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MINOR LIGNANS FROM *HAPLOPHYLLUM CAPPADOCICUM*

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Key Word Index—*Haplophyllum cappadocicum*; Rutaceae; haplomyrtoside; haplomyrtin; majidine; 1β-polygamain; vanillic acid; arylnaphthalene lignans; arylnaphthalene lignan glycosides; tetralin lignans.

Abstract—*Haplophyllum cappadocicum*, an annual herb native to Turkey, has yielded a new arylnaphthalene lignan glycoside, (−)-haplomyrtoside, which is shown to be haplomyrtin 4-*O*-β-D-apiofuranoside on the basis of detailed spectral analyses. Additionally, a known lignan diglycoside, (−)-majidine, a known tetralin lignan, (−)-1β-polygamain, and vanillic acid are reported for the first time from this species.

INTRODUCTION

Our previous studies on *Haplophyllum cappadocicum* have revealed the wide scope of its chemical composition, which comprises quinoline alkaloids [1, 2], coumarins [2] and lignans, the latter being represented by compounds of diverse subgroups. While the presence of arylnaphthalene lignans and their glycosides seems to be a characteristic feature of its lignan content [2–5], dibenzylbutyrolactones [2], unsaturated dibenzylbutyrolactones with a rather unusual trioxygenated substitution pattern on one of the aromatic rings, and their glycosides [4], have also been isolated and characterized. Furthermore, (−)-padocin isolated from the title species was shown to be the first natural example of a unique variation of an epoxylignan structure, containing an interesting C-6–C-9′ junction [6]. Recently, the isolation of (−)-3,4-bis(4-hydroxy-3-methoxybenzyl)tetrahydrofuran-2-ol appended the more rarely encountered dibenzylbutyrolactol subgroup to the chemical profile of *H. cappadocicum* [5].

Our continuing interest in the lignans of *H. cappadocicum* has resulted in the characterization of a new arylnaphthalene lignan glycoside, (−)-haplomyrtoside (**1**). The structure of **1** was established as haplomyrtin 4-*O*-β-D-apiofuranoside [=4-*O*-(β-D-apiofuranosyloxy)-7-hydroxy-3-hydroxymethyl-6-methoxy-methylenedioxyphenyl)naphthalene-2-carboxylic acid lactone] on the basis of detailed spectral analyses. High resolution ^{13}C NMR data are presented for the aglycone haplomyrtin (**2**), which was also described from this species. Majidine (**3**), a diphyllin diglycoside, originally reported from *H. buxbaumii* [7] was also obtained and identified. Isolation of (−)-1β-polygamain (**4**), a lignan recently described from *H. ptilostylum* [8, 9] has shown that the tetralin subgroup of lignans, not yet reported from *H. cappadocicum*, is also an element of the lignan content of the title species. Vanillic acid was identified as one of the polar components of *H. cappadocicum*.

RESULTS AND DISCUSSION

Compound **1**, $C_{25}H_{22}O_{11}$, was obtained as a white amorphous powder. The UV spectrum, with a prominent maximum at 261 nm and carbonyl absorption at 1745 cm^{-1}, indicated that we were once again dealing with an arylnaphthalene-type lignan, which has already been shown to be a common element of *H. cappadocicum* [2–5].

In the aromatic region of the ^{1}H NMR spectrum taken at 600 MHz, the characteristic resonances for an arylnaphthalene nucleus with a 3′,4,4′,6,7-pentasubstitution were evidenced by two singlets at δ 7.70 (H-5) and 7.05 (H-8), and signals for an ABX system. The latter gave rise to a doublet at δ 6.94 (J = 7.8 Hz) (H-5′), while the AB part, integrating for a total of two protons, displayed doubling of signals for H-2′ at δ 6.77 and 6.76 (J = 1.6 Hz) and for H-6′ at δ 6.74 and 6.73 (J = 7.9 and 1.6 Hz). The identity of the substituents were easily discernable from the signals of a methylenedioxy group at δ 6.03 and a methoxyl group at δ 4.05, predicting the presence of a phenolic group as the fifth substituent. This suggestion was confirmed by the UV spectrum of **1**, where a prominent bathochromic shift was observed upon the addition of alkali.

The more striking features of the ^{1}H NMR spectrum

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of **1**, however, were the signals accounting for the presence of a pentose unit. The doublet at δ 5.52 with a coupling constant $J_{1'',2''} = 3.6$ Hz belonged to the anomeric proton and indicated an α-configuration. H-2″ (δ 4.51) also appeared as a doublet, showing no further couplings. Of the remaining four protons of the sugar moiety, two resonated as an AB system at δ 3.71 and 3.67 ($J = 11.4$ Hz), while the signals of the other two (δ 4.34 and 3.93, $J_{gem} = 9.6$ Hz) pointed to the presence of an isolated methylene group adjacent to a deshielding oxygen atom. These NMR findings provided a unambiguous proof of the identity of the sugar as D-apiofuranose, attached to the aglycone through a β-glycosidic linkage.

As is the case with other 4-*O*-glycosidic arylnaphthalene species [4, 5, 7], the methylene protons of the lactone ring (H-10) gave rise to non-equivalent signals with coupling constants of 14.7 Hz. We have also observed the doubling of each signal, which has been previously reported as further smaller couplings of H-10 through seven bonds to H-2′ and H-6′ [10]. However, high power irradiation of the H-2′ and H-6′ signals induced no change in the H-10 signal. Likewise, irradiation of H-10 brought about no change in the H-2′ and H-6′ signals. In line with reported similar cases for other arylnaphthalene glycosides [7], we believe that the doubling of the H-10 signal, as well as those of the H-2′ and H-6′ signals previously mentioned, are probably a consequence of conformational isomerism, brought about by the slow rotation of the sugar unit around the glycosidic linkage.

Although the above data suggested on firm grounds that the site of glycosidation was C-4, A a partial nOe experiment was conducted in order to provide further proof, as well as for establishing the exact position of the methoxyl group. While irradiation of H-8 (δ 7.05) effected an enhancement of only the H-2′ and/or H-6′ protons (δ 6.75 and 6.73, respectively) of the pendent aromatic ring, the methoxyl signal at δ 4.05 showed reciprocating nOe values with H-5 (δ 7.70). Additionally, irradiation of H-5 led to enhancements of H-1″ (δ 5.52) and H-2″ (δ 4.51) of the sugar moiety. The above evidence confirmed the presence of a 6-methoxy-7-hydroxy substitution pattern of ring A, thus establishing the precise identity of the aglycone as haplomyrtin (**2**). Furthermore, these nOe results substantiated the position of the glycosidic linkage as being at C-4 of haplomyrtin rather than the alternative position at C-7.

The multiplicities of the 25 carbons accounted for in the ^{13}C NMR spectrum of **1** were determined by a DEPT experiment. Explicit assignment of all carbon chemical shifts were achieved by HSQC [11, 12] and HMBC [13] experiments (Table 1). The resonance of the anomeric carbon of apiose (δ 112.73) as well as of the remaining carbons of the sugar moiety, were in good agreement with those reported for similar glycosides having a β-D-apiofuranosyl unit as their sugars [5, 7, 10].

Due to the facile cleavage of the glycosidic linkage, the $[M]^+$, calculated as m/z 498, was not observed in the EI or CI mass spectrum of **1**. An ESI mass spectrum, however, furnished peaks at m/z 1019 $[2M + Na]^+$ and m/z 521 $[M + Na]^+$. A tandem/mass spectrum from the $[M + Na]^+$ signal furnished a quasimolecular ion at m/z 521 and a fragment ion at m/z 389 $[aglycone + Na]^+$, verifying once again the molecular formula and its structure as **1**.

Haplomyrtin (**2**), the aglycone of **1**, was originally described from *H. myrtifolium* [14]. Later its occurrence was reported in the title species [4], as well as in

Table 1. HMBC correlations for haplomyrtoside (**1**) (in MeOH-d_4 and 150.9 MHz)

Proton	δ (Hz)	Long range C–H connectivities
5	7.70	132.34 (C-8a), 146.28 (C-4), 149.22 (C-7), 152.60 (C-6)
8	7.05	127.87 (C-4a), 136.42 (C-1), 149.22 (C-7), 152.60 (C-6)
10	5.49 and 5.48 (14.7)	129.09 (C-2), 146.28 (C-4), 172.14 (C-9)
	5.56 and 5.55 (14.7)	
2′	6.77 and 6.76 (1.6)	124.70 (C-6′), 148.72 (C-4′)
5′	6.94 (7.8)	130.16 (C-1′), 148.72 (C-3′)
6′	6.74 and 6.73 (7.9; 1.6)	111.69 (C-2′), 148.72 (C-4′)
OCH_2O	6.03	148.72 (C-3′ and C-4′)
OCH_3	4.05	152.60 (C-6)
1″	5.52 (3.6)	75.85 (C-4″), 146.28 (C-4)
2″	4.51 (3.6)	64.19 (C-5″), 112.73 (C-1″)
4″α	3.93 (9.6)	78.60 (C-2″), 80.20 (C-3″), 112.73 (C-1″),
4″β	4.34 (9.6)	64.19 (C-5″)
5″	3.67 (11.4)	
		75.85 (C-4″), 78.60 (C-2″), 80.20 (C-3″)
	3.71 (11.4)	

another member of the genus, *H. telephioides* [15]. In the latter study, ^{13}C NMR values have been cited for haplomyrtin with no reference to the solvent or the instrument used. Since the high resolution ^{13}C NMR, DEPT and 2D (HSQC and HMBC) experiments we have conducted on haplomyrtin in dimethyl sulphoxide-d_6 at 600 MHz (Table 2) furnished somewhat different chemical shifts and assignments, we wish to report here our ^{13}C NMR data for this compound (see Experimental).

The spectral data for compound **3** were in full agreement with those reported for (−)-majidine, the 4 - *O* - [β - D - xylopranosyl(1 → 2)β - D - apiofuranosyl]diphyllin, the first and so far only isolation being reported from *H. buxbaumii* [7].

The structure and configuration of the third minor laevorotatory lignan (**4**) were established as (−)-(1*S*,2*R*,3*R*)-3-hydroxymethyl-6,7-methylenedioxy-1-(3′,4′-methylenedioxyphenyl)-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid lactone on the basis of its spectral analyses results (UV, IR, circular dichroism and ^1H and ^{13}C NMR). Compound **4** is identical with (−)-1β-polygamain, reported recently from *H. ptilostylum* [8,9] and is epimeric at C-1 with the already known (−)-polygamain [16]. Our spectral data are in good agreement with those reported [9], with the exception of the $J_{3,3a\alpha}$ ($J_{3,10\alpha}$) and $J_{3,3a\beta}$ ($J_{3,10\beta}$) values of the ^1H NMR data, which we believe should be reversed as being 6.5 and 10.5 Hz, respectively. A compound reported from *Heliopsis buphthalmoides* (Compositae) [17] has the same chemical structure as **4**. However, since no reference has been made either to its optical rotation or to its circulardichroic spectral data, it can only be reduced from the formula presented therein that it has apparently the opposite stereochemistry at all chiral centres, and is, therefore, enantiomeric with **4**.

Finally, another minor component was identified as vanillic acid by virtue of its spectral data. The occurrence of this simple aromatic acid has already been described in *H. telephioides* [15].

EXPERIMENTAL

General. ^1H NMR, ^{13}C NMR and 2D spectra of **1** and **2**: Bruker AMX 600; ^1H NMR of **4**: Bruker ARX 300. 70 eV EIMS 70 eV; CIMS: NH_3); MS/MS of **1** acquired with an Ar pressure of 3 mtorr and a collision offset voltage of −20 eV.

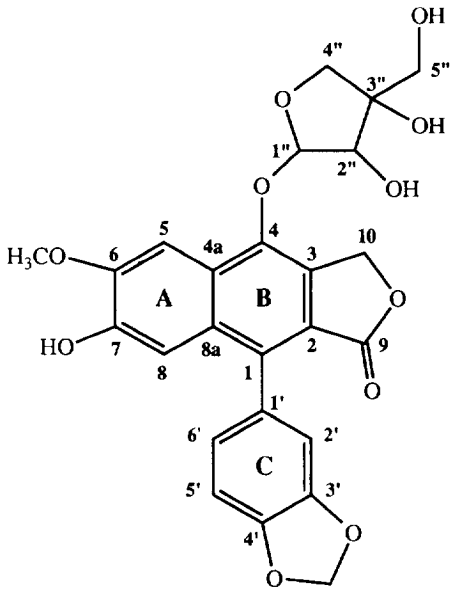
Plant material. Haplophyllum cappadocicum Spach. was collected from Old Malatya, Turkey, in July 1988. A voucher specimen, No 642, is deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University.

Extraction and isolation. Dried and powdered total plant material (27.8 kg) was extracted with EtOH (250 l) at room temp. The crude extract thus obtained was dissolved in 2% HCl (20 l) and filtered. The acidic filtrate was made alkaline with 10% aq. NH_4OH and extracted with $CHCl_3$. Evapn of the organic solvent furnished the crude basic extract (24.3 g), which was fractionated by CC using silica gel (0.063–0.200 mm, Merck) and $CHCl_3$ gradually enriched with MeOH as the mobile phase. Compound **1** (8 mg) was obtained from a fr. eluted with $CHCl_3$, whereas **3** (18.4 mg), **4** (17 mg) and vanillic acid (60.4 mg) were isolated from frns eluted with $CHCl_3$–MeOH (17:3). Further purification of the compounds was accomplished by prep. TLC on silica gel.

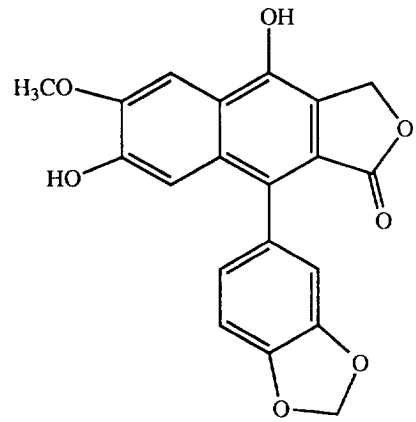
(−)-*Haplomyrtoside* (**1**). Amorphous solid. $[\alpha]_D$ −52.8° (*c* 0.125, MeOH). UV λ_{max}^{MeOH} nm (log ε) 203 (4.51), 225 (4.29), 261 (4.47), 289 (3.82), 309 (3.82), 322 sh (3.80), 350 sh (3.49); UV $\lambda_{max}^{MeOH+NaOH}$ nm (log ε) 205 (4.77), 225 sh (4.29), 282 (4.46), 330 (3.74), 389 (3.55) nm. IR (KBr) ν_{max} 3390, 2930, 1745, 1620, 1600, 1510, 1480, 1455, 1435, 1395, 1345, 1270, 1230, 1175, 1125, 1105, 1065, 1040, 1020, 960, 935, 865, 825 cm^{-1}. ^1H NMR (MeOH-d_4): δ 3.67 (1H, *d*, *J* = 11.4 Hz, H-5″), 3.71 (1H, *d*, *J* = 11.4 Hz, H-5″), 3.93 (1H, *d*, *J* = 9.6 Hz, H-4″α), 4.05 (3H, *s*, OCH_3), 4.34 (1H, *d*, *J* = 9.6 Hz, H-4″β), 4.51 (1H, *d*, *J* = 3.6 Hz, H-2″), 5.48, 5.49 (1H, *d*, *J* = 14.7 Hz, H-10),

Table 2. HMBC correlations for haplomyrtin (**2**) (in DMSO-d_6 at 150.9 MHz)

Proton	δ (Hz)	Long range C–H connectivities
5	7.59	109.01 (C-8), 122.78 (C-4a), 130.09 (C-8a), 145.03 (C-4), 147.78 (C-7), 150.23 (C-6)
8	6.94	110.95 (C-2′), 122.78 (C-4a), 128.72 (C-1), 145.03 (C-4), 147.78 (C-7), 150.23 (C-6)
10	5.33	118.34 (C-3), 120.44 (C-2), 122.78 (C-4a), 145.03 (C-4), 169.73 (C-9)
2′	6.80 (1.6)	123.76 (C-6′), 128.72 (C-1), 146.42 (C-4′)
5′	7.00 (7.9)	129.25 (C-1′), 146.67 (C-3′)
6′	6.69 (7.9; 1.6)	110.95 (C-2′), 128.72 (C-1), 146.42 (C-4′)
OCH_2O	6.07 (0.8)	
		146.42 (C-4′), 146.67 (C-3′)
	6.10 (0.8)	
OCH_3	3.92	150.23 (C-6)

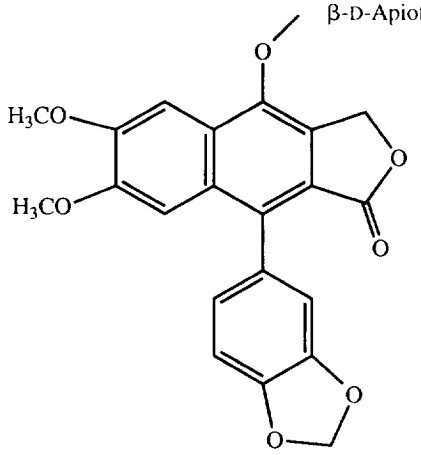


1



2

β-D-Apiofuranosyl-β-D-xylopyranosyl



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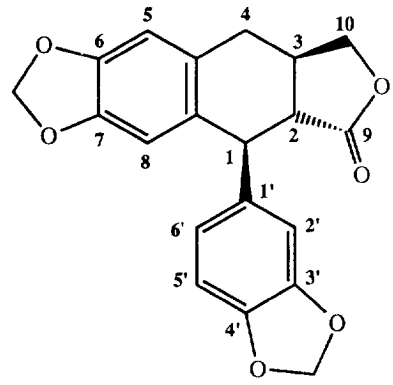

4

5.52 (1H, *d*, $J = 3.6$ Hz, H-1″), 5.55, 5.56 (1H, *d*, $J = 14.7$ Hz, H-10), 6.03 (2H, *s*, 3′,4′-OCH_2O), 6.73, 6.74 (1H, *dd*, $J = 7.9$, 1.6 Hz, H-6′), 6.76, 6.77 (1H, *d*, $J = 1.6$ Hz, H-2′), 6.94 (1H, *d*, $J = 7.8$ Hz, H-5′), 7.05 (1H, *s*, H-8), 7.70 (1H, *s*, H-5). ^{13}C NMR (MeOH-d_4): δ 56.42 (OCH_3), 64.19 (C-5″), 68.68 (C-10), 75.85 (C-4″), 78.60 (C-2″), 80.20 (C-3″), 101.77 (C-5), 102.41 (OCH_2O), 108.78 (C-5′), 110.76 (C-8), 111.69 (C-2′), 112.73 (C-1″), 119.48 (C-3), 124.70 (C-6′), 127.87 (C-4a), 129.09 (C-2), 130.16 (C-1′), 132.34 (C-8a), 136.42 (C-1), 146.28 (C-4), 148.72 (C-3′, C-4′), 149.22 (C-7), 152.60 (C-6), 172.14 (C-9). ESI MS: *m/z* 1019 $[2M + Na]^+$, 521 $[M + Na]^+$; MS/MS *m/z* 521 $[M + Na]^+$, 389 [aglycone + Na]$^+$.

Haplomyrtin (**2**). ^{13}C NMR (DMSO-d_6): δ 55.53 (OMe), 66.64 (C-10), 100.86 (C-5), 100.95 (OCH_2O), 107.79 (C-5′), 109.01 (C-8), 110.95 (C-2′), 118.34 (C-3), 120.44 (C-2), 122.78 (C-4a), 123.76 (C-6′), 128.72 (C-1), 129.25 (C-1′), 130.09 (C-8a), 145.03 (C-4), 146.42 (C-4′), 146.67 (C-3′), 147.78 (C-7), 150.23 (C-6), 169.73 (C-9).

(−)-1*β-Polygamain* (**4**). ^{1}H NMR ($CDCl_3$): δ 2.49 (1H, *dd*, $J = 13.5$, 11.0 Hz, H-2), 2.60 (1H, *m*, H-3), 2.89 (1H, *dd*, $J = 15.4$, 10.9 Hz, H-4α), 2.98 (1H, *dd*, $J = 15.3$, 5.0 Hz, H-4β), 3.98 (1H, *dd*, $J = 10.4$, 8.6 Hz, H-10β), 4.05 (1H, *d*, $J = 10.9$ Hz, H-1), 4.52

(1H, *dd*, $J = 8.6$, 6.4 Hz, H-10α), 5.88, 5.89 (2H, 2*d*, $J = 1.5$ Hz, 6,7-OCH_2O), 5.94, 5.95 (2H, 2*d*, $J = 1.5$ Hz, 3′,4′-OCH_2O), 6.33 (1H, *s*, H-8), 6.59 (2H, *s*, H-2′, H-5), 6.76 (1H, *dd*, $J = 8.0$, 1.3 Hz, H-6′), 6.79 (1H, *d*, $J = 8.0$ Hz, H-5′).

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