PERGAMON

Phytochemistry 51 (1999) 559–562

PHYTOCHEMISTRY

Dimeric guaianolides from *Daphne oleoides*

Nisar Ullah, Saeed Ahmed, Zaheer Ahmed, Pir Mohammad, Abdul Malik*

International Center for Chemical Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi, 75270, Pakistan

Received 16 November 1998; received in revised form 16 November 1998

Abstract

From the whole plant extract of *Daphne oleoides*, two dimeric guaianolides seemarin and anabsinthin were isolated and characterized as new natural products. The structures of these lactones were determined by NMR spectral studies as well as by chemical methods.

Keywords: Daphne oleoides; Thymelaeaceae; Dimeric guaianolides; Seemarin; Anabsinthin

1. Introduction

As a part of our ongoing phytochemical studies on *Daphne oleoides* Schreb, we have recently reported some lignans and triterpenoids from this species (Ullah et al., 1998; Ullah, Ahmed, Anis, & Malik, 1998). In this paper, we now report on the isolation and structural elucidation of two new dimeric guaianolides from the whole plant extract of *D. oleoides* a small multi-branched shrub, found on the Western Himalaya, from Garhwal Westward to Murree, occurring at an altitude of 3000–9000 feet (Watt, 1972). The root of this plant is a purgative, the bark and leaves are applied to skin conditions and an infusion of the leaves is used to treat gonorrhoea and applied to abscesses (Baquar, 1989).

2. Results and discussion

Compounds **1** and **2** were isolated from the chloroform-soluble fraction of the methanol extract of ground, shade dried plant material.

Seemarin (**1**) was found to have an M_r of 512 by positive ($[M+Na]^+$ 535) and negative FABMS ($[M-H]^-$ 511). Its molecular formula was determined as $C_{30}H_{40}O_7$ by negative HRFABMS ($[M\text{-}I]^+$, m/z 511.2011). The IR spectrum contained absorption bands at 1655, 1755–1765 and 3390 cm^{-1} characteristic of a double bond, the carbonyl of γ-lactone and a hydroxyl group, respectively. Further spectral data showed close agreement to absinthin, the dimeric guaianolide, isolated from *Artemisia absinthium* (Beauhaire, Fourrey, Vuilhorgne, & Lallemand, 1980).

The ^1H NMR spectrum showed two signals at δ 1.10 (3H, d, $J=6.8$ Hz) and 1.14 (3H, d, $J=7.0$ Hz) for two methyl groups (H_3-13 and H_3-13′) attached to methine carbons, three signals at δ 1.32 (3H, s), 1.42 (3H, s) and 1.80 (3H, s) attributed to H_3-14′, H_3-14 and H_3-15, and another broadened peak at δ 1.92 (3H, br. s) assigned to a vinylic methyl (H_3-15′). The signals of protons geminal to the lactone oxygen atoms (lactone protons) appeared as one proton doublets at δ 4.82 (1H, d, $J=10.2$ Hz) and 4.65 (1H, br. d, $J=10.9$ Hz). The latter was considerably broadened due to allylic coupling and, therefore, assigned to H-6′ while the former was sharp and subsequently assigned to H-6. The nature of the splitting of these signals revealed that each of the lactone protons interacts with only one vicinal proton. The spectrum further showed signals due to hydroxyl groups at δ 2.25 (1H, d, $J=2.31$ Hz) and 2.95 (1H, d, $J=1.7$ Hz). No acetylation was observed upon subjecting **1** to acetylation under nor-

* Corresponding author.

0031-9422/99/$ - see front matter
PII: S0031-9422(98)00762-6

mal acetylation conditions, suggesting the nature of these hydroxyl groups to be tertiary. This was further confirmed by treatment of **1** with trichloroacetyl isocyanate (in situ, in NMR tube) when the resonances at δ 2.25 and 2.95 were replaced by two broad singlets at δ 8.10 and 8.25, respectively. However, unlike absinthin compound **1** did not show an olefinic H-3 signal. Therefore, seemarin had to be a dimeric guaianolide with the double bond replaced by a ring because both **1** and absinthin have the same number of unsaturations (11).

In the ^{1}H–^{1}H COSY-45° spectrum, H-2 (δ 2.95) was coupled with H-2′ (δ 2.85), which was coupled with H-1′ (δ 2.40) and H-3′ (δ 2.94). H-6 (δ 4.82) was coupled with H-7 (δ 1.72), and H-6′ (δ 4.65) was coupled with H-7′ (δ 1.65).

The ^{13}C NMR and DEPT experiments displayed the signals of five methylene, 10 methine and six methyl carbon atoms. The quaternary carbon atoms were deduced by subtracting these from the BB spectrum. The low field region of the ^{13}C NMR spectrum of **1** showed four signals at δ 134.0, 148.4, 178.5 and 179.0 which were assigned to vinylic carbons C-4′ and C-5′ and lactone moieties, respectively. The presence of a tetrasubstituted double bond was also shown in the ^{1}H NMR spectrum which showed signal of a vinylic methyl at δ 1.92 (3H, br. s). The ^{13}C NMR spectrum further showed signals of two more quaternary carbons at δ 73.8 and 74.8, which could be assigned to C-10′ and C-10. Four methylenes signals at δ 24.3, 26.8, 40.4 and 44.6 were assigned to C-8′, C-8, C-9 and C-9′ through comparison of the ^{13}C NMR spectrum with that of absinthin (Bohlmann, Ang, Trinks, Jakupovic, & Huneck, 1985). The ^{13}C assignments were further confirmed by ^{1}H–^{1}H COSY and HMQC experiments. The stereochemistry of the carbons of the allylic lactone was established by comparison of the ^{1}H NMR spectrum of **1** with that of absinthin. The value of the coupling constant J 6′,7′ = 10.9 Hz as well as the upfield position of the H-6′ signal (δ 4.65) in **1** were similar to those of absinthin, strongly suggesting that in compound **1** the allylic lactone was *trans*-fused as in the case of absinthin (Beauhaire, Fourrey, Vuilhorgne, & Lallemand, 1981). The configuration of the chiral centers were further confirmed by NOE difference measurements. The remaining problem was to assign the correct chirality at C-2′ and C-3′. This was deduced by extensive NOE difference spectroscopy. Irradiation of the signal at δ 2.85 (H-2′) caused enhancement of the signal at δ 2.95 (OH) which was not only indicative of the presence of a hydroxyl group at C-1 in **1** but also showed the endo-mode of cycloaddition. This fact was also deduced from the ^{13}C NMR spectrum which showed downfield resonance for C-1 at δ 84.4. The NOEs between H-2, H-14 and H-14′ allowed the assignment of the configuration at C-10 and C-10′. Further NOE interactions were in accordance with the assigned structure and stereochemistry and are summarized in Table 1. The ^{13}C spectrum was in accordance to above assignments, showing resonances at δ 57.3 (C-1′), 43.9 (C-2), 47.8 (C-2′), 61.10 (C-3′) and 135.0 (C-4′).

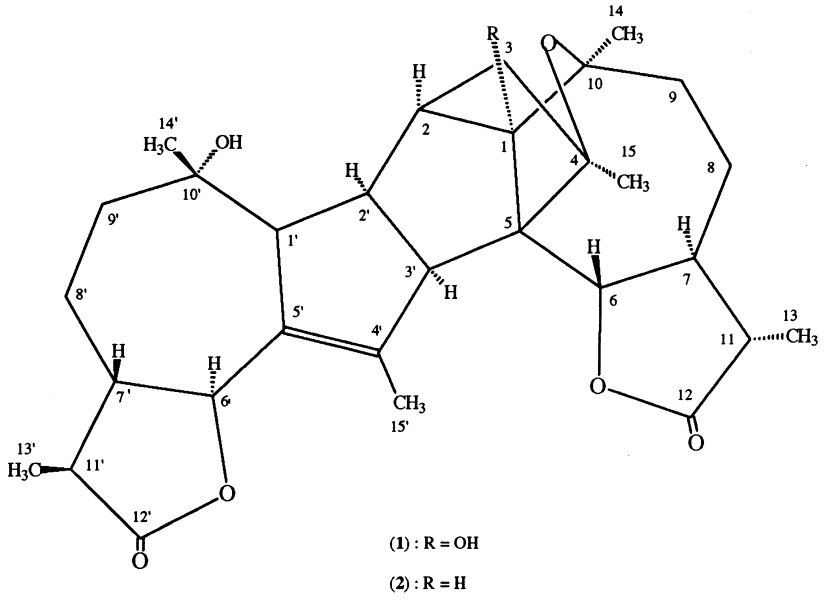
Table 1
NOEs for **1** and **2**

Compound **1**		Compound **2**	
OH-1	H-2′, H-3′	H-1	H-2′, H-3′, H-7
H-2′	OH-1, H-3′, H-14′	H-2′	H-1′, H-3′, H-14′
H-3′	OH-1	H-3′	H-I
H-7	OH-1, H-13, H-3′	H-7	H-1, H-13, H-3′
H-14	H-2	H-14	H-2
H-14′	H-2′	H-14′	H-2′
H-15′	H-3′, H-6′	H-15′	H-3′, H-6′
H-2	H-14, H-14′	H-2	H-14, H-14′

The above substitution pattern was further confirmed by HMBC experiments. The proton at δ 2.94 (H-3′) showed cross peaks to carbon atoms at δ 62.3 (C-5), 84.4 (C-1), 45.5 (C-2′) and 134.0 (C-4′). Similarly another proton at δ 2.85 (H-2′) showed cross peaks to carbons at δ 43.9 (C-2), 84.4 (C-1), 57.5 (C-1′) and 57.2 (C-3′). Likewise the methyl protons at δ 1.80 (H_3-15) showed interaction with δ 88.2 (C4), 62.3 (C-5) and 35.3 (C-3). The vinylic methyl protons at δ 1.92 (H_3-15′) showed interactions with δ 134.0 (C-4′), 148.4 (C-5′) and 57.2 (C-3′). In this way it was possible to work around the dimeric guaianolide skeleton and to assign the skeletal structure and signals of most of the carbon atoms. We were not able to assign the stereochemistry of H_3-15 on the basis of NOE measurements. A study of a Drieding model, however, showed that by placing the H_3-15 in an α configuration generated strain in its corresponding rings. The remaining choice was, therefore, to place H_3-15 in the β-configuration. On the basis of the above evidence, we have established the structure of compound **1**.

Compound **2** was assigned the molecular formula $C_{30}H_{40}O_6$ by HRMS {$[M]^+$, m/z 496.2818 (calc. for $C_{30}H_{40}O_6$ 496.2910)}. The fragmentation pattern in the mass spectrum as well as the IR spectrum were identical to compound **1**. The ^{1}H NMR and ^{13}C NMR spectra of compound **2** was also very similar to those of **1** except for the replacement of the doublet at δ 2.95 (OH-1) by δ 2.36 (1H, br. s) as well as the upfield shift of the signal of C-1 at δ 68.6, suggesting the replacement of the hydroxyl group at C-1 by a proton. Strong NOEs between H-1, H-2′, H-3′ and H-7, strongly suggested the endo-mode of cycloaddition. In the light of the above evidence, compound **2** was characterized as the 3-desoxy **1**, a compound which had been obtained synthetically by the name of anabsinthin through isomerization of absinthin in weakly

acidic medium (Beauhaire et al., 1981). This is the first instance of the isolation of anabsinthin from a natural source. To the best of our knowledge this is also the first report of the occurrence of sesquiterpene lactones in family Thymelaeaceae.



3. Experimental

MP: uncorr.; ^{1}H NMR and ^{13}C NMR: Bruker AM-500. The DEPT experiment were carried out with $\theta = 45$, 90 and 135°. Chemical shifts were reported in δ ppm, with TMS as internal standard. EIMS: Finnigan MAT-312 double focusing mass spectrometer; CC: Kieselgel 60 (35–70) mesh; TLC: silica gel using the solvent system of hexane–isopropanol (9.5:0.5). Precoated Kieselgel 60, F_{254} aluminum sheets (E. Merck, Art. No. 1.05554) were used to check the purity. Spots were visualized by spraying with ceric sulphate solution in 10% H_2SO_4 followed by heating.

3.1. Plant material

The whole plant of *D. oleoides* was collected from Hazara division of N.W.F.P. province in February, 1995. A voucher specimen (D-16995) was identified by Professor Iftikhar Hussain Shah and deposited in the Herbarium of the faculty of the Pharmacy, Gomal University, D.I. Khan, Pakistan.

3.2. Extraction and isolation

The shade dried plant material (16 kg) was extracted (×3) with MeOH. The combined methanolic extract was evaporated under reduced pressure. The residue was suspended in H_2O and extracted successively with petrol, EtOAc, $CHCl_3$ and *n*-BuOH. The $CHCl_3$ was evaporated to give 70 g residue which was subjected to

Table 2
^{1}H NMR spectral data of compounds **1** and **2** and absinthin[a]

H	**1**	**2**	Absinthin
1	–	2.14 br. s	1.98 br. s
2	2.95 m	2.40 m	2.84 br. dd
3	–	–	5.55 br. s
6	4.82 d	4.64 d	4.72 d
7	1.72 m	1.75 m	1.80 m
11	2.10 dq	2.21 dq	2.18 dq
13	1.10 d	1.12 d	1.23 d
14	1.42 s	1.40 s	1.17 s
15	1.80 s	1.82 s	1.77 d
1′	2.40 br. s	2.41 br. s	2.28 br. s
2′	2.85 ddd	2.80 ddd	2.81 ddd
3′	2.94 br. d	2.98 br.d	3.19 br.d
6′	4.65 br. d	4.69 br. d	4.59 br. d
7′	1.65 m	1.72 m	1.64 m
11′	2.25 dq	2.35 dq	2.23 dq
13′	1.14 d	1.10 d	1.19 d
14′	1.32 s	1.31 s	1.29 s
15′	1.92 br. s	1.86 br. s	1.94 br. s
1-OH	2.95	–	–
10-OH	2.25	2.19	2.15

[a] *J* (Hz) **1**: 6, 7 = 10.2, 11, 13 = 6.8; 1′, 2′ = 4.2; 2′, 3′ = 8.4; 6′, 7′ = 10.9; 11′, 13′ = 7.0; 9α, OH-10′ = 2.2; OH-1, 2 = 1.8. **2**: 1, 2 = 1.2; 6, 7 = 10.3; 11, 13 = 7.1, 1′, 2′ = 3.8; 2′, 3′ = 8.0; 6′, 7′ = 10.8; 11′, 13′ = 7.2; 9α, OH = 1.9. **3**: 1, 2 = 1; 2, 3 = 2.5; 2, 2′ = 4; 3, 15 = 1.5; 6, 7 = 10; 11, 13 = 7; 1′, 2′ = 4; 2′, 3′ = 8; 6′, 7′ = 11; 11′, 13′ = 7; 9α, OH = 2.

Table 3
^{13}C NMR spectral data of compounds **1** and **2** and absinthin

C	DEPT	**1**	**2**	Absinthin
1	C	84.4	68.6 (CH)	71.4 (CH)
2	CH	43.9	43.2	45.7
3	CH_2	35.3	34.6	122.1 (CH)
4	C	88.2	89.1	148.5
5	C	62.3	63.8	64.2
6	CH	82.5	81.9	82.7
7	CH	47.5	47.2	46.7
8	CH_2	26.8	26.2	27.5
9	CH_2	40.4	41.3	42.5
10	C	74.8	74.2	71.9
11	CH	41.6	41.7	42.0
12	C	178.5	178.2	178.4
13	CH_3	12.6	12.8	13.1
14	CH_3	30.3	29.4	32.3
15	CH_3	16.6	16.9	13.7
1′	CH	57.5	56.9	57.1
2′	CH	45.5	46.1	46.5
3′	CH	57.2	58.1	58.9
4′	C	134.0	134.3	134.9
5′	C	148.4	148.1	147.5
6′	CH	81.7	81.3	81.4
7′	CH	59.5	49.2	49.4
8′	CH_2	24.3	24.5	23.6 (CH)
9′	CH_2	44.6	44.8	43.7
10′	C	73.8	73.5	74.1
11′	CH	42.6	41.9	42.3
12′	C	179.0	178.8	178.8
13′	CH_3	12.5	12.4	12.2
14′	CH_3	30.3	30.7	29.4
15′	CH_3	17.1	17.8	18.3

CC on silica gel using a gradient of MeOH in $CHCl_3$. Mixture of compounds eluted with $CHCl_3$–MeOH (9.5:0.5) was further subjected to CC on silica gel to afford a mixture of two compounds. These compounds were finally purified by repeated CC on silica gel ($CHCl_3$–MeOH = 9.6:0.4, 9.4:0.6) to afford compound **1** (20 mg) and **2** (27 mg).

3.3. Reaction of *1* with trichloroacetyl isocyanate (TAI)

After recording the ^1H NMR spectrum of **1** in $CDCl_3$, TAI was added dropwise to the NMR tube and the mixture was left overnight, after which time the ^1H NMR spectrum was recorded again: resonances of hydroxyl groups at δ 2.25 and 2.95 replaced by downfield peaks at δ 8.10 and 8.25, respectively.

3.4. Seemarin (*1*)

Mp 256–257°, $[\alpha]_D$ + 114.5m (MeOH–$CHCl_3$; $c = 0.10$). IR ν_{max}(KBr) cm^{-1}: 1650 (double bond), 1755–1765 (CO of γ-lactone), 3390 (OH); EIMS: *m/z* 512 $[M]^+$ (100), 478 $[M\text{-}2H_2O]^+$ (43), 423 (30), 365 (11), 339 (10), 327 (6), 248 (14), 247 (13), 233 (5), 230 (4), 215 (3), 205 (5); ^1H NMR: Table 2, ^{13}C NMR: Table 3.

3.5. Anabsinthin (*2*)

Mp 267°C, $[\alpha]_D$ +113 ($CHCl_3$; $c = 0.10$). IR ν_{max}(KBr) cm^{-1}: 1660 (double bond), 1750–1765 (CO of γ-lactone), 3375 (OH); EIMS: *m/z* 496 $[M]^+$ (100), 478 $[M\text{-}H_2O]^+$ (47), 423 (27), 365 (21), 339 (6), 327 (6), 247 (23), 233 (3), 230 (7), 215 (3), 205 (5); ^1H NMR: Table 2, ^{13}C NMR: Table 3.

References

Baquar, S.R (1989). In *Medicinal and poisonous plants of Pakistan* (p. 161). Karachi, Pakistan: Printas Press.

Beauhaire, J., Fourrey, J. L., Vuilhorgne, M., & Lallemand, J. Y. (1980). *Tetrahedron Letters*, *21*, 3191.

Beauhaire, J., Fourrey, J. L., Vuilhorgne, M., & Lallemand, J. Y. (1981). *Tetrahedron Letters*, *22*, 2269.

Bohlmann, F., Ang, W., Trinks, C., Jakupovic, J., & Huneck, S. (1985). *Phytochemistry*, *24*, 1009.

Ullah, N., Ahmad, S., Anis, E., Mohammad, P., Rabnawaz, H., & Malik, A., (1998). *Phytochemistry*, in press.

Ullah, N., Ahmed, Z., Anis, E., & Malik, A. (1998). *Fitoterapia*, *LXIX*, 280.

Watt, G. (1972). *Dictionary of the economic product of India*, *III* (p. 26). India: Cosmo Publications Delhi-6.