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A pentacyclic triterpene from *Daphne oleoides*

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Abstract

From the whole plant extract of *Daphne oleoides*, a new triterpene and the known 20(30)-lupene-3,29-diol, butilin, and stigmasterol glycoside have been isolated for the first time from this source. The new triterpene was established as lup-20 (30)-ene-3α, 29-diol on the basis of homonuclear ^{1}H decoupled ^{13}C, DEPT, ^{1}H–^{13}C HMQC, long range HMBC NMR spectral studies and by chemical methods.

Keywords: Daphne oleoides; Thymelaeaceae; Lup-20(30)-ene-3α; 29-diol; Triterpenoids; Steroids

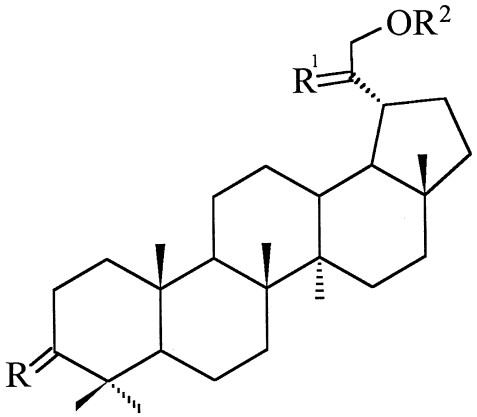
1. Introduction

As a part of our on going phytochemical studies on *Daphne oleides*, we have recently reported lignans (Ullah, Anis, Mohammed, Rabnawaz, & Malik, 1998) and dimeric guaianolides (Ullah, Ahmed, Mohammed, Ahmed, & Malik, 1998). In this paper, we now wish to report the isolation and characterisation of a new triterpene (**1**) besides the known compounds lup-20(30)-ene-3,29-diol (**2**), butilin (**3**) and stigmasterol glucoside (**4**) from the whole plant extract of *Daphne oleoides*, which is a medicinally important xerophytic shrub belonging to the family Thymelaeaceae. Roots of this plant are purgative, barks and leaves are given in cutaneous infections and infusion of the leaves is given in gonorrhoea and applied to abscesses (Baquar, 1989).

2. Results and discussion

Compound **1** was obtained as colourless needles, m.p 234–235°C. The HR mass spectrum exhibited the molecular ion peak at m/z 442.3767 corresponding to the molecular formula $C_{30}H_{50}O_2$ (calc. for $C_{30}H_{50}O_2$ 442.3562). The IR spectrum gave absorption bands at 3435, 3072, 1642 and 888 cm^{-1} characteristic of a hydroxyl and a terminal methylene group, respectively. Further spectral data showed close agreement to lupene type of triterpenes.

The ^{1}H NMR spectrum of **1** displayed signals due to six tertiary methyl groups at δ 0.66 (3H, s), 0.74 (3H, s), 0.77 (3H, s), 0.87 (3H, s), 0.98 (3H, s) 0.98 and (3H, s). The spectrum showed a signal due to carbinolic proton at δ 3.88 (1H, dd, J=4.2, 2.12 Hz). The hydroxyl group was shown to be secondary by its easy acetylation to diacetate **1b**, in which the signal at δ 3.88 was shifted downfield to δ 4.83 and the signals of H-29 and H-29′ were shifted to δ 5.05 (br. s, H-29) and δ 5.15 (br. s, H-29′). A downfield signal at δ 4.25 (2H, s) was assignable



(**1**). R = H(α), OH : $R^1 = CH_2$: R^2 = H

(**1a**). R = H(α), OH : $R^1 = CH_3$, H : R^2 = H

(**1b**). R = H(α), OAc : $R^1 = CH_2$: R^2 = OAc

(**2**). R = H(β), OH : $R^1 = CH_2$: R^2 = H

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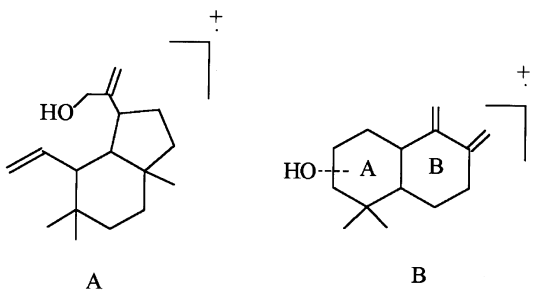
to vinylic protons, and two further downfield signals at δ 4.76 (1H, br. s) and 4.86 (1H, br. s). These signals could be assigned to H-29 and H-29′, respectively. The presence of a terminal double bond was confirmed by hydrogenation which gave the dihydro derivative (**1a**). The vinylic signal of **1** at δ 4.25 disappeared and doublets of methyl and methylene groups were observed at δ 1.1 (3*H*, d) and δ 3.72 (2H, d).

The EI mass spectrum of **1** was characteristic of lupene type triterpenes and exhibited diagnostically important peaks at *m/z* 442 (M^+), 424 (M–H_2O), 409 (M-33), 234 (A), 220 (B), 207, 203 (B-18), 191 and 189. This fragmentation pattern strongly supported that compound **1** was of the lup-20(30)-ene type, allowing the allocation of the one hydroxyl group to C-29 and that of another to ring A (Budzikiewicz, Wilson, & Jerrasi, 1963).



A B

The BB and DEPT ^{13}C NMR spectra showed the presence of six methyls, twelve methylenes, six methines and six quaternary carbon atoms. These were assigned on the basis of comparison with similar triterpenes (Mochammad, Kazuo, Ryoji, & Osamu, 1980) as well as HMQC and HMBC experiments.

The position of the hydroxyl group in ring A was assigned to C-3 by analogy. It was confirmed by long-range HMBC experiments. The geminal methyl groups at C-4 not only showed cross peaks with each other but both the methyl protons also showed cross peaks with oxygen bearing C-3 (δ 76.63). These two methyl peaks also showed cross peaks to a methine carbon C-5 (δ 54.89), which also showed a third cross peak from a methyl proton signal, assigned to the C-25 (δ 15.74) methyl group. In this way, it was possible to work around the triterpene skeleton, assigning both the skeletal structure and most of the carbon signals.

Having established the site of the hydroxyl group as C-3, the axial orientation followed from the small coupling constant ($J \sim 2$) of the methine proton in the ^1H NMR spectra of compounds **1**–**1b** (Herz, Santhanam, & Inger, 1972), which was reconfirmed by ^1H-J resolved experiment. On the basis of the above evidences, the structure of compound **1** was assigned as lup-20(30)-ene-3α, 29-diol. The corresponding 3β-epimer has earlier been isolated from *Flourensia heterolepis* (Ferdinand & Jasmin, 1979).

3. Experimental

Mps (uncorr) were determined on a Buchi 535 melting point apparatus. $[\alpha]_D$ values were on a JASCO DIP-360 instrument. ^1H NMR and ^{13}C NMR were obtained on a Bruker AM-300 spectrometer. The DEPT experiments were carried out with $\theta = 45°$, 90° and 135°. Chemical shifts were reported in δ ppm, with TMS as internal standard. EIMS on Finnigan MAT-312 double focusing mass spectrometer. IR JASCO A-302. Kieselgel 60 (35–70) mesh was used for CC. TLC was carried out on silica gel plates using the solvent system of $CHCl_3$–MeOH (9.5:0.5). Precoated Kieselgel 60, F_{254} aluminium sheets (E. Merck, Art. No. 1.05554) was used to check the purity. Spots were visualised by spraying with ceric sulphate solution in 10% H_2SO_4 followed by heating.

The whole plant of *Daphne oleoides* was collected from Hazara division of NWF province in February, 1995. A voucher specimen was identified by Professor Iftikhar Hussain Shah and deposited in the Herbarium of the faculty of the Pharmacy, Gomal University, D.I. Khan, Pakistan.

3.1. Extraction and isolation

The shade dried plant material (16 kg) was extracted 3× with MeOH. The combined methanolic extract was evaporated under red. pres. The residue obtained was suspended in H_2O and extracted successively with petrol, EtOAc, $CHCl_3$ and *n*-BuOH. The hexane fraction was subjected to VLC eluting with pure $CHCl_3$ and $CHCl_3$–MeOH gradient systems to obtain subfractions A, B, C, D, E and F, respectively. The fraction C obtained by eluting with $CHCl_3$:MeOH (9.8:0.2), was further subjected to repeated CC eluting by $CHCl_3$:MeOH gradient system to afford compound **3** (9.75:0.25), **2** (9.7:0.3), **1** (9.6:0.4) and **4** (9:1), respectively.

3.2. Compound *1*

Colorless needles, $C_{30}H_{50}O_2$; m.p 234–235°C; $[\alpha]_D^{CHCl_3}$ -17° (*c* 1.0); IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 3072, 1642, and 888 (terminal methylene); EIMS *m/z* (rel. int): 442 [M^+] (74), 424 [M–H_2O] (100), 409 [M-33] (27), 234 [A] (23), 220 [B] (31), 207 (11), 203 [B–OH] (8), 191 (8), and 189 (5). ^1H NMR (300 MHz, DMSO-d_6), δ 0.66 (3H, s), 0.74 (3H, s), 0.77 (3H, s), 0.87 (3H, s), 0.91 (3H, s), 0.98 (3H, s), 3.88 (1H, dd, $J = 4.2$, 2.12 Hz, H-3), 4.25 (2H, br. s, H-30), 4.76 (br. s, H-29), and 4.85 (br. s, H-29′). ^{13}C NMR is presented in Table 1.

3.3. Acetylation of compound *1*

Compound **1** (8 mg) was dissolved in pyridine (1 ml) and treated with Ac_2O (3 ml) at room temp. overnight. Ice was added to the reaction mixture which was

Table 1
^{13}C NMR assignments of compound **1**. (75.43 MHz, DMSO-d_6)

C#	DEPT	^{13}C	C#	DEPT	^{13}C
1	CH_2	38.27	16	CH_2	35.02
2	CH_2	27.16	17	C	42.52
3	CH	76.63	18	CH	48.07
4	C	38.48	19	CH	43.21
5	CH	54.89	20	C	155.05
6	CH_2	17.96	21	CH_2	28.96
7	CH_2	33.93	22	CH_2	39.35
8	C	40.46	23	CH_3	28.09
9	CH	49.90	24	CH_3	15.74[a]
10	C	36.71	25	CH_3	15.74[a]
11	CH_2	20.55	26	CH_3	15.88
12	CH_2	26.0	27	CH_3	14.35
13	CH	37.58	28	CH_3	17.45
14	C	42.34	29	CH_2	62.70
15	CH_2	27.05	30	CH_2	105.69

[a]Assignments may be interchanged.

Table 2
^{13}C NMR assignments of compound **2**. (75.43 MHz, pyridine-d_5)

C#	DEPT	^{13}C	C#	DEPT	^{13}C
1	CH_2	39.14	16	CH_2	35.87
2	CH_2	27.44	17	C	42.86
3	CH	78.05	18	CH	49.05
4	C	39.31	19	CH	43.51
5	CH	55.72	20	C	155.36
6	CH_2	18.16	21	CH_2	29.03
7	CH_2	34.67	22	CH_2	39.20
8	C	41.11	23	CH_3	28.49
9	CH	50.65	24	CH_3	16.03[a]
10	C	37.32	25	CH_3	16.03[a]
11	CH_2	20.95	26	CH_3	16.22
12	CH_2	25.64	27	CH_3	14.81
13	CH	37.47	28	CH_3	18.10
14	C	42.35	29	CH_2	63.05
15	CH_2	28.03	30	CH_2	106.08

[a]Assignment may be interchanged.

extracted with EtOAc and H_2O. The EtOAc layer was evaporated to yield the diacetate **1b**. IR ν_{max}^{KBr} cm^{-1}: 2850, 1750 ($OCOCH_3$), 3065, 1622, and 873 (terminal methylene); EIMS *m/z* (rel. int): 526 [M^+] (3), 466 [M^+-AcOH] (100), 276 (27), 258 (29), 198 (7), 186 (9). ^1H NMR ($CDCl_3$, 300 MHz), δ 0.69 (s, CH_3),), 0.75 (s, CH_3), 0.81 (s, CH_3), 0.85 (s, CH_3), 0.89 (s, CH_3), 1.02 (s, CH_3), 2.02 ($OCOCH_3$), 4.41 (2H, br. s, H-30), 5.05 (br. s, H-29), and 5.15 (br. s, H-29′).

3.4. Compound (*2*)

Colorless needles, m.p 235–236°C, $[\alpha]_D^{CHCl_3}$ −16°. The physical and spectral data coincided with literature values of Ferdinand & Jasmin (1979). In the present work ^{13}C NMR spectrum of **2** has been recorded and assigned for the first time (Table 2).

3.5. Butilin (*3*)

Cryst, $C_{30}H_{50}O_2$; m.p 251–252°C; $[\alpha]^{Py}{}_D$ +20°. The physical and spectral data matched with the published data of Mochammad et al. (1980).

3.6. Stigmasterol-3-O-β-D-glucopyranoside (*4*)

Cryst, m.p 299°C. The physical and spectral data coincided with the published data of Wright, Burton, & Berry (1962).

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