Carbon-13 NMR Study of (20,24)-Epoxydammarane Triterpenes

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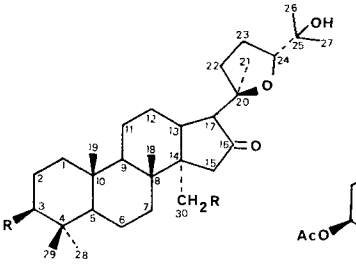
Assignments of the ^{13}C NMR signals of the dammarane triterpenes, 3β,25,30-trihydroxy-(20*R*,24*R*)-epoxydammaran-16-one 3,30-diacetate (trevoagenin A diacetate) (2), its 20*S*-isomer (trevoagenin B diacetate) (3) and their related (20*R*)-3β,30-diacetoxy-16-oxo-25,26,27-trisnordammarane-24,20-lactone (4) and its 20*S*-isomer (5) have been achieved. Suitable tetrahydrofuran models have been synthesized in order to aid the ^{13}C NMR assignments of the side-chain carbons of the above-mentioned compounds. The remarkable chemical shift differences observed for C-21 and C-22 between each pair of the C-20 epimers (2, 3 and 4, 5) allowed the confirmation of the C-20 stereochemistry of these ocotillol-type dammarane triterpenes.

INTRODUCTION

The ^{13}C NMR chemical shifts of triterpenoids with the dammarane skeleton have been described[1–4] and this technique has proved valuable for structural elucidation of this type of natural product. However, the ^{13}C NMR spectra of (20,24)-epoxydammarane triterpenes of the ocotillol type have received less attention and only chemical shifts for the 20*S* series have been reported.[2–4] In this paper we describe a ^{13}C NMR study on the dammarane triterpenes, in particular the acetates of trevoagenin A (**2**) and B (**3**) isolated from *Trevoa trinervis*[5] (Rhamnaceae) and their oxidation products, lactones **4** and **5**, respectively. As compounds **2** and **4** have 20*R* stereochemistry and compounds **3** and **5** show 20*S* stereochemistry some remarkable differences exist between the ^{13}C NMR chemical shifts of the two series of stereoisomers. In this paper we show the usefulness of this technique in the establishment of the stereochemistry at C-20, which is difficult to determine by other spectroscopic and chemical procedures.



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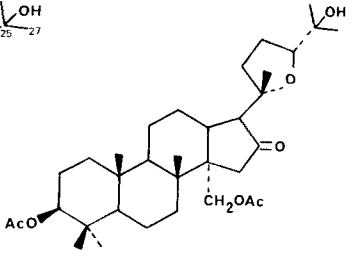
2 R = OAc



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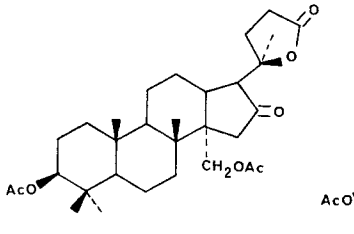

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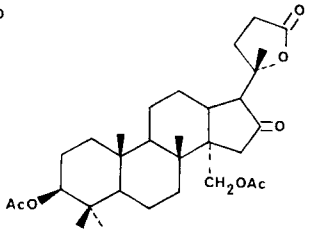

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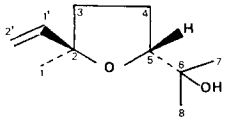

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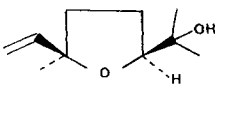

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RESULTS AND DISCUSSION

The carbon resonances of compounds **2–5** were assigned as shown in Table 1 and were deduced from the proton-noise decoupled and off-resonance decoupled spectra. Assignments for carbons 1–12 and the methyl groups 18, 19, 28 and 29 are closely related with those of several dammarane[3,4] and 3β-acetyl derivatives of ursane and oleane[6] triterpenes. The triols obtained by hydrolysis of **2–5** give rise to complex ^{13}C NMR spectra as a consequence of the equilibrium between the 16-oxo-30-ol and its hemiketal form, e.g. the C-16 resonance of alcohol **1** appears at δ 216.4 (C-16 carbonyl) and simultaneously at δ 109.4 (C-16 hemiketal).

In order to assign the values corresponding to the side-chain carbons we synthesized *trans*- and *cis*-linalool oxide, **6** and **7**, respectively, and *trans*- and *cis*-2-phenyl-2-methyl-5-(2-hydroxyisopropyl)tetrahydrofuran, **10** and **11**, respectively, and 2-phenylpentane-5,2-lactone (**12**). Compounds **6** and **7** were

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CCC-0030–4921/84/0022–0034$02.50

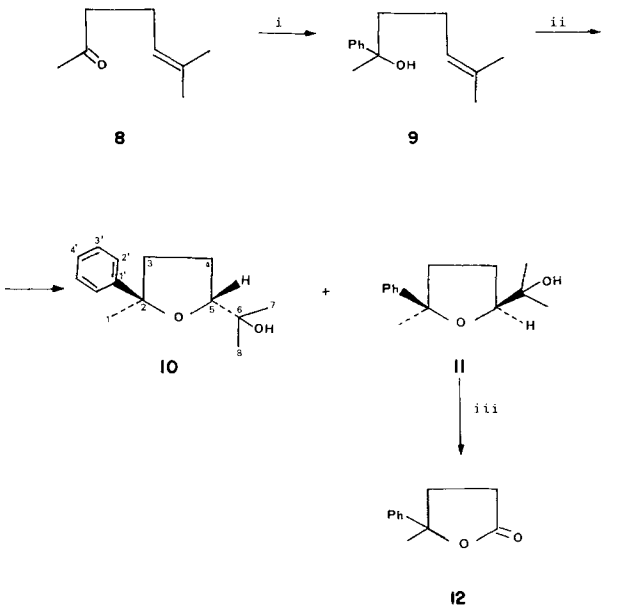
Table 1. Carbon-13 chemical shift data of dammarane triterpenes 2–5

Carbon	2	3	4	5
1	38.5	38.45	38.5	38.45
2	23.6	23.6	23.6	23.6
3	80.6	80.5	80.5	80.5
4	37.9	37.9	38.0	37.9
5	55.8	55.8	55.8	55.8
6	18.2	18.2	18.2	18.2
7	35.85	35.9	35.8	35.7
8	40.7	40.6	40.8	40.6
9	51.6	51.6	51.4	51.4
10	37.5	37.5	37.5	37.5
11	21.25	21.5	21.15	21.3
12	25.8	25.4	25.9	26.5
13	42.2	41.9	42.0	42.0
14	47.6	47.7	47.75	47.6
15	43.8	44.6	43.7	44.1
16	216.4	216.4	214.4	214.3
17	57.45	56.8	58.05	57.4
18	16.5[a]	16.5[a]	16.55[a]	16.4[a]
19	16.4[a]	16.5[a]	16.4[a]	16.5[a]
20	84.5	84.6	87.05	87.0
21	22.7	25.4	21.75	27.3
22	38.5	34.3	34.55	30.3
23	26.2	26.9	28.0	28.5
24	85.3	84.1	176.2	176.1
25	70.7	71.5		
26	24.3	24.8		
27	27.4	27.25		
28	27.9	27.9	28.0	27.95
29	16.7[a]	16.7[a]	16.9[a]	16.9[a]
30	64.4	65.0	64.3	64.6
$OCOCH_3$	21.25	21.2	21.15	21.3
$OCOCH_3$	20.9	20.9	20.85	20.8
$OCOCH_3$	170.8	170.75	170.4	170.8
$OCOCH_3$	170.7	170.6	170.5	170.4

[a] Assignments may be reversed.

prepared by the previously described[7] oxidation of linalool with monoperphthalic acid. The *trans*- and *cis*-tetrahydrofuran derivatives **10** and **11** and lactone **12** were synthesized from the commercially available 6-methylhept-5-en-2-one (**8**) through the reaction sequence shown in Scheme 1 (see Experimental). The establishment of the relative stereochemistry on C-2 and C-5 of compounds **10** and **11** was accomplished by the study of the ^1H NMR spectra with the aid of a lanthanide shift reagent. Figure 1 shows the relationship between the magnitude of the induced shift for the 1-Me and the amount of added $Eu(fod)_3$. As expected, the 1-Me group in **10** (*cis* relationship between the dimethylcarbinol at C-5 and the methyl group at C-2) experiences a higher rate of deshielding than that in its isomer, **11**.

The assignment of the ^{13}C NMR chemical shifts of **6–12** are shown in Table 2. Selective deuteriation (see Experimental) of carbons 1 and 3 in compounds **8–12** was also used as an assignment aid. The signals of the methylene and the methyl group in compounds **10–12** that are absent from the spectra of the corresponding 1,1,1,3,3-pentadeuteriated compounds are assigned to C-3 and C-1, respectively. Quaternary carbons 2 and 6 are easily assigned by comparing the spectra of **10** and **11** with **12**. Special mention should be made of the



Scheme 1. Reagents: i, PhMgBr; ii, perbenzoic acid; iii, Jones's reagent. Although one enantiomer is shown, both compounds **10** and **11** are racemic.

difference between the chemical shifts (*ca* 3 ppm) of the two methyl groups of the isopropyl carbinol in **6**, **7**, **10** and **11**. This can only be explained in terms of a strong hydrogen bond between the tertiary hydroxyl group and the tetrahydrofuran oxygen, resulting in one methyl group being shielded by the presence of

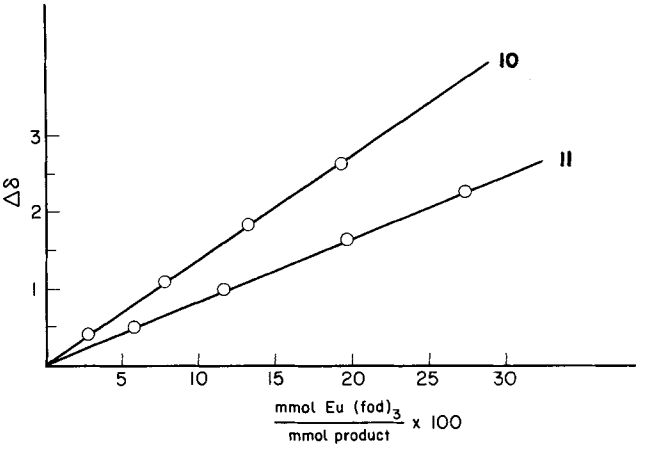


Figure 1. Lanthanide-induced shift for 1-Me in the ^1H NMR spectra of compounds **10** and **11**.

Table 2. Carbon-13 chemical shift data of compounds 6–12

Carbon	6	7	8	9	10	11	12
1	26.9	26.05	29.55	30.4	30.6	29.4	29.4
2	83.05	82.8	207.95	74.9	84.7	84.55	86.95
3	37.5	38.0	43.45	43.85	39.65	39.0	36.2
4	26.4	26.5	22.45	23.0	26.5	26.3	28.95
5	85.6	85.6	122.8	124.3	85.5	85.2	177.1
6	71.1	71.2	132.25	132.0	71.1	71.5	
7	27.2	27.4	25.4	25.7	27.3	27.2	
8	24.3	24.4	17.4	17.6	24.3	24.6	
1′	143.8	144.4		148.0	148.3	148.3	144.4
2′	111.3	111.5		124.85	124.6	124.5	124.1
3′				128.1	128.2	128.25	128.6
4′				126.5	126.4	126.5	127.65

two γ-*gauche* interactions with C-4 and the oxygen atom of the tetrahydrofuran ring. As expected,[8] no significant differences were observed between the chemical shifts of each pair of *trans* and *cis* isomers, **6**–**7** and **10**–**11**.

The side-chain and D-ring carbon atoms of the triterpenes **2** and **3** and the trisnor-lactones **4** and **5** (Table 1) were assigned on the basis of the chemical shift data shown in Table 2. As with **6**, **7**, **10** and **11**, the isopropyl carbinol methyl groups C-26 and C-27 in the acetates of trevoagenins A and B, **2** and **3**, respectively, show different chemical shifts (*ca* 3 ppm). In this case (24*R* configuration) the *pro*-*S*-methyl group at C-25 absorbs at higher field than the 25-*pro*-*R*-methyl group; this could be of interest in biosynthetic investigations in this type of dammarane triterpene. Previously reported[4] assignments of ^{13}C NMR chemical shifts of these methyl groups in several (20,24)-epoxydammarane triterpenes should be revised on this basis.

Carbon-23 in **2** and **3** absorbs at higher field than C-22 (12.3 and 7.4 ppm, respectively) as a consequence of two γ-*gauche* interactions with the methyl groups at C-25 and another upfield shift[9] associated with the anti-periplanar hydroxyl group at C-25. A similar situation is observed for C-4 and C-3 in **6**, **7**, **10** and **11**. The variations observed between the chemical shifts of the carbons adjacent to C-17 and C-20 in each pair of the C-20 epimers are shown in Table 3. The chemical shift differences observed for C-21 and C-22 can only be explained if the rotation around the C-17—C-20 bond is restrained to the conformations indicated in Fig. 2 for the two isomers. This allowed us to distinguish between the C-20 epimers of this type of dammarane triterpene; the C-21 methyl group resonance in the 20R series is more shielded than that of the corresponding 20*S* series, while the C-22 signal is more deshielded in the 20R series than that of its counterpart. It should be noted that the conformation indicated for trevoagenin A (20R) in Fig. 2 is also supported by x-ray crystallographic analysis[5] of **1**. A similar situation is observed[3] for 12β,20-dihydroxydammaranes such as **A** (Fig. 3), where the rotation around the C-17—C-20 linkage is restricted by strong hydrogen bonding between the hydroxyl groups at C-12 and C-20. Determination of the C-20 stereochemistry of these ocotillol-type dammarane triterpenes is difficult to achieve by IR, ^1H NMR, mass spectrometry and chemical methods, hence the importance of ^{13}C NMR spectroscopy in the resolution of this problem.

EXPERIMENTAL

The ^{13}C NMR spectra were recorded on a Varian CFT-20 NMR spectrometer operating at 20.1 MHz in the FT mode. The compounds were submitted to proton-noise decoupling and single-frequency off-resonance decoupling (SFORD) by offsetting the ^1H decoupler frequency by *ca* 6 ppm upfield of TMS to establish the carbon shifts and degree of protonation. The quaternary carbons were exclusively observed by setting the ^1H decoupler frequency *ca* 15 ppm upfield from TMS, with a noise band width of 500 Hz. The samples were recorded in 5 mm o.d. tubes using $CDCl_3$ as solvent as well as internal lock signal. All solutions were 0.1–0.2 M in concentration. The chemical shifts reported are in δ (ppm) downfield from internal TMS. The spectra were recorded over 5000 Hz (4000 Hz for **6**–**12**), a pulse width of 12 μs and 8K data points. ^1H NMR spectra were recorded with a Perkin–Elmer R-12B (60 MHz) or R-32 (90 MHz) instrument in $CDCl_3$ with TMS as internal reference. IR spectra were measured on a Perkin–Elmer 402 spectrophotometer. Mass spectra were recorded with a Hewlett–Packard 5930A instrument. Thin-layer chromatography (TLC) was performed on Merck silica gel (0.063–0.2 mm), the spray reagents being iodine or vanillin (1 g)–H_2SO_4 (160 ml)–EtOH (40 ml).

Trevoagenin A 3,30-diacetate (**2**) and trevoagenin B 3,30-diacetate (**3**), isolated from *Trevoa trinervis* Miers (Rhamnaceae), and the trisnor-lactones **4** and **5**, prepared by Jones's oxidation of **2** and **3**, respectively, have been described previously.[5]

Trans- and *cis*-linalool oxides **6** and **7** were prepared according to a previously reported method.[7]

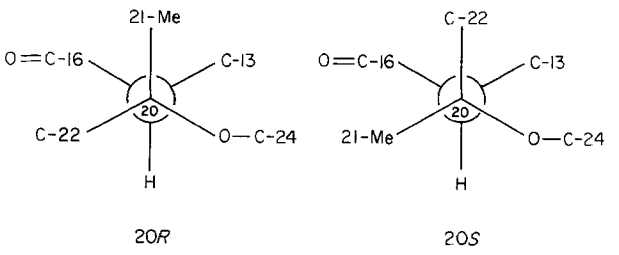


Figure 2. Conformation around the C-17—C-20 linkage of 20*R* compounds **2** and **4**, and their 20*S* epimers **3** and **5**.

Table 3. Carbon-13 chemical shifts differences between (20*R*) and (20*S*) epimers

(20*R*)–(20*S*)	C-13	C-16	C-17	C-20	C-21	C-22
2–3	+0.3	0.0	+0.65	−0.1	−2.7	+4.2
4–5	0.0	+0.1	+0.65	+0.05	−5.55	+4.25

Treatment of 6-methylhept-5-en-2-one (8) with phenylmagnesium bromide

To a cold (−20 °C) solution of **8** (12.6 g, 0.1 M) in diethyl ether (40 ml) was added dropwise, under nitrogen, phenylmagnesium bromide (0.15 M) in diethyl ether (60 ml). After stirring for 3 h at room temperature, aqueous NH_4Cl was added to the reaction mixture, which was then extracted with diethyl ether. The organic phase was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure, and the

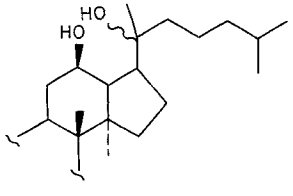


Figure 3. Partial structure of **A**.

crude material was purified by distillation to give **9** (12.2 g, 60%), b.p. 70 °C (0.1 mmHg) [lit.,[10] b.p. 155–156 °C, (19 mmHg)]; $\nu_{max.}$ (film) 3360, 3080, 3050, 3020, 1590, 760 and 705 cm^{-1}; δ_H 1.46 (3H, br s, $W_{1/2}$ 4 Hz, *trans*-6-Me), 1.50 (3H, s, 2-Me), 1.62 (3H, br s, $W_{1/2}$ 4 Hz, *cis*-6-Me), 5.1 (1H, m, $W_{1/2}$ 13 Hz, H-5) and 7.3 (5H, m, $W_{1/2}$ 20 Hz, Ph).

Oxidation of 9 with perbenzoic acid

To a cold (0 °C) solution of the olefin **9** (10 g) in chloroform (250 ml) was added perbenzoic acid (13.5 g) in chloroform (150 ml), and the reaction mixture was kept at this temperature for 5 h. The mixture was poured into ice–water, and the organic phase was washed with aqueous sodium carbonate and water, dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography [benzene–ethyl acetate (90 : 10) as eluant] of the crude material gave *trans*-2-phenyl-2-methyl-5-(1-hydroxyisopropyl)tetrahydrofuran (**10**) (3.5 g) and *cis*-2-phenyl-2-methyl-5-(1-hydroxyisopropyl)tetrahydrofuran (**11**) (4 g).

Compound **10** had b.p. 80 °C (0.15 mmHg); *m/z* 220 (1%, M^+), 205 (2%, M^+-Me), 187 (3%, M^+-Me-H_2O), 162 (14%), 161 (9%), 143 (20%, M^+-Ph), 119 (100%); $\nu_{max.}$ (film) 3560, 3440, 3080, 3040, 3020, 765 and 700 cm^{-1}; δ_H 1.17, 1.29 (2×3H, s, 6-Me_2), 1.50 (3H, s, 2-Me), 3.81 (1H, t, *J* 7 Hz, H-5) and 7.3 (5H, m, $W_{1/2}$ 20 Hz, Ph).

Compound **11** had b.p. 75 °C (0.15 mmHg); *m/z* 220 (1%, M^+), 205 (2%, M^+-Me), 187 (3%, M^+-Me-H_2O), 162 (14%), 161 (7%), 143 (20%, M^+-Ph), 119 (100%); $\nu_{max.}$ (film) 3540, 3440, 3075, 3040, 3010, 765 and 700 cm^{-1}; δ_H 1.13, 1.28 (2×3H, s, 6-Me_2), 1.49 (3H, s, 2-Me), 3.98 (1H, t, *J* 7 Hz, H-5) and 7.3 (5H, m, $W_{1/2}$ 20 Hz, Ph).

Oxidation of 11 with Jones's reagent

Compound **11** (1 g) in acetone (60 ml) was treated at 0 °C with excess of Jones's reagent. The usual work-up gave 2-phenylpentane-5,2-lactone (γ-phenyl-γ-valerolactone) (**12**) (0.48 g), b.p. 80 °C (0.15 mmHg) [lit.,[11] b.p. 123 °C (1 mmHg)]; δ_H 1.71 (3H, s, 2-Me), 2.5 (4H, m, $W_{1/2}$ 9 Hz, H-3 and H-4), 7.32 (5H, br s, $W_{1/2}$ 5 Hz, Ph).

6-Methylhept-5-en-2-one-1,1,1,3,3-$^2H_5$ (8-d_5)

Clean sodium (0.3 g) was allowed to react with CH_3OD (10 ml). Deuterium oxide (10 ml) and the ketone **8** (0.2 g) were added to this solution, and the mixture was refluxed for 3 h. After cooling, the reaction mixture was diluted with diethyl ether, and the organic phase washed with water and dried over sodium sulphate. Evaporation of the solvent gave **8**-d_5 with percentages of deuteriated species (mass spectrometry) as follows: d_1 (1%), d_2 (7%), d_3 (22%), d_4 (35%) and d_5 (35%); δ_H 1.64 (3H, br s, *trans*-6-Me), 1.68 (3H, br s, *cis*-6-Me), 5.0 (1H, m, $W_{1/2}$ 18 Hz, H-5); δ_C 211.0 (C-2), 132.7 (C-6), 122.85 (C-5), 25.7 (C-7), 22.5 (C-4), and 17.6 (C-8).

Trans- and *cis*-2-phenyl-2-methyl-5-(1-hydroxyisopropyl)tetrahydrofuran-1,1,1,3,3-$^2H_5$ (10-d_5 and 11-d_5, respectively)

These compounds were obtained from **8**-d_5 in the same way as **10** and **11** were prepared from **8**.

Compound **10**-d_5, δ_H 1.17, 1.29 (2×3H, s, 6-Me_2), 3.81 (1H, t, *J* 7 Hz, H-5), 7.35 (5H, m, $W_{1/2}$ 20 Hz, Ph); δ_C 148.3 (C-1′), 128.2 (C-3′), 126.4 (C-4′), 124.6 (C-2′), 85.55 (C-5), 84.5 (C-2), 71.1 (C-6), 27.3 (C-7), 26.3 (C-4) and 24.3 (C-8).

Compound **11**-d_5, δ_H 1.13, 1.28 (2×3H, s, 6-Me_2), 3.99 (1H, t, *J* 7 Hz, H-5), 7.4 (5H, m, $W_{1/2}$ 20 Hz, Ph); δ_C 148.3 (C-1′), 128.25 (C-3′), 126.5 (C-4′), 124.5 (C-2′), 85.2 (C-5), 71.6 (C-6), 27.2 (C-7), 26.2 (C-4) and 24.6 (C-8).

2-Phenylpentane-5,2-lactone-1,1,1,3,3-$^2H_5$ (12-d_5)

This compound was obtained from **11**-d_5 as previously described for the preparation of lactone **12** from compound **11**. Compound **12**-d_5, δ_H 2.44 (2H, m, $W_{1/2}$ 6 Hz, H-4), 7.4 (5H, br s, $W_{1/2}$ 5 Hz, Ph); δ_C 144.4 (C-1′), 128.6 (C-3′), 127.6 (C-4′), 124.1 (C-2′), 86.9 (C-2) and 28.8 (C-4).

Acknowledgement

Part of this work was supported by the Investigation Programme of the Comisión Asesora de Investigación Científica y Técnica.

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Received 30 April 1983; accepted (revised) 2 July 1983