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A LUPANE DERIVATIVE AND THE ^{13}C NMR CHEMICAL SHIFTS OF SOME LUPANOLS FROM *PLEUROSTYLIA OPPOSITA*

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Key Word Index—*Pleurostylia opposita*; Celastraceae; triterpenes; new lupane derivatives; ^{13}C NMR chemical shifts.

Abstract—A new lupane derivative isolated from *Pleurostylia opposita* has been assigned the structure 6β-hydroxy-lup-20(29)-en-3-one, using spectral evidence and chemical interconversions. The ^{13}C NMR spectral assignments of 20-hydroxy-lupan-3-one, 6β, 20-dihydroxy-lupan-3-one, 6β, 28-dihydroxy-lup-20(29)-en-3-one and 20-hydroxy-lupane-3, 6-dione previously isolated from the same plant are also reported.

INTRODUCTION

We have previously reported [1] the isolation of α-amyrin, sitosterol, pristimerin, 20-hydroxy-lupan-3-one (**1**), 3β, 20-dihydroxy lupane (**2**) and four oxygenated lupane derivatives (**3–6**) from the stem bark of *Pleurostylia opposita* (Wall) Alston (Celastraceae). We now report the isolation and characterization of a new oxygenated lupane derivative, 6β-hydroxy-lup-20(29)-en-3-one (**7**) from the same plant. Although ^{13}C NMR spectral data of lupenone [2], lupeol [2] and some other lupane triterpenes including 1, 3-, 1, 23- and 1, 28-dioxygenated lupane derivatives [3] is well documented, ^{13}C NMR chemical shifts of 6-oxygenated derivatives of lupane, hopane, ursane or oleanane triterpenes have not been recorded. Here we report the ^{13}C NMR chemical shifts of some lupane derivatives.

RESULTS AND DISCUSSION

A new oxygenated lupane derivative [1] was isolated from both the the benzene and methanol extracts of the stem bark of *P. opposita*. Purification by conventional CC and prep. TLC yielded a pure crystalline sample of **7**.

IR evidence (ν_{max} cm^{-1}: 3450, 1690) indicated the presence of a hydroxy and a carbonyl group. The ^1H NMR spectrum showed signals attributable to the presence of a lup-20(29)-ene system [4] [δ4.70 (1H, *d*), 4.60 (1H, *d*) and 1.68 (3H, *br s*)]. A multiplet at δ 4.50 due to a carbinol methine proton was overlapped by the signal at δ 4.60. The chemical shift of the carbinol methine proton was identical to the chemical shift of the carbinol methine proton at C-6 in the 6β-hydroxy lupane derivatives reported previously [1] and hence suggested the presence of a 6β-hydroxy group in compound **7**. Signals due to six tertiary methyl groups were observed (Table 1). The *t*-methyl region of the spectrum was identical to that of 6β, 28-dihydroxy-lup-20(29)-en-3-one (**6**) reported previously except for the presence of a signal at δ0.81 in **7** (Table 1). The signal at δ0.8 is known to be due to the tertiary methyl group at C-28 [4]. The observed deshielding of C-24–C-26 tertiary methyl resonances (Table 1) which has been attributed to 1, 3-diaxial interactions [1, 5–7] with a hydroxy group, further supported the presence of an axial β-hydroxy group at C-6. Hence **7** was assigned the structure 6β-hydroxy-lup-20(29)-en-3-one.

Table 1. ^1H NMR methyl resonances (δ $CDCl_3$)

Compound	C-23	C-24 C-25 C-26	C-27	C-28	C-29/C-30
4	1.06	1.40 1.44 (3H) (6H)	0.93	0.83	1.10/1.20
6	1.13	1.43 (9H)	0.95	—	—
7*	1.14	1.40 (9H)	0.91	0.81	—

*Solvent: CCl_4.

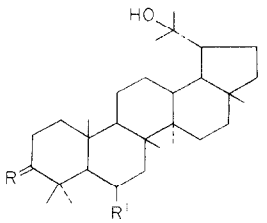
The lupene, **7**, was related to the lupane derivatives reported previously [1] by dehydration to give lupa-5, 20(29)-dien-3-one (**3**) or oxidation to yield lup-20(29)-ene-3,6-dione (**8**) (see Experimental). Compound **7** was also isolated from the reaction mixture resulting from the attempted acetylation of **4**.

Assignment of the carbon chemical shifts in the ^{13}C NMR spectra of compounds **1**, **4**, **6**, **7** and **10** was made by comparison with the ^{13}C NMR spectral data of known lupane derivatives such as lupenone and lupeol, the use of observed multiplicities of signals in the off-resonance decoupled spectra, the known chemical shift rules due to hydroxy substitution and also steric γ- and δ-effects [8].

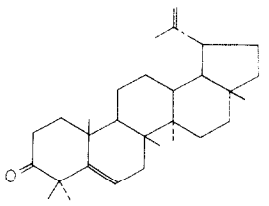
The ^{13}C NMR chemical shifts of compounds **1**, **4**, **6** and **7** were found to closely resemble those of lupenone, **9**, but with predictable differences (Table 2). The lowfield quaternary carbon signal at δ 73.4 in the spectra of **1** and **4** was assigned to C-20. Two methyl resonances due to C-29 and C-30 appeared at lowfield (δ24.8–25.2 and 31.6) and were absent in the ^{13}C NMR spectrum of lupenone [2]. The signal at δ28.7, assigned to C-12, was found to be deshielded in both **1** and **4** by δ 3.5 compared to the chemical shift of this carbon in lupenone. Examination of Dreiding models revealed that the molecule could exist in a preferred conformation in which the oxygen atom (of the hydroxy group at C-20) with its lone pair of electrons lies in very close proximity to C-12 resulting in the observed shift.

In the 6β-hydroxy lupanes **4**, **6** and **7** the doublet due to C-6 appeared at δ69.6–69.7 which was δ50 to lower field of the C-6 signal in **1** confirming the presence of a hydroxy group at C-6. It was observed that the β-effect of the hydroxy group in these compounds was greater at C-7 (δ8.3) than at C-5 (δ1.7) as in the case of 6β-androstanol [9]. The γ-effect of the 6β-hydroxy group was seen to be negligible at C-4, C-8 and C-10. This can be explained in terms of the syn-diaxial interaction of the hydroxy group with the axial methyl groups C-24–C-26, which brings about a deformation of the hydroxy group, moving it away from the γ-carbons and thus reducing its influence on these carbons. Evidence for syn-diaxial interactions of the hydroxy group with these tertiary methyl groups was also observed in the ^1H NMR spectra of the 6β-hydroxy lupane derivatives (Table 1). Zero shifts of a similar nature have been observed [7] for steroidal alcohols where the carbon γ to the hydroxy group is quaternary. The signals due to the axial methyl groups C-24–C-26 have been shifted δ1.0–2.7 downfield when compared to those of lupenone (**9**) due to the δ-effect of the axial hydroxy group at C-6.

The ^{13}C NMR spectrum of **6** showed two signals in the δ60–70 region corresponding to two carbons bearing hydroxy substituents. By comparison with **4** the signals at δ 69.6 and 60.5 were assigned to C-6 and C-28 respectively. Comparison with **4** and **7** showed that C-17 in **6** had been shifted downfield by δ 3.20–4.0 due to the β-effect of the hydroxy group at



1 R=O, R¹=H
2 R=α-H, β-OH, R¹=H
4 R=O, R¹=OH



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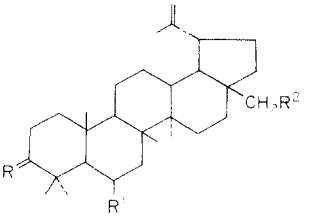
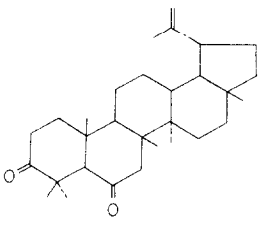


5 R=α-H, β-OH, R¹=OH, R²=H
6 R=O, R¹=R²=OH
7 R=O, R¹=OH, R²=H
9 R=O, R¹=R²=H



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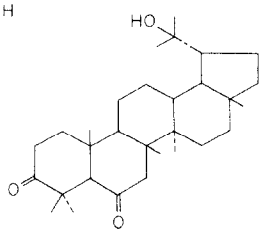

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Table 2. ^{13}C NMR chemical shifts of lupane derivatives*

Carbon no.	1	4	6	7	9	10
C-1	39.6	42.5	42.2	43.0	39.6	43.4
C-2	34.6	34.4	34.5	34.5	34.1	33.8
C-3	217.8	216.8	216.6	216.7	217.9	214.6
C-4	47.2	48.9	48.9	49.0	47.3	48.5
C-5	54.9	56.6	57.0	56.6	55.0	65.1
C-6	19.7	69.7	69.6	69.7	19.6	211.6
C-7	33.9	42.1	42.2	42.2	33.6	52.2
C-8	41.4	40.7	40.2	40.0	40.8	41.0
C-9	50.0	50.6	50.7	50.7	49.8	50.2
C-10	36.8	36.8	36.5	36.8	36.9	46.2
C-11	22.0	21.8	21.2	21.3	21.5	21.7
C-12	28.7	28.7	25.4	25.2	25.2	28.6
C-13	37.7	36.8	34.0	37.2	38.2	37.3
C-14	43.6	43.9	43.1	42.2	42.9	44.4
C-15	27.6	27.7	27.2	27.5	27.4	27.6
C-16	35.6	35.6	36.8	35.5	35.6	35.4
C-17	44.6	44.6	47.8	43.2	42.9	43.9
C-18	48.3	48.5	47.8	48.3	48.3	48.1
C-19	49.7	50.1	47.9	48.0	47.9	50.1
C-20	73.4	73.4	150.3	150.8	150.7	73.3
C-21	29.7	29.1	29.8†	29.8	29.9	28.2
C-22	40.2	40.2	29.2†	39.9	40.0	39.9
C-23	26.7	24.9	25.1	25.0	26.6	24.1
C-24	21.0	23.7	23.8	23.7	21.0	21.9
C-25	16.0	17.5	17.0	17.0	15.8	16.4†
C-26	16.0	17.0	17.1	17.1	15.4	16.2†
C-27	14.8	15.2	15.1	14.8	14.4	15.2
C-28	19.2	19.2	60.5	18.0	18.0	19.2
C-29	31.6	31.7	109.8	109.4	109.2	32.0
C-30	24.8	25.2	19.5	19.3	19.3	24.5

*Chemical shifts (in δ values) relative to internal TMS; error $\pm \delta$ 0.1.

†Values may be interchanged.

C-28. The γ-gauche effect of the C-28 hydroxy group on C-22 had resulted in shielding of the signals due to C-22 by δ10, when compared with the chemical shift of this carbon in **1**, **4** and **7**. The signals due to C-16 and C-18, both of which are γ to the hydroxy group were found to show only small shifts and as such the hydroxy group probably has a γ-*trans* conformation to these carbons. Examination of a Dreiding model of **6** showed that the hydroxy group at C-28 could well be γ-*trans* to C-16 and C-18 due to their axial hydrogens on the β-face, whereas C-22 has only a quasi-equatorial hydrogen on this face. This would favour the γ-gauche conformation between the C-28 hydroxy group and C-22.

^{13}C NMR signals of **7** were found to show a close relationship to those of lupenone and **4**, thus confirming the assigned structure. The chemical shifts of the signals in the ^{13}C NMR spectrum of **10** were assigned by comparison with **4**. Oxidation of the secondary hydroxy group at C-6 to C=O in **10** was seen to result in predictable changes in chemical shifts of the neighbouring carbon atoms.

EXPERIMENTAL

Mps were determined on a Kofler hot stage apparatus and are uncorr. Identity of compounds was established by mmp, co-TLC, IR and NMR comparison. Petrol refers to the fraction having boiling range 40–60°. TLC and prep. TLC were carried out on Merck Kiesel gel 60 $PF_{254+365}$. Optical rotations were measured at 25° in $CHCl_3$. IR spectra were recorded using KBr discs. ^1H NMR spectra were recorded at 60 MHz with TMS as int. standard. MS were determined at the Research School of Chemistry, The Australian National University. ^{13}C NMR spectra were recorded in $CDCl_3$ solvent, on a JEOL FX 100 instrument operating at 25.2 MHz at the Swedish University of Agricultural Sciences. Microanalyses were performed by the CSIRO, Microanalytical Service, Melbourne.

Extraction and isolation of compounds. The C_6H_6 extract (28.0 g) of the stem bark of *P. opposita* yielded a triterpene **7** (0.4 g) after chromatography on Si gel. The MeOH extract (18.0 g) of the same plant material after Si gel chromatography (petrol–$CHCl_3$) yielded the same triterpene **7** from the $CHCl_3$ fraction along with sitosterol. Sitosterol and **7** were separated by prep. TLC ($CHCl_3$), **7** being identified as 6β-hydroxy-lup-20(29)-en-3-one. The lupene **7** was recrystallized (0.2 g) from petrol as white crystals, mp 233–234°, $[\alpha]_D - 14.0°$ (*c* 2.0); (found C, 81.79; H, 10.94%; $C_{30}H_{48}O_2$ requires C, 81.75; H, 10.99%); IR ν_{max} cm^{-1}: 3450, 1690 and 880; ^1H NMR ($CDCl_3$): δ 4.70 (1H, *br d*, H-29), 4.60 (2H, *m*, H-6 and H-29), 1.68 (3H, *br s*, vinylic CH_3), 1.40 (9H, *s*, 3 × *t*-Me), 1.14, 0.91 and 0.81 (each 3H, *s*, *t*-Me); MS *m/z*

(rel. int.): 440 $[M]^+$ (10), 258(7), 218(100), 205(32), 204(29), 203(78), 189(43), 175(24), 161(30), 149(31), 145(35), 135(40), 133(43), 121(57).

Characterization of 6β-*hydroxy-lup*-20(29)-*en*-3-*one* (**7**). (*a*) *Oxidation of* **7**. A mixture of **7** (0.07 g), CrO_3 (0.07 g) and pyridine (5 ml) was kept at room temp. for 12 hr. The mixture was poured into cold H_2O and extracted with Et_2O. Prep. TLC ($CHCl_3$–petrol, 3:1) and crystallization from MeOH gave colourless crystals (0.05 g) of lup-20(29)-ene-3, 6-dione (**8**), mp 180–182° (lit. 180–181° [1]); (found M^+ 438.350; $C_{30}H_{46}O_2$ requires M^+ 438.398); IR ν_{max} cm^{-1}: 1725, 1710 and 880; ^1H NMR (CCl_4): δ 4.69 (1H, *br d*, H-29), 4.50 (1H, *br d*, H-29), 1.66 (3H, *br s*, vinylic Me), 1.43, 1.23, 1.09, 1.03, 1.00, 0.80 (each 3H, *s*, 6 × *t*-CH_3); MS *m/z* (rel. int.): 438 $[M]^+$ (81), 423(40), 370(30), 327(18), 233(10), 218(100), 205(46), 204(18), 203(42), 189(31), 177(24), 175(14), 161(16), 149(25), 135(26), and 121(32).

(*b*) *Dehydration of* **7**. Pyridine (5 ml), $POCl_3$ (0.5 ml) and **7** (0.05 g) were stirred at room temp. for 30 min, then poured into cold H_2O and extracted with Et_2O. Prep. TLC and crystallization from MeOH yielded colourless crystals mp 214–216° of lupa-5, 20(29)-dien-3-one (**3**) shown to be identical to the natural product described previously [1].

(*c*) *Acetylation of* 6β,20-*dihydroxy-lupan*-3-*one* (**4**). Pyridine, Ac_2O and **4** were heated at 100° for 20 hr. The usual work-up was followed by prep. TLC ($CHCl_3$–petrol, 3:1). 6β-Hydroxy-lup-20(29)-en-3-one (**7**) was isolated from the reaction mixture, and shown to be identical with the natural product.

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