

Lignan and Coumarin Glycosides from *Haplophyllum suaveolens*

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Two new lignan glycosides of dibenzylbutyrolactol type (haplopharin) and aryl-naphthalene type (haploborin) were isolated from the aerial parts of *Haplophyllum suaveolens* together with the known aryl-naphthalene lignan glycoside arabelline and hydroxycoumarin glycoside xeroboside. So far arabelline is found only in *H. buxbaumii*. This is the first report of the isolation of xeroboside from *Haplophyllum*.

Introduction

Members of the genus *Haplophyllum* (Rutaceae) are known for their use in the traditional medicine, as well as for synthesizing a variety of chemical constituents of diverse structures – lignans, coumarins, alkaloids etc. A number of these compounds exhibit significant biological activity. There are three *Haplophyllum* species native to Bulgaria, namely *H. suaveolens*, *H. tesioides* and *H. balcanicum*. Of them *H. suaveolens* is the most widely spread and the best studied. Previous studies on *H. suaveolens* have shown the presence of quinoline alkaloids, lignans and flavonoids (Ionescu *et al.*, 1971; Ulubelen, 1984; 1986; Kostova *et al.*, 2000). However, no coumarins have been found so far.

In continuation of our investigation on *H. suaveolens* we report here the isolation and structure elucidation of a new dibenzylbutyrolactol lignan haplopharin (**1**) and a new aryl-naphthalene lignan haploborin (**2**) from the aerial parts of this plants. Known compounds isolated from the same source in this study are the lignan arabelline (**3**) and the coumarin xeroboside (**4**).

Material and Methods

General

All NMR spectra were recorded in CD₃OD on a Bruker DRX 250 spectrometer (TMS as internal standard) using Bruker standard software; Mass

spectra: Varian MAT 311A, APCI: Finnigan TSQ 700; TLC: aluminum sheets, silica gel 60 F₂₅₄ (Merck), bands were detected under UV light, by exposure to J₂ vapour, or by spraying with Dragendorff reagent; liquid vacuum chromatography (LVC): silica gel 60 (Merck); medium – pressure liquid chromatography (MPLC): LiChroprep RP – 8 (40–63 µm, 31 × 2.5 cm i. d., Merck).

Plant material

Haplophyllum suaveolens (DC.) G. Don fil. (aerial parts) was collected in July 1998 in the region of Plovdiv (Gara Ognyanovo), Bulgaria. The plant material was authenticated by Dr. A. Vitkova, and a voucher specimen (No. SOM/CO 340) was deposited in the Herbarium of the Institute of Botany, BAS, Sofia.

Extraction and isolation

The dried and powdered aerial parts (1.5 kg) of *H. suaveolens* were extracted with 95% EtOH at room temperature (3 × 24 h × 3.5 l). Concentration of the combined EtOH solutions to a small volume and treatment with charcoal gave the crude ethanolic extract (CEE, 24.7 g). Solvent – solvent partition of CEE gave the petrol (1.7 g), EtOAc (3.0 g) and water – ethanol (15.0 g) extracts. LVC of the water – ethanol extract over silica gel (150 g) using CHCl₃-MeOH-H₂O (10 : 1 : 0.1 → 1 : 1 : 0.1) yielded fractions F1 – F16. The combined

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F10 and F11 (600 mg) were subjected to medium – pressure liquid chromatography using MeOH-H₂O (1: 5 → 1 : 1.25) to give fractions S1 – S16. The fractions S6 – S8 (102 mg) were further worked up by multiple CC over silica gel with CHCl₃-MeOH-H₂O (5 : 1 : 0.1) to afford the lignans haplomarín (**1**, 5.2 mg), haploborín (**2**, 12.2 mg) and arabellín (**3**, 7.3 mg), and the coumarin xeroboside (**4**, 5.0 mg).

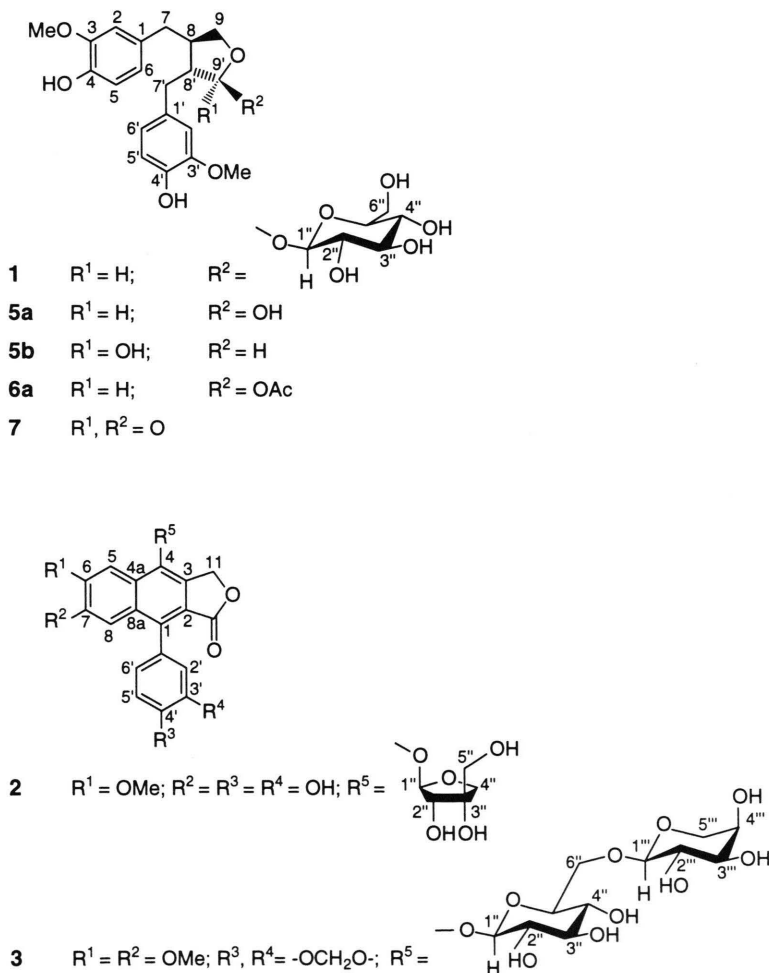
Haplomarín (1), Amorphous powder. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 208, 230, 280; IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3442, 1603, 1517, 1462; ¹H and ¹³C NMR spectra: see Table I; FABMS m/z : 545 [M+Na]⁺, 522 [M]⁺.

Haploborín (2), Amorphous powder. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 225, 262, 292, 315, 360; IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3421, 1746, 1623, 1510, 1484; ¹H and ¹³C NMR spectra: see Table II; APCI, m/z (rel. int.): 487 [M

+ H]⁺ (52.0), 355 [(M + H) – 132]⁺ (100), 133 (14.0).

Results and Discussion

From the ethanolic extract of the aerial parts of *H. suaveolens* four glycosides have been isolated. Two of them are the new lignans haplomarín (**1**) and haploborín (**2**) of dibenzylbutyrolactol and aryl-naphthalene type. The remaining two glycosides are identified as the known compounds arabellín (**3**) and xeroboside (**4**). The first and so far the only isolation of the aryl-naphthalene lignan **3** is from *H. buxbaumii* in 1990 (Al – Abed *et al.*, 1990). The coumarin glycoside **4** is also a rare natural compound (Sibanda *et al.*, 1989) and this is the first report of its occurrence in *Haplophyllum*.



The assignment of the signals in the ^{13}C and ^1H spectra of **1** – **3** was achieved by a combination of 1D (NOED, selective COSY) and 2D (H-H COSY, HMQC, HMBC) techniques.

Haplomarin (**1**) showed IR absorption bands for aromatic rings (1603, 1517, 1462 cm^{-1}) and hydroxyl group (3442 cm^{-1}). Its molecular formula $\text{C}_{26}\text{H}_{34}\text{O}_{11}$ was established according to the FAB mass spectrum ($[\text{M} + \text{Na}]^+$ at m/z 545 and $[\text{M}]^+$ at m/z 522) and NMR data. The ^1H and ^{13}C spectra (Table I) indicated two 1, 2, 4 – trisubstituted aromatic rings, two phenolic methoxys, three methylene and two methyne groups and two oxygenated quaternary carbons. A lack of carbonyl function was evident from the IR and ^{13}C NMR spectra. In the ^1H NMR spectrum a *brs* at δ 5.30 (δ_{c} 106.5) for an acetalic proton (H-9') was clearly visible. These data provided evidence for a lignan structure of butyrolactol type. The NOE enhancement of the doublets for H-2 (δ 6.48) and H-2' (δ 6.60) on irradiation of the OMe signals at δ 3.74 and 3.77 placed the respective OMe groups at C-3 and C-3'.

The MS and the signals left in the ^1H and ^{13}C spectra after assigning the lignan skeleton suggested a presence of a hexose sugar unit. Its being a glucose was unambiguously confirmed by selective COSY experiments and HMQC spectra. The attachment of the glucose at C-9' of the lactol ring was determined by the cross peaks H-9'/C-1'' and H-1''/C-9' in the HMBC spectrum and by the mutual NOE enhancement between H-1'' and H-9'. In this way the arrangement shown in structure **1** was unambiguously assigned to haplomarin.

The aglucone of **1** was found to be the known lignan **5** isolated from *Abies pinsapo* (Barrero *et al.*, 1994). A presence of two epimers **a** and **b** with one multiplet for both hemiacetalic protons H-9' at δ 5.23 was described for this compound in solution. The observed chemical shifts of C-8 (δ 45.74 for **5a**; δ 42.87 for **5b**), C-7' (δ 39.21 for **5a**; δ 33.46 for **5b**) and C-9' (δ 103.46 for **5a**; δ 98.92 for **5b**) for **5a** and **5b** were very different and useful for assigning of their stereochemistry. On acetylation only one acetate **6a** was obtained with $J_{8',9'} = 1.5$ Hz and chemical shifts of C-8 (44.83), 7' (39.22) and 9' (103.05) close to those of **5a** suggesting a β -orientation of the 9'-OAc. The authors confirmed the 8R,

Table I. ^1H and ^{13}C NMR data for compound **1** in CD_3OD , δ in ppm (J in Hz).

Position	^1H	^{13}C
Aglucone		
1		133.8 s
2	6.48 d (1.5)	113.2 d
3		148.8 ^a s
4		145.8 ^b s
5	6.62 d (8.0)	115.9 d
6	6.47 dd (8.0, 2.0)	122.1 d
7	2.52 m, 2H	39.9 t
8	2.20 m	46.8 d
9	3.95 m *	73.5 t
	3.75 m *	
1'		132.6 s
2'	6.60 d (1.5)	113.5 d
3'		148.9 ^a s
4'		145.6 ^b s
5'	6.68 d (8.0)	115.9 d
6'	6.54 dd (8.0, 2.0)	122.6 d
7'	2.76 dd (12.5, 5.0)	39.4 t
	2.34 m *	
8'	2.25 m	53.3 d
9'	5.30 bs	106.5 d
3-MeO	3.74 s	61.1 q
3'-MeO	3.77 s	62.0 q
Glucose		
1''	4.55 d (7.9)	99.2 d
2''	3.21 dd (8.0) **	75.0 d
3''	3.37 dd (8.0) **	78.3 ^c d
4''	3.25 dd (8.0) **	71.7 d
5''	3.30 m **	78.1 ^c d
6''	3.85 dd 12.0, 6.0) **	62.8 t
	3.65 dd (12.0, 5.8) **	

a,b,c The values with the same indexes could be interchanged; * Overlapped signals. The coupling constants not observed; ** Assigned with the help of selective COSY experiments.

8'R stereochemistry of **5a,b** by oxidation to the corresponding butyrolactone **7**, which was proved identical with the known lignan matairesinol isolated from the same plant.

Several natural lignans of dibenzylbutyrolactol type have been reported to exist as a mixture of two epimers (8R, 8'R, 9'R and 8R, 8'R, 9'S) in solution. On the basis of detailed 1D and 2D NMR investigations Gözler *et al.* (1996b) assigned the 9'- β OH configuration to the epimer having the smaller $J_{8',9'}$ (1.5 Hz) in the ^1H NMR spectrum and the lower field chemical shifts of C-8, 7' and 9' in the ^{13}C NMR spectrum. The same authors observed ^1H – ^{13}C long-range correlations between H-9' and the three γ – carbon atoms C-8, 9, 7' for the 9'- β OH epimer and between H-9' and

the two γ – carbon atoms C-8, 9 for the 9' – α OH epimer.

The ^1H NMR spectrum of **1** gave no enough evidence for its stereochemistry as H-8, 8' and 7' appeared as multiplets. However, a comparison of the ^{13}C NMR data of **1**, **5a** (8*R*, 8'*R*, 9'*R*) and **5b** (8*R*, 8'*R*, 9'*S*), **6a** (8*R*, 8'*R*, 9'*R*) and the two lactol epimers described by Gözler *et al.* (1996b) clearly indicated the 8*R*, 8'*R*, 9'*R* configuration of **1**. The HMBC spectrum revealed the expected cross-peaks between H-9' and the three γ -carbon atoms

7', 8 and 9 (Gözler *et al.*, 1996b). The 8*R*, 8'*R*, 9'*R* configuration of **1** is also supported by the broad singlet for H – 9' at δ 5.30 and proved by the NOE enhancement of the two H – 7' protons on irradiation of H – 9'.

Haplomarin (**1**) belongs to group of the rare dibenzylbutyrolactol lignans. The natural occurrence of their 9'-OMe and 9'-OEt derivatives has already been described in the literature (Badheka *et al.*, 1987; Marco *et al.*, 1996). To the best of our knowledge, 9'-O-glycosides of the dibenzylbutyro-

Table II. ^1H and ^{13}C NMR data for compounds **2** and **3** in CD_3OD , δ in ppm (*J* in Hz).

Position	^1H	2	^{13}C	^1H	3	^{13}C
Aglycone						
1			137.6 <i>s</i>			137.5 <i>s</i>
2			129.4 <i>s</i>			128.7 <i>s</i>
3			119.4 <i>s</i>			120.0 <i>s</i>
4			145.9 <i>s</i>			146.0 <i>s</i>
5	7.66 <i>s</i>		101.8 <i>d</i>	8.14 <i>s</i>		102.2 <i>d</i>
6			152.7 <i>s</i>			153.1 <i>s</i>
7			149.1 <i>s</i>			151.4 <i>s</i>
8	7.10 <i>s</i>		111.3 <i>d</i>	7.06 ^a <i>s</i> ; 7.07 ^a <i>s</i>		106.9 <i>d</i>
4a			128.0 <i>s</i>			120.0 <i>s</i>
8a			132.6 <i>s</i>			132.5 ^a , 131.7 ^a <i>s</i>
9			172.4 <i>s</i>			172.3 <i>s</i>
11	5.49, 2H, ABq (15.0) ^d		68.7 <i>t</i>	5.80 ^a ; 5.47 ^a <i>d</i> (15.5) 5.79 ^a ; 5.46 ^a <i>d</i> (15.5)		69.4 <i>t</i>
1'			128.2 <i>s</i>			129.7 <i>s</i>
2'	6.74 ^a <i>d</i> (1.9) 6.73 ^a <i>d</i> (1.9)		118.7 <i>d</i>	6.84 ^a <i>d</i> (1.9) 6.79 ^a <i>d</i> (1.9)		111.5 ^a , 111.7 ^a <i>d</i>
3'			146.1 <i>s</i>			148.7 <i>s</i>
4'			146.3 <i>s</i>			148.8 <i>s</i>
5'	6.88 <i>d</i> (8.0)		116.0 <i>d</i>	6.95 <i>d</i> (8.0)		108.8 <i>d</i>
6'	6.60 ^a <i>dd</i> (8.0, 1.9) 6.59 ^a <i>dd</i> (8.0, 1.9)		123.1 <i>d</i>	6.80 ^a <i>dd</i> (8.2, 1.4) 6.77 ^a <i>dd</i> (8.2, 1.4)		124.5 ^a , 124.6 ^a <i>d</i>
MeO	4.03 <i>s</i>		56.5 <i>q</i>			
O-CH ₂ -O				6.05 <i>m</i> , 2H		102.4 <i>t</i>
Sugar units						
1''	5.50 <i>d</i> (3.6)		112.9 <i>d</i>	4.80 ^a , 4.79 ^a <i>d</i> (7.8)		106.6 <i>d</i>
2''	4.50 <i>d</i> (3.6)		78.7 <i>d</i>	3.62 <i>t</i> (7.8)		75.2 <i>d</i>
3''			80.4 <i>s</i>	3.45 ^c		76.8 <i>d</i>
4''	4.38 <i>d</i> (10.0) 3.92 <i>d</i> (10.0)		75.9 <i>t</i>	3.50 ^b		71.1 <i>d</i>
5''	3.68, 2H, ABq (2.0) ^d		64.3 <i>t</i>	3.80 <i>dd</i> (15.0, 4.0) 3.45 ^c		77.7
6''				4.11 <i>m</i> 4.07 <i>m</i>		69.7 <i>t</i>
1'''				4.22 ^a , 4.21 ^a <i>d</i> (6.7)		104.8 <i>t</i>
2'''				3.57 <i>m</i>		72.1 <i>d</i>
3'''				3.45 ^c		74.0 <i>d</i>
4'''				3.70 <i>m</i>		69.3 <i>d</i>
5'''				3.50 ^b		66.6 <i>t</i>

^a The signals are doubled due to hindered rotation; ^b, ^c Overlapped signals. Coupling constants not observed; ^d Observed splitting.

lactol lignans have not been reported so far. Haplomarin is the first example of such natural compound.

Haploborin (**2**), $C_{24}H_{22}O_{11}$, exhibited UV and IR spectra indicative of an aryl-naphthalene type lignan. The 1H and ^{13}C NMR data (Table II) revealed the characteristic singlets for H-5 (δ 7.66) and H-8 (δ 7.10), suggested 4, 6, 7, 3', 4' - substitution and a presence of an apiofuranose sugar unit. The position of the OMe at C-6 was determined by the NOE enhancement of H-5 (δ 7.66) on irradiation of the OMe signal (δ 4.03). The appearance of the ABq at δ 5.49 fixed the position of the lactone methylene group at C-11 (Okigawa *et al.*, 1970).

The position of the aromatic ring at C-1 was further confirmed by HMBC cross peaks between H-6', H-2' and C-1, as well as by the NOE enhancement of H-2' and H-6' on irradiation of H-8. The HMBC cross peaks H-8 (δ 7.10)/C-4a (δ 128.0) and H-5 (δ 7.66)/C-8a (δ 132.6) confirmed the assignments of C-4a and C-8a. This is in accordance with the observation that in the naphthalene sys-

tem the values of $^3J_{C-H}$ (5–9 Hz) are bigger than those of $^2J_{C-H}$ (0.6–1.8 Hz) (Hansen, 1979). The position of the apiose at C-4 was suggested by the cross peak H-1''/C-4 in the HMBC spectrum and confirmed by the NOE between H-5 and H-1''. Based on these data, the structure **2** is determined for this compound.

Lack of 1H and ^{13}C NMR spectra in CD_3OD for arabelline (**3**) prompted us to report our spectral data for this compound (Table II). The chemical shifts of C-2 and C-3 in **2** and **3** were assigned on the basis of the expected substituent effects in the aromatic ring. They are close to the values reported for other aryl-naphthalene lignans (Gözler *et al.*, 1996a) and reverse to those previously assigned to C-2 and C-3 in arabelline (Al-Abed *et al.*, 1990).

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