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LIGNANS, ALKALOIDS AND COUMARINS FROM *HAPLOPHYLLUM VULCANICUM**

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Key Word Index—*Haplophyllum vulcanicum*; Rutaceae; (−)-haplomyrfolol; (−)-syringaresinol; dibenzylbutyrolactols; dibenzylbutyrolactone lignans; arylnaphthalene lignan; arylnaphthalene lignan glycoside; furofuran lignan; furoquinoline alkaloids; coumarins.

Abstract—Six lignans, (−)-haplomyrfolol, (−)-haplomyrfolin, (−)-kusunokinin, (−)-syringaresinol, diphyllin and (−)-tuberculatin, five furoquinoline alkaloids, dictamnine, γ-fagarine, haplopine, robustine and skimmianine, and two coumarins, scopoletin and umbelliferone, have been isolated from *Haplophyllum vulcanicum*. (−)-Haplomyrfolol, a novel dibenzylbutyrolactol, exists as an epimeric mixture in solution. Complete assignments of the proton and carbon chemical shifts for the individual epimers were accomplished on the basis of high resolution 1D and 2D NMR data. This is the first report of (−)-syringaresinol from the genus *Haplophyllum* and of (−)-haplomyrfolin, (−)-tuberculatin, dictamnine, haplopine and umbelliferone from the title species.

INTRODUCTION

The members of the genus *Haplophyllum*, represented by *ca* 70 species widespread from the Mediterranean area to Siberia [1], are prolific sources of quinoline alkaloids, lignans of diverse structures and coumarins. In Turkey, the genus comprises 14 species, of which seven are endemic [2]. Prior to the present study, we have reported on the chemical composition of *H. vulcanicum*, an annual herb native to central Anatolia. The aerial parts of this species were shown to elaborate two novel furoquinoline alkaloids, (+)-nigdenine and (+)-nkolbisine [3], accompanied by the already known furoquinolines, delbine, γ-fagarine, kokusaginine, robustine and skimmianine [3, 4]. Furthermore, it yielded a novel lignan, konyanin, which is the first example of a 1,4-dihydronaphthalene lignan incorporating a lactone carbonyl at C-3, rather than at C-2 [4]. Two other lignans also obtained were diphyllin [3, 4] and (−)-kusunokinin [4], the latter being the first report of the occurrence of this dibenzylbutyrolactone type of lignan in *Haplophyllum*. A simple coumarin, scopoletin, was also described [4].

Our recent investigation of the title species afforded a novel compound, (−)-haplomyrfolol (**1**), a dibenzylbutyrolactol lignan which exists as an epimeric mixture in solution. High resolution 1D and 2D NMR studies allowed complete assignments of the proton and carbon shifts of the individual epimers. (−)-Haplomyrfolin (**2**), the dibenzylbutyrolactone counterpart of **1**, was also obtained in our study, along with another compound of the same subgroup, (−)-kusunokinin [4]. Of the ubiquitous arylnaphthalene subgroup of lignans, diphyllin [4] and its 4-*O*-β-D-apiofuranoside, (−)-tuberculatin [5, 6], were shown to be present in good abundance, while the furofuran lignan (−)-syringaresinol [7–10] is reported for the first time from *Haplophyllum*. In addition to the re-isolation of the known furoquinoline alkaloids, γ-fagarine [11], robustine [12] and skimmianine [12, 13], two further compounds with the same nucleus, dictamnine [13, 14] and haplopine [15], were also isolated and characterized. *Haplophyllum vulcanicum* also afforded two simple coumarins, scopoletin [4] and umbelliferone [16], the latter being its first isolation from this species. Both compounds are common elements of the chemical compositions of rutaceaeous plants [17]. The characterization of all compounds was accomplished by extensive spectral analyses.

RESULTS AND DISCUSSION

The EI mass spectrum of the laevorotatory compound **1**, $C_{20}H_{22}O_6$, with two prominent peaks at *m/z* 137 (100%) and 135 (72%), accounting for a hydroxymethoxybenzyl, and a methylenedioxybenzyl cation, respectively, resembled closely that of **2**, a known dibenzylbutyrolactone also found in *H. vulcanicum*. However, the $[M]^+$ of **1** (*m/z* 358, 11%) was found at

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2 mu higher than that of **2**, suggesting the incorporation of a γ-butyrolactol structure, instead of a γ-butyrolactone. The lack of a carbonyl absorption in the IR spectrum of **1** also favoured this proposition.

Preliminary information gathered from the ^{1}H NMR spectrum of **1** lent further support to its tentative structure as the lactol counterpart of **2**. Signals for two aromatic ABX systems, a methoxyl and a methylenedioxy group and nine aliphatic protons, including a relatively high frequency signal for the acetalic proton, were clearly discernable. The identity of the fourth substituent as a phenolic hydroxyl was demonstrated by the UV spectrum, where the addition of the alkali induced a prominent bathochromic shift.

Of greater interest, however, was the doubling of the signals in both the ^{1}H and the ^{13}C NMR spectra of **1**, indicating the presence of an epimeric mixture, as is the case with a number of known dibenzylbutyrolactones [6, 18–21]. The ratio of the epimers, as deduced from the integrations in the ^{1}H NMR spectrum, was 3:2 (**1a**:**1b**). Since the high resolution ^{1}H NMR spectrum furnished well-resolved signals for both the major and the minor isomers, assignments of the chemical shifts for the individual epimers were attempted.

Initially, a selective nOe experiment correlated the methoxyl signals of **1a** (δ 3.83) and **1b** (δ 3.87) to the respective H-2′ *meta*-coupled protons at δ 6.51 and 6.61, respectively. With these H-2′ chemical shifts as points of reference, a subsequent ^{1}H TOCSY experiment allowed the precise assignments of the 12 aromatic protons comprising the four ABX systems.

The signal of the acetalic proton (H-9) for **1a** was observed at δ 5.22 as a doublet (J = 1.6 Hz), while the corresponding proton of **1b** resonates also as a doublet at δ 5.24 (J = 4.5 Hz). Another set of chemical shifts belongs to the methylene protons at C-9′, which are observed at δ 4.0 and 3.80 for the major (**1a**) and at δ 4.09 and 3.58 for the minor (**1b**) isomers, respectively. Correlations of the above-mentioned well-defined high frequency aliphatic signals with the remaining more upfield signals were accomplished by a subsequent ^{1}H–^{1}H COSY experiment, which allowed a complete map of the proton shifts assigned individually for each epimer (Table 1).

The presence of two epimers was also discernable from the ^{13}C NMR spectrum of **1**, which accounts for 40 carbons. With the well-established proton chemical shifts in hand, it was then possible to make unambiguous associations of these protons to the protonated carbons from the data obtained from a HSQC experiment. The assignments of the non-protonated carbons, as well as the verification of the previous assignments, were accomplished on the basis of information gathered from a HMBC experiment. The individual carbon chemical shifts thus established for **1a** and **1b** are cited in detail in Table 1. Noteworthy is the fact that the corresponding carbon chemical shifts of the epimers are nearly identical, with the exception of C-7, C-9 and C-8′, all of which resonate at relatively higher frequencies in **1a** as compared to those in **1b**. The magnitude of the chemical shift difference between the corresponding signals of the isomers is *ca* 5 ppm for the former two signals, while a Δδ of *ca* 3 ppm is observed for those of C-8′, reflecting the different configurations at the C-9 chiral centre.

To establish the configuration of the chiral centres at C-8 and C-8′, compound **2**, of established (8*R*, 8′*R*) configuration, was subjected to DIBAH reduction [20,

Table 1. ^{1}H and ^{13}C chemical shifts (ppm) and J (^{1}H, ^{1}H) coupling constants (Hz) of (−)-haplomyrfolol (**1a** and **1b**)

	1a		**1b**	
	^{1}H	^{13}C	^{1}H	^{13}C
1		133.32		134.53
2	6.55 *d* (1.5)	109.14	6.74 *d* (1.5)	109.29
3		147.60		147.49
4		145.85		145.68
5	6.68 *d* (7.6)	107.98	6.72 *d* (7.9)	108.10
6	6.54 *dd* (8.0, 1.5)	121.71	6.68 *dd* (7.9, 1.5)	121.56
7	2.42 *m*, 2.65 *m*	38.39	2.61 *m*, 2.78 *m*	33.61
8	2.15 *m*	53.10	2.00 *m*	52.04
9	5.22 *d* (1.6)	103.36	5.24 *d* (4.5)	98.83
1′		132.23		131.92
2′	6.51 *d* (1.8)	111.07	6.61 *d* (1.8)	111.03
3′		146.34		146.46
4′		143.85		144.00
5′	6.79 *d* (8.0)	114.25	6.82 *d* (7.9)	114.32
6′	6.56 *dd* (8.0, 2.0)	121.17	6.63 *dd* (8.0, 1.8)	121.20
7′	2.59 *m*, 2.62 *m*	39.15	2.45 *m*, 2.77 *m*	38.82
8′	2.17 *m*	45.99	2.45 *m*	42.88
9′α	3.80 *t* (8.3),	72.24	4.09 *t* (8.2),	72.63
9′β	4.00 *dd* (8.6, 7.1)		3.58 *t* (8.3)	
OCH_3	3.83	55.79	3.87	55.87
OCH_2O	5.905, 5.908	100.83	5.911, 5.914	100.74

21] to yield an epimeric mixture identical in all respects with natural **1**. Furthermore, comparison of the circular dichroic spectral data for **1** with those for analogous compounds of established stereochemistry confirmed the aforementioned configuration [6, 22].

A survey of the literature in an attempt to establish the configuration of **1a** and **1b** at C-9 revealed that a H-9β configuration has often been assigned to the isomer with the higher frequency C-7, C-9 and C-8′ signals in the ^{13}C NMR spectrum and with a smaller coupling constant of $J_{8,9}$ in the ^{1}H NMR spectrum [20, 23, 24]. However, Barrero *et al.* claimed the opposite stereochemistry according to the interpretation of their ^{13}C NMR data for C-7 and C-9 [21]. Therefore, an effort has been made to ascertain the orientation of the hydroxyl group at C-9.

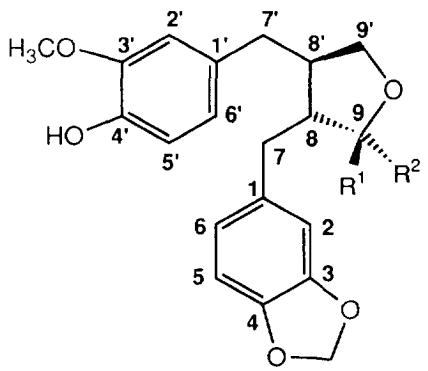
In the ^{1}H–^{13}C long-range correlation experiment, where a coupling constant of 9.1 Hz had been selected, H-9 of the major epimer (**1a**) correlates with the γ-carbon atoms 7 (δ 38.39), 8′ (δ 45.99) and 9′ (δ 72.24). A correlation with the β-carbon atom 8 (δ 53.10) is also observed. On the other hand, H-9 of the minor isomer (**1b**) correlates with two γ-carbon atoms 8′ (δ 42.88) and 9′ (δ 72.63) and a β-carbon atom 8 (δ 52.04), while a correlation with the γ-carbon atom 7 (δ 33.61) is missing. The lack of this correlation indicates the presence of a substantially smaller ^{3}J coupling constant between C-7 and H-9 in **1b**, and consequently a C(7)–C(8)–C(9) dihedral angle of nearly 90°. Studies using Dreiding models show that these conditions are fulfilled only when there is a *cis*-relationship between H-8 and H-9. Since the configuration of H-8β has previously been established, it follows that the orientation of H-9 of **1b** is also β. Thus, **1b** is characterized as (8*R*, 8′*R*, 9*S*)-4′-hydroxy-3′-methoxy-3,4-methylenedioxy-9,9′-epoxylignan, while **1a** is its epimer at C-9 with an (8*R*, 8′*R*, 9*R*) configuration.

At this point, a further partial nOe experiment allowed the precise assignment of the chemical shifts to the non-equivalent protons at C-9′. An irradiation of H-8 of the major isomer (**1a**) (δ 2.15) brought about a prominent enhancement of the high frequency signal at δ 4.0, indicating that this proton is on the same side of the five-membered-ring plane as H-8. On the other hand, irradiation of H-8 of the minor isomer (**1b**) (δ 2.0) led to the enhancement of the signal at δ 3.58 confirming the orientation of this proton as being on the same side of the ring plane as H-8 and H-9 (δ 5.24). Therefore, it follows that H-9′ with the higher frequency signal lies on the same side as the hydroxyl group at C-9.

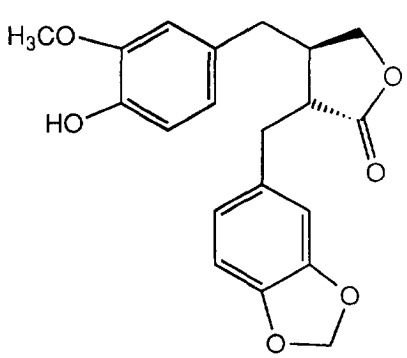
Compound **2**, which is the butyrolactone counterpart of **1**, has also been isolated from *H. vulcanicum* in high yield as one of the major lignans. The first and so far only isolation of **2** had been from *H. myrtifolium* by one of the authors [22]. However, since no ^{13}C NMR data could have been reported then, we wish to report here the relevant data to complete the spectral information on this dibenzylbutyrolactone lignan.

While dibenzylbutyrolactones and their congeners seem to be common elements of the chemical compositions of *Haplophyllum* species, it has been observed that the lignan content of *H. vulcanicum* is dominated by the arylnaphthalene lignan glycoside, (−)-tuberculatin, and its aglycone, diphyllin, which together comprise *ca* 15% of the crude basic extract. (−)-Tuberculatin, already reported from a number of *Haplophyllum* species [5, 6], is described for the first time from the title species.

One of the minor lignans of *H. vulcanicum* has been identified as (−)-syringaresinol ($[\alpha]_D$ −13.0°, c 0.40, $CHCl_3$) on the basis of its physical and spectral data, which are in good accord with those reported in the literature [7–10]. Both (+)- and (−)-enantiomers, as well as the racemic form of syringaresinol, have been described previously from some rutaceaeous plants [25–27]. However, the present study is the first report of syringaresinol from *Haplophyllum*, which provides further chemotaxonomical evidence for the relationship between *Haplophyllum* and other genera in the Rutaceae. Moreover, its occurrence appends the furofuran nucleus to the lignan content of *H. vulcanicum*.



1a R^1 = OH; R^2 = H
1b R^1 = H; R^2 = OH



2

The presence of the furoquinoline alkaloids, dictamnine and haplopine, and of the coumariun, umbelliferone, are described for the first time from the title species.

EXPERIMENTAL

General. ^{1}H NMR (600.13 MHz), ^{13}C NMR (150.9 MHz) and 2D correlation spectra of **1**: Bruker AMX 600; ^{13}C NMR (75.45 MHz) spectrum of **2**: Bruker ARX 300. EIMS: 70 eV; CIMS, NH_3.

Plant material. Halophyllum vulcanicum Boiss. et Heldr. was collected from Nigde, Ulukisla, Turkey, in June 1994. A voucher specimen, No 1177, is deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University.

Extraction and isolation. Dried and powdered total plant material (40 kg) was extracted with EtOH (800 l) at room temp. The crude extract thus obtained was dissolved in 5% aq. HCl (20 l) and filtered. The acidic soln was made alkaline with 10% aq. NH_4OH and then extracted with $CHCl_3$. The organic solvent was evapd to furnish the crude basic extract (67.14 g). Initial fractionation was by CC on silica gel (0.063–0.200 mm, Merck) using $CHCl_3$ gradually enriched with MeOH as mobile phase. Similar frs (1 l) were combined. For subsequent CC fractionation of the combined frs, a finer adsorbent (silica gel, 0.015–0.040 mm, Merck) was used. Further purifications were either by prep. TLC on silica gel or by recrystallization from suitable solvents.

Elution of the preliminary column with $CHCl_3$ afforded frs 9 and 10 (14.42 g), further fractionated by CC using C_6H_6–Me_2CO (19:1). The following compounds were obtained in order of elution: robustine (116 mg), dictamnine (98 mg), (−)-kusunokinin (16 mg), (−)-haplomyrfolin (**2**) (2.4 g) and γ-fagarine (4.5 g). Frs 14 and 15 (4.5 g) were composed mainly of skimmianine, which recrystallized from MeOH to give 3.7 g pure alkaloid. Frs 19–23 (1.42 g) obtained by elution with 1% MeOH in $CHCl_3$ was subjected to further sepn on a silica gel column using C_6H_6–Me_2CO (4:1). Prep. TLC of a faster moving band (241 mg) furnished 48 mg haplopine and 73 mg **1**. Prep. TLC of two of the slower moving bands (62.1 and 77.6 mg) yielded scopoletin (15 mg) and (−)-syringaresinol (63 mg), respectively. CC sepn of frs 28–33 (7.92 g) using C_6H_6–EtOAc–MeOH (8:1:1) gave 2.5 g diphyllin and 125 mg umbelliferone. (−)-Tuberculatin was the main component of frs 49–62 (8.75 g) from which it pptd as an amorphous solid (7.5 g).

(−)-*Haplomyrfolol* (**1**). Amorphous solid. $[\alpha]_D$ −48.0° (*c* 0.10, MeOH). UV λ_{max}^{MeOH} nm (log ε) 206 (4.59), 230 (4.10), 284 (3.91); UV $\lambda_{max}^{MeOH+NaOH}$ nm (log ε) 208 (4.66), 237 (4.01), 289 (3.85). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3050, 2940, 2880, 2850, 1610, 1515, 1505, 1490, 1465, 1445, 1370, 1270, 1240, 1220, 1190, 1150, 1125, 1100, 1040, 935, 865, 810. ^{1}H NMR ($CDCl_3$): Table 1. ^{13}C NMR ($CDCl_3$): Table 1. EIMS *m/z* (rel. int.) 358 [M^+, 11], 203 (6), 163 (13), 138 (35), 137 (100), 136 (54), 135 (72), 131 (13), 123 (12), 122 (11), 106 (13). CD (MeOH) Δε (nm): 0 (303), −1.2 (289), 0 (250), −1.71 (230), −7.5 (207).

(−)-*Haplomyrfolin* (**2**). ^{13}C NMR ($CDCl_3$): δ 34.68 (C-7), 38.22 (C-7′), 41.23 (C-8′), 46.37 (C-8), 55.75 (OCH_3), 71.15 (C-9′), 100.93 (OCH_2O), 108.12 (C-5), 109.41 (C-2), 110.98 (C-2′), 114.44 (C-5′), 121.22 (C-6′), 122.19 (C-6), 129.68 (C-1′), 131.33 (C-1), 144.37 (C-4′), 146.37 (C-4), 146.54 (C-3′), 147.78 (C-3), 178.46 (C-9).

DIBAH reduction of **2**. Compound **2** (119 mg, 0.33 mmol) was dissolved in 12 ml CH_2Cl_2 and 1 ml 1M DIBAH in CH_2Cl_2 was added dropwise while stirring under N_2 at −78°. Stirring was continued at the same temp. for 3 hr. After addition of MeOH, the reaction mixt. was warmed to room temp. and 3 ml Et_2O, 3 ml satd NaCl soln, 3 ml 0.5 N HCl and a further 3 ml Et_2O were added consecutively. After sepn of the organic phase, the aq. phase was extracted with 3 × 15 ml $CHCl_3$. The organic phase was dried (Na_2SO_4), filtered and distilled *in vacuo* to yield 78 mg crude product. Subsequent prep. TLC on silica gel using C_6H_6–EtOAc–MeOH (17:2:1) yielded 35 mg **1**, identical in all respects with natural **1**, as well as 32 mg unreacted **2**.

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