BUSALIOL AND BUSALICIFOL, TWO NEW TETRAHYDROFURAN LIGNANS FROM *BUPLEURUM SALICIFOLIUM*

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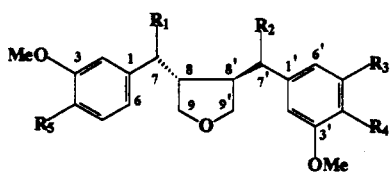
ABSTRACT.—Three tetrahydrofuran lignans [**1–3**] were obtained from the leaves of *Bupleurum salicifolium*, of which busaliol [**1**] and busalicifol [**2**] are novel. Their structures were determined by spectral and chemical methods. Also obtained in this investigation were a number of other lignans, coumarins, a polyacetylene, and a triterpenoid, all of known structure.

Bupleurum salicifolium Soll. ex Lowe (Umbelliferae) is endemic to the Canary Islands and is a species highly specialized in the biosynthesis of secondary metabolites derived from shikimic acid (1–7). This paper reports the isolation and structure elucidation of two new tetrahydrofuran lignans, busaliol [**1**] and busalicifol [**2**] from *B. salicifolium*. Although the biological activity of these lignans has yet to be evaluated, other tetrahydrofuran lignans have been shown to be CNS stimulants and to act synergistically with the insecticide pyrethrum (8–10). Several metabolites were also isolated from the EtOH extract of the leaves after repeated chromatography on Si gel and Sephadex LH-20; inclusive of the polyacetylene, 8*S*-heptadeca-2(*Z*)-9(*Z*)-diene-4,6-diyne-1,8-diol (11); the triterpenoid, betulin; six coumarins comprised of 6,7,8-trimethoxycoumarin, herniarin, scopoletin, scoparone, and limettin (12–16); and nineteen lignans, consisting of bursehernin, matairesinol dimethyl ether, kaerophyllin, guayadequiol, pluviatolide, guamaroline, bupleurol, matairesinol, epipinoresinol, (−)-arctigenin, (−)-nortrachelogenin, thujaplicatin methyl ether, guayarol, salicifolin, isosalicifolin, (−)-epinortrachelogenin, 2-hydroxythujaplicatinmethyl ether, 2,5-dehydrothujaplicatinmethyl ether, and 3-(2,4-dihydroxy-3-methoxybenzyl)-4-(4-hydroxy-3-methoxybenzyl)tetrahydrofuran [**3**] (2, 5, 7, 17–21).



1 R_1=H, R_2=R_4=R_5=OH, R_3=OMe
2 R_1=OEt, R_2=R_4=R_5=OH, R_3=H
3 R_1=H, R_2=R_4=R_5=OH, R_3=H
4 R_1=H, R_2=R_4=R_5=OAc, R_3=OMe
5 R_1=OEt, R_2=R_4=R_5=OAc, R_3=H

Repeated chromatography on Si gel and Sephadex LH-20 of the EtOH extract of the leaves of *B. salicifolium* gave three tetrahydrofuran lignans [**1–3**] along with the other constituents of known structure listed above. Compound **3** was identified as 3-(2,4-dihydroxy-3-methoxybenzyl)-4-(4-hydroxy-3-methoxybenzyl)tetrahydrofuran which was isolated previously from *Tinospora cordifolia* Miers (Menispermaceae) (21).

Compound **1** was isolated as an oil, showing a positive optical rotation and a $[M]^+$ at *m/z* 390.1679, consistent with the molecular formula $C_{21}H_{26}O_7$. Its uv (234, 280 nm) and ir (3420, 3012, 1612, 1514 cm^{-1}) data indicated its aromatic nature and the presence of one or more hydroxy groups. The ^{1}H-nmr spectrum of **1** (Table 1) exhibited the general features of tetrahydrofuran lignans (21–23) and was similar to that of **3**. A multiplet at δ 2.45, double doublets at δ 2.40, 2.74, and a multiplet at δ 2.60 were attributed to H-8′, H-7α, H-7β, and H-8, respectively, while four double doublets at δ 3.48, 3.52, 3.82, and 4.09 were assigned to H-9α,β and H-9′α,β. Sig-

TABLE 1. ^{1}H-Nmr Data of Compounds **1–5**.[a]

Proton	Compound				
	1	**2**	**3**	**4**	**5**
2′	6.68 s	6.94 d (1.8)	6.92 d (1.9)	6.70 s	6.83 d (1.8)
5′	—	7.01 d (7.8)	7.05 d (8.1)	—	7.13 d overlapping
6′	6.68 s	6.87 dd (8.2, 1.8)	6.82 dd (8.0, 1.9)	6.70 s	6.70 dd (8.1, 1.9)
2	6.46 d (1.9)	6.72 d (1.8)	6.45 d (1.9)	6.52 m	7.04 d (1.9)
5	6.97 d (8.0)	6.92 d (7.8)	6.94 d (8.1)	6.95 d (8.5)	6.95 d (8.0)
6	6.56 dd (1.9, 8.0)	6.62 dd (7.9, 1.8)	6.55 dd (8.2, 1.8)	6.52 m	7.13 d overlapping
7α	2.40 dd (12.7, 10.3)	4.05 d (8.2)	2.37 dd (12.9, 10.4)	2.20–2.60 m	4.09 (8.4)
7β	2.74 dd (12.6, 4.5)		2.78 dd (12.9, 4.6)	2.20–2.60 m	
7′	4.93 d (6.1)	4.63 d (7.50)	4.86 d (6.2)	4.87 d (5.4)	4.57 d (8.8)
8	2.60 m	2.57 m	2.59 m	2.20–2.60 m	2.25 m
8′	2.45 m	2.04 m	2.22 m	2.20–2.60 m	2.26 m
9α	3.48 dd (6.2, 8.6)	4.07 dd (8.8, 7.6)	3.42 dd (6.8, 8.5)	3.70 dd (6.3, 8.6)	3.96 dd (7.0, 9.2)
9β	3.52 dd (6.1, 8.5)	4.37 dd (8.9, 4.8)	3.51 dd (8.5, 7.0)	3.99 dd (6.2, 8.6)	4.56 dd (9.1, 4.0)
9′α	3.82 dd (11.0, 7.0)	3.28 dd (6.7, 7.1)	3.80 dd (6.4, 10.4)	4.01 dd (7.3, 11.3)	3.81 dd (6.0, 9.0)
9′β	4.09 dd (6.3, 11.0)	3.08 dd (7.0, 9.2)	4.05 dd (6.5, 8.6)	4.30 dd (6.4, 11.3)	3.81 dd (6.0, 9.0)
OMe	3.37 s (×2), 3.18 s	3.17 s, 3.21 s	3.13 s, 3.16 s	3.36 s (×2), 3.30 s	3.42 s, 3.43 s
OH/OAc	1.40 s, 5.34 s, 5.38 s	1.58 s, 5.61 s	5.36 s, 5.44 s	1.70 s, 1.93 s, 2.00 s	1.56 s, 1.85 s, 1.88 s
CH_3CH_2O-	—	1.05 t (6.1)	—	—	1.03 t (6.1)
CH_3CH_2O-	—	3.18 m overlapping	—	—	3.22 q (6.9) 3.05 q (6.9)

[a]Recorded in C_6D_6 at 200 MHz with TMS as internal standard; values in δ (ppm); coupling constants (Hz) in parentheses.

nals for two singlets at δ 3.18 (3H) and δ 3.37 (6H) corresponded to three methoxy groups, and two broad singlets at δ 5.34 and δ 5.38 to phenolic OH groups. An ABX spin system (δ 6.46, 6.56, 6.97) was attributed to a 1,3,4-trisubstituted aryl group and a singlet at δ 6.68 was assigned to a 1,3,4,5-tetrasubstituted aromatic ring. The main difference with respect to **3** was the signal of H-8′, which shifted 0.23 ppm downfield for **1** with respect to the same signal in **3**, and the presence of an additional methoxy group.

The ^{1}H-^{1}H COSY experiment was particularly helpful in elucidating the structure of compound **1**. The coupling between the singlet at δ 6.68 for H-2′,H-6′ with the doublet at δ 4.93 for H-7′ established that the syringyl group (3′,5′-dimethoxy-4′-hydroxyphenyl) was attached to C-7′ rather than C-7. No coupling between H-8 and H-8′ could be discerned, which suggested a *trans* stereochemistry with a dihedral angle between these hydrogens close to 90°.

The ms of **1** gave prominent fragment peaks at *m/z* 390 (M^+), 181 (100%) ($C_9H_9O_4$), 167 (22%) ($C_9H_{10}O_3$), 153 (26%) ($C_8H_9O_3$), 137 (81%) ($C_8H_8O_2$), 123 (19%) ($C_7H_7O_2$), as in similar lignans

TABLE 2. ^{13}C-Nmr Data for Compounds **1–5**.[a]

Carbon	Compound					Carbon	Compound				
	1	**2**	**3**	**4**	**5**		**1**	**2**	**3**	**4**	**5**
1	132.20	131.49	133.45	138.20	139.05	1′	133.95	133.50	136.68	141.06	140.46
2	111.14	108.76	113.09	112.73	110.04	2′	102.40	109.61	110.40	101.99	110.54
3	146.50	146.61	148.35	151.02	151.07	3′	147.02	146.77	148.23	152.11	151.32
4	143.96	145.17	145.76	138.83	139.36	4′	143.96	145.41	146.55	138.83	139.36
5	114.40	114.09	116.29	122.80	122.66	5′	147.02	114.13	114.88	152.11	122.66
6	121.14	120.98	121.86	120.53	119.53	6′	102.40	119.32	119.36	101.99	118.14
7	33.28	83.01	34.06	33.45	82.71	7′	83.03	83.86	83.72	83.04	83.40
8	42.31	49.87	43.55	42.06	48.93	8′	52.55	51.61	53.91	49.00	49.61
9	72.91	64.06	73.76	72.80	64.41	9′	60.92	62.95	61.05	62.71	63.74
OMe	2×56.31	55.95	57.48	2×56.16	55.89	OCH_2CH_3...	—	70.65	—	—	70.50
OMe	55.81	55.86	57.48	55.84	55.89	OCH_2CH_3...	—	15.14	—	—	15.19
OAc	—	—	—	20.46/168.83	20.66/168.88	OAc	—	—	—	20.87/171.00	20.66/170.75
OAc	—	—	—	20.65/169.00	20.66/169.04						

[a]Recorded in $CDCl_3$ at 50 MHz; chemical shifts are given in δ (ppm).

(21), and was consistent with the other data described above.

^{13}C-Nmr assignments (Table 2) were made for **1** on the basis of chemical shift calculations and by comparing the values with those reported for other tetrahydrofuran lignans like **3** (21). The ^{13}C-nmr spectrum and DEPT experiment of **1** revealed the presence of three methoxy (3q, δ 56.31×2, 55.81), three methylene (t, δ 42.31, 52.55, 83.03), and twelve aromatic carbons, of which seven were quaternary (s, δ 132.2, 133.95, 143.96×2, 147.2×2, 146.50), and the remaining five were unsubstituted aromatic carbon atoms (d, δ 102.40×2, 111.14, 114.40, 121.14). The main differences with respect to **3** were the signals attributable to aromatic carbons, with the remaining signals (C-8, C-8′, C-7, C-7′) being very similar. Compound **1** formed a triacetate [**4**] when it was treated with an excess of Ac_2O in the presence of pyridine [δ 1.70 s (3H), 1.93 s (3H), 2.00 s (3H)]. The foregoing data all indicated structure **1** for the new lignan, which was given the trivial name busaliol.

The second lignan, busalicifol [**2**], was isolated as an oil with positive optical activity, and a $[M]^+$ at *m/z* 404, consistent with a molecular formula of $C_{22}H_{28}O_7$. Its ir spectrum revealed the presence of hydroxy groups (3425 cm^{-1}) and an aromatic nucleus (1608, 1460 cm^{-1}). Uv absorption bands appeared at 238 and 264 nm. Its ^{1}H-nmr spectrum (Table 1) was similar to that of busaliol and **3**, with the most important differences being the presence of a triplet at δ 1.05 (3H) and a multiplet at δ 3.09 (2H), characteristic of an ethoxy group and an additional doublet at δ 4.05 (1H) typical of a methine hydrogen at a carbinol carbon. The ^{1}H-nmr spectrum of **2** also had two singlets at δ 3.21 and 3.17 for two methoxy groups, a broad singlet at 5.61 (2H) due to a phenolic OH, a group of signals between δ 6.60–7.00 attributable to six aromatic protons, and the upfield signals (δ 3.08–4.05) characteristic of aliphatic protons in tetrahydrofuran lignans.

The ^{1}H-^{1}H COSY nmr experiment performed on **2** showed the following correlations: the doublet at δ 4.05 coupled with the multiplet at δ 2.57 and with the double doublet at δ 4.37 which established the assignments of H-7, H-8, and H-9β, respectively; the doublet at δ 4.63 coupled with the multiplet at δ 2.04 and with the double of doublets at δ 3.28 which established the assignments of H-7′, H-8′, and H-9′α. The H-2 and H-2′ protons were also seen to be coupled with the OMe groups, indicating the presence of two 3-methoxy-4-hydroxyphenyl groups. The ms showed a fragment ion at *m/z* 151 ($C_8H_7O_3$) as the base peak and another significant peak at *m/z* 181 ($C_{10}H_{13}O_3$). The ^{13}C-nmr data were similar to those of **1** and **3**, with the presence of an additional methyl carbon at δ 15.4 and a methylene carbon at δ 70.65, corresponding to CH_3CH_2O- and CH_3CH_2O-, respectively.

Compound **2** formed a triacetylated derivative [**5**] when treated with Ac_2O/pyridine. The most significant ^{1}H-nmr data of **5** were the three singlets at δ 1.56 (OAc), 1.85 (PhOAc), and 1.88 (PhOAc), and a very significant 0.32 ppm upfield shift of the H-8 signals (δ 2.25) in comparison with the analogous data in **2**. This was due to the proximity of the acetate group on C-7′ and confirmed the *trans* stereochemistry of H-8 and H-8′. The novel compound **2** was given the trivial name busalicifol.

The absolute stereochemistry of **1–3** could not be determined because only very limited amounts of these lignans were isolated.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir and uv spectra were recorded on Perkin-Elmer model 681 and 550 SE spectrophotometers, respectively. ^{1}H- and ^{13}C-nmr spectra were run on a Bruker WD spectrophotometer at 200 (C_6D_6) and 50 ($CDCl_3$) MHz, respectively, with TMS as internal standard. Eims were obtained on a Micromass

ZAB-2AF spectrometer. Specific rotations were measured on a Perkin-Elmer model 141 polarimeter with $CHCl_3$ in 5-cm cells; tlc was carried out on precoated Si gel (Schleicher & Schüll F-100/LS 254). Merck Si gel (particle size 0.063–0.2 mm) and Sephadex LH-20 were used for cc.

PLANT MATERIAL.—The leaves of mature specimens of *B. salicifolium* were collected at Barranco Rio Badajoz, Güimar, Tenerife, Canary Islands, Spain in August 1988. A voucher specimen was lodged in the TFC file in the Department of Botany of the University of La Laguna.

EXTRACTION AND ISOLATION.—Dried leaves (3.2 kg) were extracted with cold EtOH. The dried EtOH extract was treated with H_2O (300 ml). Solvent was removed from the insoluble fraction which was then extracted with *n*-C_6H_{14} (3 times, 250 ml). The *n*-hexane extracts were removed and the insoluble residue was extracted with C_6H_6 (3 times, 250 ml). All C_6H_6 extracts were collected, and subsequently evaporated to dryness, yielding 112.8 g of dark residue. This residue was repeatedly chromatographed on a Si gel column using mixtures of *n*-C_6H_{14}/EtOAc of increasing polarity and on Sephadex LH-20 eluted with *n*-C_6H_{14}-$CHCl_3$-CH_3OH (2:2:1). The following compounds were isolated: salicifolin (10 mg), isosalicifolin (8 mg), (−)-epinortrachelogenin (16 mg), betulin (0.4 g), 6,7,8-trimethoxycoumarin (6 mg), bursehernin (89 mg), 8*S*-heptadeca-2(*Z*)-9(*Z*)-diene-4,6-diyne-1,8-diol (0.15 g), kaerophyllin (16 mg), matairesinol dimethyl ether (0.5 g), guayadequiol (9.3 mg), herniarin (6.7 mg), pluviatolide (3 mg), guamaroline (5 mg), *p*-hydroxyphenethyl alcohol (6 mg), bupleurol (43 mg), matairesinol (0.1 g), epipinoresinol (24 mg), scopoletin (10 mg), (−)-arctigenin (18 mg), scoparone (3 mg), limettin (3.4 mg), (−)-nortrachelogenin (67.3 mg), guayarol (10 mg), thujaplicatin methyl ether (0.1 g), allohydroxymatairesinol (9.2 mg), syringaresinol (15 mg), 2-hydroxythujaplicatin methyl ether (10 mg), 2,5-dehydrothujaplicatinmethyl ether (7.8 mg), **3** (5 mg), and the new lignans busaliol [**1**] (8 mg) (R_f 0.6; EtOAc-$CHCl_3$, 3:1) and busalicifol [**2**] (7 mg) (R_f 0.3; EtOAc-$CHCl_3$, 3:1).

Busaliol [**1**].—Yellow oil: $[\alpha]^{20}D$ +25° (c=0.2, $CHCl_3$); uv (EtOH) λ max 234, 280 nm; ir ν max (film) 3420, 2937, 2380, 1612, 1514, 1463, 1428, 1371, 1330, 1214, 1154 cm^{-1}; eims *m/z* $[M]^+$ 390 (24), 181 (100), 167 (22), 137 (81); hreims *m/z* 390.16788 ($C_{21}H_{26}O_7$ requires 390.16785), 181.06536 ($C_9H_9O_4$ requires 181.06573), 137.06040 ($C_8H_9O_2$ requires 137.06025); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

Acetylation of busaliol [**1**].—A quantity of Ac_2O (0.3 ml) was added to a solution of **1** (2.5 mg) in pyridine (1 drop), and the resulting solution was left at 20° for 24 h to form a triacetate; eims *m/z* $[M]^+$ 516 (4), 474 (2), 181 (32), 137 (100); hreims *m/z* 516.19954 ($C_{27}H_{32}O_{10}$ requires 516.19955), 474.18887 ($C_{25}H_{30}O_9$ requires 474.18898), 181.04999 ($C_9H_9O_4$ requires 181.05008), 137.06012 ($C_8H_9O_2$ requires 137.06025); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

Busalicifol [**2**].—A pale yellow oil; $[\alpha]^{20}D$ +45° (c=0.2, $CHCl_3$); uv (EtOH) λ max 238, 264 nm; ir ν max (film) 3425, 2940, 1608, 1515, 1460, 1372, 1270, 1154, 1117, 1029, 867 cm^{-1}; eims *m/z* $[M]^+$ 404 (15), 181 (100), 152 (13), 137 (14); hreims *m/z* 404.18348 ($C_{22}H_{28}O_7$ requires 404.18340), 181.08651 ($C_{10}H_{13}O_3$ requires 181.08647), 152.04747 ($C_8H_8O_3$ requires 152.04734); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

Acetylation of busalicifol [**2**].—Busalicifol acetate [**5**] was prepared from 4 mg of **2** as described previously for **4**. Eims *m/z* $[M]^+$ 530 (5), 223 (20), 181 (100), 183 (13), 151 (51); hreims *m/z* 530.21533 ($C_{28}H_{34}O_{10}$ requires 530.21520), 223.09685 ($C_{12}H_{15}O_4$ requires 223.09703), 181.08618 ($C_{10}H_{13}O_3$ requires 181.08647), 151.03944 ($C_8H_7O_3$ requires 151.03952); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

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