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HAPLOMYRTIN AND (−)-HAPLOMYRFOLIN: TWO LIGNANS FROM *HAPLOPHYLLUM MYRTIFOLIUM*

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Key Word Index—*Haplophyllum myrtifolium*; Rutaceae; whole plant; haplomyrtin; haplomyrfolin; arylnaphthalide lignans; dibenzylbutyrolactone lignans.

Abstract—Haplomyrtin, a new 1-aryl-2-3-naphthalide lignan, and (−)-haplomyrfolin, a new dibenzylbutyrolactone lignan, have been obtained from Turkish *Haplophyllum myrtifolium*.

INTRODUCTION

The genus *Haplophyllum* (Rutaceae) is represented by about 70 species spread out from the Mediterranean to Eastern Siberia [1], and has been shown to elaborate lignans [2–8] along with a range of other secondary constituents [9]. In the present study, we report the isolation and structure elucidation of two new lignans, haplomyrtin (**1**) and (−)-haplomyrfolin (**4**) from *Haplophyllum myrtifolium* Boiss., a perennial herb endemic to Turkey, and hitherto uninvestigated for its chemical content.

RESULTS AND DISCUSSION

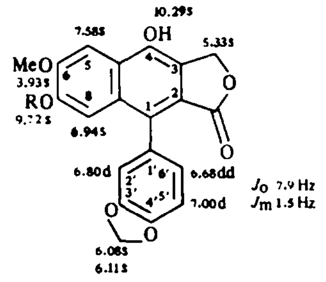
Haplomyrtin (**1**), $C_{20}H_{14}O_7$, was obtained as a white amorphous powder. The mass spectral molecular ion, m/z 366, was also the base peak. The 360 MHz NMR spectrum in DMSO-d_6, summarized around expression **1**, closely resembled that of the known arylnaphthalide lignan diphyllin (**2**) [6]. A prominent feature of the spectrum was a two-proton singlet at δ5.33 representing the methylene protons of the γ-lactone ring. This chemical shift was diagnostic of a 1-aryl-2-naphthoic acid rather than of the alternate 1-aryl-3-naphthoic acid arrangement [14–16].

Also present in the NMR spectrum were one-proton singlets at δ9.72 and 10.29 which disappeared upon addition of D_2O, and which indicated the presence of two exchangeable and phenolic protons. Consonant with this finding were the UV bathochromic shift upon addition of alkali, as well as the strong IR stretching absorptions between 3000 and 3500 cm^{-1}. Upon treatment with diazomethane, haplomyrtin (**1**) furnished the di-*O*-methyl derivative **3** which corresponded with the compound obtained by *O*-methylation of authentic diphyllin (**2**). Compound **3** was in fact the known lignan justicidin A [2], thus confirming that the methylenedioxy function in **1** was situated on the lower phenyl ring.

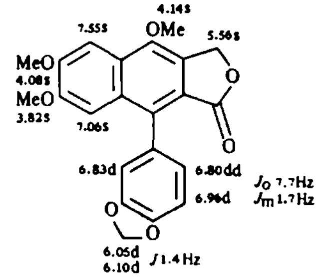
At this stage, it was necessary to carry out NMR NOEDS experiments on haplomyrtin (**1**) to assign the positions of the phenolic functions as well as the methoxyl group. The methylene protons of the lactone ring (δ5.33) showed reciprocating NOEs with the phenolic proton at δ10.29, thus establishing the position of the latter at C-4. In turn, irradiation of this phenolic proton produced an enhancement of the aromatic proton at δ7.58, making it possible to assign this chemical shift to H-5. The H-5 absorption also exhibited strong reciprocating NOEs with the methoxyl protons at δ3.93, thereby confirming the position of the methoxyl at C-6. Additionally, the singlet absorption at δ6.94 displayed reciprocating NOEs with the phenolic proton at δ9.72 (Experimental).

For further confirmation of our assignments, an NMR NOEDS study was also carried out with the methylated product **3**, namely justicidin A. The H-2′ absorption (δ6.83) recorded an enhancement upon irradiation of H-8 (δ7.06), whereas no enhancement of the H-6′ signal (δ6.80) was observed. In this case, the pendant aryl ring is sterically constrained and is not perpendicular to the naphthalene system as sometimes assumed [10–12]. Rather, this ring, while still definitely out of the naphthalene plane, lies in a conformation where H-8 is proximate to H-2′. Conversely, the less hindered side of the pendant ring (i.e. H-6′) lies close to the relatively bulky lactone carbonyl. This conformational preference is made even more evident when one considers the chemical shifts for H-6′ in haplomyrtin (**1**) on the one hand, and justicidin A (**3**) on the other. The proton in question in haplomyrtin (**1**) absorbs at δ6.68. But in justicidin A, where the relatively bulky methoxyl is present at C-7, the free rotation of the pendant ring is further impeded, so that H-6′ is more strongly under the deshielding influence of the oxygen of the lactone carbonyl. The net result is that H-6′ in justicidin A falls further downfield at δ6.80.

Our second lignan, (−)-haplomyrfolin (**4**), $C_{20}H_{20}O_6$, exhibited a strong carbonyl absorption at 1765 cm^{-1} in its IR spectrum, suggesting the presence of a butyrolactone system. The 360 MHz NMR spectrum in $CDCl_3$, summarized around expression **4**, displayed an aromatic methoxyl singlet, a methylenedioxy group as two close doublets, and six aromatic protons as two ABX systems. The presence of a phenolic function was discernible from



1 R = H (with NMR)
2 R = Me



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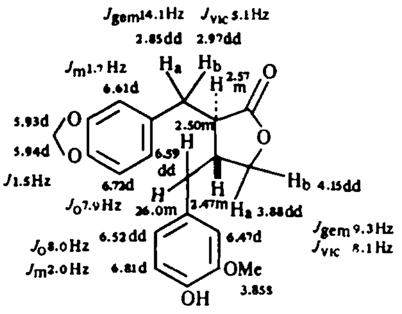

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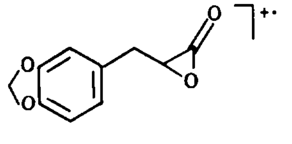

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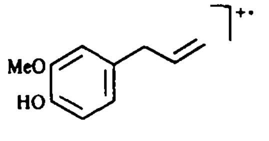

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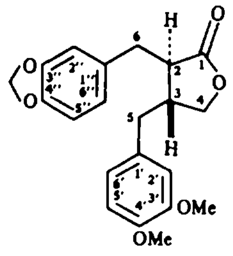

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the bathochromic shift in the UV spectrum upon addition of alkali, and from the strong IR absorption at 3540 cm^{-1}.

In the mass spectrum, the major ions resulting from facile benzylic cleavages were *m/z* 135 and 137. These data pointed to a butanolide substituted at C-2 and C-3 with two benzylic moieties bearing a methylenedioxy substituent in one case, and a methoxyl and a hydroxyl in the other. Furthermore, ions *m/z* 192 and 164, as represented by expressions **5** and **6**, supplied strong support for the substitution pattern in haplomyrfolin (**4**) which must incorporate a 2-(3″,4″-methylenedioxybenzyl)butyrolactone, rather than the alternate 3-(3″,4″-methylenedioxybenzyl)butyrolactone arrangement [13]. (For the numbering system, refer to expression **7**.)

NMR spin decoupling experiments were run to confirm each of the chemical shift assignments. Furthermore, NMR NOEDS studies were carried out to complement the data. In particular, the H-2′ doublet (δ6.47) exhibited strong reciprocating NOEs with the methoxyl signal (δ3.85), thus confirming the site of the methoxyl group.

The relative configuration at C-2 and C-3 for haplomyrfolin (**4**) could be established from the NMR spectrum. The ring fusion is *trans* because of the non-equivalence of the C-4 methylene protons, H-4a and H-4b being at δ3.88 and 4.15 [13, 14]. The coupling constants $J_{3,4a}$ and $J_{3,4b}$ are essentially identical ($J \simeq 8.1$ Hz), which lends further support to a *trans* relationship between the two benzylic groups [15]. Finally, the absolute configuration (2*R*,3*R*) was derived from the CD curve in methanol (Experimental) which was closely related to those reported for analogous compounds of established stereochemistry [16].

As expected, diazomethane *O*-methylation of haplomyrfolin (**4**) supplied the known (−)-kusunokinin (**7**) [6]. It should be pointed out in conclusion that species **4** had been previously prepared as a racemate [17]. The NMR, mass and IR spectra for the levorotatory natural product proved to be essentially identical with those reported for the synthetic racemate.

EXPERIMENTAL

Plant material. The whole plant was collected near Malatya, Turkey, in early July, 1983, by one of the authors. A voucher specimen was deposited in the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Ege University.

Isolation of haplomyrtin (**1**) *and* (−)*-haplomyrfolin* (**4**). The dried and finely powdered plant material (8.77 kg) was extracted with EtOH at room temp. The crude alcoholic extracts (1 kg) was acidified with 2.5% HCl and extracted with CHCl$_3$. Upon evaporation of the solvent, a 'non-basic' fraction (16 g) was obtained which was fractionated on a silica gel column, using CHCl$_3$ and CHCl$_3$–MeOH mixtures as eluents. Of the 75 250 *ml* fractions collected, fraction 10 furnished **4** (0.39 g), which was further purified by TLC on silica gel glass plates, using the solvent system C$_6$H$_6$–CHCl$_3$–MeOH (80:17:3) satd with NH$_3$ vapour. Compound **1** (42 mg) precipitated from a CHCl$_3$–MeOH soln of fraction 36.

Haplomyrtin (**1**). UV λ_{max}^{MeOH} nm (log ε): 228 (4.32), 268 (4.48), 309 (3.81), 322 (3.79), 3.61 (355); $\lambda_{max}^{MeOH+OH^-}$: 211 (4.78), 242 (4.34), 264 (4.29), 290 (4.30), 348 (3.72); IR ν_{max}^{KBr} cm^{-1}: 3420, 3260, 3180, 1705, 1595, 1455, 1340, 1150, 990; IR ν_{max}^{nujol} cm^{-1}: 1705; EIMS *m/z* (rel. int.): 366 [M]$^+$ (100), 365 (5), 337 (4), 321 (4), 308 (6), 307 (12), 293 (6), 279 (22), 264 (4); NMR NOEDS: 6-MeO to H-5 (13%), H-5 to 6-OMe (16%), 4-OH to H-5 (5%), 4-OH to CH$_2$ (6%), CH$_2$ to 4-OH (2%), 7-OH to H-8 (9%), H-8 to 7-OH (4%).

Methylation of **1**. A methanolic soln of **1** was treated with ethereal CH$_2$N$_2$ and kept near 4°C for 16 hr. Work-up furnished **3** in near quantitative yield. An authentic specimen of diphyllin

(2) was also methylated by the same procedure to furnish 3, again in almost quantitative yield.

Justicidin A. NMR NOEDS: 6-OMe to H-5 (39%), H-5 to 6-OMe (19%), H-5 to 4-OMe (16%), 4-OMe to H-5 (15%), 4-OMe to CH_2 (7%), CH_2 to 4-OMe (33%), 7-OMe to H-8 (32%), H-8 to 7-OMe (18%), H-8 to H-2′ (11%).

Haplomyrfolin (4). $[\alpha]_D^{25} - 39°$ (MeOH; *c* 0.069) $[\alpha]_D^{25} - 31.6°$ ($CHCl_3$; *c* 0.11); UV λ_{max}^{MeOH} nm (log ε): 230 (4.18), 284 (4.00); $\lambda_{max}^{MeOH + OH^-}$: 215 (4.78), 242 (4.26), 290 (4.07); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540, 1765, 1605, 1480, 1440, 1220, 1015, 910; EIMS *m/z* (rel. int.): 356 $[M]^+$ (33), 192 (6), 164 (9), 163 (5), 138 (16), 137 (46), 136 (14), 135 (100); CD(MeOH) Δε (nm): −0.7 (280), −0.2 (255), −4.0 (229); NMR NOEDS: H-2″ to H-6a (2%), H-6a to H-2″ (7%), H-6a to H-6″ (2%), H-6a to H-2 (8%), 3′-OMe to H-2′ (6%), H-2′ to 3′-OMe (11%).

Methylation of 4. Haplomyrfolin was methylated with CH_2N_2 by the above procedure to furnish (−)-7 in near quantitative yield.

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REFERENCES

1. Willis, J. C. (1980) *A Dictionary of the Flowering Plants and Ferns*, 8th edn, revised by Airy Shaw, H. K., p. 532. Cambridge Universtiy Press, Cambridge.
2. Khalid, S. A. and Waterman, P. G. (1981) *Planta Med.* **43**, 148.
3. Gonzales, A. G., Darias, V. and Alonso, G. (1979) *Planta Med.* **36**, 200.
4. Razakova, D. M. and Bessonova, I. A. (1981) *Khim. Prir. Soedin.* 516.
5. Batsuren, D., Batirov, E. Kh., Malikov, V. M., Zemlyanskii, V. N. and Yagudaev, M. R. (1981) *Khim. Prir. Soedin.* 295.
6. Gözler, T., Gözler, B., Patra, A., Leet, J. E., Freyer, A. J. and Shamma, M. (1984) *Tetrahedron* **40**, 1145.
7. Ulubelen, A. (1985) *Phytochemistry* **24**, 372.
8. Sheriha, G. M. and Abou Amer, K. M. (1984) *Phytochemistry* **23**, 151.
9. Waterman, P. G. and Grundon, M. F. (eds) (1983) *Chemistry and Chemical Taxonomy of the Rutales*. Academic Press, London.
10. Horii, Z., Tsujiuchi, M. and Momose, T. (1969) *Tetrahedron Letters* 1079.
11. Holmes, T. L. and Stevenson, R. (1971) *J. Org. Chem.* **36**, 3450.
12. Okigawa, M., Maeda, T. and Kawano, N. (1970) *Tetrahedron* **26**, 4301.
13. Corrie, J. E. T., Green, G. H., Ritchie, E. and Taylor, W. C. (1970) *Aust. J. Chem.* **23**, 133.
14. Lopes, L. M. X., Yoshida, M. and Gottlieb, O. R. (1983) *Phytochemistry* **22**, 1516.
15. Harmatha, J., Budezinsky, M. and Irka, A. (1982) *Collect. Czech. Chem. Commun.* **47**, 644.
16. Nishibe, S., Chiba, M. and Hisada, S. (1977) *Yakugaku Zasshi* **97**, 1366.
17. Ganeshpure, P. A. and Stevenson, R. (1982) *Aust. J. Chem.* **35**, 2175.