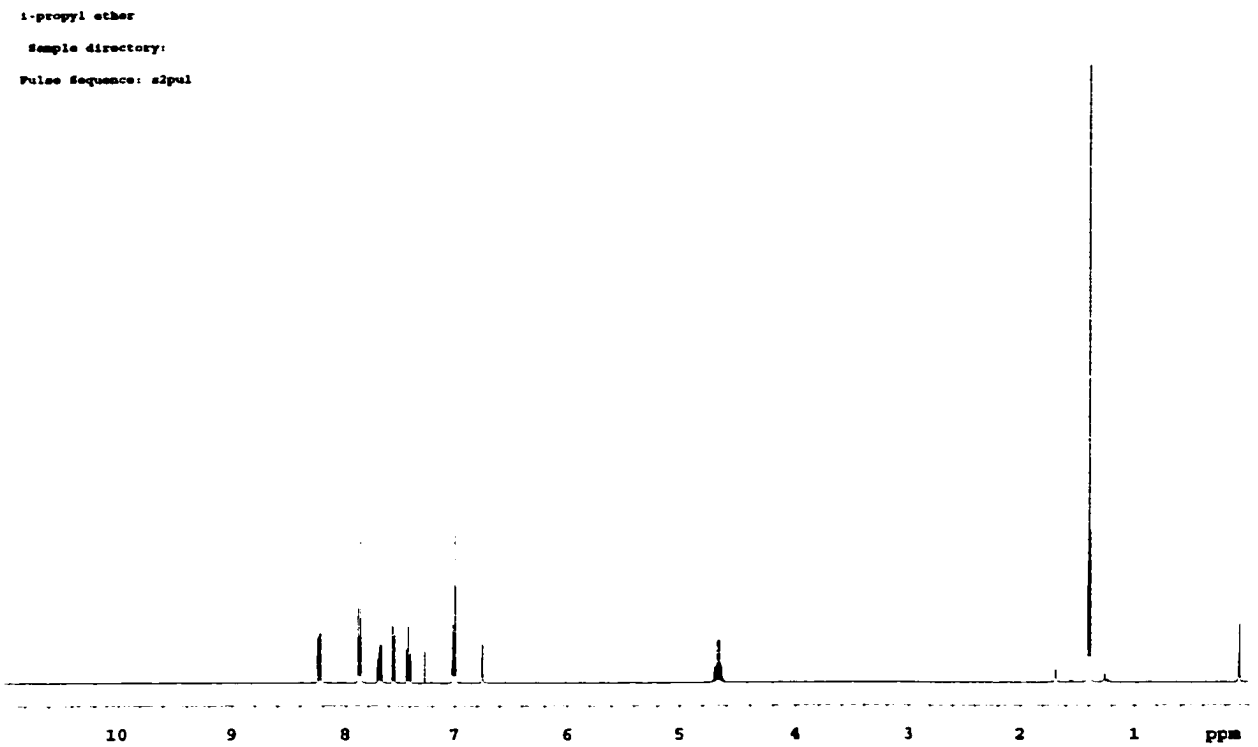

Figure 2.19. ^{1}H spectrum of 4'-*i*-propoxyflavone (**39**).

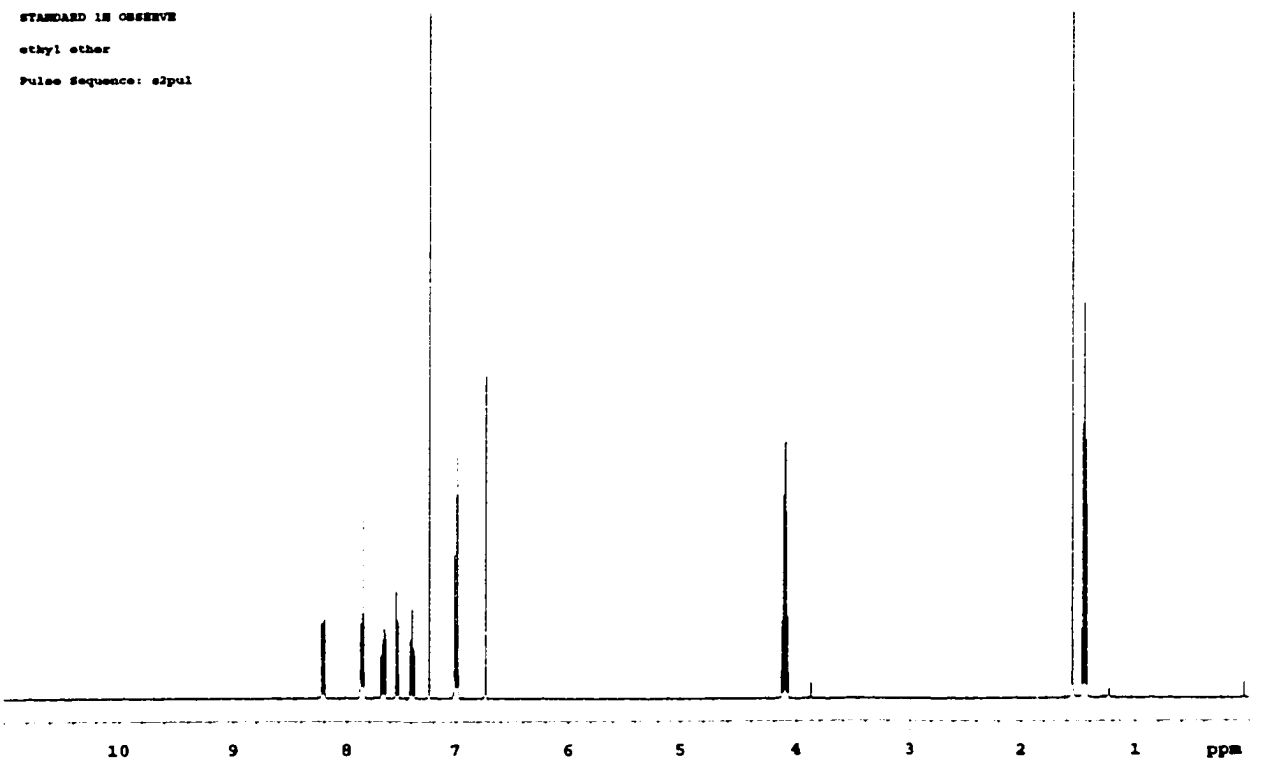

Figure 2.20. ^{1}H spectrum of 4'-ethoxyflavone (**40**).

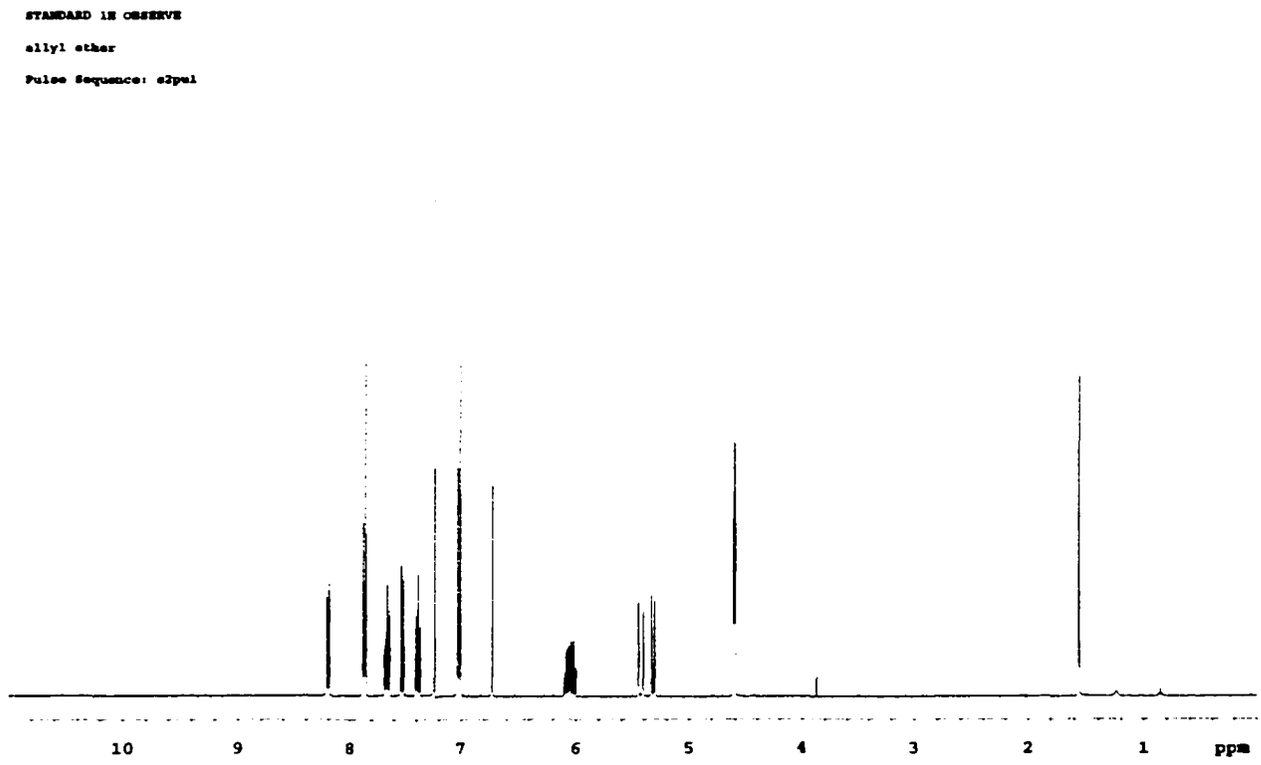

Figure 2.21. ^{1}H spectrum of 4'-allyloxyflavone (**41**).

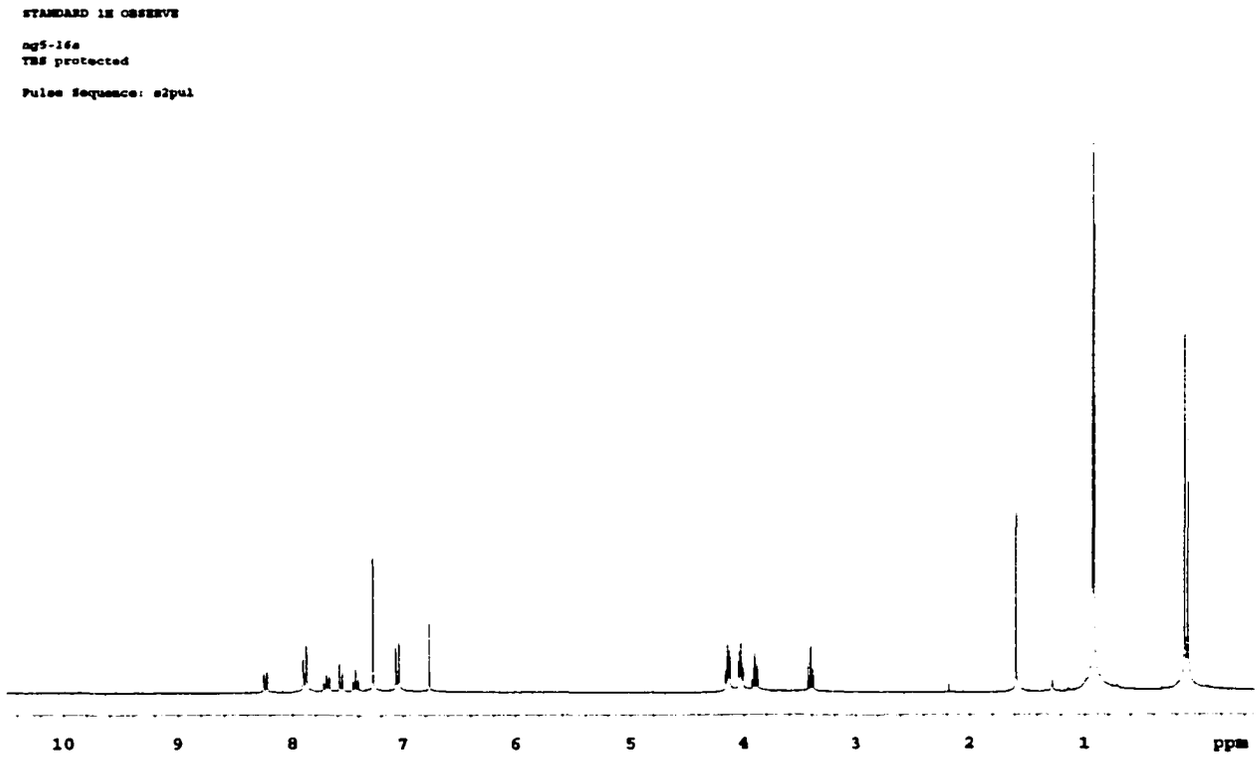

Figure 2.22. ^{1}H spectrum of 4'-(2"-*t*-butyl-dimethylsilyloxyethyl)-flavone (**42** precursor).

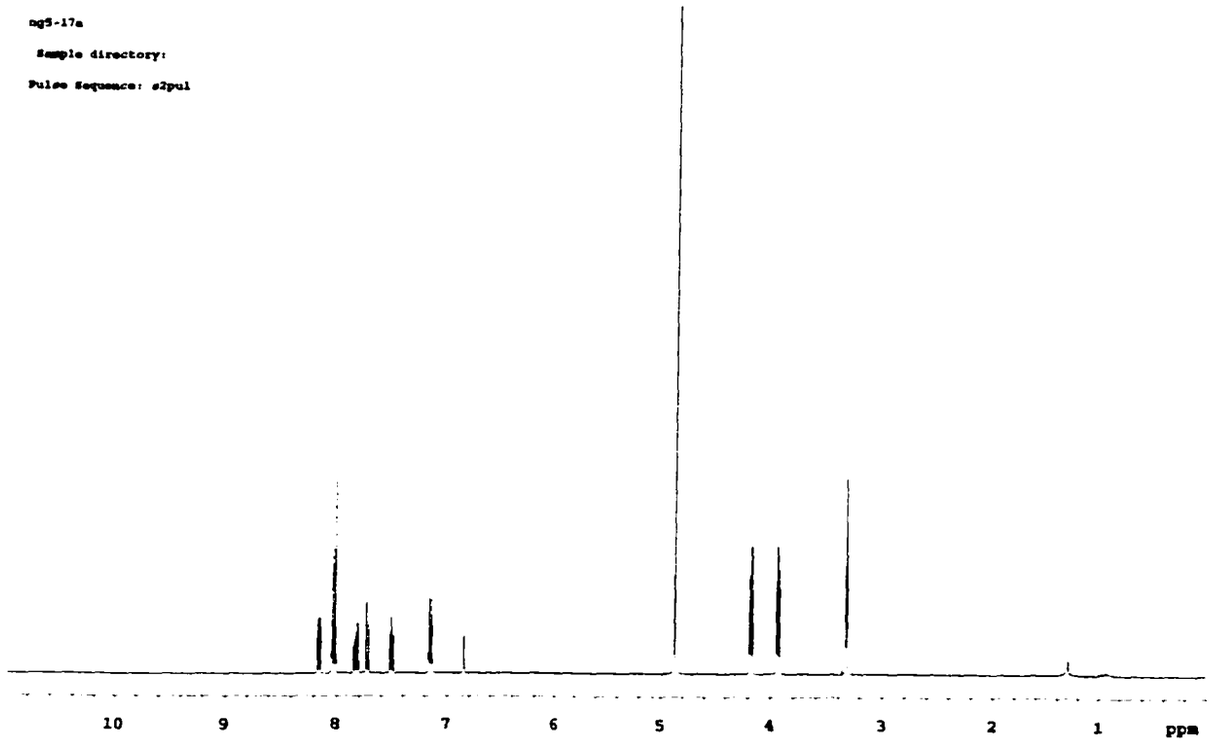

Figure 2.23. ^{1}H spectrum of 4'-(2"-hydroxyethyl)-flavone (**42**).

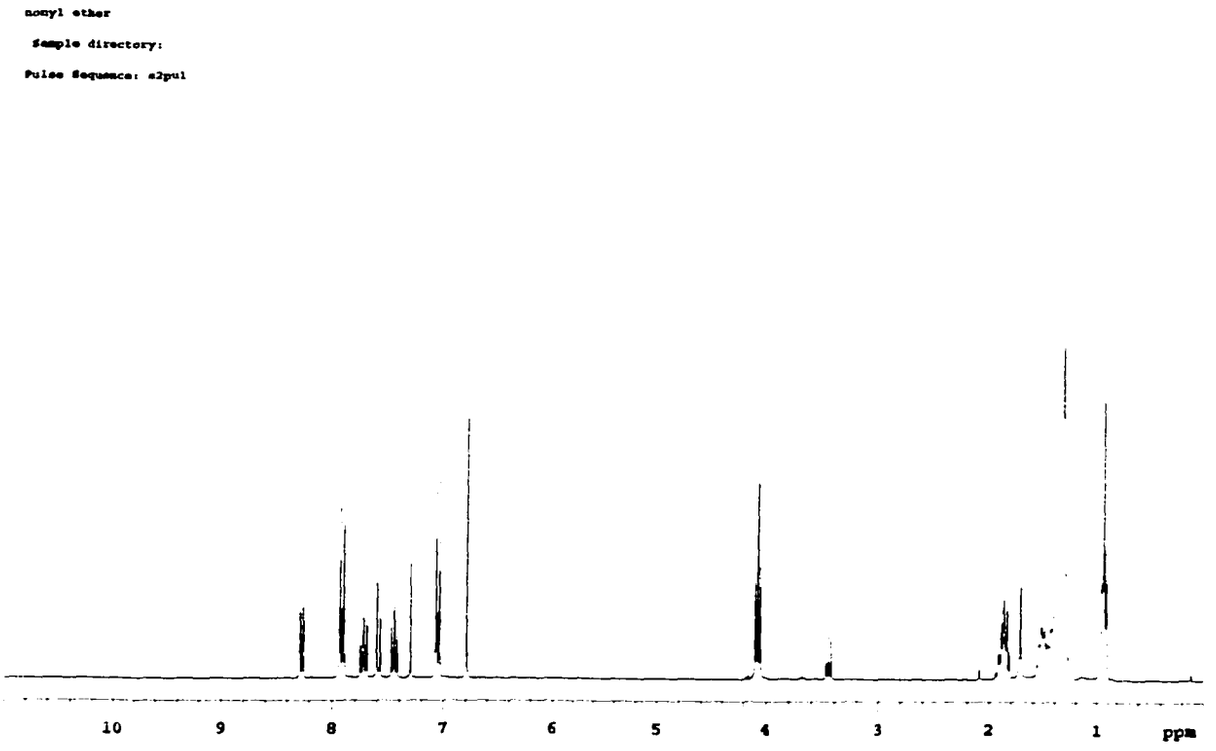

Figure 2.24. ^{1}H spectrum of 4'-nonyloxyflavone (**43**)

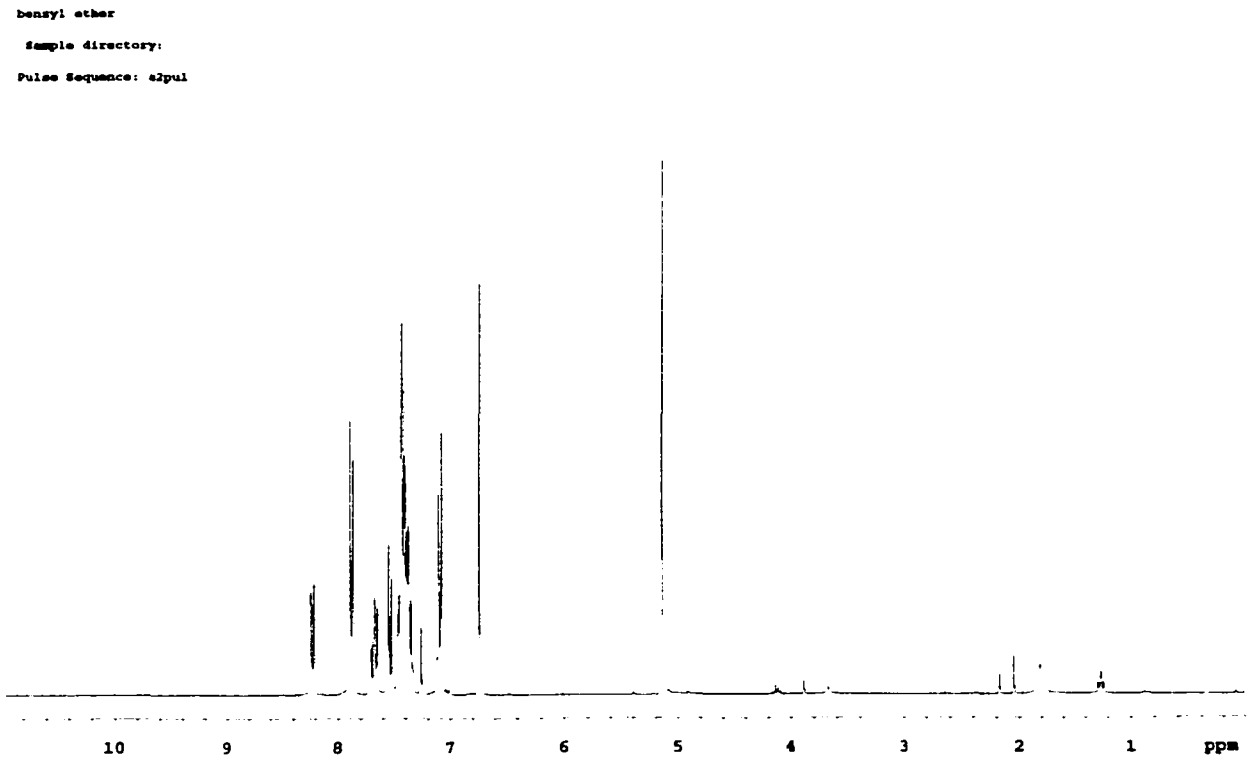

Figure 2.25. ^{1}H spectrum of 4'-benzyloxyflavone (**44**).

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Chapter 3

New Coumarins from *Harbouria trachypleura*: Isolation and Synthesis

Introduction

In contrast to single cell walled gram positive bacteria, gram-negative bacteria contain an additional outer wall membrane that provides extra protection to the cell. The outer membrane contains specialized proteins called porins that enable small hydrophilic nutrients to enter cells but restricts entry of antibiotics and cell toxins. Gram-negative bacteria have evolved a unique three-protein system to remove toxins. In *Escherichia coli*, for example, the EmrA, EmrB and TolC proteins protect the cell against uncouplers.[1] Any hydrophobic weak acid that can permeate the outer membrane in the anionic form is an uncoupler. These compounds shuttle protons across from the inner cell to the inner membrane and disrupt cell potential.[2] Extruding the uncoupler to the inner/outer membrane interlude would be counterproductive as the uncoupler can easily pass back into the cell. The EmrB pump protects the cell by extruding uncouplers via the EmrA accessory protein, a protein found in the inner/outer membrane interlude. This protein then passes the uncoupler to the TolC protein, a special porin-like outer envelope transmembrane protein, and extrudes the uncoupler.[1] A similar mechanism exists in *E.*

coli for the AcrB efflux pump, using both the AcrA and TolC proteins. The AcrB efflux pump extrudes basic dyes, mitomycin C, and tetracyline in the same manner as the EmrAB/TolC system.[3] It appears that this advanced efflux mechanism is wide spread among gram-negative bacteria and genetic homologs have been found in *Haemophilus influenzae* and *Vibrio cholerae*.[1]

In parallel with his research with *S. aureus* mutants lacking the NorA efflux pump, Lewis and coworkers are currently investigating *E. coli* mutants lacking the TolC protein.[4] Without this outer membrane protein, MDR efflux pumps that utilize the TolC protein to extrude antibiotics and toxins are rendered ineffective. Preliminary results show that the anthraquinone rhein is a potent antibiotic against *E. coli* mutants but has no activity against wild type *E. coli*. This result is excellent evidence that rhein is a substrate of EmrAB and/or AcrAB. The search for an inhibitor of these efflux pumps is currently underway. Some early results suggested that coumarins may be possible inhibitors of the EmrAR and/or AcrAB efflux pumps. *Harbouria trachypleura* (Gray) Coult. & Rose belongs to the *Apiaceae*, a family of plants known to often contain coumarins. The plant was tested with rhein and wild type *E. coli* but the bioassay was negative. In very preliminary studies, an extract *of H. trachypleura* appears to be active in an MDR reversal bioassay in *Saccharomyces* species.[4]

H. trachypleura, however, belongs to a monotypic genus and this distinction warranted its further investigation. The subject is a commonly known as whiskbroom parsley and is approximately a third to a half meter tall (Figure 3.1). This plant is found mainly on the foothills of the Colorado and Wyoming Front Range but has also been identified in Utah and northern New Mexico. Collection occurred at Horsetooth

Mountain Park, west of Fort Collins, Colorado on 5 May, 1998. A voucher specimen (#77735) is deposited in the Department of Biology, Colorado State University and identified by Jun Wen, Department of Biology.

Figure 3.1. *Harbouria trachypleura* from the Colorado Front Range. Photo courtesy of Professor Wayne H. Whaley, Utah Valley State College.

Results and Discussion

Isolation and Structure Elucidation

The air-dried aerial parts of *H. trachypleura* (185.6 g) were immersed in 1L of distilled MeOH for one day, the solvent filtered, and the process repeated one other time. A portion (9.61 g) of the total crude MeOH extract (18.10 g) was subjected to VLC (vacuum liquid chromatography, gradient hexanes to EtOAc) to yield 14 fractions. Fractions 5-7 contained coumarins as determined by TLC and ^{1}H NMR. Fraction 7 was subjected to flash column chromatography (CC, 97:3 $CH_2Cl_2/(CH_3)_2CO$) to yield 10 mg of umbelliferone (**1**). Combined fractions 5 and 6 were also subjected to CC (97:3 to 4:1 $CH_2Cl_2/(CH_3)_2CO$) to yield two fractions A (frs. 5-10) and B (frs. 11-16). Both frs. A and B were chromatographed separately on C-18 VLC (1:1 MeOH/H_2O to 100% MeOH) to provide twelve fractions each. Fr. A1 yielded 4 mg of a new coumarin, (+)-**2**. Fr. A2 yielded 7 mg of epoxysuberosin ((+)-**3**). Fr. A3 yielded a mixture of a new coumarin (**4**) and saxalin (**5**). These isolates were separated by preparative TLC using 3:2 hexanes/EtOAc to yield 3 mg of (±)-**4** and 2 mg of (+)-**5**. Fr. B1 yielded 75 mg of

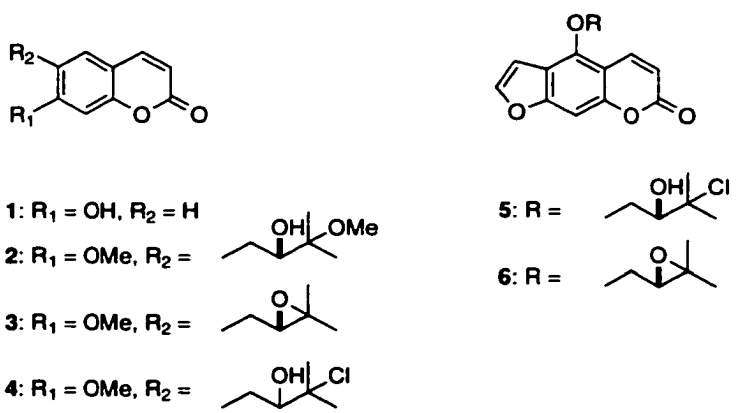
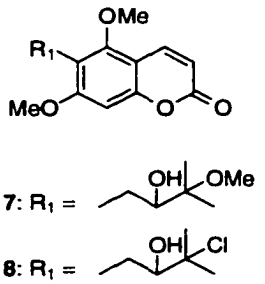


Figure 3.2. Coumarins and furanocoumarins from *Harbouria trachypleura*.

oxypeucedanin ((+)-**6**). Final isolates (**2**, **3**, and **6**) were subjected to preparative TLC (97:3 $CH_2Cl_2/(CH_3)_2CO$) to remove minor impurities (Figure 3.2).

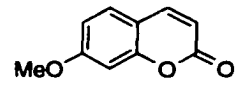
Isolates were analyzed by ^1H, ^{13}C, COSY and DEPT NMR as well as ES^- and FAB^+ mass spectrometry. Spectral data for the 7-methoxycoumarins with prenyl derived side chains are well documented in the literature. The structures for the unknown coumarins were tentatively assigned as **2** and **4** based upon data for similar compounds **7** and **8**.[5,6] Compounds **1**, **3**[7,8], **4**[9,10] and **6**[11,12] are well characterized in the literature and all spectra were in excellent agreement with previously reported isolations.



Synthesis and Structure Verification of (+)-2, (+)-3 and (±)-4

The synthesis of **2, 3** and **5** is outlined in Scheme 3.1. The commercially available 2-hydroxy-4-methoxy-benzaldehyde (**9**) was used as a starting point. A Williamson ether reaction with 4-bromo-2-methyl-2-butene afforded the phenyl prenyl ether **10** in 80% yield. Still's reagent[13] was used under thermodynamic conditions to smoothly yield the corresponding ester **11** in 96% yield and 77:23 *E/Z* selectivity. The optimization of *E/Z* selectivity was not investigated because of the subsequent cyclization reaction of **11.** This cyclization was achieved by refluxing **11** in $PhN(Et)_2$ to yield the corresponding 6-prenylated coumarin suberosin (**12**) in good yield. This reaction utilizes the well-known sequential [3,3] Claisen-Cope rearrangement of phenyl prenyl ethers.[14,15] The coumarin

12 was targeted using a similar strategy by Mali and coworkers under less efficient conditions.[16] Cairns, Harwood and Astles also synthesized **12** in their investigations of 6-substituted prenyl, geranyl, and farnesyl analogues of **1**.[17] They, however, used the coumarin herniarin (**13**) as a starting point. This sequential [3,3] Claisen-Cope rearrangement has also been utilized in the syntheses of other natural products as well.[17,18,19,20]



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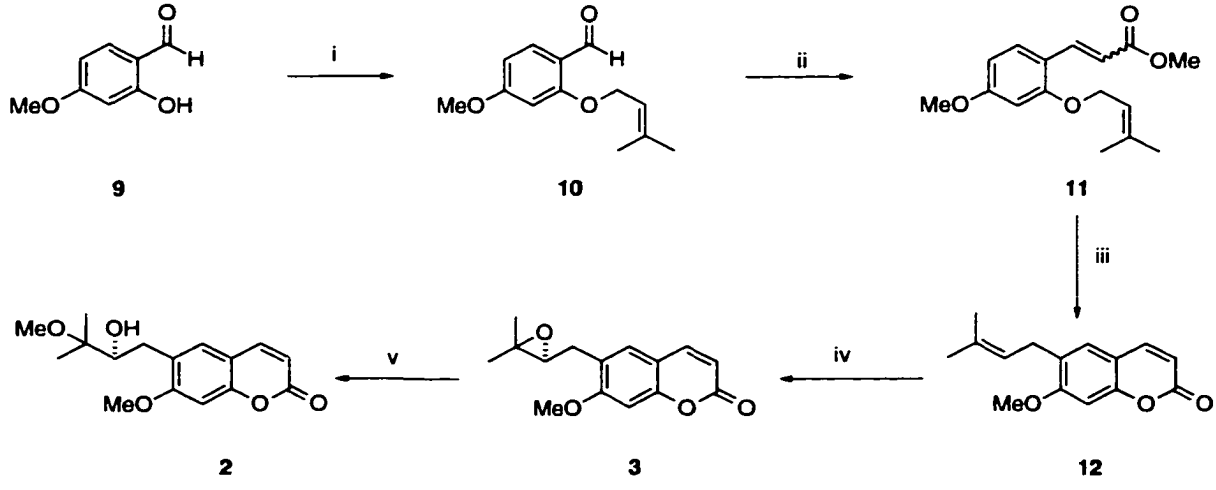


i. 4-bromo-2-methyl-2-butene, K_2CO_3, acetone, reflux, 80%; ii. $(CF_3CH_2)_2P(O)CH_2CO_2Me$, NaH, THF, - 30°C, 98% 77:23 *E/Z*; iii. N'-N'-diethylaniline, reflux, 4 hrs, 81%; Shi asymmetric epoxidation, 82% ee, 85% yield; v. TsOH, MeOH, 99%

Scheme 3.1. Synthesis of *R*-(+)-Trachypleuranin A.

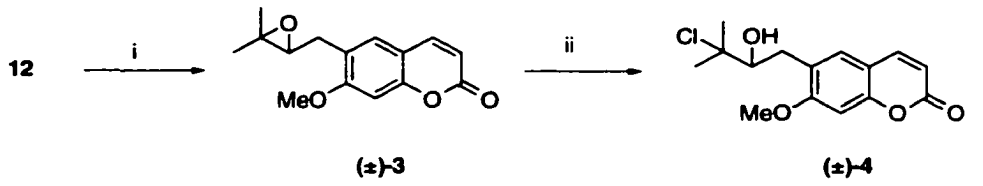
With **12** in hand, the olefin was subjected to Shi asymmetric epoxidation conditions[21] to yield the desired epoxide (*R*)-(+)-**3** in 82% yield. The ^1H and ^{13}C spectral data of the synthetic epoxide were essentially identical to those in a literature report and the authentic sample isolated from *H. trachypleura*. The optical rotation of the synthetic was +17.3 (c=0.010, $CHCl_3$). Gray reports an optical rotation of +34.3 (c=0.070, $CHCl_3$) for a sample of epoxysuberosin isolated from *Coleonema album*[8] and the isolate from *H. trachypleura* had an optical rotation of +27 (c=0.006, $CHCl_3$). While the optical rotation

of the synthetic epoxide was in fair agreement with the isolates, the determination of the isolate's absolute configuration was the desired goal of the asymmetric epoxidation. Extensive attempts to determine the % ee of the epoxidation reaction using chiral HPLC, GC and europium-based chiral NMR shift reagents failed. It was thus decided to determine the % ee of the epoxide opened final product by using the same chiral analytical techniques or the creation of a pair of diastereomers.

To synthesize the final product, the epoxide was stirred in distilled methanol and catalytic tosic acid to give (*R*)-(+)-**2** in 99% yield. The epoxide opening should occur without racemization of the secondary alcohol because of the tertiary carbocation formed as a reactive intermediate. The data for synthetic **2** were essentially the same as those of the natural product. The optical rotation of the isolate was +34 (c=0.003, $CHCl_3$) and the synthetic +27.7 (c=0.010, $CHCl_3$) thus confirming the absolute structure of the isolate as (*R*)-(+)-**2**.

The final % ee of **2** was also unobtainable after extensive attempts at chiral HPLC and GC and europium-based chiral NMR shift reagents. A Mosher ester was thus synthesized using **2**, (+)-MTPA, oxalyl chloride and catalytic DMF in dry CH_2Cl_2. The product was analyzed by C-18 HPLC (HP 1090a, Adsorbosphere C-18 5 micro, 250 mm x 4.5 mm column, 1:1 acetonitrile/water, 330 nm detection) to give an 82% ee for **2** (Figure 3.3, after experimental section).

The synthesis of (±)-**4** was elaborated through a racemic epoxidaton of suberosin with *m*CBPA in 89% yield (Scheme 3.2). The chlorohydrin (±)-**4** was synthesized using concentrated HCl in $CHCl_3$ for 30 minutes (72% yield). The ^1H and ^{13}C NMR spectral data of synthetic **4** were identical to those of the natural product.



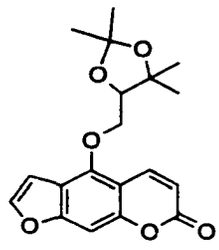
(±)-3 (±)-4

i: mCPBA, CH_2Cl_2, 30 min, rt, 89%; ii: conc. HCl, $CHCl_3$, 30 min, rt, 72%.

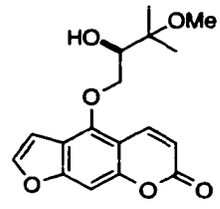
Scheme 3.2: Synthesis of (±)-trachypleuranin B

It is interesting to note that of the pro-chiral isolates (**2**, **3**, **4**, **5** and **6**), all but one (**4**) possessed optical activity. Because of the small quantity of isolated **4**, it was possible that the sample concentration of **4** was not high enough to obtain an optical rotation. To verify the presence or lack of optical rotation, a circular dichroism experiment was performed scanning from 250 to 400 nm. The spectrum obtained from this experiment showed no circular dichroism and it was thus concluded that **4** was a racemic isolate (Figure 3.4).

There has been some debate in the literature on whether epoxide-opened coumarins are actually constituents of the plant or artifacts of isolation. Large quantities of toddalolactone (**7**), an analog of **2**, were found in a Soxhlet extraction of *Toddalia asiatica* with refluxing methanol.[22] It was believed that a thermal initiated opening of 5-OMe epoxysuberosin and subsequent trapping of the epoxide-opened intermediate yielded **7**. The authors showed in a reextraction of *T. asiatica* with supercritical CO_2 that **7** was in fact a component of the plant, although smaller quantities of **7** were isolated with this extraction method. This problem was not anticipated with *H. trachypleura* because the extraction was at room temperature. Moreover, a literature report details the isolation of **14a**, the acetonide of **14**, from the acetone extracts of *Eremocitrus glauca*.[23]



14a



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It was shown experimentally that the naturally occurring citric acid in *E. glauca* catalyzed the formation of the acetonide of **14**. In the extraction of *H. trachypleura*, a similar acid catalyzed mechanism could have opened the epoxide of **3** to yield **2**. It was assumed that if this mechanism occurred with **3** it would have also occurred with the major furanocoumarin constituent of the plant (**6**) and thus **15** to be easily found in the plant extract. The furanocoumarin **15**, however, was not isolated or seen in any ^{1}H NMR spectra of the crude plant extracts. It was also possible that **2** was formed by a silica-gel catalyzed reaction with methanol, but stirring a sample of **3** with methanol and silica gel for 24 hours at room temperature gave only starting material by TLC and ^{1}H NMR. We concluded that **2** and by the same reasoning **4** are in fact constituents of *H. trachypleura* and not artifacts of isolation.

Experimental

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded at 25 °C on a Varian Inova spectrometer at 400 and 100 MHz, respectively, using $CDCl_3$ as the solvent and internal reference. Melting points were determined on a Laboratory Device's Mel-Temp and are uncorrected. All solvents were distilled prior to use. THF was freshly distilled from benzophenone-ketyl and dichloromethane was freshly distilled from CaH_2. ACS acetone was stored over 4 Å molecular sieves. All non-aqueous reactions were performed in dry glassware under an argon atmosphere. All column chromatography separations (CC) were performed with normal phase silica gel (Scientific Adsorbents

Incorporated, 32-63 μm particle size, 60 Å pore size). All starting materials were used as received from Aldrich Chemical Co. ^{1}H and ^{13}C NMR spectra of final products and selected intermediates are presented after the experimental section.

4-Methoxy-2-(3-methylbut-2-enyloxy)-benzaldehyde (10). To a 100 mL three-neck round-bottom flask was added 0.770 g (5.06 mmol) of 2-hydroxy-4-methoxy-benzaldehyde (**9**), 25 mL of dry acetone and 3.50 g (25.3 mmol) of anhydrous K_2CO_3. After stirring 5 min, 0.981 g (6.58 mmol, 0.76 mL) of prenyl bromide was syringed into the reaction flask. The reaction was heated at reflux temperature for 1.5 h, allowed to cool, and the reaction washed with brine and extracted with EtOAc. The EtOAc was dried with $MgSO_4$, filtered, and removed by rotary evaporation to yield a yellow oil that partially solidified upon standing. The crude product was subjected to CC using 4:1 hexanes/EtOAc to yield 0.983 g (80%, very pale yellow microcrystalline solid, mp=38-39 °C , lit=41-42 °C[24]) of pure **10**. ^{1}H NMR ($CDCl_3$): δ 1.76 (*s*, *gem*-Me), 1.81 (*s*, *gem*-Me), 3.87 (*s*, OMe), 4.61 (*d*, *J*=6.8 Hz), 5.50 (*br t*, *J*=6.8 Hz), 6.25 (*d*, *J*=2.0 Hz), 6.54 (*dd*, *J*= 8.4, 2.0 Hz), 7.82 (*d*, *J*= 8.4 Hz), 10.31 (*s*). ^{13}C NMR ($CDCl_3$): δ 18.3, 25.8, 55.6, 65.4, 99.0, 105.8, 118.8, 119.3, 130.3, 138.7, 163.1, 166.0, 188.5. See Figure 3.9 for ^{1}H NMR spectrum.

Methyl 4'-methoxy-2-(3-methylbut-2-enyloxy)-cinnamate (11). To a 100 mL three-neck round-bottom flask was added 0.500 g of **10** (2.27 mmol), 25 mL of dry THF and 0.983 g (2.95 mmol) of bis(2,2,2-trifluoroethyl)-(methoxycarbonylmethyl) phosphonate. The reaction was cooled to –30 °C using a Dry-Ice/acetone bath and 71 mg of NaH

(washed with hexanes prior to addition) was then added. The reaction was stirred for 1 h, quenched with ice, and washed with brine. The THF was removed by rotary evaporation and the organics extracted with EtOAc. The EtOAc was dried with $MgSO_4$, filtered and removed by rotary evaporation to yield 0.602 g (96%, clear oil) of **11**. The oil was a 77:23 mixture of *E/Z* isomers (by ^{1}H NMR) but was otherwise pure. A comparison of ^{1}H NMR resonances with a literature report proved the desired α,β unsaturated ester was formed.[25] See Figure 3.10 for ^{1}H NMR spectrum.

Suberosin (12). To a 50 mL round-bottom flask was added 0.136 g (0.492 mmol) of the cinnamate ester (**11**) and 20 mL of freshly distilled N,N-diethylaniline. The contents of the flask were heated at reflux temperature for 3 h, allowed to cool and poured into a separatory funnel with 20 mL of brine. Next, 45 mL of 1**N** HCl and 45 mL of EtOAc were added to the separatory funnel and extracted with 3 x 45 mL EtOAc. The EtOAc was dried with $MgSO_4$, filtered and removed by rotary evaporation to yield a pale yellow oil. The oil was subjected to CC using 4:1 hexanes/EtOAc to yield 0.097 g of **12** (81%, white solid, mp=86-87 °C, lit=86.5-88 °C,[25] 87-88 °C[26]). ^{1}H and ^{13}C NMR resonances were identical to those reported in the literature.[25,26]

(+)-Epoxysuberosin (3). To a 25 mL round-bottom flask was added 0.045 g (0.184 mmol) of **12**, 5 mL of buffer (0.05M $Na_2B_4O_7 \cdot 10\ H_2O$ in 4 x 10^{-4} **M** aqueous Na_2(ETDA)), 7 mL of acetonitrile, a catalytic amount (1 mg) of tetrabutylammonium hydrogen sulfate, and chiral ketone catalyst (2 mg). A solution of Oxone (0.85 g, 1.38 mmol) in aqueous Na_2(EDTA) (4 x 10^{-4} **M**, 6.5 mL) and a solution of K_2CO_3 (0.8g, 5.8

mmol) in water (6.5 mL) were added dropwise by two separate addition funnels at the rate of 1 drop/20 seconds. After 2 hours, TLC (7:3 hexanes/EtOAc) showed that the reaction was complete. The reaction was washed with brine and extracted with EtOAc. The EtOAc was dried with $MgSO_4$, filtered and removed by rotary evaporation to yield a off-white solid that was subjected to CC (7:3 hexanes/EtOAc) to yield 0.042 g of a bright white solid (88%, mp=110-111 °C). ^1H and ^{13}C NMR resonances were identical to those reported in the literature[7] and those of an authentic sample isolated from *H. trachypleura*. See Figure 3.11 for ^1H NMR spectrum.

(+)-Trachypleuranin A (2). To a 25 mL round-bottom flask was added 0.020 g of (+)-**3**, 10 mL of distilled methanol and a catalytic amount (1 mg) of tosic acid. The reaction was stirred for 10 min, washed with a saturated $NaHCO_3$ solution and the methanol removed by rotary evaporation. The organics were extracted with EtOAc and the EtOAc dried with $MgSO_4$, filtered and removed by rotary evaporation to yield a off-white solid. This solid was subjected to CC (2:3 hexanes/EtOAc) to yield 0.022 g of desired product as a bright white solid (99%, mp = 113-114 °C, $[\alpha]_D$= +27.7 c=0.010, $CHCl_3$; authentic sample mp =110-111 °C, $[\alpha]_D$= +34 c=0.003, $CHCl_3$). H^1 NMR: δ 1.22 (*s*, *gem*-Me), 1.25 (*s*, *gem*-Me), 2.48 (*bs*, OH), 2.51 (*dd*, *J*=14.0, 10.0 Hz), 2.99 (*dd*, *J*= 14.0, 1.6 Hz), 3.28 (*s*, OMe), 3.72 (*br d*, *J*=10 Hz), 3.91 (*s*, ArOMe), 6.25 (*d*, *J*=9.4 Hz), 6.81 (*s*, ArH), 7.37 (*s*, ArH), 7.64 (*d*, *J*=9.4 Hz); ^{13}C NMR δ 19.2, 20.7, 31.9, 49.2, 55.9, 76.0, 98.7, 112.0, 113.0, 125.5, 129.5, 143.6, 154.8, 160.7, 161.5; HRFAB$^+$ calc. 293.1389, found 293.1399. ^1H and ^{13}C NMR resonances were identical to those of an authentic sample isolated from *H.*

trachypleura. See Figures 3.12, 3.13, 3.14, and 3.15 for ^{1}H, ^{13}C, COSY, and DEPT NMR spectra, respectively.

(±)-Epoxysuberosin (3). To a 25 ml round-bottom flask was added 0.021 g (0.086 mmol) of **12**, 10 mL of CH_2Cl_2, and 0.030 g (approx. 1.5 eq, 85% tech grade) of *m*CPBA. The reaction was stirred for 30 min., quenched with a dilute solution of $NaHSO_3$ and $NaHCO_3$, and the organics extracted with CH_2Cl_2. The CH_2Cl_2 was dried with $MgSO_4$, filtered and removed by rotary evaporation to yield a white solid that was subjected to CC (7:3 hexanes/EtOAc) to yield a bright white solid of the desired epoxide (93%, mp =111-112 °C, lit =111.4-114.5 °C[7]). ^{1}H and ^{13}C NMR resonances were identical to those reported in the literature[7] and those of an authentic sample isolated from *H. trachypleura*.

(±)-Trachypleuranin B (4). To a 25 mL round-bottom flask was added 10 mg of (±)-**3**, 5 mL of $CHCl_3$ and 1 drop of concentrated aqueous HCl. The reaction was stirred for 30 min. and quenched with 10 mL of a saturated $NaHCO_3$ solution. The organics were extracted with $CHCl_3$, dried with $MgSO_4$, filtered, and removed by rotary evaporation to yield a white solid that was subjected to CC (7:3 hexanes/EtOAc) to yield 8.2 mg of pure **4** (72%, mp = 121-122 °C , authentic sample =118-119 °C). ^{1}H NMR δ 1.67 (*s*, *gem*-Me), 1.71 (*s*, *gem*-Me), 2.25 (*d*, *J*=5.2 Hz, OH), 2.62 (*dd*, *J*=14, 10 Hz), 3.16 (*dd*, *J*=10, 2 Hz), 3.77 (*m*), 3.92 (*s*, OMe), 6.27 (*d*, *J*=9.6 Hz), 6.83 (*s*), 7.34 (*s*), 7.64 (*dd*, *J*=9.6 Hz). ^{13}C NMR: δ27.6, 29.0, 32.6, 56.0, 75.2, 78.2, 98.9, 112.2, 113.3, 124.5, 129.7, 143.4, 154.9, 160.6, 161.3. HRFAB^{+} calc'd 297.0894, found 297.0884. ^{1}H and ^{13}C NMR resonances

were identical to those of an authentic sample isolated from *H. trachypleura*. See Figures 3.16, 3.17, and 3.18 for ^{1}H, ^{13}C, and COSY NMR spectra, respectively.

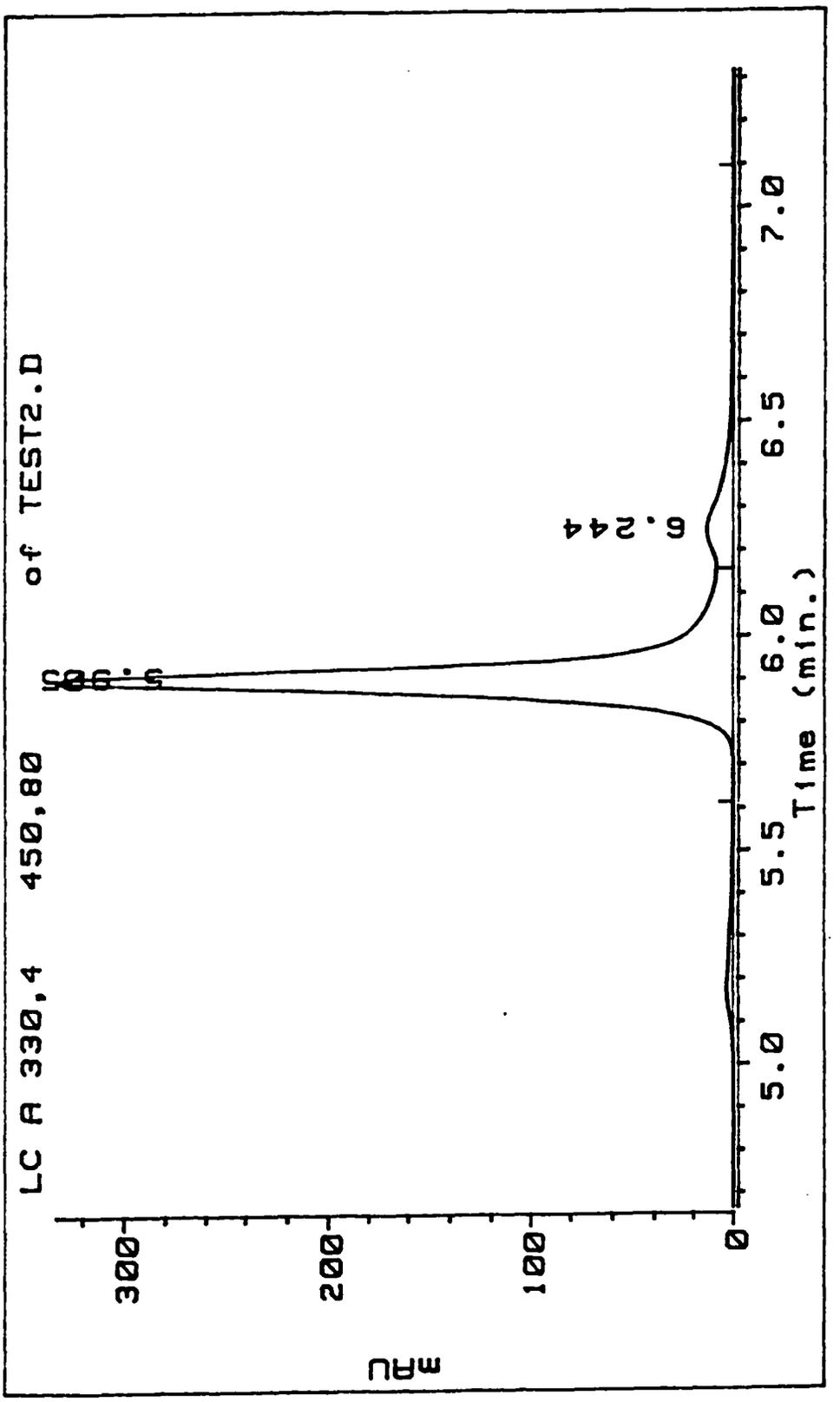

Figure 3.3 HPLC chromatogram of (+)-MTPA diastereomers of 2.

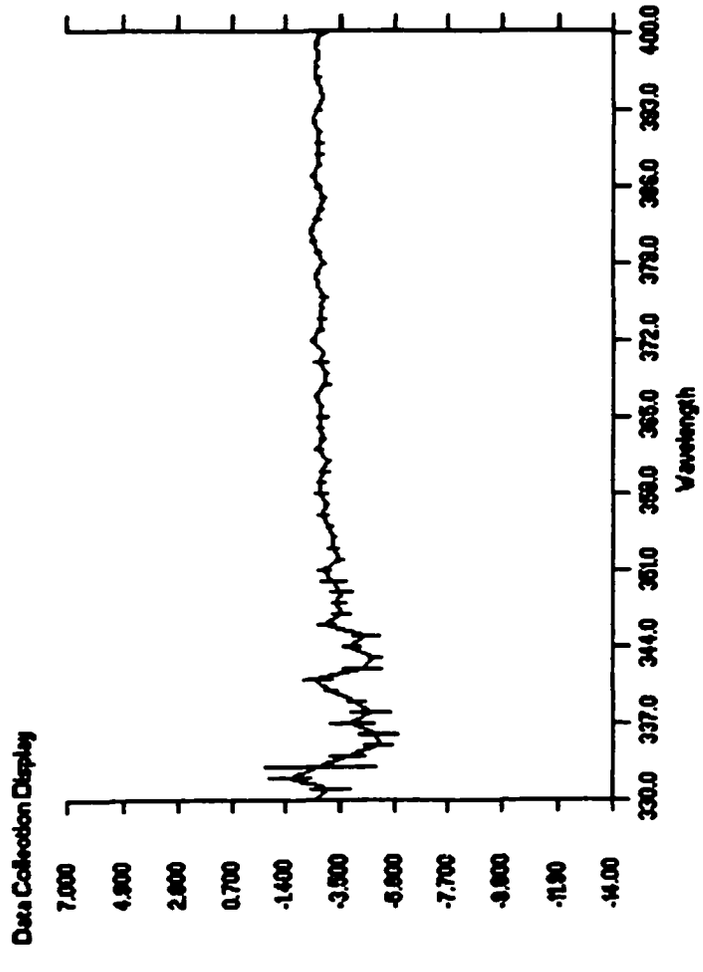

Figure 3.4. Circular dicroism spectrum (scan from 330 to 400 nm) showing the lack of optical rotation for isolate 4.

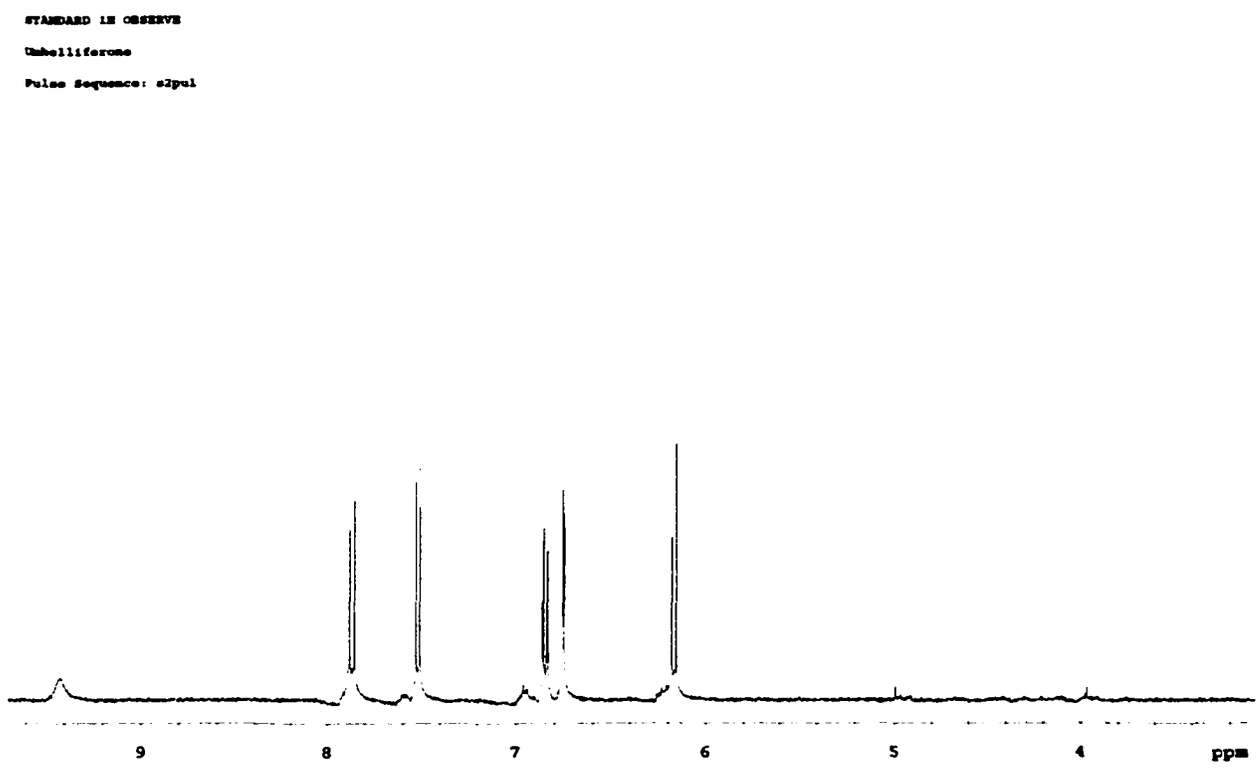

Figure 3.5. ^{1}H NMR spectrum of umbelliferone (**1**).

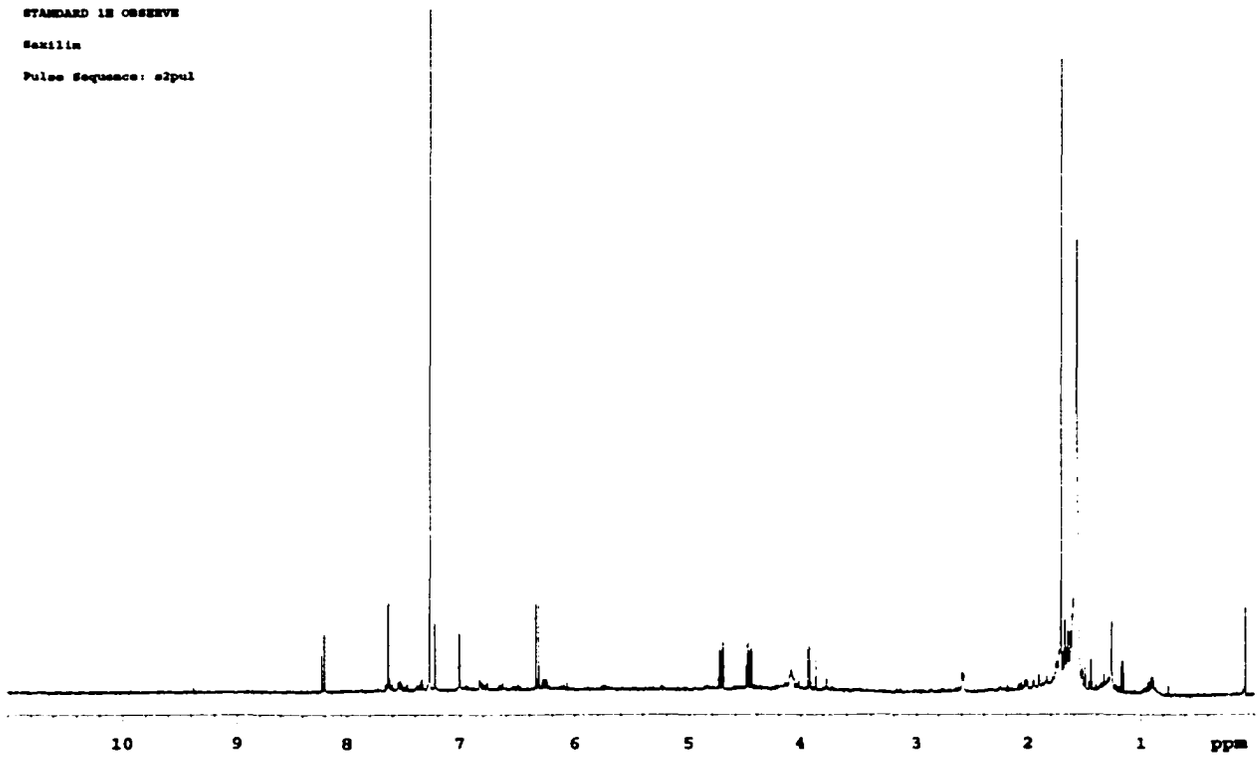

Figure 3.6. ^{1}H NMR spectrum of saxalin (**5**).

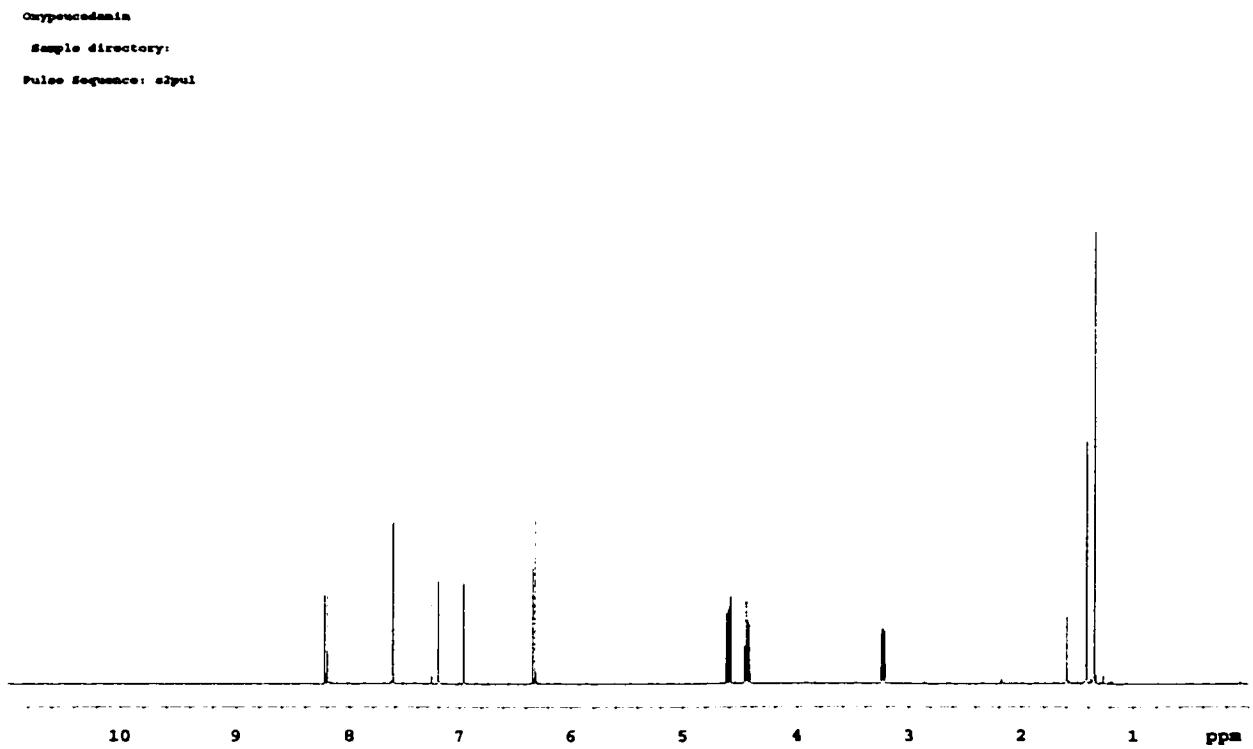

Figure 3.7. ^{1}H NMR spectrum of oxypeucedanin (**6**).

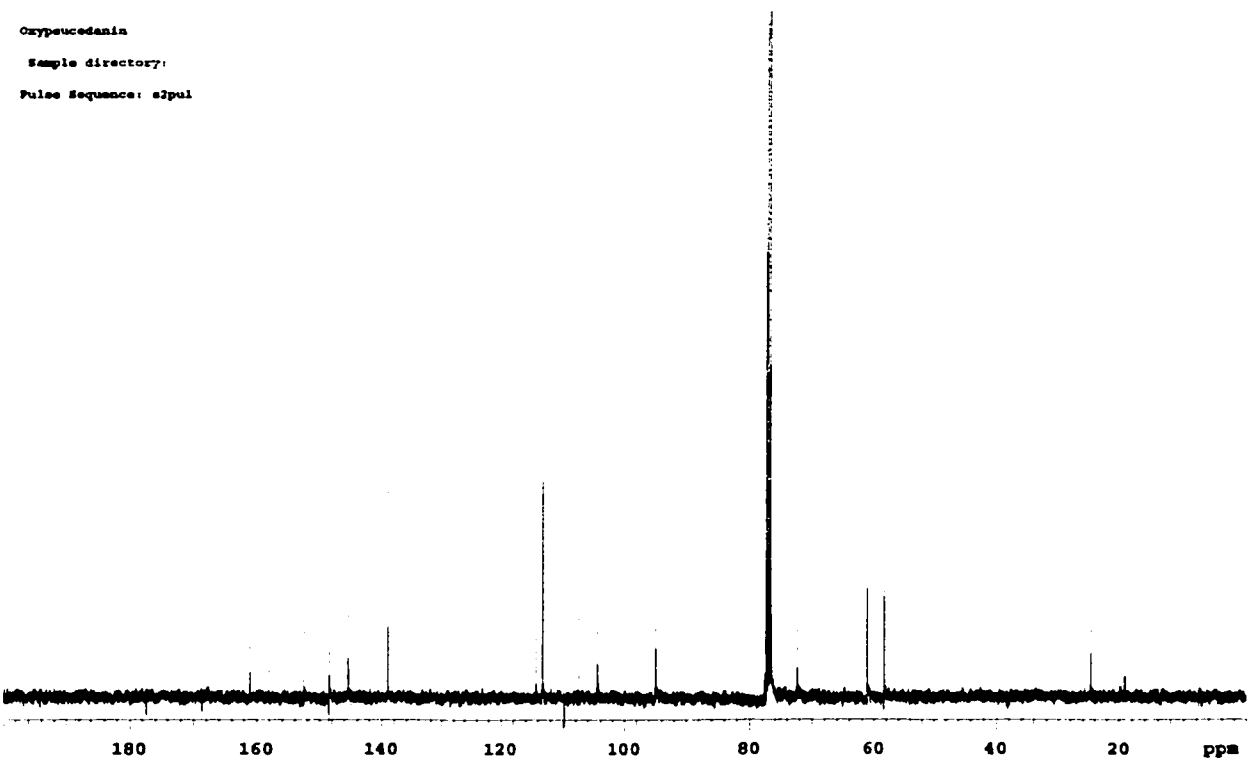

Figure 3.8. ^{13}C NMR spectrum of oxypeucedanin (**6**).

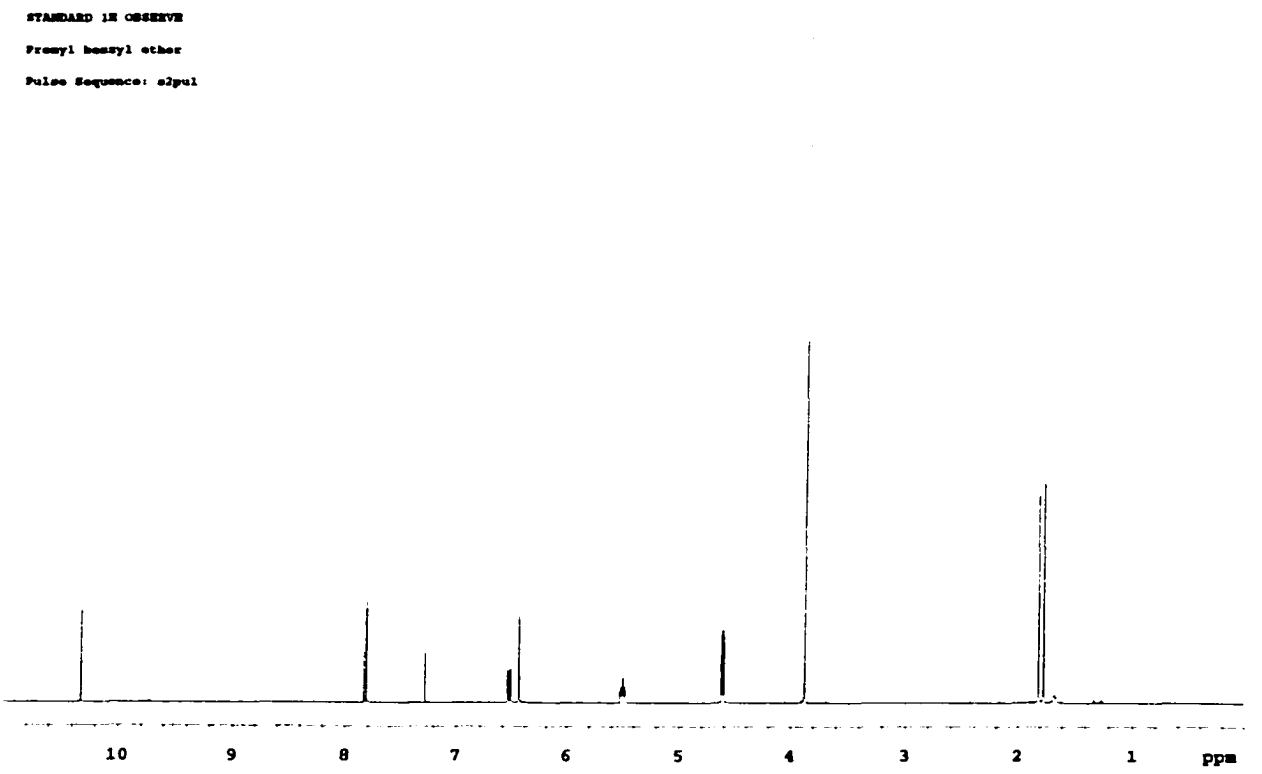

Figure 3.9. ^{1}H NMR spectrum of synthetic benzyl prenyl ether (**10**).

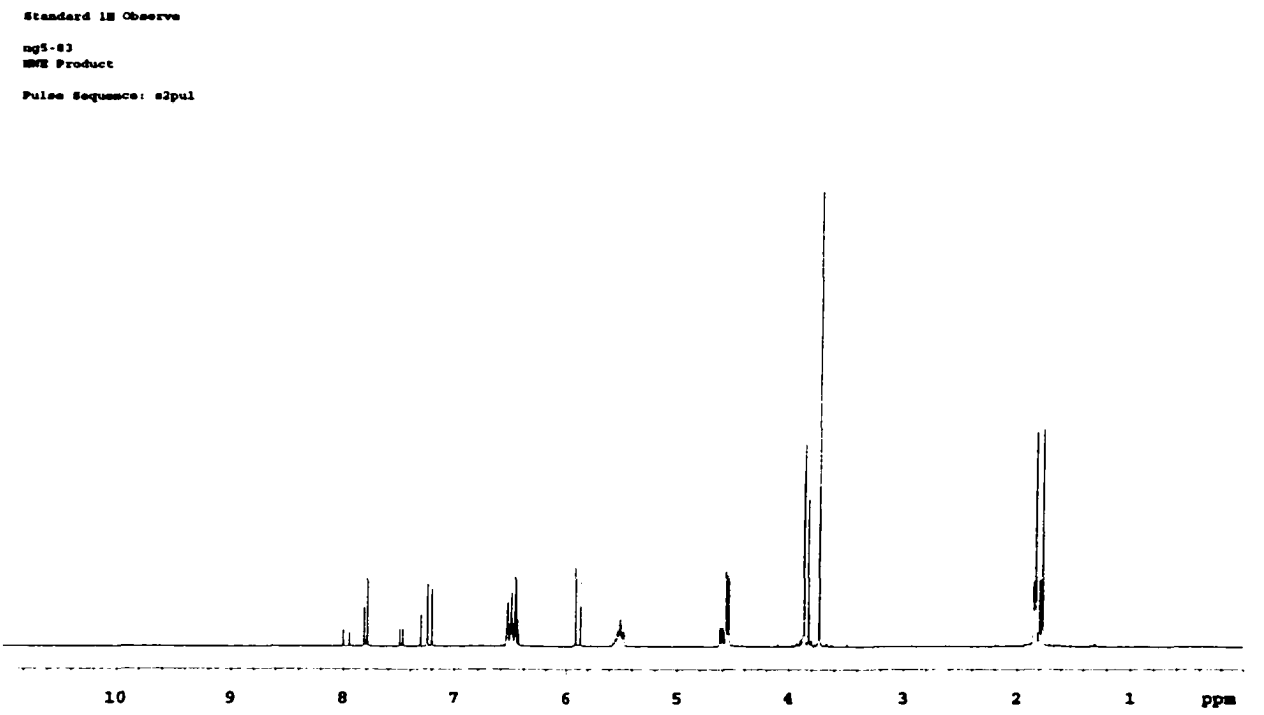

Figure 3.10. ^{1}H NMR spectrum of Horner-Wadsworth-Emmons reaction product; 77:23 *E/Z* isomers (**11**).

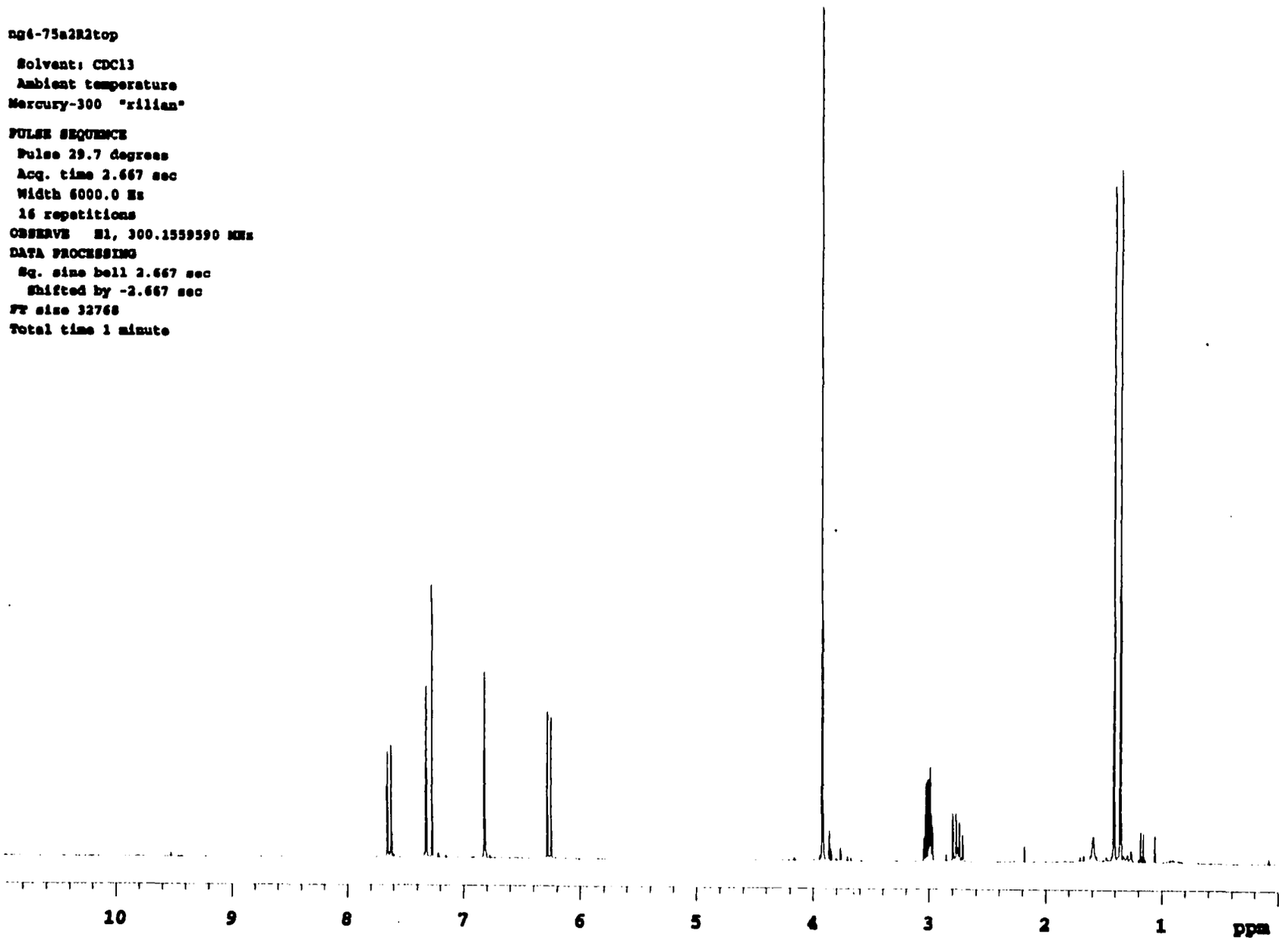

Figure 3.11. ^{1}H NMR spectrum of epoxysuberosin (3).

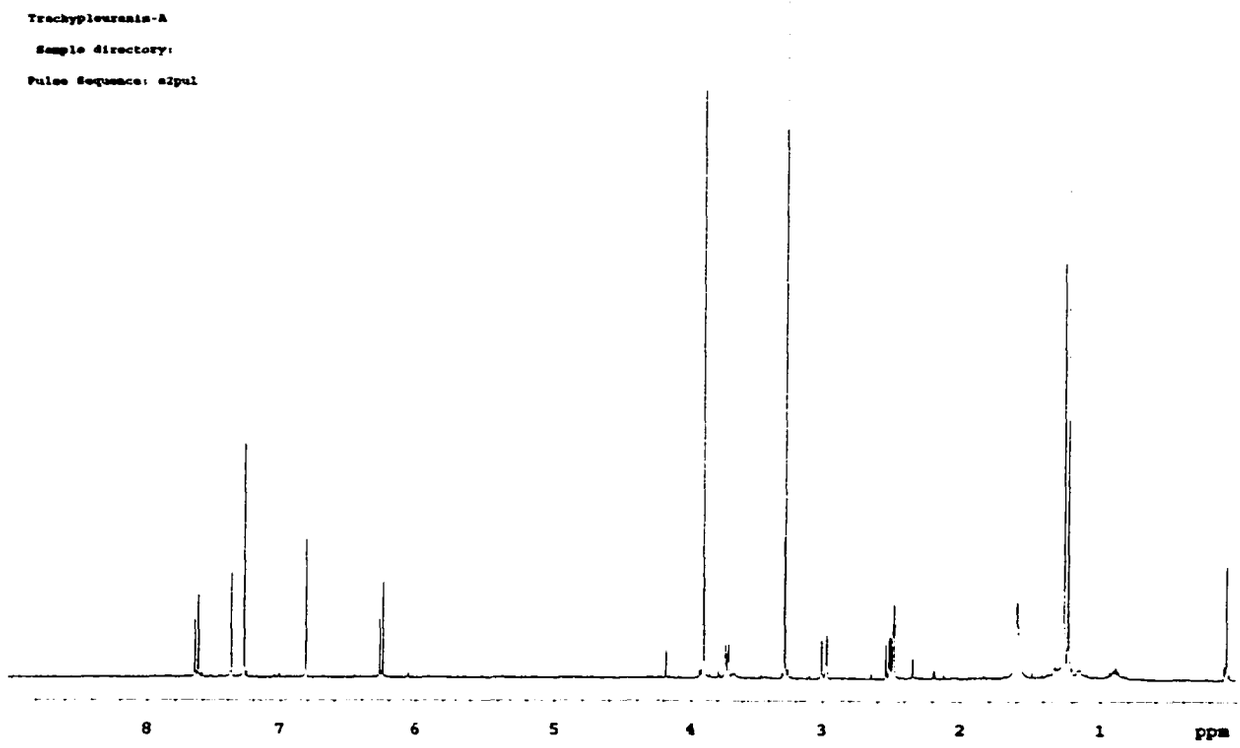

Figure 3.12. ^{1}H NMR spectrum of trachypleuranin-A (**2**).

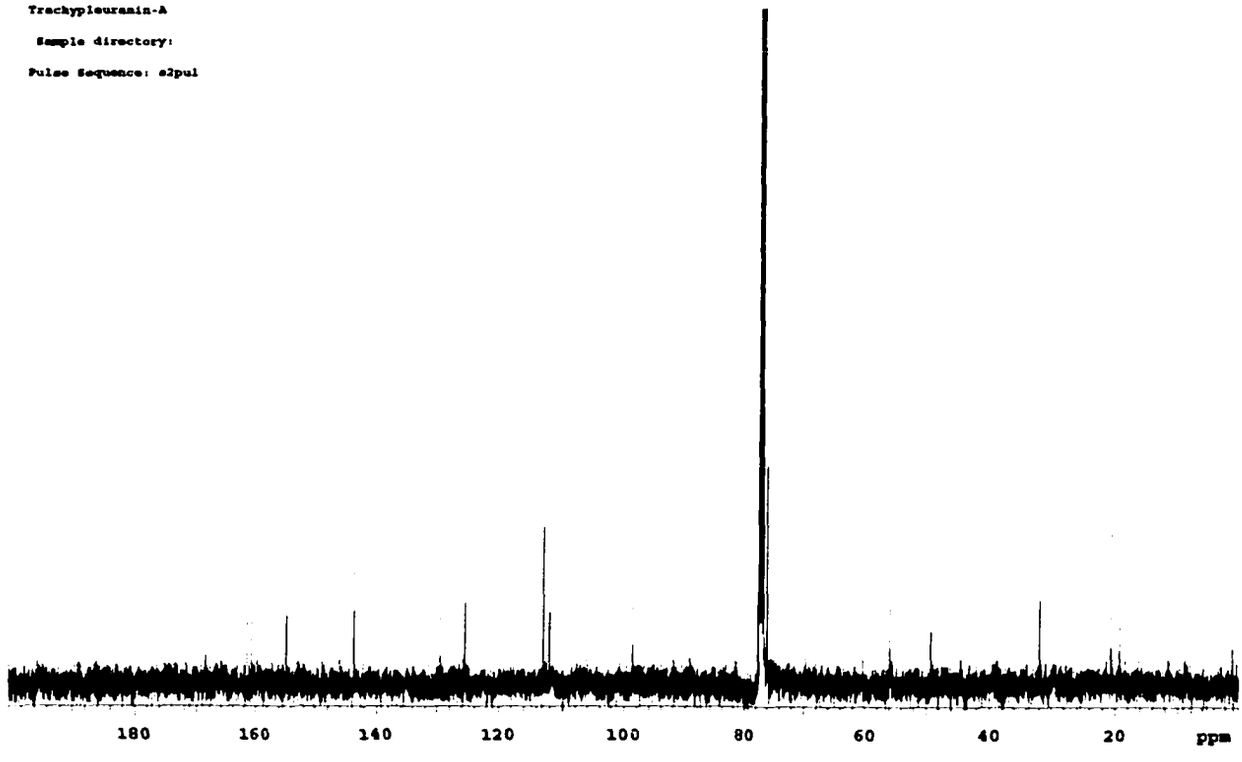

Figure 3.13. ^{13}C NMR spectrum of trachypleuranin-A (**2**).

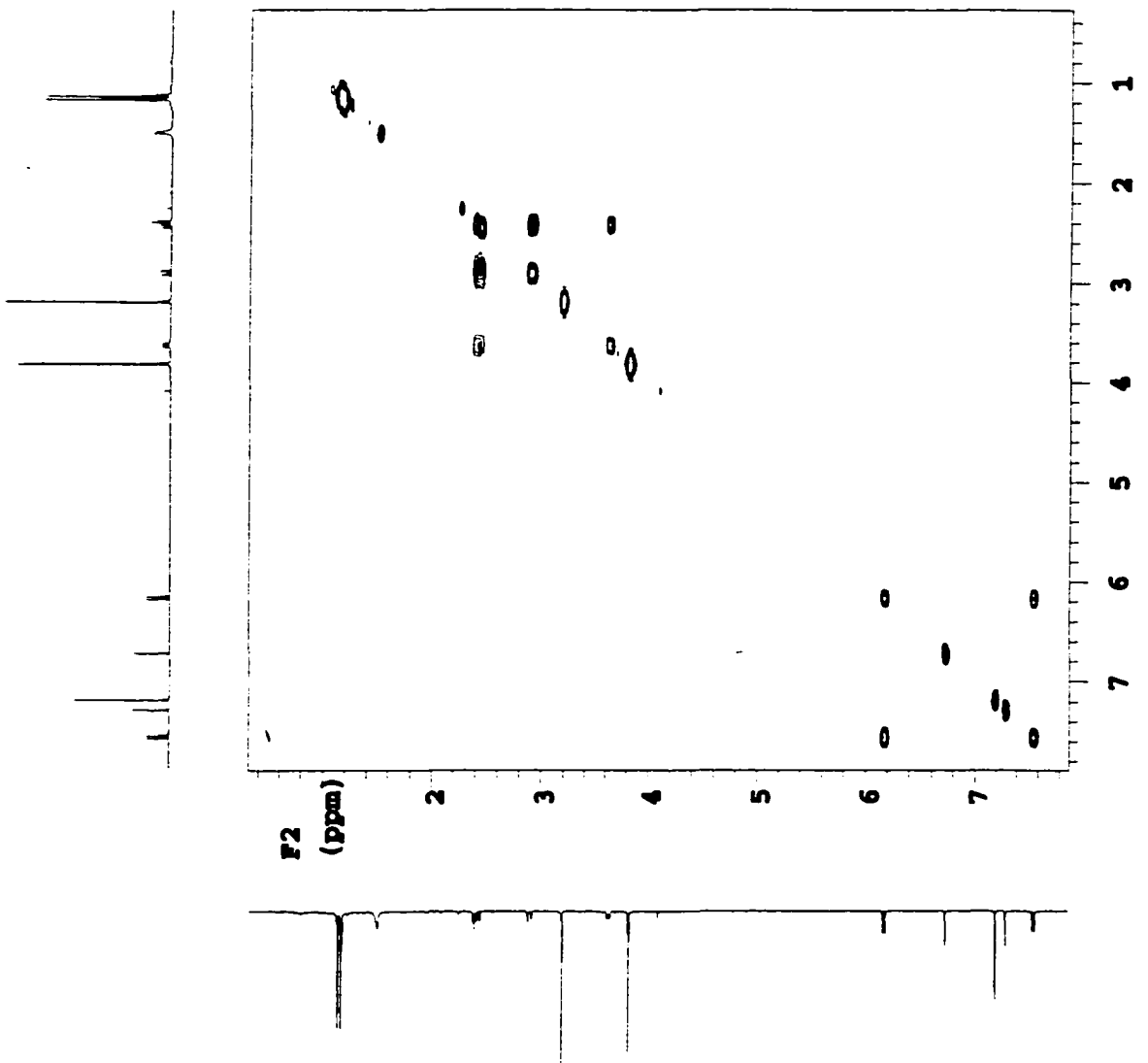

Figure 3.14. COSY NMR spectrum of trachypleuranin-A (2).

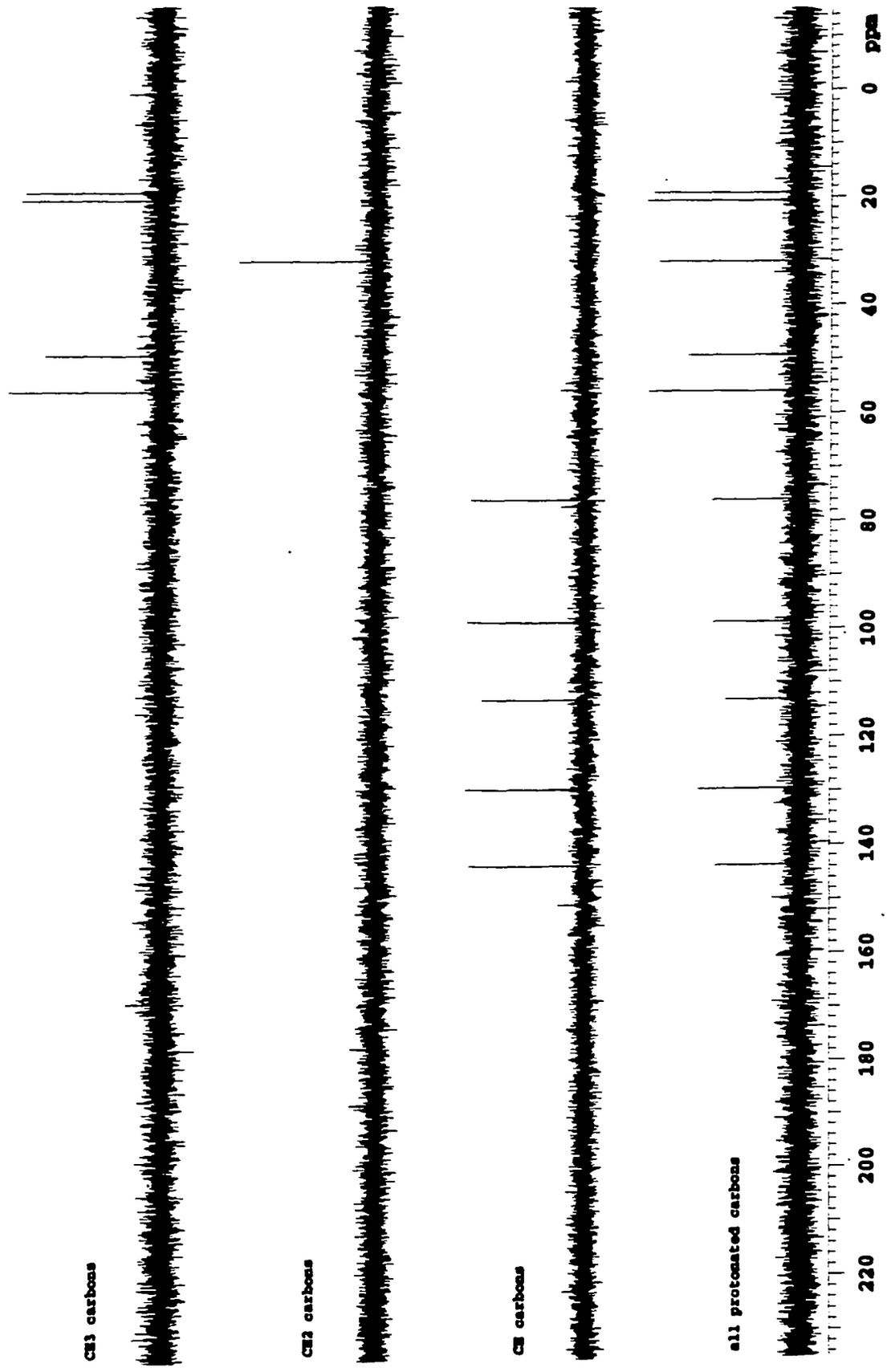

Figure 3.15. DEPT NMR spectrum of trachypleuranin-A (2).

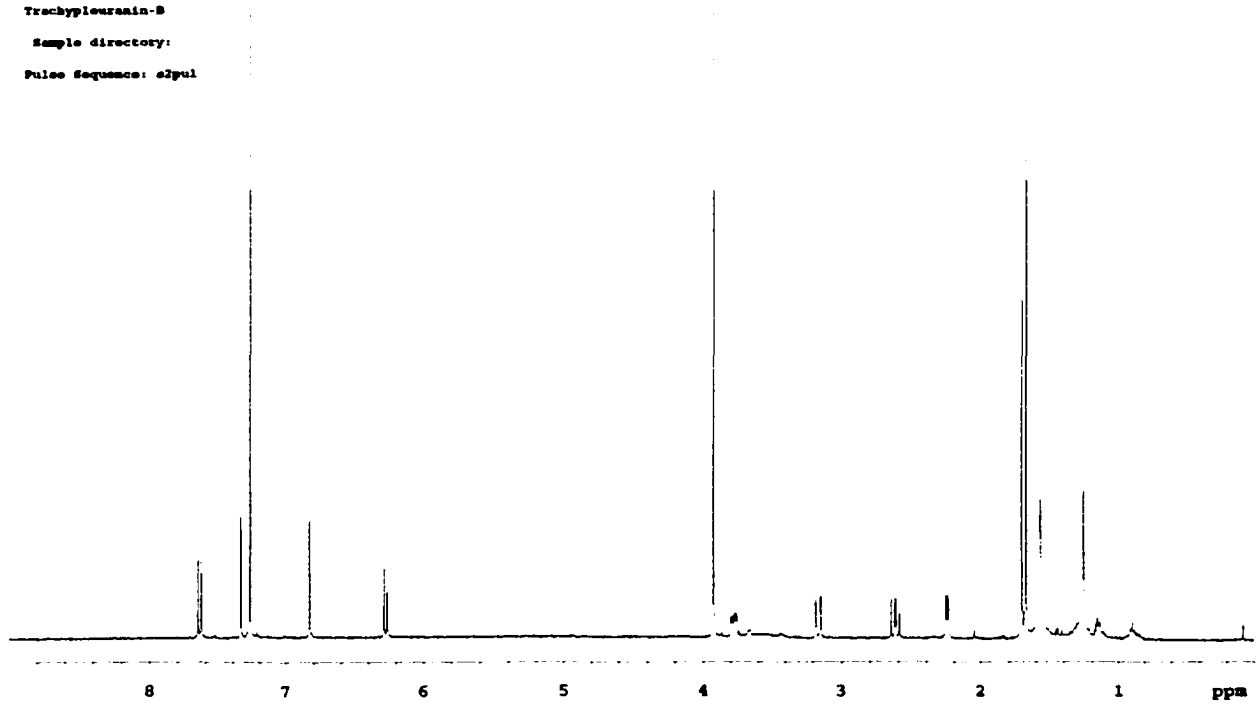

Figure 3.16. ^{1}H NMR spectrum of trachypleuranin-B (4).

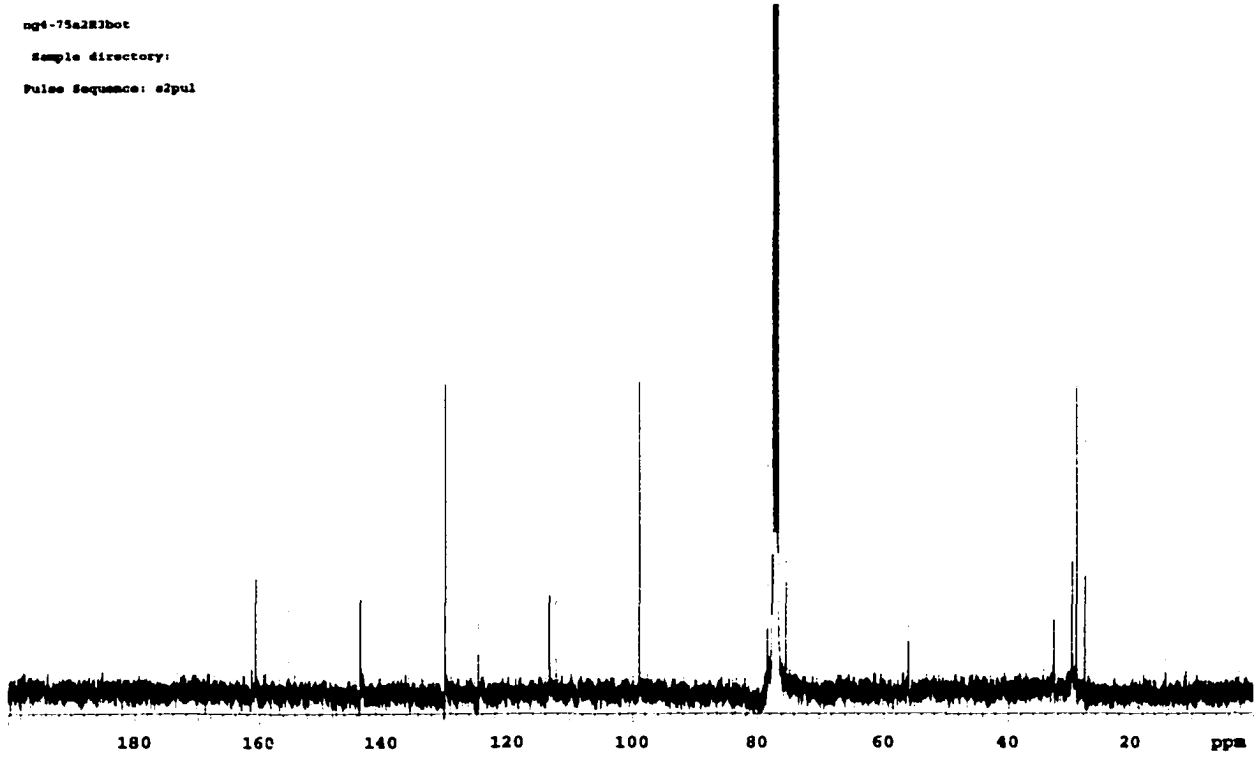

Figure 3.17. ^{13}C NMR spectrum of trachypleuranin-B (4).

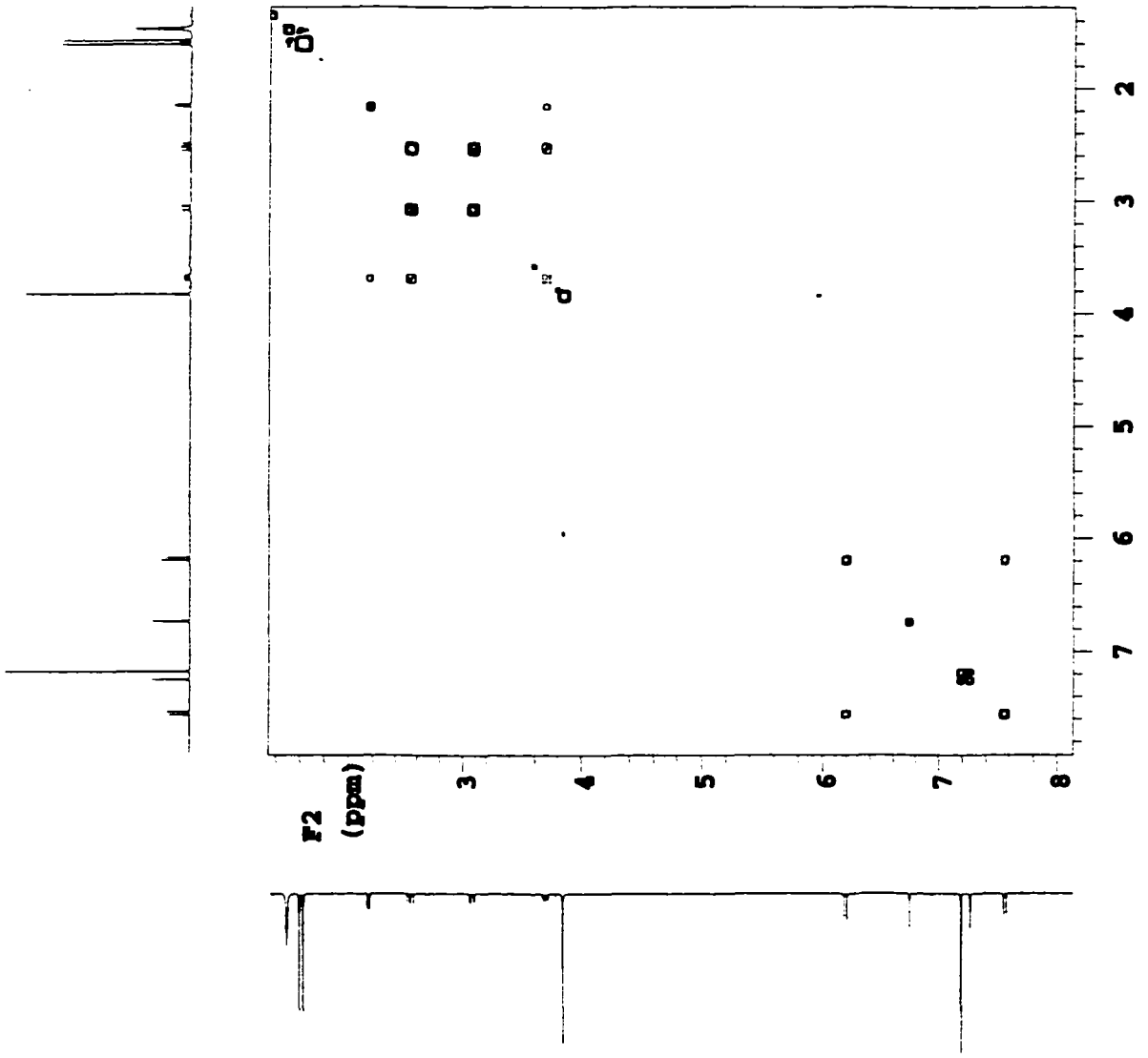

Figure 3.18. COSY NMR spectrum of trachypleuranin-B (4).

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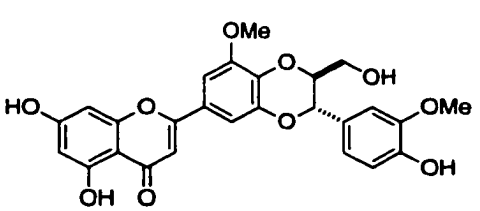
Chapter 4

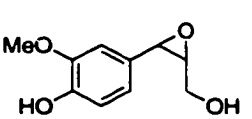
Spectral Comparisons of Coniferyl and Cinnamyl Alcohol Epoxide Derivatives with a Purported *cis*-Epoxyconiferyl Alcohol Isolate.

Introduction

Previous to the final structure identification of 5'-methoxyhydnocarpin-D (**1**), a flavonolignan isolated from *Berberis fremontii*, it was believed that the isolate included an epoxide functional group. The isolate contained both ^{1}H and ^{13}C NMR resonances that typified those of an epoxide.[1] To verify the presence of an epoxide, samples of epoxyconiferyl or epoxycinnamyl alcohols were required for NMR spectral comparisons. There were no literature reports of epoxyconiferyl alcohols as synthetics and only a single isolation reference to *cis*-epoxyconiferyl alcohol (**2**) as an isolate from the bark of *Fraxinus oxycarpa*.[2] Examination of the reported NMR data for this *F. oxycarpa* isolate, however, did not seem consistent with literature data for some epoxycinnamyl alcohols. The synthesis of some epoxy derivatives was therefore attempted which, along with literature data, might confirm or negate the isolation report.



1



2

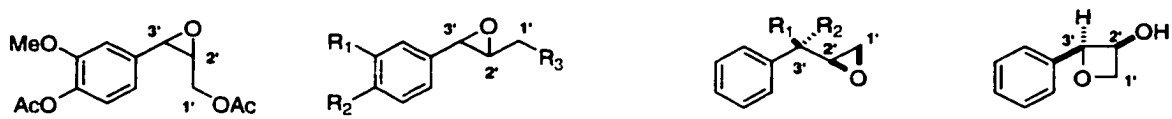
Figure 4.1. *Fraxinus oxycarpa*, commonly known as Raywood or claret ash.

Results and Discussion

Epoxidations of *cis*- and *trans*-coniferyl alcohols were undertaken with *m*-chloroperoxybenzoic acid (*m*CPBA), transition metal catalyzed reactions,[3] *in situ* generation of dimethyldioxirane (DDO),[4] and dilute DDO solutions.[5] Arsonium salt mediated condensations/epoxidations with 4-benzyl-3-methoxy-benzaldehyde were also tried[6] as well as epoxidations of coniferyl alcohol with dilute DDO in a NMR tube with dry, alumina washed CD_2Cl_2. A ring opened condensation of coniferyl alcohol and *meta*-chlorobenzoic acid resulted from epoxidation attempts with *m*CPBA, a result similar to that of Koerner *et al.* with *anti*-sesquinorbornene and *m*CPBA.[7] The other methods also failed to provide the desired epoxide and a multitude of unidentified products resulted

from these attempts. The first four described methods, however, were successful for the preparation of *trans*-epoxycinnamyl alcohol and the diacetates of *cis*- and *trans*-epoxyconiferyl alcohols. Attempts to epoxidize *cis*- or *trans*-coniferyl alcohols and then generate the acetate *in situ* failed, but subjecting the prior acetylated coniferyls to epoxidation produced the desired acetate derived epoxyconiferyl alcohols. Deacetylations of the epoxyconiferyl alcohol acetates were unsuccessful and again a multitude of unidentified products resulted from this endeavor. It is felt that the para phenolic OH on **2** creates a labile benzylic position that makes epoxidation extremely difficult if not impossible.

The NMR spectral data (Table 4.1) for the prepared epoxides, in comparison with the *Fraxinus oxycarpa* isolate NMR spectral data,[2] clearly indicate that the isolate cannot be *cis*-epoxyconiferyl alcohol. Data from the literature for a few isomeric epoxides (**3-8**)



3

4: R_1 = OMe, R_2 = R_3 = OAc
5: R_1 = R_2 = H, R_3 = OH
6: R_1= OMe, R_2 = H, R_3 = OH

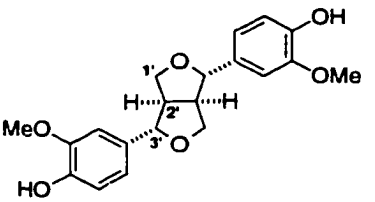
7: R_1 = H, R_2 = OH
8: R_1 = OH, R_2 = H

9

Figure 4.2. Structures of isomeric epoxides and a oxetanol compared to the purported isolate (**2**).

and an oxetanol (**9**) which might be considered structures for the isolate (Figure 4.2 and Table 4.1) were in some cases partially closer to the reported data for **2**, but still not within good agreement. When the data for this report were submitted to the literature, a reviewer suggested that the purported isolate's NMR spectral data be compared with that of pinoresinol, a coniferyl alcohol dimer.[8] A sample of (±)-pinoresinol (**10**) was prepared by a procedure described by Quideau and Ralph[9] and compared to the ^{1}H and ^{13}C NMR

data of the purported epoxide (Table 1). These data are essentially identical as were the IR and UV peaks for **2** and **10**.[10] An optical rotation of +51.65 ($CHCl_3$) was reported for **2** and +84.0 (acetone) for **10**.[10] The 70eV mass spectrum for **2** was reported as (*m/z*, rel. int.) 196(8), 165(8), 151(100), 137(38). The direct probe EI mass spectrum of our synthetic sample **10** showed a strong M^+ at 358(70), no 196, but 163(38), 151(100), and 137(72). Thus, there is an optical rotation difference between the purported **2** and **10**, which could be accounted for by the solvent difference, but if the isolate **2** was really **10**, the *m/z* 358 peak should have been easily observable. It is clear from the NMR data that **2** cannot be the purported isolate, and its positive identification as **10** was somewhat clouded by the nonidentity of mass spectra, at least as reported for **2**. We did not receive an answer upon request for a standard sample of **2.** A communication was received from the author of the isolation report[2] after the above data were published,[8] and the identity of the purported isolate was confirmed as **10**.[11]



10

Table 4.1. Comparison of ^{1}H and ^{13}C NMR spectra between **2**, synthetics, and literature data. All couplings are measured in Hertz. Side chains are denoted C-1', etc. to remain consistent with the Kostova *et. al* publication.

Position: Compound	1'		2'		3'	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
2	71.7	3.88 (*dd*) *J*=9.0, 3.7 4.25 (*dd*) *J*=9.0, 7.0	54.2	3.10 (*m*)	85.9	4.74 (*d*) *J*=4.2
3[12]	62.2	3.85 (*m*) 4.09 (*dd*) *J*=12.4, 4.0	55.9	3.45 (*m*)	56.1	4.15 (*d*) *J*=4.0
4[12]	63.9	4.10 (*dd*) *J*=12.2, 5.7 4.45 (*dd*) *J*=12.3, 3.3	56.2	3.20 (*m*)	59.3	3.81 (*m*)
5[12,13]	62.5	3.81 (*dd*) *J*=13, 5 4.18 (*dd*) *J*=13, 3	55.6	3.25-3.30 (*m*)	61.2	3.95 (*d*) *J*=3
6[14]	62.3	3.80 (*ddd*) *J*=12.8, 7.9, 3.8 4.07 (*ddd*) *J*=12.8, 5.2, 2.3	55.6	3.21 (*ddd*) *J*=3.8, 2.3, 2.1	61.3	3.92 (*d*) *J*=2.1
7[15]	43.6	4.85 (*d*) *J*=2.9	55.0	3.16-3.19 (*m*)	70.8	2.72 (*dd*) *J*=5.4, 3.9 2.92 (*dd*) *J*=5.4, 2.9
8[15]	45.3	4.40 (*t*) *J*=4.9	56.0	3.16-3.19 (*m*)	74.5	2.76 (*dd*) *J*=4.9, 2.9 2.77-2.81 (*m*)
9[16]	79.3	4.40 (*m*) 4.8 (*m*)	67.5	4.8 (*m*)	88.6	5.77 (*d*) *J*=5.6
10[12]	71.7	3.88 (*dd*) *J*=9.0, 3.2 4.25 (*dd*) *J*=9.0, 6.8	54.2	3.10 (*m*)	85.9	4.74 (*d*) *J*=4.4

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded at 25 °C on a Varian Inova spectrometer at 300 and 75 MHz, respectively, using $CDCl_3$ as the solvent and internal reference unless otherwise noted. All solvents were distilled prior to use. Dichloromethane was freshly distilled from CaH_2. ACS acetone was stored over 4 Å molecular sieves. All reactions were performed in dry glassware under an argon atmosphere. All column chromatography separations (CC) were performed with normal phase silica gel (Scientific Adsorbents Incorporated, 32-63 μm particle size, 60 Å pore size). *trans*-Coniferyl alcohol was purchased from Aldrich Chemical Company and *cis*-coniferyl alcohol was prepared according to a previously published procedure.[17] The diacetates of coniferyl alcohol were prepared using standard pyridine/acetic anhydride conditions. Acetylation of *cis*- and *trans*-coniferyl alcohols produced a clear oil that was

used directly in the epoxidations. Epoxidation with the neutral species DDO[5] (approximately 0.06 **M** in acetone), was performed by adding an equivalent of DDO to an acetone solution of the coniferyl diacetates or cinnamyl alcohol. An equivalent of DDO was added every fifteen minutes for 1.5 hours. In each case, removal of the solvent *in vacuo* gave pure epoxides as clear oils in excellent yield (>95%). The diacetate coniferyl epoxides rapidly degraded when not stored in solution ($CHCl_3$ for these experiments).

***cis*-Epoxyconiferyl alcohol diacetate (3).** ^{1}H NMR: δ 2.08 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 3.47 (m, 1H, CH), 3.85 (s, 3H, OCH_3), 3.87 (m, 1H, CH), 4.09 (dd, 2H, J = 12.0, 4.0 Hz, CH_2), 4.17, (d, 1H, J = 4.0 Hz, CH), 6.92 (d, 1H, J = 2.0 Hz, CH), 6.95 (dd, 1H, J = 12.0, 2.0 Hz, CH), 7.03 (d, 1H, J = 8.0 Hz, CH). ^{13}C NMR: δ 20.55 (CH_3), 20.65 (CH_3), 55.85 (OCH_3), 55.91 (CH), 56.12 (CH), 62.22 (CH_2), 110.09 (CH), 118.37 (CH), 122.72 (CH), 132.94 (C), 139.33 (C), 151.01 (CH), 168.83 (C=O), 170.65, (C=O). HRFAB MS^+: calc'd 281.1020; found 281.1017. See Figure 4.3 for ^{1}H NMR spectrum.

***trans*-Epoxyconiferyl alcohol diacetate (4).** ^{1}H NMR: δ 2.16 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 3.20 (m, 1H, CH), 3.81 (m, 1H, CH), 3.82 (s, 3H, OCH_3), 4.10 (dd, 1H, CH, J = 12.2, 5.7 Hz), 4.45 (dd, 1H, CH, J = 12.3, 3.3 Hz), 6.84 (d, 1H, CH, J = 1.8 Hz), 6.89 (dd, 1H, CH, J = 8.1, 1.8 Hz), 7.01, (d, 1H, CH, J = 8.1 Hz). ^{13}C NMR: δ 20.57 (CH_3), 20.70 (CH_3), 55.83 (OCH_3), 56.19 (CH), 59.31 (CH), 63.95 (CH_2), 108.94 (CH), 118.16 (CH), 122.80 (CH), 135.19 (C), 139.71 (C), 151.33 (C), 168.96 (C=O), 170.61 (C=O). HRFAB$^+$ MS: calc'd 281.1020; found 281.1020. See Figure 4.4 for ^{1}H NMR spectrum.

(±)-Pinoresinol (10). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.10 (m, 2H, CH), 3.88 (dd, 2H, CH, J = 9.2, 3.2 Hz), 3.91 (s, 6H, OCH_3), 4.25 (dd, 2H, CH, J = 9.0, 6.8), 4.74 (d, 2H, CH, J = 4.4 Hz), 5.59 (bs, 2H, OH), 6.82 (dd, 2H, CH, J = 8.0, 2.0), 6.88 (d, 2H, CH, J = 8.0), 6.90 (d, 2H, J = 1.6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 54.17 (CH), 55.95 (OCH_3), 71.67 (CH_2), 85.87 (CH), 108.58 (CH), 114.26 (CH), 118.96 (CH), 132.94 (C), 145.24 (C), 146.69 (C). MS EI^+ (direct probe, m/z rel. int.): 358(70), 327(11), 235(15), 205(30), 163(38), 151(100), 137(72). See Figures 4.5 and 4.6 for ^{1}H and ^{13}C NMR spectra, respectively.

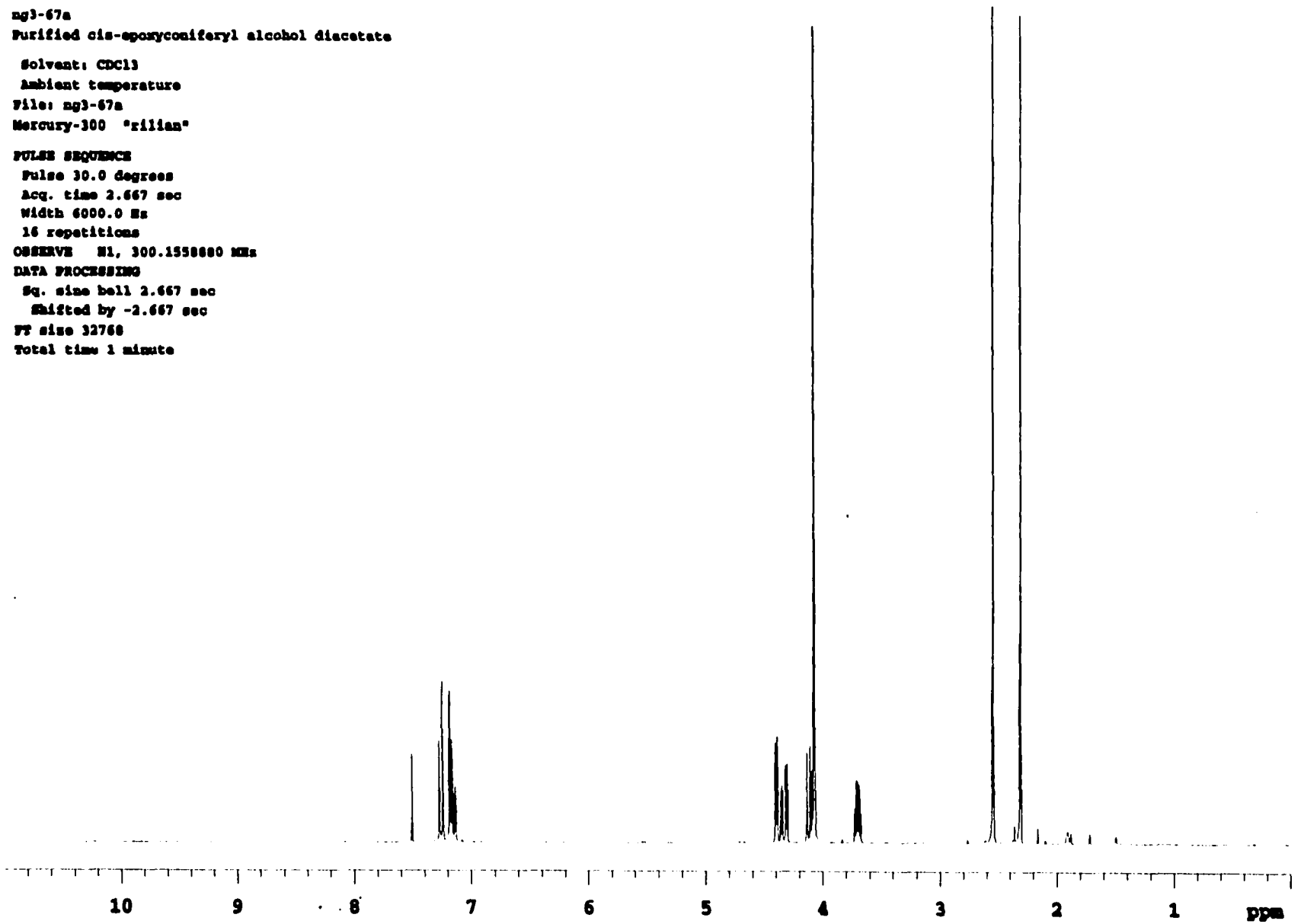


Figure 4.3. ^{1}H NMR spectrum of *cis*-epoxyconiferyl alcohol diacetate (**3**).

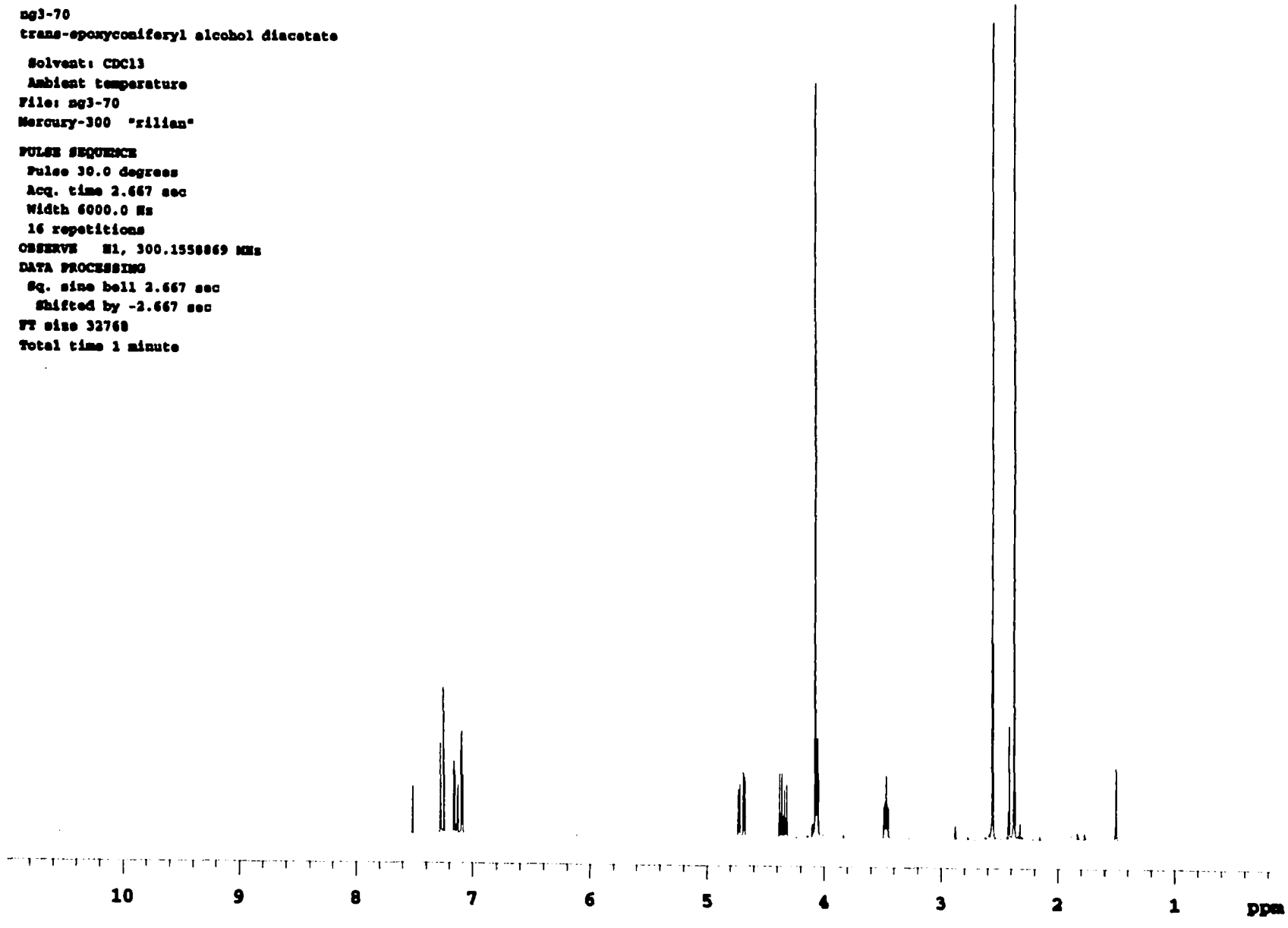


Figure 4.4. ^{1}H NMR spectrum of *trans*-epoxyconiferyl alcohol diacetate (**4**).

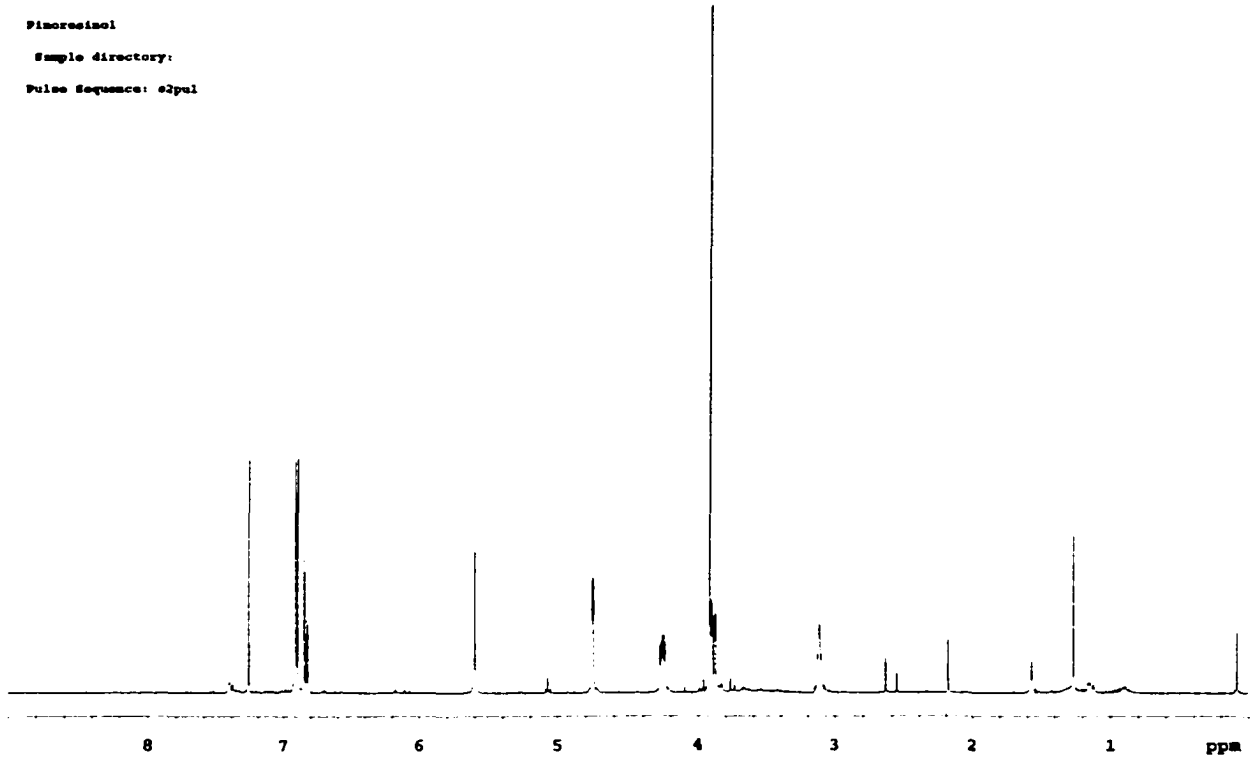


Figure 4.5. ^{1}H NMR spectrum of (±)-pinoresinol (**10**).

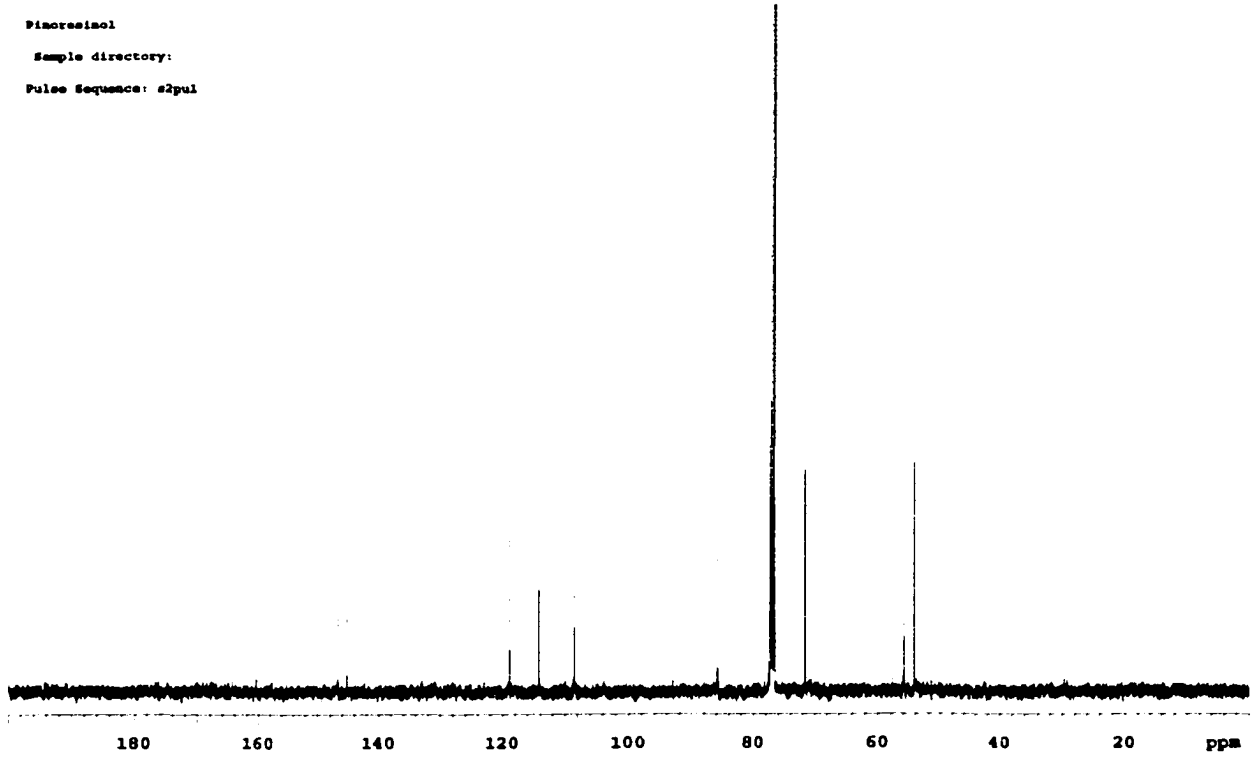


Figure 4.6. ^{13}C NMR spectrum of (±)-pinoresinol (**10**).

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