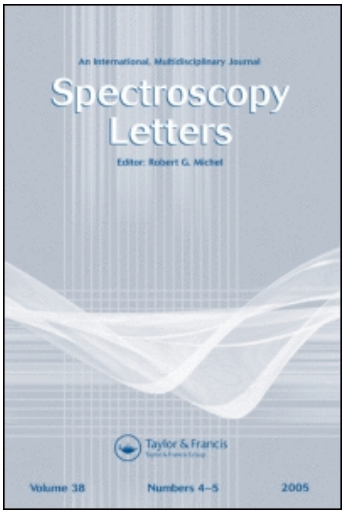

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Carbon-13 Assignments and Revision of the Stereostructures of the Cadinanes 2-Hydroxy-8α-angeloyloxycalamenene and 2-Hydroxy-8α-hydroxycalamenene

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Carbon-13 Assignments and Revision of the Stereostructures of the Cadinanes 2-Hydroxy-8α-angeloyloxycalamenene and 2-Hydroxy-8α-hydroxycalamenene

Key Words: *Heterotheca subaxillaris*; 2-hydroxy-8α-angeloyloxycalamenene; 2-hydroxy-8α-hydroxycalamenene; cadinanes; ^{1}H nuclear magnetic resonance spectroscopy; ^{13}C nuclear magnetic resonance spectroscopy; COSY; NOESY; DEPT; HETCOR; COLOC.

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Abstract: The two known cadinanes 2-hydroxy-8α-hydroxycalamenene and 2-hydroxy-8α-angeloyloxycalamenene were isolated from a Louisiana population of *Heterotheca subaxillaris*. Their ^{13}C NMR spectra were fully assigned by the application of HETCOR, COLOC, COSY, NOESY and DEPT experiments. It was shown on the basis of NOESY experiments that both cadinanes require revision at the stereogenic centre C-7.

INTRODUCTION

The polymorphic *Heterotheca subaxillaris* (Lam.) Britton & Rusby (family Asteraceae; tribe Astereae), commonly referred to as camphorweed, is widespread, spaning the coastal plain of the Southern and Eastern United States. Although the chemistry of many Asteraceae

has been investigated, members of the tribe Astereae have received only limited attention[1]. These taxa appear to be characterized by the absence of sesquiterpene lactones or alkaloids, but commonly accumulate mono-, sesqui- and diterpenoids. The five species of the North American genus *Heterotheca*, which have so far been investigated chemically are rich in cadinanes which exhibit diverse biological activities with remarkable dependence on minor structural and stereochemical changes[2,3]. Cadinanes, which are common in higher plants, have been further divided into four subclasses based on the nature of the ring fusion and the orientation of the isopropyl group at C(10)[4]. Due to complex stereochemistry of these compounds, many of the previous structural and configurational assignments appear to be uncertain[3]. It is also evident that the present classification of cadinane-type compounds needs a revision of nomenclature[5].

^{13}C NMR represents an important tool for structure determination, since ^{13}C chemical shifts are far more sensitive to their chemical environment than proton shifts, providing valuable structural information[6]. The normal and DEPT-edited experiment[7] can be used to partially assign ^{13}C spectra in terms of the number of attached protons. Furthermore, the recent development of two-dimensional NMR spectroscopy has provided a number of new NMR assignment techniques which are useful in the area of natural products chemistry[8,9].
In particular, the direct heteronuclear (^{1}H-^{13}C) chemical shift-correlated spectra allow simultaneous determination of ^{1}H and ^{13}C chemical shifts for directly bonded $^{13}C^{1}H_n$ units[10].

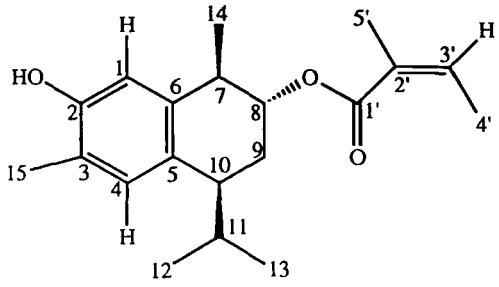
As part of an ongoing study of 2-hydroxy-8α-angeloyloxycalamenene (**1**) and 2-hydroxy-8α-hydroxycalamenene (**2**) as germination stimulants for *Striga* seeds, it was deemed important to report their complete ^{13}C NMR assignments, information which is invaluable for the structure elucidation of other terpenoids in general and calamenene-type compounds in particular. The calamenenes are A-ring-aromatized cadinanes with a methyl group at the C-7 position[4]. It is of some medicinal interest to note that the skeleton of **1** and **2** is the same as that of the spermicidal gossypol[11]. Unambiguous assignments for all skeletal carbon resonances of **1** and **2** were made primarily by the application of 2D ^{13}C-^{1}H heteronuclear correlated spectroscopy (HETCOR)[10] and long-range heteronuclear correlation experiments (COLOC)[12].

RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of the crude dichloromethane extract of *H. subaxillaris* showed absorptions both in the aromatic region as well as aliphatic methyl signals. From fractions of the medium polarity fractions, the cadinanes, 2-hydroxy-8α-angeloyloxycalamenene (**1**) and 2-hydroxy-8α-hydroxycalamenene (**2**) were isolated. Both compounds had previously been isolated from *H. subaxillaris* of an unspecified collection site and their structures were determined by spectroscopic methods[13].

^{13}C NMR spectral assignments in 2-hydroxy-8α-angeloyloxycalamenene (1)

The spectral data (^{1}H NMR, IR, MS) of compound **1** were in full agreement with the reported values[13]. Only the homonuclear coupling constants in the ^{1}H NMR spectrum showed marginal differences. The ^{13}C NMR spectrum of **1** indicated the presence of 20 carbon signals in the molecule. Its relative stereochemistry was determined from NOESY[10] experiment and proton coupling data which allowed the unambiguous assignment of carbon and proton resonances (Table 1). The DEPT spectrum of **1** (Fig. 1) exhibited six methyls, one methylene and seven methine signals, while the remaining 6 signals in the broad-band spectrum were due to the quaternary carbon atoms. The methyl protons were assigned by analyses of COSY[14], NOESY[10], and 2D one-bond heteronuclear correlation (HETCOR)[10] contour diagrams shown in Figures 2, 3, and 4, respectively. The aromatic methine protons H-1 and H-4 at 6.58 and 6.92 ppm showed cross peaks with carbon resonances at 113.6 and 130.3 ppm, respectively (Fig. 4a). The upfield region of the HETCOR spectrum (Fig. 4b) showed cross peaks of the methyl protons H-12, H-13, H-14, H-15, H-4' and H-5' at 17.2, 21.1, 17.3, 15.6, 15.5, and 20.5 ppm, respectively. The signal at 72.3 ppm was due to the oxygen-bearing carbon, C-8[15]. The ^{13}C NMR resonances of one methylene carbon (C-9) and the remaining 5 methine carbons were similarly assigned from the correlation diagram (Fig. 4a) and are depicted in Table 1. The only difficulty in assignment of the ^{13}C NMR resonances came from the non-protonated carbons, C-2, C-3, C-5, C-6, C-1', and C-2' which do not have cross peaks in the HETCOR diagram. To unambiguously assign these ^{13}C NMR signals, a three-bond long-range



Structure (1). 2-hydroxy-8α-angeloyloxycalamenene

heteronuclear correlation experiment (COLOC) was carried out. Figure 5 shows the non-protonated carbons at the downfield region of the COLOC contour plot for **1**.

The carbonyl resonance at 168.1 ppm showing long-range correlation with the H-5' was assigned to C-1'. Based on the correlation with the H-5' proton signal, the carbon resonance at 128.2 ppm could be assigned to C-2'. Three carbon resonances at 130.3 (C-4), 152.0, and 121.7 ppm showed correlation with H-15, indicating either a two-bond or three-bond coupling to H-15. The carbon resonances at 121.7 and 152.0 ppm were tentatively assigned to C-3 and C-2, respectively. The carbon resonance at 152.0 ppm showed correlation peaks with H-1, H-4 and H-15 could be unambiguously assigned to C-2, whereas the carbon at 121.7 ppm was assigned to C-3, values in accord with chemical shift considerations. Three correlation peaks were observed between the H-4 proton signal at 6.92 ppm and carbon resonances at 130.4 (C-4), 152.0 (C-2) and 138.8 ppm. The carbon signal at 138.8 ppm showed a three-bond correlation with C-4 and was unambiguously assigned to C-6. The remaining ^{13}C signal at 130.0 ppm showed a three-bond correlation peak with the H-1 signal at 6.58 ppm and was therefore assigned to C-5.

^{13}C NMR spectral assignments in 2-hydroxy-8α-hydroxycalamenene (2)

The structure of 2-hydroxy-8α-hydroxycalamenene (**2**) was illustrated to be a cadinane sesquiterpenoid with a secondary hydroxyl group at C-8. Its spectral data resembled those of **1**

Table 1. ^{13}C-^{1}H correlation of 2-hydroxy-8α-angeloyloxycalamenene (**1**) (400 MHz, $CDCl_3$)

$\delta^{13}C$	Mult.[a]	$\delta_{attached}H$	Assignment
113.6	CH	6.58 s	1
152.0	C[d]	-	2
121.7	C[d]	-	3
130.4	CH	6.92 s(br)	4
130.0	C[d]	-	5
138.8	C[d]	-	6
36.1	CH	3.05 dq	7
72.3	CH	5.37 ddd	8
25.2	CH_2	1.85 ddd; 2.05 ddd	9
40.8	CH	2.80 ddd	10
32.9	CH	2.10 dqq	11
17.2[b]	CH_3	0.99 d	12
21.1[c]	CH_3	0.75 d	13
17.3	CH_3	1.21 d	14
15.6	CH_3	2.10 s(br)	15
168.1	C[d]	-	1'
128.2	C[d]	-	2'
137.6	CH	6.02	3'
15.5	CH_3	1.89	4'
20.5	CH_3	1.85	5'

J(Hz): 7α, 8β, = 4.4; 7α, 14 = 6.8; 8β, 9β = 8.5; 9α, 9β = 14; 9α, 10α = 9β, 10α = 10α, 11 = 6; 11, 12 = 11, 13 = 6.8.

[a] Carbon multilplicities determined through DEPT experiments.

[b,c] Assignments may be interchanged in each vertical column.

[d] Assignments based on COLOC experiment.

occurring in the same plant. The IR spectrum of **2** showed the presence of hydroxyl and double bond (C=C) absorptions at 3500 and 1650 cm^{-1}, respectively. The high resolution ^{1}H NMR spectrum provided considerable information (Table 2) and the chemical shift values were in close agreement (differences of < 0.01 ppm) with the literature data[13]. There were 1H singlets at 6.95 and 6.61 ppm indicating the aromatic hydrogens (H-4 and H-1, respectively) in

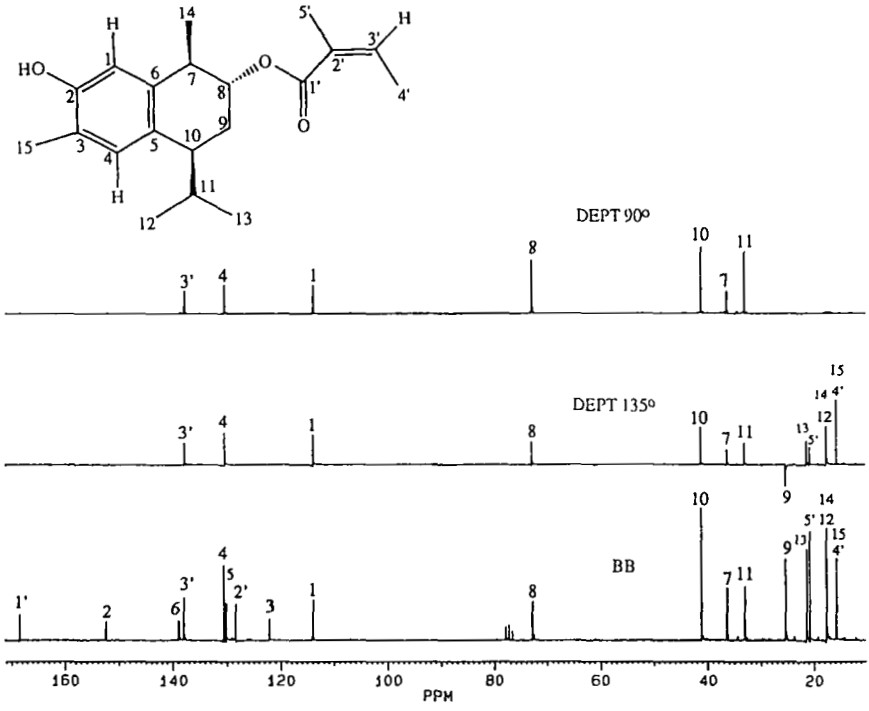
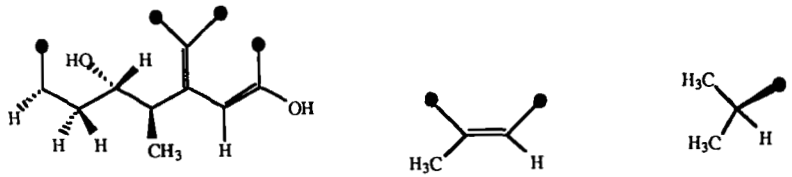


Figure 1. Broad Band ^{13}C NMR Spectrum and DEPT 135 and DEPT 90 Subspectra ($CDCl_3$) of 2-hydroxy-8α-angeloyloxycalamenene (1).

a *para* relationship. A singlet for an aromatic methyl group appeared at 2.20 ppm and an OH group signal at 6.15 ppm. The remaining two positions of the benzene ring are occupied by benzylic MeCH< and Me_2CHCH< groups (the underlined protons being vicinal to the phenyl ring).

The 2D ^1H-^1H COSY and NOESY experiments revealed and clarified the presence and connectivities of each of the three partial segments, A - C:



A B C

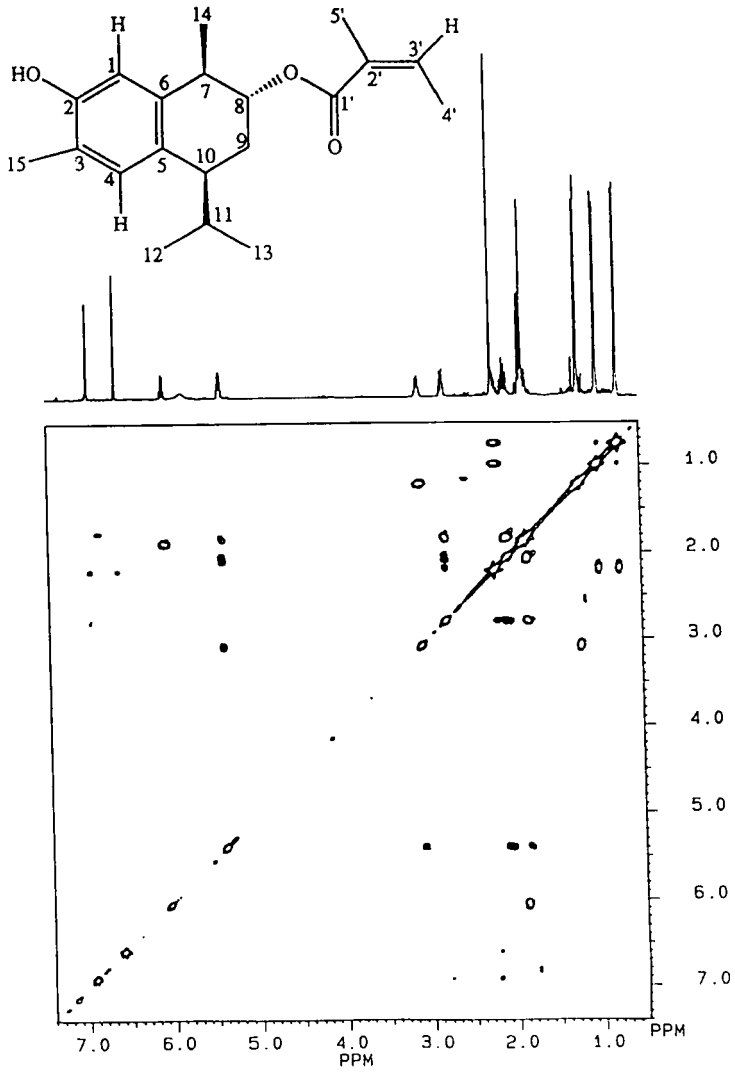


Figure 2. The 2D ^{1}H-^{1}H Shift Correlation of 2-hydroxy-8α-angeloyloxycalamenene (1) by COSY-45 at 400 MHz in $CDCl_3$.

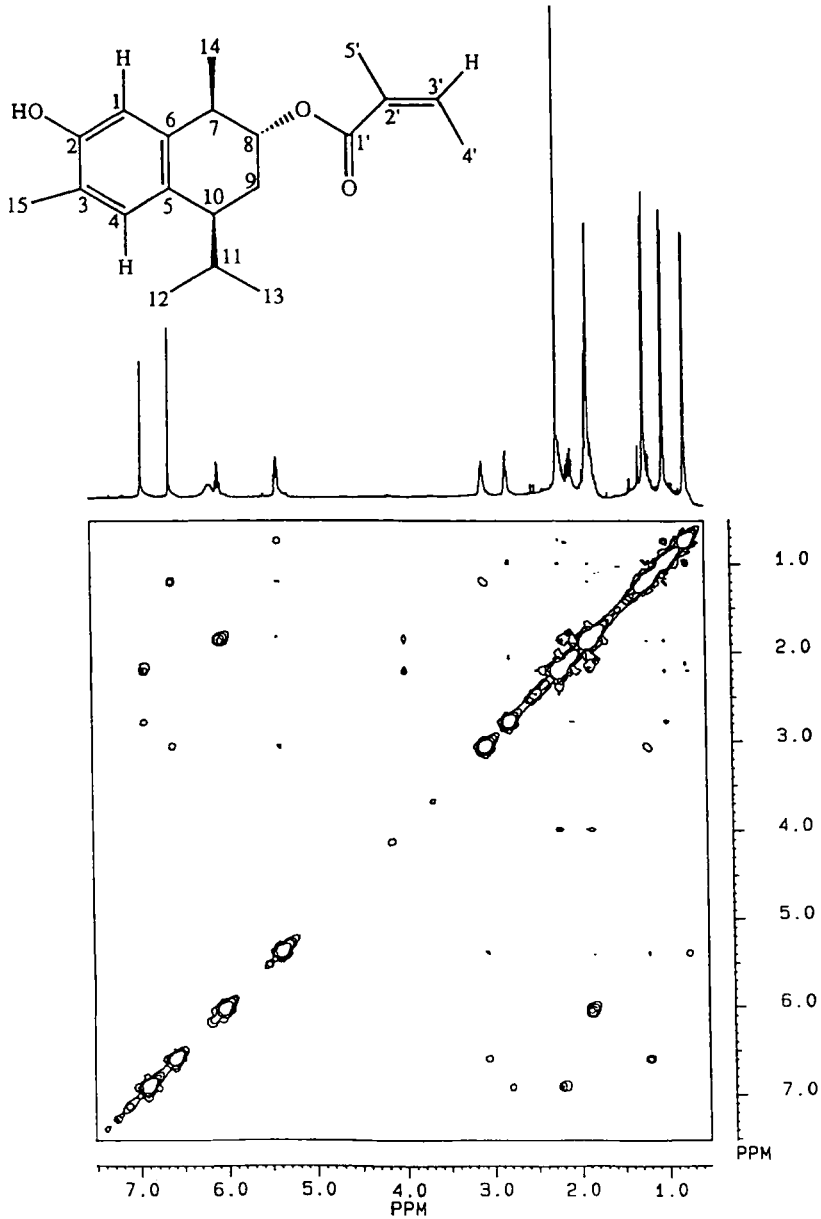


Figure 3. 2D NOESY spectrum of 2-hydroxy-8α-angeloyloxycalamenene (1).

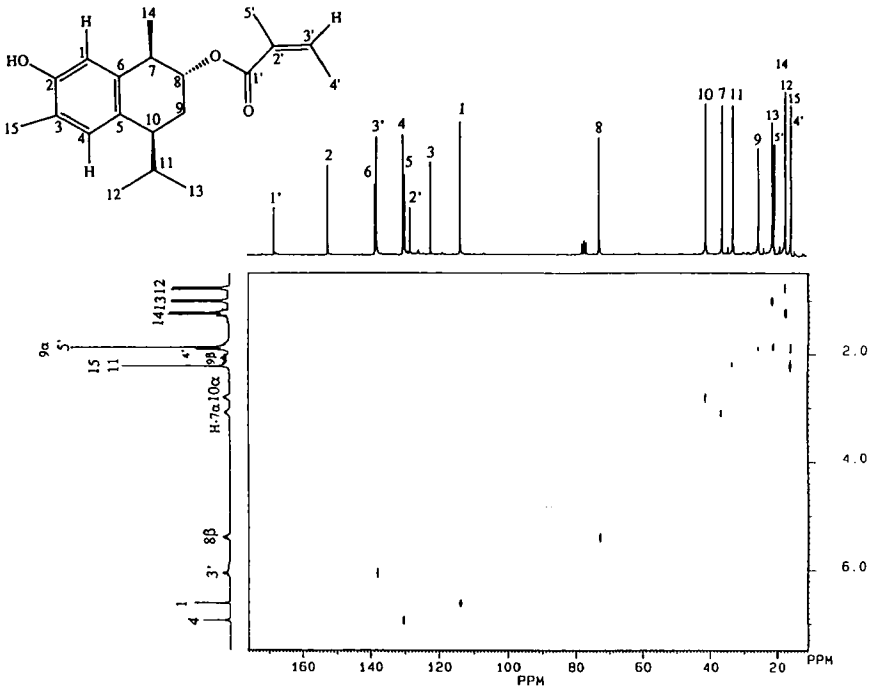


Figure 4a. The 2D ^{13}C-^{1}H Correlation (HETCOR) of 2-hydroxy-8α-angeloyloxycalamenene (1).

One oximethinic proton (H-8) located downfield at 4.12 ppm correlated with both methylene protons at C-9 carbon and a secondary methyl (H-14) in segment A. The C-4 proton (H-4) correlated with a tertiary methyl (H-15) in segment B and H-1 and in segment A. Two secondary isopropyl methyl groups (H-12 and H-13) were correlated with the methine proton (H-11) in segment C and with H-14 methyl group in segment A. A pair of 3H doublets (J = 6.8 Hz) at 0.66 and 0.98 ppm, which were scalar-coupled to a 1H multiplet at 2.20 ppm in COSY spectrum (Figure 6) confirmed the presence of an isopropyl moiety (segment C). Moreover, the EI-mass spectrum established a molecular weight of 234 $[M]^+$ (corresponding to $C_{15}H_{22}O_2$) with major fragment ions resulting from loss of isopropyl group.

The stereochemistry of **2** was deduced by the NOESY experiment. Several significant NOEs observed were not in agreement with the previously assigned stereostructures of

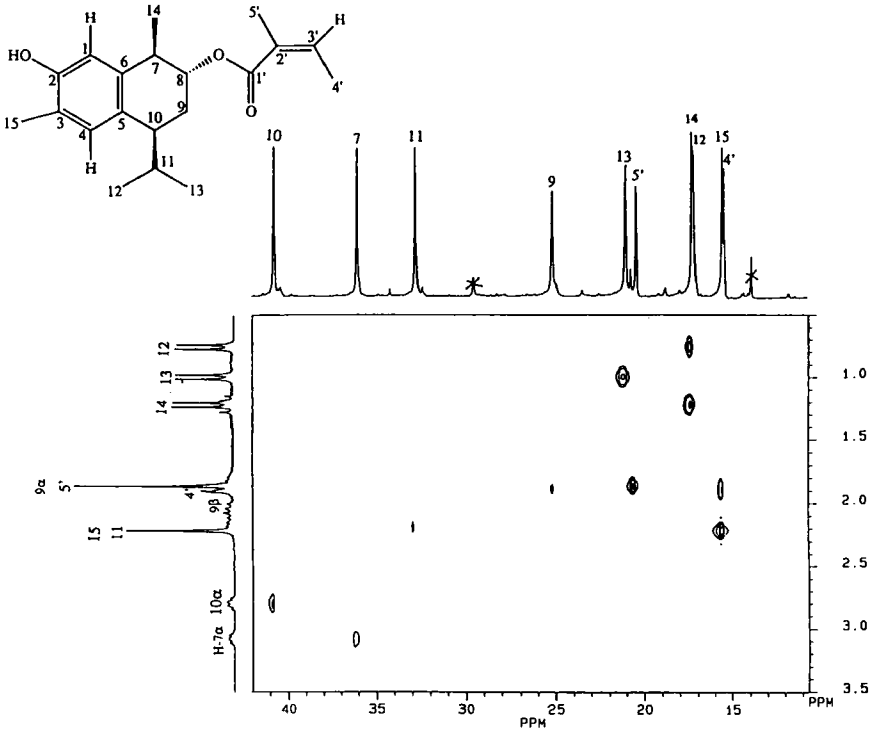


Figure 4b. Contour plot of upfield region of HETCOR spectrum of 2-hydroxy-8α-angeloyloxycalamenene (1).

2-hydroxy-8α-angeloyloxycalamenene (**1**) and 2-hydroxy-8α-hydroxycalamenene (**2**)[13]. As noticeable in Figures 3 and 7, cross peaks between H-12 and H-11, H-13 and H-14, between H-14 and H-11 strongly suggested that the C-7 methyl group and the C-10 isopropyl group in both **1** and **2** are *cis*-oriented. Chemical shifts correlations, integrals and determination of the relevant coupling constants (e.g. $J_{9,10}$ and $J_{7,8}$) further supported the relative configurations at C-7, C-8 and C-10.

The ^{13}C NMR spectrum of 2-hydroxy-8α-hydroxycalamenene (**2**) showed signals of fifteen carbon atoms, four of them being quaternary. The protonated carbon signals were assigned by the polarization transfer experiment (DEPT) (Figure 8) which indicated the presence of four methyl groups, one methylene and six methines (one directly bonded to an

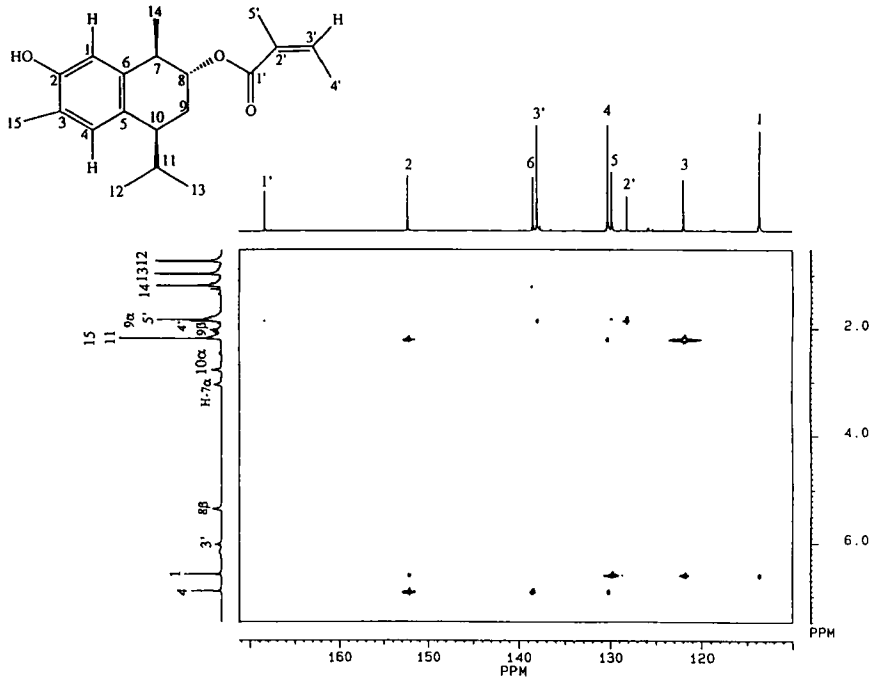
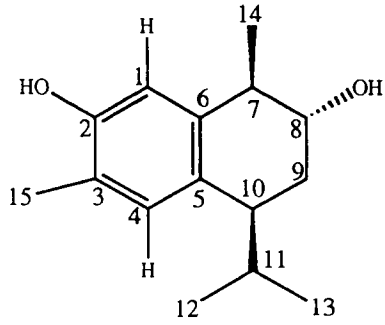


Figure 5. Downfield region of 2D ^{1}H-^{13}C long range correlation (COLOC) of 2-hydroxy-8α-angeloyloxycalamenene (1).



Structure (**2**). 2-hydroxy-8α-hydroxycalamenene

Table 2. ^{1}H- and ^{13}C NMR spectral data (δ) for 2-hydroxy-8α-hydroxycalamenene (**2**) (400 MHz, $CDCl_3$)

$\delta^{13}C$	Mult.[a]	δ attached^{1}H	Assignment
113.6	CH	6.61 s	1
152.2	C[b]	-	2
121.8	C[b]	-	3
130.3	CH	6.95 s(br)	4
130.6	C[b]	-	5
138.0	C[b]	-	6
37.7	CH	2.84 dq	7
70.7	CH	4.12 ddd	8
28.9	CH_2	1.50 ddd;1.95 ddd	9
39.4	CH	2.85 ddd	10
33.0	CH	2.20 dqq	11
16.6[c]	CH_3	0.98 d	12
20.8[c]	CH_3	0.66 d	13
16.6	CH_3	1.29 d	14
15.5	CH_3	2.20 s(br)	15

J (Hz): 7α, 8β, = 3.5; 7α, 14 = 7; 8β, 9β = 3; 9β, 9α = 13.5; 9α, 10α = 9β, 10α = 7; 10α, 11 = 6.

[a] Carbon multilplicities determined through DEPT experiments.

[b] These carbon number assignments were made by comparison with assignments for **1** and related compounds[11].

[c] Assignments may be interchanged in each vertical column.

oxygen function at 70.7 ppm). Two sets of aromatic carbon signals arose at δ 113.6, 130.3 and were attributed to (-C=CH) and at δ 121.8, 130.6, 138.0, 152.2 due to quaternary aromatic carbons. Two-dimensional ^{13}C-^{1}H COSY spectral data (Figures 9a and 9b) was employed in the assignments of chemical shifts of protonated carbons in the ^{13}C NMR spectrum. Quaternary carbons (C-2, C-3, C-5 and C-6) were assigned by comparison with the spectral data for **1** and for similar skeletal structures[11].

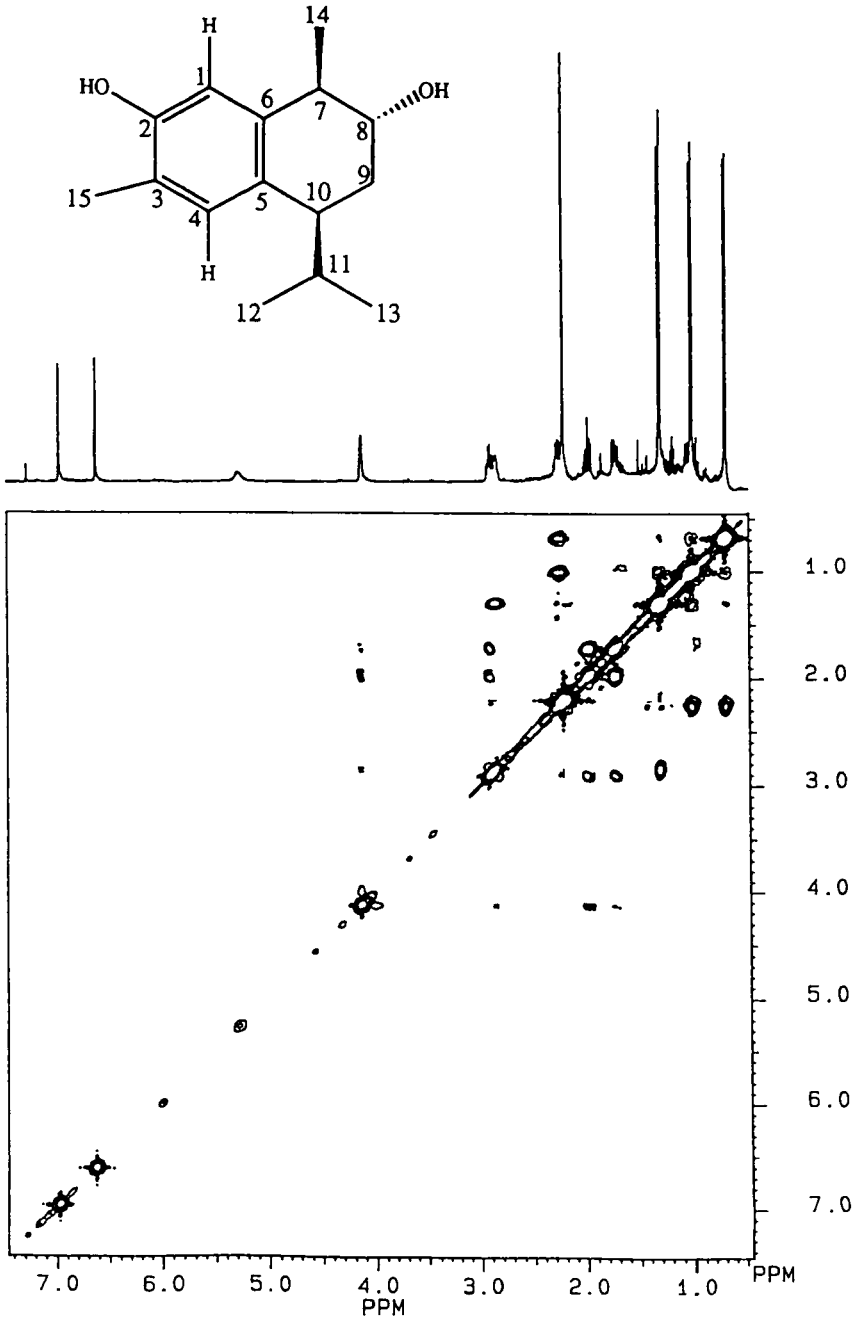


Figure 6. The 2D ^{1}H-^{1}H Shift Correlation of 2–Hydroxy-8α-hydroxycalamenene (2) by COSY-45 at 400 MHz in $CDCl_3$.

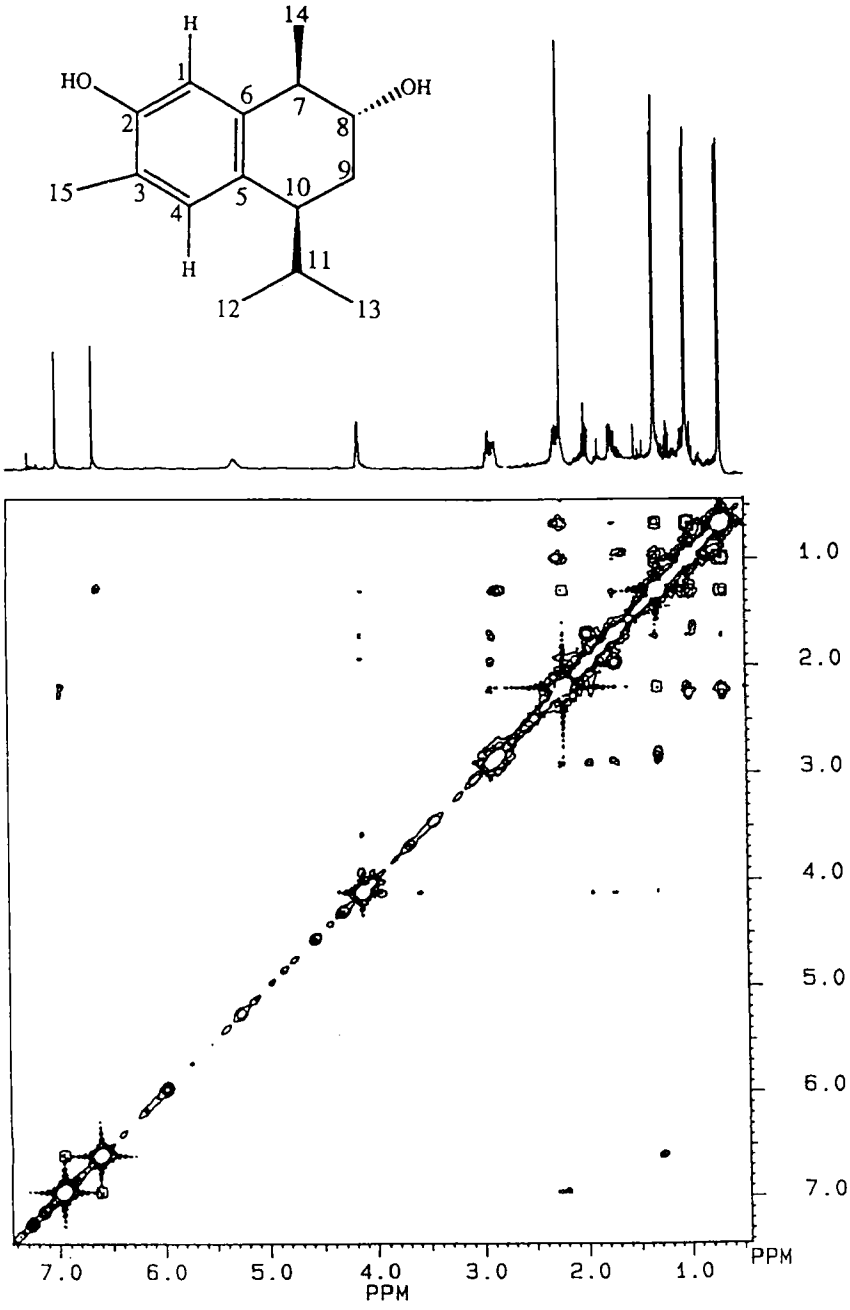


Figure 7. 2D NOESY spectrum of 2–Hydroxy-8α-hydroxycalamenene (2).

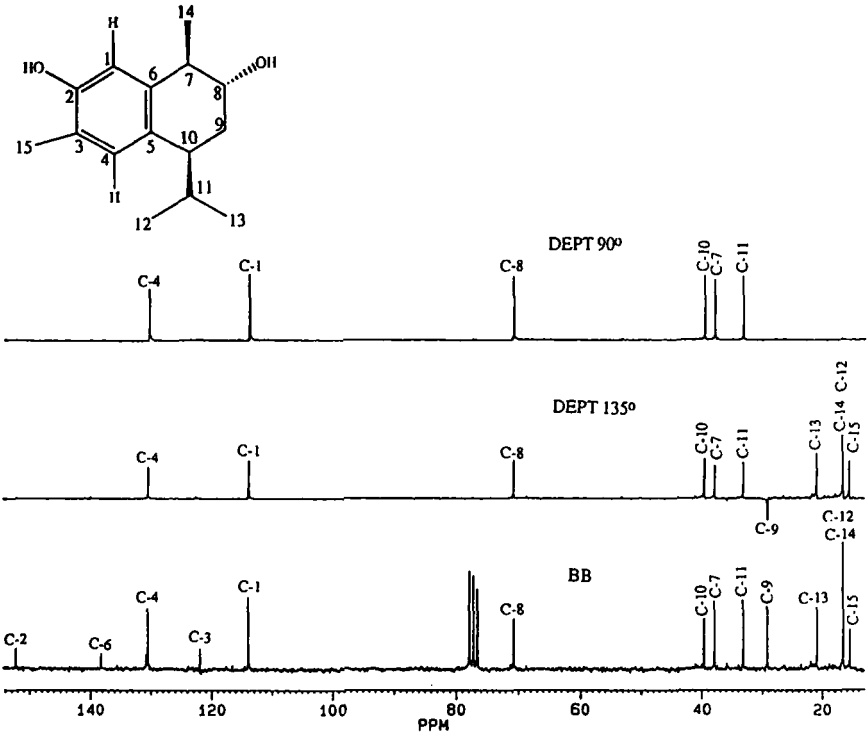


Figure 8. Broad Band ^{13}C NMR Spectrum and DEPT 135 and DEPT 90 Subspectra ($CDCl_3$) of 2-Hydroxy-8α-hydroxycalamenene (2).

EXPERIMENTAL

Plant materials. The aerial parts of *H. subaxillaris* were collected by N. H. Fischer, H. D. Fischer and L. Quijano on February 2, 1989, in Jefferson Parish along the beach at Grand Isle, Louisiana, U.S.A. (Voucher No. Fischer 375; voucher deposited at the Louisiana State University Herbarium). IR spectra were recorded on a Perkin-Elmer 1760x FT-IR spectrometer as a film on KBr plate using $CHCl_3$ solution. Mass spectra were run on a Hewlett-Packard 5971 A GC-MS spectrometer. Comparison by TLC[16] (EtAOc-hexane, 2:5), GC-MS and ^1H NMR of the crude extract of stems and leaves showed that they contained the same compounds with minor quantitative differences.

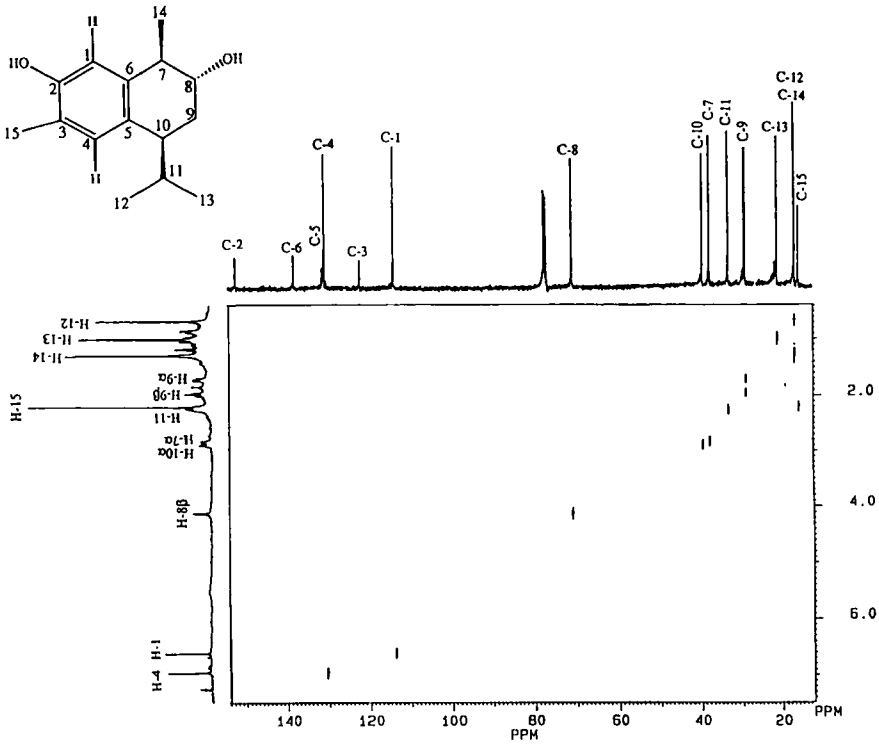


Figure 9a. The 2D ^{13}C-^{1}H Correlation (HETCOR) of 2–Hydroxy-8α-hydroxycalamenene (2).

Extraction and isolation. Powdered leaves (502 g) were extracted with CH_2Cl_2 (6 x 900 ml) at room temperature for 72 hours. Evaporation of the combined CH_2Cl_2 extracts under reduced pressure gave 5.6 g of crude extract which was chromatographed by VLC[17] using 100 g silica gel (MN Kieselgel G) and ethyl acetate-hexane as eluent. The percentage of EtOAc in hexane was gradually increased {(EtOAc:hexane (v/v): 0:1; 1:9; 1:4; 3:7; 1:1; 7:3; 9:1; 1:0, 50 ml each)}. Forty fractions of 10 ml each were collected and monitored by TLC performed on precoated MN Sil-G 25 UV_{254} plates having a layer thickness of 0.25 mm. Fraction 12 provided 14 mg of 2-hydroxy-8α-angeloyloxycalamenene (**1**) [R_f 0.86 (SiO_2, EtAOc-hexane, 2:5)] as a yellow oil. The medium polar fractions 20-25 eluted with 25% EtAOc-hexane were combined and evaporated to afford 120 mg of 2-hydroxy-8α-hydroxycalamenene (**2**) [R_f 0.45 (SiO_2, EtOAc-hexane, 2:5)] as a yellow oil.

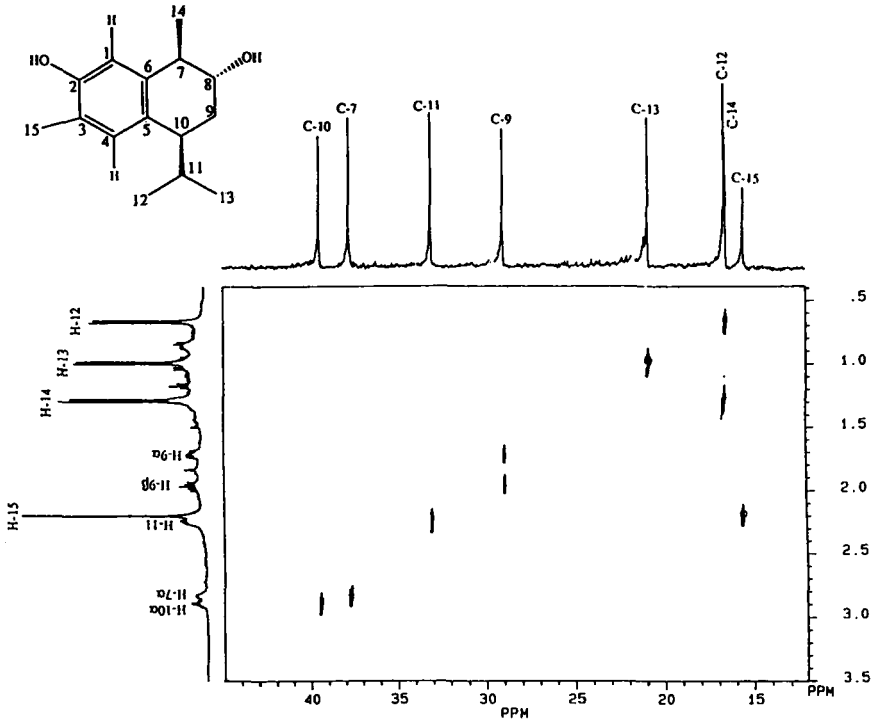


Figure 9b. Contour plot of upfield region of HETCOR of 2-Hydroxy-8α-hydroxycalamenene (2).

NMR. One and two-dimensional NMR spectra were obtained in a Bruker AMX 400 NMR spectrometer at room temperature. Samples of 6 mg of 1 or 2 were dissolved in 0.7 ml of $CDCl_3$ (99.8% isotopically pure in deuterium, from Aldrich Chemical Company Inc., Milwaukee, U.S.A) in 5 mm diameter NMR tubes. The ^1H and ^{13}C chemical shifts are reported in δ-values (ppm) and referenced to the signal of $CDCl_3$ at 7.26 and 77.00 ppm, respectively. To make unambiguous assignments of carbon signals additional ^1H-^1H COSY[14], NOESY[10], DEPT[7], ^{13}C-^1H correlation (HETCOR)[10] and COLOC[12] experiments were performed. The 2D experiments were acquired and processed with the software provided by Bruker on AMX 400. Typical acquisition time and processing conditions for COSY and NOESY experiments were: relaxation delay of 1 and 2 seconds, 512 t_1 increments; 1024 to

2048 t_2 points; sweep width of 2 ppm. Sine bell squared and shifted ($\pi/4$, $\pi/6$ and $\pi/8$) apodization functions were used for processing. The mixing time in NOESY experiments, generally set at 1.2-1.5 seconds, was also varied between 0.8 and 2 seconds, without substantial change in the results. For ^1H-^{13}C (^{13}C detected) correlations, the same relaxation delays were used, 256 to 512 t_1 increments, 1024 to 2048 t_2 points, the sweep width being 60 ppm for ^{13}C. Lorentz and Gaussian deconvolution were generally used in the processing. The number of scans was set for an overall acquisition time of about 12 to 16 hours. HETCOR was carried out at a ^{13}C frequency of 100.62 MHz using 4K data points in the t_2 dimension and 256 increments on t_1. A Gaussian window function was applied to the t_2 dimension and a sinebell window function was applied to the t_1 dimension. Zero filling to 1K was also applied in the ^1H dimension. Three-bond long-range ^1H-^{13}C shift correlations (COLOC) experiment were performed using a pulse sequence reported by Kessler *et al*[12]. The data were acquired in 12 hours using 256 experiments, each with a block size of 4K. The following parameters were used: D1 = 2 sec, Df = 3 msec, D3 = 0.071 sec, D4 = 0.036 sec. The D3 and D4 values were computed using the observed three-bond coupling constant, $^3J_{CH}$ = 7Hz. The data were processed using sinebell multiplication in both dimensions (SSB1 = SSB2 = 0) and Gaussian multiplication in the second dimension (WDW2 = G; LB2 = 2.0) before Fourier transformation. The contour plot was plotted at the 256 K level.

Acknowledgements

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REFERENCES

1. Lincoln, D. E. and Lawrence, B. M. The volatile constituents of camphorweed, *Heterotheca subaxillaris*. *Phytochemistry*, **1984**, 23, 933-934.

2. Kalsi, P. S. and Talwar, K. K. A dramatic effect of stereochemistry on the plant growth activity of the cadinane group of terpenoids. *Phytochemistry*, **1981**, 20, 511-513.

3. Borg-Karlson, A-K. and Norin, T. Configurations and conformations of torreyol (δ-cadinol), α-cadinol, T-muurolol and T-cadinol. *Tetrahedron*, **1981**, 37, 425-430.

4. Bordoloi, M., Shukla, V. S., Nath, S. C. and Sharma, R. P. Naturally occurring cadinenes. Review article number 45. *Phytochemistry*, **1989**, 28, 2007-2037.

5. Vlahov, R., Holub, M. and Herout, V. The structure of two hydrocarbons of cadalene type isolated from *Mentha piperita* oil of Bulgarian origin. *Collect. Czech. Chem. Commun.*, **1967**, 32, 823-829.

6. Bax, A., Aszalos, A., Dinya, Z. and Sudo, K. Structure elucidation of the antibiotic desertomycin through the use of new two-dimensional NMR techniques. *J. Am. Chem. Soc.*, **1986**, 108, 8056-8063.

7. Doddrell, D. M. Pegg, D. T. and Bendall, M. R. Distortional enhancement of NMR signals by polarization transfer. *J. Magn. Reson.*, **1982**, 48, 323-327.

8. Derome, A. E. The use of N.M.R. spectroscopy in the structure determination of natural products: Two-dimensional methods. *Nat. Prod. Rep.*, **1989**, 6, 111-141.

9. Shoolery, J. N. Recent developments in ^{13}C- and proton-NMR. *J. Nat. Prod.*, **1984**, 47, 226-259.

10. Bax, A. and Morris, G. A. An improved method for heteronuclear chemical shift correlation by two-dimensional NMR. *J. Magn. Reson.*, **1981**, 42, 501-505.

11. Davila-Huerta, G., Hamada, H., Davis, G. D., Stipanovic, R. D., Adams, C. M. and Essenberg, M. Cadinane-type sesquiterpenes induced in *Gossypium* cotyledons by bacterial inoculation. *Phytochemistry*, **1995**, 39, 531-536.

12. Kessler, H., Griesinger, C., Zarbrock, J. and Loosli, H. R. Assignment of carbonyl carbons and sequence analysis in peptides by heteronuclear shift correlation via small couplings constants with broad band decoupling in t_1 (COLOC). *J. Magn. Reson.*, **1984**, 57, 331-336.

13. Bohlmann, F. and Zdero, C. Neue cadinan-derivate aus *Heterotheca subaxillaris*. *Phytochemistry*, **1979**, 18, 1185-1187.

14. Aue, W. P., Bartholdi, E. and Ernst, R. R. Two-dimensional spectroscopy. Application to nuclear magnetic resonance. *J. Chem. Phys.*, **1976**, 64, 2229-2246.

15. Silverstein, R. M.; Basler, G. C. and Morrill, T. C. *Spectrometric Identification of Organic Compounds*, 5th Edn, John Wiley & Sons Inc., New York, 1991, pp. 227-248.

16. Fischer, N. H. Sesquiterpenoid Lactones. "Methods in Plant Biochemistry". Ed. by B. V. Charlwood and D. V. Banthorpe, Academic Press, London, 1991, vol. 7, Chapter 5, pp. 187-211.

17. Pelletier, S. W., Chokshi, H. P. and Desai, H. K. Separation of diterpenoid alkaloid mixtures using vacuum liquid chromatography. *J. Nat. Prod.*, **1986**, 49, 892-900.

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