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Lignans and Alkaloids from *Haplophyllum suaveolens*

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Summary. A new lignan of the dibenzylbutyrolactone type, haplotonin, was isolated from the aerial parts of *Haplophyllum suaveolens* together with five known compounds-the alkaloids flindersine, 6-methoxyflindersine, N-acetoxymethylflindersine, and evoxine and the lignan 4-acetyldiphyllin. This is the first report on the occurrence of N-acetoxymethylflindersine in the genus *Haplophyllum* and on 6-methoxyflindersine and 4-acetyldiphyllin as constituents of the investigated species.

Keywords. Alkaloids; *Haplophyllum suaveolens*; Haplotonin; Lignans; Phytochemistry.

Introduction

Haplophyllum species (*Rutaceae*) are a rich source of quinoline alkaloids, lignans, and coumarins. Previous studies on the aerial parts of *Haplophyllum suaveolens* (DC.) G. Don *fil* have shown the presence only of quinoline alkaloids [1, 2] and flavonoids [3]. This prompted us to start a phytochemical investigation of *H. suaveolens* of Bulgarian origin which has not been studied so far. In this paper we describe the isolation and structure elucidation of the novel lignan haplotonin (**1**) and five known compounds. The work is connected with our interest in the chemistry of rutaceous plants [4–6].

Results and Discussion

From the aerial parts of *H. suaveolens*, five known compounds and the new lignan haplotonin (**1**) were isolated. The known constituents were identified as the pyrano-2-quinolone alkaloids flindersine (**2**), 6-methoxyflindersine (**3**), and N-acetoxymethyl-flindersine (**4**), the furoquinoline alkaloid evoxine (**5**), and the diarylnaphtalene lignan 4-acetyldiphyllin (**6**) on the basis of their NMR spectra and by comparison with literature data [1, 7–9]. Flindersine (**2**) and evoxine (**5**) have already been isolated from *H. suaveolens* [1, 2]. This is the first report on the isolation of **4** from the genus *Haplophyllum*, and of **3** and **6** from the title species.

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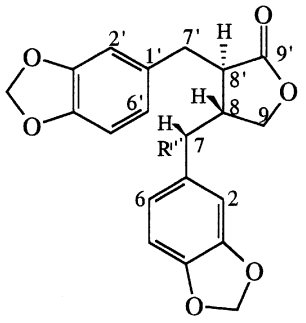
The structure of the novel compound **1** was deduced from a detailed study of its 1D and 2D NMR and mass spectra.



	R
1	$OCOCH_2CHMe_2$
7	OAc
8	OH
9	H

Haplotonin (**1**) was obtained as an amorphous powder. A molecular formula of $C_{25}H_{26}O_8$ was confirmed from its 70 eV MS (M^+, $m/z = 454$) as well as from its NMR spectra (^1H, ^{13}C, DEPT). The UV and IR spectra suggested a lignan structure of the dibenzylbutyrolactone type. In addition to the C = O group in the lactone ring (1750 cm^{-1}, $\delta_C = 178.1$ ppm), an ester carbonyl group was indicated by the IR (1733 cm^{-1}) and ^{13}C NMR (171.9 ppm) spectra. The signals of aromatic ABX systems ($\delta = 6.52$–6.69 ppm, m, 4H, H-2, H-6, H-2′ H-6′; $\delta = 6.70$ ppm d, $J = 8.4$ Hz, H-5; $\delta = 6.72$ ppm, d, $J = 8.1$ Hz, H-5′) and two methylenedioxy groups ($\delta = 5.94$ ppm, m, 4H; $\delta_C = 101.1$ and 101.4 ppm) were clearly visible in the ^1H and ^{13}C NMR spectra.

The ^1H, ^{13}C, COSY, and HMQC spectra proved an CH_2CHMe_2 unit. The HMBC correlation from the protons of the CH_2 group at 2.24 ppm (d, $J = 7.0$ Hz) to the carbon atoms at 25.6, 22.4, and 171.9 ppm confirmed the existence of this unit and its attachment to the ester carbonyl group. The downfield doublet at 5.72 ppm ($J = 6.5$ Hz, 1H) was assigned to H-7 ($\delta_C = 76.0$ ppm) because of its coupling to H-8 ($\delta_H = 2.72$ ppm, m; δ_C 43.4 ppm) observed in the COSY spectrum. H-8 was in turn coupled to H-8′ (2.80 ppm, m, 1H), H_a-9 (3.92 ppm, dd, $J = 10.0$ and 6.0 Hz, 1H), and H_b-9 (3.99 ppm, dd, $J = 10.0$ and 7.8 Hz, 1H); H-8′ was further coupled to H-7′ (2.90 ppm, m, 2H). The following long range heteronuclear shift correlations confirmed the proposed arrangement in structure **1** (chemical shifts in ppm): H-7 (5.72)→C-8 (43.4), C-9 (68.1), C-1 (131.1), C-2 (106.4), C-6 (119.9); H_a-9 (3.92) and H_b-9 (3.99)→C-8 (43.4), C-7 (76.0), C-9′ (178.1); methylene group at C-7′ (2.90, m; 35.1)→C-8′ (43.8), C-9′ (178.1), C-1′ (130.6), C-2′ (109.6), C-6′ (122.6); H-8′ (2.80, m)→C-7′ (35.1), C-1′ (130.6), C-8 (43.4), C-7 (76.0), C-9′ (178.1).

In the 70 eV mass spectrum of **1** the characteristic ions resulting from the facile benzylic cleavages were found at $m/z = 135$ and 235 as expected. The base peak at $m/z = 175$ suggested the formation of ring-opened ions **a** (ef. Experimental) at $m/z = 219$ and further easy loss of CO_2 [10]. Elimination of isovaleric acid (102

mass units) from M^+ to $m/z = 352$ was also in full accordance with the proposed structure.

The trans ring fusion was indicated by NOE difference experiments. Irradiation of H_b-9 caused an enhancement of H-8, whereas irradiation of H_a-9 led to enhancements of H-8′ and H-7. NOE enhancements of H_a-9, H-8, H-8′, H-2, and H-6 upon irradiation of H-7 were also observed.

The amount available of compound **1** was not enough for measurement of its optical rotation or/and for chemical transformation to the known compounds **7**–**9**, whose absolute stereochemistry (8*R*, 8′*R*) has been established by CD spectra [11]. However, the ^{1}H NMR data of **1** were in good agreement with those reported for **7** and **8**, and the chemical shift and the coupling constant of H-7 in **1** (5.72 ppm, 6.5 Hz) were identical with those reported for **7** [11, 12]. This indicated that, most probably, haplotonin has the same stereochemistry as compounds **7** and **8**.

Experimental

General procedures

NMR spectra were obtained in $CDCl_3$ on a Bruker DRX 250 spectrometer using *TMS* as internal standard; 70 eV MS; Varian MAT 311A; TLC: aluminum sheets, silica gel 60 F_{254} (Merck), bands detected under UV light, by exposure to J_2 vapour, or by spraying with *Dragendorff* reagent of H_2SO_4; liquid vacuum chromatography (LVC): silica gel 60 (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 ($3 \times 24\,h \times 3.5\,dm^3$). 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 with *PE* and EtOAc afforded *R*-1 (1.7 g) and *R*-2 (3.0 g), respectively. *R*-2 was subjected to LVC over silica gel (45 g) using $CHCl_3$-EtOAc = 30:1 → 5:1 to give fractions F1–F10. From F3 (650 mg), **1** (8.6 mg) and **4** (1.6 mg) were isolated by CC over silica gel (25 g) eluting with hexane:EtOAc = 6:1 → 1:1 and subsequent pTLC ($CHCl_3$). F4 (720 mg) was treated in the same way to give **2** (7.3 mg), **3** (5.0 mg), **5** (2.6 mg), and **6** (3.0 mg).

Haplotonin (**1**; $C_{25}H_{26}O_8$)

Amorphous powder; IR: $\nu_{max}^{KBr} = 1750, 1733, 1606, 1500, 1493, 1440, 1250, 1093, 1033, 1006, 926, 807\ cm^{-1}$; UV: $\lambda_{max}^{MeOH} = 207, 234, 288$ nm; EIMS: $m/z = 454$ ($M^{+\cdot}$, 37.0), 370 (1.0), 352 (M-$Me_2CHCH_2COOH^{+\cdot}$, 11.5), 307 (6.0), 280 (1.6), 235 (4.0), 230 (4.0), 219 (**a**, Ar-CH_2-CH = CH-CH_2-O-C ≡ O^+, 1.6), 217 (4.0), 192 (3.0), 178 (4.5), 175 (**a**-$CO_2{}^+$, 100.0), 151 (16.0), 135.0 (75.0), 131 (12.0), 85 (44.0), 57 (39.0); ^{1}H NMR (250 MHz, δ, $CDCl_3$): 6.72 (1H, d, $J = 8.1$ Hz, H-5 or H-5′), 6.70 (1H, d, $J = 8.4$ Hz, H-5 or H-5′), 6.69-6.52 (4H, m, H-2, H-2′, H-6, H-6′), 5.94 (4H, m,

2xOCH$_2$O), 5.72 (1H, d, $J = 6.5$ Hz, H-7), 3.99 (1H, dd, $J = 10.0$ and 7.8 Hz, H$_b$-9), 3.92 (1H, dd, $J = 10$ and 6.0 Hz, H$_a$-9), 2.90 (2H, m, CH$_2$-7′), 2.80 (1H, m, H-8′), 2.72 (1H, m, H-8), 2.24 (2H, d, $J = 7.0$ Hz, COC*H*$_2$CHMe$_2$), 2.09 (1H, m, COCH$_2$C*H*Me$_2$), 0.94 (3H, d, $J = 6.5$ Hz, COCH$_2$CH*Me*), 0.92 (3H, d, $J = 6.5$ Hz, COCH$_2$CH*Me*) ppm; ^{13}C NMR (62.89 MHz, δ, CDCl$_3$): 178.1 (C-9′), 171.9 (*C*OCH$_2$CHMe$_2$), 148.0 (C-4 or C-4′), 147.8 (C-4 or C-4′), 147.6 (C-3 or C-3′), 146.5 (C-3 or C-3′), 131.1 (C-1), 130.6 (C-1′), 122.6 (C-6′), 119.9 (C-6), 109.6 (C-2′), 108.3 (C-5 or C-5′), 108.2 (C-5 or C-5′), 106.4 (C-2), 101.4, 101.1 (2xOCH$_2$O), 76.0 (C-7), 68.1 (C-9), 43.8 (C-8′), 43.4 (C-8, CO*C*H$_2$CHMe$_2$), 35.1 (C-7′), 25.6 (COCH$_2$*C*HMe$_2$), 22.4 (COCH$_2$CH*Me*$_2$) ppm.

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