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THREE LIGNANS FROM *BUPLEURUM SALICIFOLIUM*

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Key Word Index—*Bupleurum salicifolium*, Umbelliferae, lignans, dibenzyl-γ-butyrolactone; 2,3-dibenzyl-2-buten-4-olide; arylnaphthalide lignan

Abstract—Three new lignans, 2β-hydroxy-2α-3′,4′-dimethoxybenzyl-3α-3″,4″-methylenedioxybenzyl-γ-butyrolactone, 2β-acetyl-2α-3′,4′-dimethoxybenzyl-3α-3″,4″-methylenedioxybenzyl-γ-butyrolactone and 2-3′,4′-dimethoxybenzyl-3-3″,4″-methylenedioxybenzyl-2,3-dehydro-γ-butyrolactone, were isolated from the leaves of *Bupleurum salicifolium*. Three other lignans were identified as 2β-3′,4′-dimethoxybenzyl-3α-3″,4″-methylene-dioxybenzyl-γ-butyrolactone, 2β,3α-bis(3′,4′-dimethoxybenzyl)-γ-butyrolactone and 1-3′,4′-dimethoxyphenyl-2,3-naphthalide-γ-butyrolactone. The triterpene betulin was also obtained

INTRODUCTION

Salicifoliol [1] and isokaerophyllin [2], two lignans from *Bupleurum salicifolium* Soland, an Umbelliferae species endemic to the Canary Islands, have already been reported. In the course of an intensive study of this plant, the new lignans, 2β-hydroxy-2α-3′,4′-dimethoxybenzyl-3α-3″,4″-methylenedioxybenzyl-γ-butyrolactone (**1**) (guayadequiol), 2β-acetyl-2α-3′,4′-dimethoxybenzyl-3α-3″,4″-methylenedioxy-γ-butyrolactone (**2**) (guayadequiol acetate) and 2-3′,4′-dimethoxybenzyl-3-3″,4″-methylenedioxybenzyl-2,3-dehydro-γ-butyrolactone (**3**) (guayadequiene) have been isolated and identified by spectroscopic and chemical means. The known lignans 2β-3′,4′-dimethoxybenzyl-3α-3″,4″-methylenedioxybenzyl-γ-butyrolactone (methylpluviatolide) (**6**), 2β,3α-bis(3′,4′-dimethoxybenzyl)-γ-butyrolactone (dimethylmatairesinol) (**7**) and 1-3′,4′-dimethoxyphenyl-2,3-naphthalide-γ-butyrolactone (chinensin) (**8**) were also obtained, as was the triterpene betulin

RESULTS AND DISCUSSION

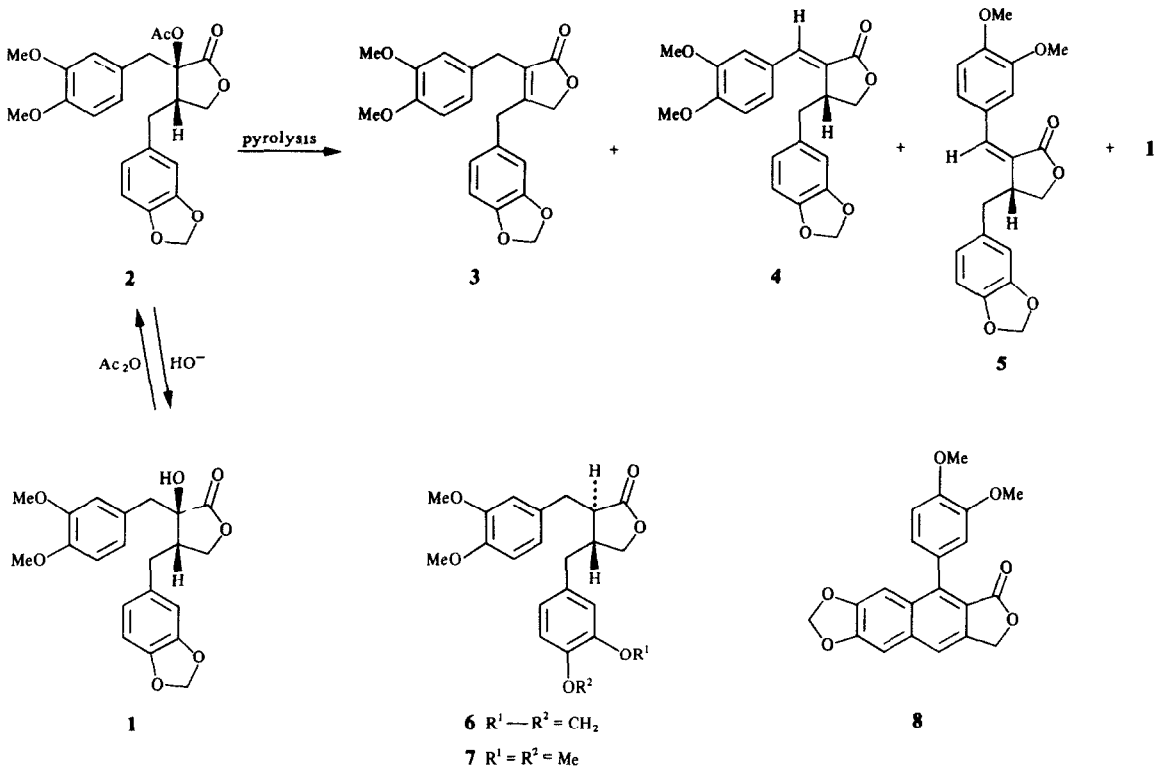
The *n*-hexane fraction of the ethanol extract of the leaves of *B. salicifolium* was subjected to preparative column chromatography and six γ-butyrolactones were isolated. Three proved to be new compounds **1** {$[M]^+$ 386, $C_{21}H_{22}O_7$, ν_{max} 3450 cm^{-1} (OH)}; **2** ($[M]^+$ 428, $C_{23}H_{24}O_8$) and **3** ($[M]^+$ 368, $C_{21}H_{20}O_6$) The lignans were characterized from their chemical and spectral data, and by chemical correlation with other natural [3] or synthetic [4] lignans with similar structures The ^1H NMR spectra for the new lignans contained signals for veratrylmethyl and piperonylmethyl groups (Table 1) and these groups were confirmed by mass spectrometry (base peaks at *m/z* 151 and 233 for **1** and **2** and at *m/z* 135 and 233 for **3**).

Two-dimensional ^1H–^1H homonuclear COSY established the β orientation of H-3 and showed it to be

Table 1 ^1H NMR spectral data (200 MHz, $CDCl_3$) for compounds **1–3** (TMS as int standard)

H	1	2	3
3	2.80–2.90 *m*	3.55–3.70 *m*	—
4α	4.19 *dd* (7.7, 9.3)	4.10–4.30 *m* (peak δ4.21)	—
4β	3.85 *dd* (10.1, 9.3)	3.54 *dd* (10.1, 6.8)	4.53 *s*
5a		3.04 *d* (14.3)	
	2.96 *s*		3.64 *s*
5b		3.24 *d* (14.3)	
6a	2.64 *dd* (13.2, 11.3)	2.73 *dd* (13.8, 9.6)	
			3.66 *s*
6b	3.11 *dd* (13.1, 3.8)	3.01 *dd* (13.8, 4.9)	
2′	6.67 *d* (1.6)	6.57 *br s*	6.46–6.83 *m*
5′	6.74 *d* (1.7)	6.74 *s*	6.46–6.83 *m*
6′	6.62 *dd* (6.8, 1.6)	6.55 *dd* (7.7, 1.8)	6.46–6.83 *m*
2″	6.74 *d* (1.7)	6.70 *s*	6.46–6.83 *m*
5″	6.83 *br s*	6.81 *br s*	6.46–6.83 *m*
6″	6.78 *dd* (3.8, 1.9)	6.81 *br s*	6.46–6.83 *m*
OMe	3.87 *s*	3.86 *s*	3.83 *s*
OMe	3.87 *s*	3.86 *s*	3.84 *s*
OCH_2O	5.95 *s*	5.93 *s*	5.93 *d* (0.3)
OAc	—	2.06 *s*	—

*J in Hz in parentheses



Scheme 1

coupled to H-4β. The data for the H-5 signals in **1** and **2** (Table 1), the results of 2D ^1H–^{13}C heteronuclear correlation experiments and the chemical transformation of **2** to **1** by saponification, or of **1** to **2** by acetylation indicated the orientation of the hydroxyl or acetoxy group to be the same as that of H-3 Pyrolysis of **2** (Scheme 1) led to the formation of **3** (53%), **4** (24%) and **5** (16%) by a *cis* type elimination of acetic acid, and of **1** (7%) by loss of CH_2CO These chemical transformations confirm the position of the acetate group on C-2 in **2** with the same disposition as H-3 [4, 5]. Compounds **1** and **2** are the first natural dibenzyl-γ-butyrolactone type lignans to be found with an *O*-substituent at C-2 with the same *cis* disposition as H-3

The IR spectrum of **3** showed bands at ν_{max} 1760 and 1670 cm^{-1} indicating the presence of an α,β-unsaturated-γ-lactone ring in the molecule There are no signals for vinylic protons in the ^1H NMR spectrum of **3**. Three two-proton singlets can be attributed to the benzylic CH_2 and lactone CH_2 groups These appear at quite low field in relation to those for the dibenzyl-γ-butyrolactone lignans such as **6** or **7** or the benzylidenebenzyl-γ-butyrolactones such as **4** and **5** The ^{13}C NMR spectrum shows the C-2 peak at low field as in other 2-buten-4-olides [6], the C-4 signal is found further downfield than it is in **4** or **5**, (Table 2) These data, taken in conjunction with the facts that **3** is formed from **2** by deacetylation and does not hydrogenate under the same conditions as **4** or **5** confirm the structure of guayadequiene as 2-veratrylmethyl-3-piperonylmethyl-2,3-dehydro-γ-butyrolactone (**3**). This is

Table 2 ^{13}C NMR spectral data (50 MHz, $CDCl_3$) for compounds **1**–**3** (TMS as int standard)*

C	1	2	3
1	177 92	173 29	174 92
2	75 95	81 67	159 73
3	48 29	43 14	127 19
4	69 44	69 27	71 40
5	32 30	32 27	29 43
6	38 40	37 32	33 38
1′	131 66	131 35	130 78
2′	108 71	108 70	108 99
3′	144 90	146 61	148 09
4′	148 35	148 18	148.09
5′	113 72	113 97	112.23
6′	122.81	123 12	121 31
1″	125 57	125.34	129.51
2″	108 86	108 62	108 80
3″	148 81	148 67	149 36
4″	149 35	148 79	149 36
5″	111 36	111 07	111 56
6″	121 50	121 34	120 63
OMe	56 03	55 93	55 03
OMe	56 13	56 04	56 13
OCH_2O	101 24	101 20	101 39
OCOMe		21 27, 169 38	

*Assignments are based on DEPT experiments [12] and correlations with other products

the first recorded instance of a natural lignan with a 2,3-dibenzyl-2-buten-4-olide moiety.

The other three lignans were identified variously as methylpluviatolide (**6**) [7], dimethylmatairesinol A (**7**) [8] and chinensin (**8**) [9], all previously obtained from *Bupleurum frutiscescens* L. [10]. The pentacyclic terpene, betulin [11], was also obtained.

EXPERIMENTAL

Mps uncorr UV EtOH ^{1}H and ^{13}C NMR 200 and 50 MHz, respectively, with TMS as int standard; TLC Schleicher–Schull F-1500/LS 254 and G 1510/LS 254 silica gel foils, CC· silica gel (0 2–0 063 mm dia)

Plant material and isolation of compounds Dry leaves (1 9 kg) from wild specimens of *Bupleurum salicifolium*, gathered in the Barranco de Guayadeque, Gran Canaria, were extracted with EtOH The extract was fractionated under reflux with C_6H_{14} and C_6H_6. The study of the C_6H_6 fraction has already been reported [2] The C_6H_{14} fraction gave a residue (12 1 g) which was chromatographed on a 700 g column and then washed with mixtures of C_6H_{14}–C_6H_6 (5 l), C_6H_6 (1 l) and C_6H_6–$CHCl_3$ (2 l) giving unexamined oil and colouring matter With $CHCl_3$ (2 l) (fr. B), a substance was sepd (182 mg), mp 249–250°, [M]$^+$ 442.3810 ($C_{30}H_{50}O_2$ requires 442 3811), which formed a diacetate ([M]$^+$ 364) which was identified as the triterpene betulin (IR, ^{1}H and ^{13}C NMR and MS) [11]

CC of fr A (120 mg), with C_6H_6–EtOAc (19·1) as eluent gave three frs· A-1 (30 ml), **6** (80 mg) {*m/z* 370 [M]$^+$, $C_{21}H_{22}O_6$ IR UV, MS (base peak at *m/z* 151), ^{1}H and ^{13}C NMR in accordance with the data given in the lit. [13]}, A-2 (150 ml), **3** (24 mg), and A-3 (680 ml), a mixture (248 mg) of various compounds. This mixture was re-crystallized with $CHCl_3$–C_6H_{14} to give **8** (128 mg) {mp 227–228°, [M]$^+$ 364 0939 ($C_{21}H_{16}O_6$ requires 364 0947), IR UV and ^{1}H NMR spectral data as reported elsewhere [9, 10]} ^{13}C NMR ($CDCl_3$) δ55 93 (OMe), 56 04 (OMe), 68.03 (lactonic CH_2), 101.89 (OCH_2O), 103.73 (C-8 or C-5), 103 81 (C-5 or C-8), 110.92 (C-5′), 113.59 (C-2′), 119.04 (C-4), 122 56 (C-6′), 127.32 (C-9), 130.56 (C-1′), 134.72 (C-10), 139 98 (C-1 or C-3), 140.48 (C-3 or C-1), 148 73 (C-3′) 149.04 (C-4′), 149.23 (C-6 or C-7), 150.01 (C-7 or C-6), 169.96 (C=O). The mother liquor from the recrystallization left a residue from which prep. TLC gave **2** (78 mg), **1** (23 mg) and **8** (16 mg) With C_6H_6–EtOAc (7 3), **7** (172 mg) was separated: (mp 128–129°, [M]$^+$ 386, $C_{22}H_{26}O_6$, IR, UV, ^{1}H and ^{13}C NMR, MS as given in ref [14]).

Guayadequiol (**1**) IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3450, 1770, 1600, 1585, 1508, 1495, 1435, 1135, 1083, 1020 UV λ_{max} nm 232, 283 ^{1}H NMR: Table 1, ^{13}C NMR Table 2. MS *m/z* (rel int.)· 386 1367 ([M]$^+$ 24, $C_{21}H_{22}O_7$ requires 386.1366), 151.0765 [(100), $C_9H_{11}O_2$ requires 151.0759], 137 (9), 135 (51), 107 (26). When **1** (6.2 mg) was acetylated with Ac_2O (0.5 ml) and pyridine (0.5 ml) at 100° for 1 hr, an acetate (4.4 mg) was formed which was identical (TLC, spectra) to **2** obtained directly from *B salicifolium*

Guayadequiol acetate (**2**). Mp 128–129°. IR ν_{max}^{nujol} cm^{-1} 1795, 1745, 1509, 1498, 1380, 1260, 1248, 1230, 1175, 1085, 1040, 1010, 930, 828 UV λ_{max} nm. 234, 286. ^{1}H NMR. Table 1, ^{13}C NMR Table 2. MS *m/z* (rel. int.): 428.1449 {[M]$^+$ (6), $C_{23}H_{24}O_8$ requires 428.1471}, 368 (3), 233 0824 [(100), $C_{13}H_{13}O_4$ requires 233.0814], 151 (74), 131 (57). When **2** (20 2 mg) was hydrolysed with 4% KOH in EtOH at room temp for 14 hr and then acidified with 4 M HCl and extracted with Et_2O, an amorphous substance (15.8 mg) was obtained which was identified (TLC and spectra) as **1** described above

Guayadequene (**3**) Uncrystallizable, IR ν_{max}^{film} cm^{-1}· 1760, 1670, 1610, 1593, 1510, 1252, 1039, 928 UV λ_{max} nm 256, 274 ^{1}H NMR: Table 1, ^{13}C NMR· Table 2 MS *m/z* (rel. int). {[M]$^+$ 368 1255 (17), $C_{21}H_{20}O_6$ requires 368 1260}, 233 0820 [(100), $C_{13}H_{13}O_4$ requires 233 0813], 177 (30), 146 (11), 135.0424 [(100), $C_8H_7O_2$ requires 135.0446] **3** was not hydrogenated under the same conditions as those used for **4** or **5** [2, 3]

Pyrolysis of guayadequiol acetate (**2**). Compound **2** (19 8 mg) was pyrolysed at red. pres. under Ar by careful heating over a Bunsen burner The dark residue was refluxed with $CHCl_3$ and a reddish product extracted (14 7 mg) which TLC analysis (EtOAc–C_6H_{14}–C_6H_6, 5 6 2) showed to be a mixture of various substances. Prep. TLC with the same eluent mixture afforded **3** (5 mg), **4** (2 3 mg), **5** (1.5 mg), **2** (1 9 mg) and **1** (0 7 mg) which were identified by spectroscopy, TLC and comparison with authentic samples

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