Lignans from *Bupleurum handiense*

H. López* and A. Valera

Centro de Productos Naturales Antonio González, Instituto Universitario de Bioorgánica, Universidad de La Laguna, 38206 La Laguna, Tenerife, Canary Islands, Spain

J. Trujillo

Instituto de Productos Naturales y Agrobiología, Carretera de la Esperanza 2, C.S.I.C., 38206 La Laguna, Tenerife, Canary Islands, Spain

Received July 13, 1995[⊗]

From the ethanolic extract of the leaves of *Bupleurum handiense* three lignans have been isolated, one of which is described for the first time, while two are already known. The structure of **1**, 9-(3,4-dimethoxyphenyl)-5,9-dihydro-8*H*-furo[3′,4′:6,7]naphtho[2,3-*d*][1,3]dioxol-6-one, was determined by spectroscopic methods.

Bupleurum handiense Bolle (Umbelliferae) is an endemic species in the Canary Islands. This plant is a bush found mainly in the Jandía region (Fuerteventura Island) as well as in some areas in Lanzarote Island.[1] Some species of the genus *Bupleurum* have been shown to accumulate lignans,[2,3] compounds that are reported to possess various biological properties such as insect-feeding stimulation and antineoplastic and neuroleptic effects.[4−6]

In this paper we report the isolation and structure elucidation of a previously undescribed lignan, 9-(3,4-dimethoxyphenyl)-5,9-dihydro-8*H*-furo[3′,4′:6,7]-naphtho[2,3-*d*][1,3]dioxol-6-one (**1**), along with the known chinensin[7] and isodiphyllin,[8] from *B. handiense.*

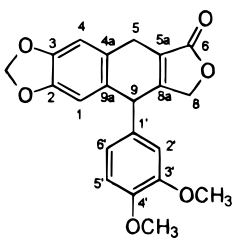
Compound **1** was isolated in small amounts (4 mg) as an oily substance of mol wt 366 Da and exhibited an elemental formula of $C_{21}H_{18}O_6$ (HREIMS). A chemical shift at 172.24 ppm in the ^{13}C-NMR spectrum typical of a lactone carbonyl carbon,[9] signals for methoxy (*δ* 3.81, 3.83) and methylenedioxy (*δ* 5.92) groups, and a series of aromatic protons (*δ* 6.58−6.80) in the ^{1}H-NMR spectrum, in addition to its molecular weight, indicated that this compound was a lignan. The DEPT experiment[10] showed in **1** three methylene groups (*δ* 29.22, 70.98, 101.25), six methine groups (*δ* 42.17, 109.57, 107.71, 111.98, 111.25, 120.17), 10 quaternary carbons (*δ* 147.24, 146.95, 130.05, 128.30, 157.00, 123.68, 135.38, 148.02, 149.00, 172.24) including the above-mentioned lactone carbonyl carbon, and two methyl carbons belonging to two methoxy groups. All these values were considered consistent with the presence of a lignan of the arylnaphthalene type with the B ring partially hydrogenated, thereby permitting the assignment of the three methylene groups [lactone methylene (70.98 ppm), methylenedioxy (101.25), and C-5 methylene (29.22 ppm)].[11] Thus, in the aromatic proton region, in a suitably expanded ^{1}H-NMR spectrum, shifts for a veratric system could be clearly observed [*δ* 6.80 (d, J = 2.0 Hz), 6.73 (d, J = 8.4 Hz), 6.58 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz)], along with two singlets (*δ* 6.70 and 6.61) accounting for H-4 and H-1, respectively. Two doublets (*δ* 4.80 and 4.87) were due to the lactone methylene. Superimposed on these doublets was an additional signal (*δ* 4.82), which was assigned to H-9. A doublet (*δ* 5.92) was ascribed to the methylenedioxy group. The signals at *δ* 3.64 (double doublet) and 3.86−3.97 (complex) were attributed to the protons attached to C-5.

Table 1. ^{1}H-NMR, ^{13}C-NMR, and HMBC Data of Compound **1**[a]

position	^{1}H	^{13}C	HMBC
1	6.61 (s)	109.57	
2		147.24	H-4, H-10
3		146.95	H-1
4	6.71 (s)	107.71	
4a		130.05	
5	α = 3.64 (dd, J_1 = 28.0, J_2 = 3.6) β = 3.86−3.97 (m)	29.22	H-4
5a		128.30	H-8, H-9
6		172.24	H-5
8	α = 4.87 (d, J = 16.0) β = 4.80 (d, J = 16.0)	70.98	
8a		157.00	H-5
9	4.82 (br s)	42.17	H-1, H-2′
9a		123.68	H-5
10	5.92 (d, J = 8.8)	101.25	
1′		135.38	H-5′
2′	6.80 (d, J = 2.0)	111.98	H-6′
3′		149.00	H-5′
4′		148.02	H-2′, H-6′
5′	6.73 (d, J = 8.4)	111.25	
6′	6.58 (dd, J_1 = 8.4, J_2 = 2.0)	120.17	H-2′
3′-OMe	3.83 (s)	55.86	
4′-OMe	3.81 (s)	55.97	

[a] Assignments were confirmed by COSY, DEPT, HMQC-NMR spectra.

A 2D-COSY experiment showed that the methylene protons at C-5 and H-9 were clearly related; this type of long-range coupling has already been observed in similar compounds.[11] The remainder of the correlations in this spectrum were in agreement with the proposed structure **1**.



1

The C-5a, C-8a double bond was located on the basis of ^{13}C-NMR spectral data (Table 1), because the compound having a double bond at C-8a, C-9 could also

[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1996.

S0163-3864(96)00133-4 CCC: $12.00

explain the ^{1}H-NMR spectrum. We were unable to find any ^{13}C-NMR data for model compounds in the literature. For this reason, we simulated the ^{13}C-NMR spectrum[12] for the two possible structures for **1**, obtaining shifts at 153.5 ppm and 132.2 ppm for C-8a in the first and second possibility, respectively. Our value (δ 157.0) was more in agreement with the first alternative. If the carbonyl group were at C-8, the simulated values for C-8a would be 132.7 and 125.3 ppm, respectively, which points to our compound having the lactone carbonyl group at C-6.

The structure of **1** was also supported by the HMBC NMR spectrum[10] (Table 1). There was a connectivity between C-9 and H-1 and H-2′, which definitively established the double bond at C-5a, C-8a. The connectivity between C-6 and the methylene protons at C-5 ruled out the carbonyl being at C-8. The relationship between the protons of the methylene group at C-8 and C-5a confirmed the structure of the lactone ring.

The two methoxy groups were assigned to the C-3′ and C-4′ positions using the NOE-DIF technique data. Irradiation of the methoxy group at δ 3.81 resulted in the appearance of only the H-5′ doublet, whereas irradiation of the signal at δ 3.83 gave a spectrum showing H-2′ as the only signal enhanced. This experiment enabled us not only to establish these methoxy groups at C-3′ and C-4′ but also to fix these specifically as OMe-4′ (δ 3.81) and OMe-3 (δ 3.83).

Experimental Section

General Experimental Procedures. ^{1}H- and ^{13}C-NMR spectra were measured on a Bruker AMX-400 spectrometer using TMS as internal standard. MS were obtained in a VG Micromass ZAB-2F spectrometer. HPLC were run on a Shimadzu LC-9A instrument. Si gel (Kieselgel 60-Merck) and Sephadex LH-20 were used for column chromatography, and Kieselgel $60F_{254}$ (Merck) was employed for TLC. Anhydrous Na_2SO_4 was used for drying solvents.

Plant Material. *B. handiense* was collected from a cultivated stand at the garden of the Centro de Productos Naturales Antonio González. It was authenticated by Dr. Marcelino del Arco, Department of Botany, Faculty of Pharmacy, University of La Laguna, Tenerife, Spain, and a voucher specimen was deposited in the Department of Botany of the University of La Laguna.

Extraction and Isolation. The air-dried, powdered aerial parts of the plant (825 g) were extracted with hot EtOH. After the solvent was removed *in vacuo*, a dark brown syrup remained. The raw extract was absorbed on silica (30−70 mesh) and percolated through Si gel (>230 mesh) under vacuum with *n*-C_6H_{12} and increasing amounts of EtOAc, and finally with pure EtOAc. From the medium-polarity fractions a fraction (3.95 g) was obtained which, after a series of Si gel column chromatography and HPLC steps, three compounds were isolated: **1** (4 mg), chinensin (10 mg), and isodiphyllin (17 mg).

9-(3,4-Dimethoxyphenyl)-5,9-dihydro-8*H*-furo[3′,4′:6,7]naphtho[2,3-*d*][1,3]dioxol-6-one (**1**) was obtained as a viscous oil: $[\alpha]^{25}_D$ +4° (*c* 0.3, $CHCl_3$); UV (EtOH) λ max (log ϵ) 223 (4.03), 259 (3.81) nm; IR ($CHCl_3$) ν max 2927−2850 (CH, aliphatic), 1757 (C=O, lactone), 1515, 1507, 1487, 1463, 1288, 1262, 1139, 1041, 1006 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR spectral data, see Table 1; HR-EIMS *m/z* 366.1527 ($C_{21}H_{18}O_6$); EIMS (70 eV) *m/z*, $[M]^+$ 366 (2), 199 (25), 189 (25), 185 (82), 177 (27), 176 (33), 171 (28), 165 (32), 151 (74), 138 (100), 115 (35), 79 (60).

Chinensin was obtained as colorless crystals (MeOH/$CHCl_3$): mp 220−222 °C (lit.[7] mp 220−221 °C), with IR characteristics as reported.[7]

Isodiphyllin was isolated as crystals (Me_2CO): mp 254−256 °C dec (lit.[8] mp 256 °C (dec)), with IR and ^{1}H-NMR data as reported.[8]

Acknowledgment. This work was supported by Grant P. I. No. 92/136 from the Gobierno de Canarias. The authors are grateful to Dr. Marcelino del Arco, Department of Botany, Faculty of Pharmacy, University of La Laguna, Tenerife, Spain, for authenticating the plant material.

References and Notes

(1) Bramwell, D.; Bramwell, Z. *Flora Silvestre de las Islas Canarias*, Editorial Rueda: Madrid, 1990; p 199.
(2) Whiting, D. A. *Nat. Prod. Rep.* **1985**, *2*, 191−211.
(3) Ward, R. S. *Nat. Prod. Rep.* **1993**, *7*, 1−28.
(4) Gottlieb, O. R. In *Proceedings of the First International Congress on Medical Plant Research, Section A. University of Munich, Sept. 1976*. Wagner, H.; Wolff, P., Eds.; Springer-Verlag: New York, 1977; p 227.
(5) Koppaka, V. R.; Alvarez, F. M. *Tetrahedron Lett.* **1983**, *24*, 4947−4950.
(6) El-Feraly, F. S.; Cheatham, S.; Breedlove, R. L. *J. Nat. Prod.* **1983**, *46*, 493−498.
(7) Ghosal, S.; Chauhan, R. P. S.; Srivastava, R. S. *Phytochemistry* **1974**, *13*, 2281−2284.
(8) González, A. G.; Pérez, J. P.; Trujillo, J. M. *Tetrahedron* **1978**, *34*, 1011−1013.
(9) Abraham, R. J.; Loftus, P. *Proton and Carbon C-13 NMR Spectroscopy*. John Wiley & Sons: Chichester, 1985; Chapter 2, p 32.
(10) Kusumi, T.; Iwashita, T.; Naoki, H. *One-dimensional and Two-dimensional NMR Spectra by Modern Pulse Techniques*. Nakanishi, K., Ed.; Kodansha Ltd.: Tokyo, 1990; Part III, pp 203−226.
(11) Gözler, T.; Gözler, B.; Patra, A.; Leet, J. E.; Freyer, A. J.; Shamma, M. *Tetrahedron* **1984**, *40*, 1145−1150.
(12) *C-13 NMR Module for ChemWindow v.3.0*; SoftShell International: 1994.

NP960133R