PERGAMON

PHYTOCHEMISTRY

Phytochemistry 51 (1999) 99–101

A dicoumarin glycoside from *Daphne oleoides*

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Received in revised form 18 October 1998

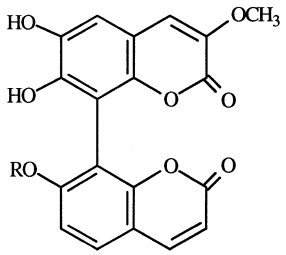
Abstract

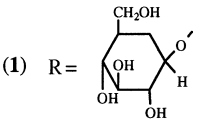
From the whole plant extract of *Daphne oleoides*, a new dicoumarin glycoside gulsamanin, has been isolated. Its structure was established as 6,7-dihydroxy-3-methoxy-8-[2-oxo-2H-1-benzopyran-7-(*O*-*β*-D-glucopyranosyl)-8-yl]-2H-1-benzopyran-2-one, on the basis of extensive NMR spectral studies, as well as by chemical methods.

Keywords: Daphne oleoides; Thymelaeaceae; Gulsamanin

1. Introduction

As a part of our ongoing phytochemical studies on *Daphne oleoides*, we have recently reported some lignans, and coumarins from this species (Ullah, Anis, Mohammed, Rabnawaz, & Malik, 1998; Ullah, Ahmed, Anis, & Malik, 1998). In this paper, we report the isolation and structural elucidation of a new dicoumarin glycoside, gulsamanin (**1**), from the whole plant extract of *D. oleoides*. This species is a small multi-branched shrub, found on the Western Himalayas, from Garhwal Westward to Murree, occurring at an altitude of 3000–9000 feet (Watt, 1972). Its roots are used as a purgative, the bark and leaves are given in cutaneous affections and an infusion of leaves is given in gonorrhoea and applied to abscesses (Baquar, 1989).





(2) R = H

2. Results and discussion

The dried and ground whole plant was extracted three times with methanol. The combined methanolic extract was subjected to VLC eluting with a gradient system ($CHCl_3$–MeOH) of increasing polarity to obtain fractions A–F, respectively. Fraction D, which eluted with $CHCl_3$–MeOH (8.2:1.8) was further subjected to repeated CC eluting with $CHCl_3$–MeOH to obtain compound (**1**). Analysis by positive ($[M+Na]^+$, m/z 553) and negative ($[M-H]^-$, m/z 529) FAB mass spectrometry showed the M_r of 530. The molecular formula of **1** was determined as $C_{25}H_{22}O_{13}$ by negative HRFAB mass spectrometry. The IR spectrum showed absorptions at 3380, 1715, 1625, 1525 and 1440 cm^{-1}, and the close similarity of the UV spectrum with that of umbelliferone (Macias, Massanet, Rodriguez-Luis, & Salva, 1989), suggested a coumarin structure for **1**.

The ^1H NMR was in accordance with this showing resonances of two AX-systems at δ 6.18 (1H, d, $J=9.5$ Hz) and 7.95 (1H, d, $J=9.5$ Hz) and 7.12 (1H, d, $J=8.5$ Hz) and 7.60 (1H, d, $J=8.5$ Hz) and a methoxyl group at δ 3.84 (3H, s). The spectrum further showed four peaks at δ 7.42, 7.90, 10.35 and 10.50 (1H, each s) and an anomeric proton signal at δ 4.95 (1H, d, $J=7.42$ Hz), showing the presence of a sugar moiety in **1** in the β-configuration. Acid hydrolysis of **1** yielded an aglycone and a sugar, the latter being identified as D-glucose by PC and also from the retention time of its TMS ether in GC (Markham, 1982).

The ^{13}C NMR and DEPT experiments exhibited the

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0031-9422/99/$ - see front matter
PII: S0031-9422(98)00726-2

presence of 11 methine, one methylene and one methyl carbon atoms. The quaternary carbon atoms were deduced by subtracting these from the BB spectrum. From ^{13}C NMR chemical shifts and multiplicities, a glycosidic subunit and two coumarin moieties could be identified. Using the ^{13}C shift values of umbelliferone (Macias et al., 1989), daphnoretin (Gordell, 1984) and methoxycoumarins (Gunther, Prestien, & Joseph-Nathan, 1975; Macias et al., 1989) as reference data, it could be deduced that a linear linked dimeric coumarin unit must be present, with one of the subunits being substituted by the glucose moiety. To prove the substitution pattern, ^1H NMR correlated spectroscopy (COSY) and $^3J_{HH}$ coupling constants were used to assign all resonances in the ^1H spectra as far as possible. The substitution pattern and linkages were recognized by spin pattern analysis showing two pairs of vicinal protons of the AX-type, with different coupling constants and chemical shifts, and five singlets corresponding to 11 protons of the dicoumarin aglycone. Further confirmation of the substitution pattern was achieved using 1-D and 2-D NOE difference measurement spectroscopy. For coumarin unit A, the H-4 singlet at δ 7.90 was used to assign the H-5 (δ 7.42) by 2-D NOE correlation between H-4 and H-5. The linkage of the glucose unit to the dicoumarin aglycone was located by 1-D NOE measurement. Irradiation of the anomeric proton at δ 4.95 caused a 10.5% enhancement of the signal coupled with an *ortho*-proton at δ 7.12 (H-6′), confirming the site for glucose moiety at C-7′. Irradiation of the methoxyl group at δ 3.84 caused 9.2% enhancement of the singlet at δ 7.90 (H-4), showing the presence of a methoxyl group at C-3. Another NOE difference was observed between the hydroxyl group at δ 10.50 and the anomeric proton of the glucose residue. This is indicative of the presence of a hydrogen bridge to the glycosidic oxygen.

^1H–^{13}C HMQC and HMBC experiments were not only useful for the attribution of the chemical shifts of several protonated and nonprotonated carbon atoms, but also helpful for confirming the above assignments. The methoxyl protons at δ 3.84 showed cross-peaks to carbons at δ 139.2 (C-3), 159.8 (C-2) and 129.8 (C-4). Likewise, proton at δ 7.90 (H-4) showed cross-peaks to carbons at δ 111.7 (C-10), 139.2 (C-3), 149.1 (C-9) and 159.8 (C-2). In this way, it was possible to work around the dicoumarin skeleton and to assign the skeletal structure and signals of most of the carbon atoms. These results led to the structure of **1** as 6,7-dihydroxy-3-methoxy-8-[2-oxo-2H-1-benzopyran-7-(*O*-β-D-glucopyranosyl)-8-yl] 2H-1-benzopyran-2-one.

Hydrolysis of **1** with hydrochloric acid yielded the corresponding aglycone (**2**). The negative HRFAB mass spectrum showed the $[M]^+$ at m/z 367.2115, corresponding to the molecular formula $C_{19}H_{12}O_8$ (calcd for $C_{19}H_{12}O_8$ 367.2110). The IR spectrum showed absorptions at 3440, 1720, 1635 and 1520 cm^{-1}, which were characteristic of coumarins with additional phenolic functionalities.

The ^1H NMR of **2** showed the presence of two pairs of vicinal protons at δ 6.25 (1H, d, $J=9.5$ Hz), 7.98 (1H, d, $J=9.5$Hz) and δ 7.22 (1H, d, $J=8.56$ Hz), 7.55 (1H, d, $J=8.56$ Hz), as well as five singlets at δ 3.82, 7.40, 7.92, 10.32 and 10.45. This evidence and the corresponding ^{13}C NMR, identified compound **2** as the corresponding aglycone of **1**.

3. Experimental

3.1. General

Mps are uncorr. ^1H and ^{13}C NMR were obtained on a Bruker AM-300 spectrometer. DEPT were carried out with $\theta-45$, 90 and 135°. Chemical shifts are in δ, with TMS as int. standard and J in Hz. ^1H NMR COSY were taken at 300 MHz, NOESY and 1-D-difference spectra (NOEDIF) at 400 MHz. NOEs were detected by 2-D phase-sensitive NOESY correlation (Kessler, Gehreke, & Griesinger, 1988) and determined quantitatively by a 1-D difference technique (Kinns & Sanders, 1984). Silica gel 60 (35–70 mesh) was used for CC. TLC was carried out on silica gel plates using $CHCl_3$–MeOH (19:6). Precoated Kieselgel 60, F_{254} aluminium sheets (Merck) were used to check the purity. Spots were visualized by spraying and subsequent heating with a soln of ceric sulphate in 10% H_2SO_4.

3.2. Plant material

Whole plants of *D. oleoides* were collected from Hazara division of N.W.F.P., Pakistan, in February, 1995. A voucher specimen was identified by Professor Iftikhar Hussain Shah and is deposited in the Herbarium of the Faculty of the Pharmacy, Gomal University, D. I. Khan, Pakistan.

3.3. Extraction and isolation

Shade-dried and ground plant material (16 kg) was extracted $\times 3$ with MeOH. The combined MeOH extracts were evap. under red. pres. The resulting residue was suspended in H_2O and extracted successively with petrol. ether, EtOAc, $CHCl_3$ and *n*-BuOH. The *n*-BuOH fr. was subjected to VLC eluting with a $CHCl_3$–MeOH gradient system of increasing polarity, to obtain frs A–F, respectively. Fr. D which eluted with $CHCl_3$–MeOH (41:9) was further subjected to repeated CC, eluting with $CHCl_3$–MeOH (19:6) to obtain **1** (45 mg).

3.4. Acid hydrolysis of compound (*1*)

Compound **1** (20 mg) was refluxed for 4 h with 1N HCl in MeOH (5 ml). Soln was concd under red. pres.

Table 1
^{1}H NMR and ^{13}C NMR of compounds **1** and **2**

	^{1}H and ^{13}C NMR of compound **1**				^{13}C NMR of compound **2**	
#H	1H (*J* in Hz)	#C	DEPT	^{13}C	DEPT	^{13}C
–	–	2	C	159.8	C	160.3
–	–	3	C	139.2	C	138.8
4	7.90 (1H, s)	4	CH	129.8	CH	129.6
5	7.42 (1H, s)	5	CH	116.4	CH	116.4
OH	10.35 (1H, s)	6	C	144.1	C	144.3
OH	10.50 (1H, s)	7	C	141.2	C	142.1
–	–	8	C	115.6	C	115.3
–	–	9	C	149.1	C	149.7
–	–	10	C	111.7	C	110.8
–	–	2′	C	160.1	C	161.5
3′	6.18 (1H, d, $J=9.5$ Hz)	3′	CH	112.6	CH	113.1
4′	7.95 (1H, d, $J=9.5$ Hz)	4′	CH	144.5	CH	144.3
5′	7.60 (1H, d, $J=8.5$ Hz)	5′	CH	128.2	CH	129.1
6′	7.12 (1H, d, $J=8.5$ Hz)	6′	CH	112.5	CH	114.9
–	–	7′	C	158.1	C	153.2
–	–	8′	C	110.3	C	111.1
–	–	9′	C	110.7	C	111.7
–	–	10′	C	152.4	C	151.6
1″	4.95 (1H, d, $J=7.42$ Hz)	1″	CH	101.5	–	–
–	–	2″	CH	74.2	–	–
–	–	3″	CH	75.1	–	–
–	–	4″	CH	70.3	–	–
–	–	5″	CH	76.0	–	–
–	–	6″	CH_2	61.8	–	–
OCH_3	3.84 (3H, s)	OCH_3	CH_3	56.4	CH_3	56.5

and dil. with 5ml H_2O. It was extracted with EtOAc and the residue recovered from the organic phase was subjected to prep. TLC to obtain **2** which was identified as the corresponding aglycone of **1**. The aq. phase was concd and glucose was identified by PC on Schleicher and Schuell 2043b with the solvent system, *n*-BuOH–HOAc–H_2O (4:1:5) and detection with aniline–phthalic acid. Identification was further confirmed by comparing the R_t of its TMS ether with an authentic sample by GC.

3.5. Compound (*1*)

Mp 192–193°C. UV MeOH λmax nm ($\log\varepsilon$) 225sh (4.30), 260 (3.92), 312sh (4.15), 322sh (4.30), 338 (4.35). IR ν_{max} (KBr) cm^{-1}: 3380, 2930, 1625, 1525, 1440 and 1270. Pos. FABMS, *m/z* (rel. int.): 553 $[M+Na]^+$ (60), 391 (78), 376 (100). Neg. FABMS, *m/z* (rel. int.) 529 $[M-H]^-$ (51), 367 (71), 352 (100). ^{1}H NMR (DMSO-d_6, 300 MHz) and ^{13}C NMR (DMSO-d_6, 75.4 MHz): Table 1.

3.6. Compound (*2*)

Mp 179–180°C. IR ν_{max} (KBr) cm^{-1}: 3380, 2930, 1625, 1440 and 1270. Neg. FABMS, *m/z* (rel. int.): 367 $[M-H]^-$ (61), 352 (100). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.45 (2H, s), 10.32 (1H, s), 7.98 (1H, d, $J=9.5$ Hz), 7.92 (1H, s), 7.55 (1H, d, $J=8.56$ Hz), 7.40 (1H, s), 7.22 (1H, d, $J=8.56$ Hz), 6.25 (1H, d, $J=9.5$ Hz) and 3.82 (3H, s, OMe). ^{13}C NMR (DMSO-d_6, 75.4 MHz): Table 1.

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