Pergamon

Tetrahedron Vol. 50, No. 17, pp. 5203-5210, 1994

0040-4020/94 $6.00+0.00

0040-4020(94)E0182-S

Structural Elucidation and Conformational Analysis of New Lignan Butenolides from the Leaves of *Bupleurum salicifolium.*

Ana Estévez-Braun*, Rafael Estévez-Reyes and Antonio G. González.

C.P.N.O. Antonio González, Instituto Universitario de Bio-Orgánica, Universidad de La Laguna, Avda. Astrofísico Francisco Sánchez 2, La Laguna, 38206, Tenerife. Canary Islands. Spain.

Abstract: From the leaves of *Bupleurum salicifolium* two new benzylidene-benzyl-γ-butyrolactone-type lignans salicifoline (2) and isosalicifoline (3) were isolated. The dibenzyl-butyrolactone (-)-epinortrachelogenin (1) was also isolated and it is the first time than this lignan has been obtained from a natural source. Their structures were determined by means of ^{1}H and ^{13}C NMR spectroscopic studies, including ^{1}H-^{13}C heteronuclear correlation (HETCOR), long range correlation spectra with inverse detection (HMBC), and chemical evidence. A study of the conformational analysis of these lignans using Molecular Mechanics Calculations is also included.

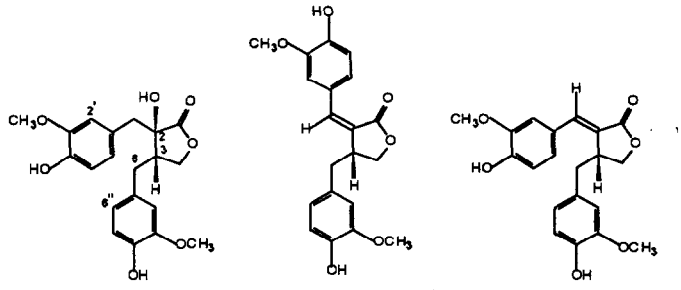
Bupleurum salicifolium, a species endemic to the Canary Islands, is a species highly specialized in biosynthesizing secondary metabolites derived from shikimic acid. Until now, most of the compounds isolated from *B. salicifolium* have been lignans, coumarins or flavonols.[1-6] This paper describes the isolation, structural elucidation and study of the conformational analysis of three lignans, isolated for the first time as natural products: (-)-epinortrachelogenin (1) [2-(3'-methoxy-4'-hydroxy-benzyl)-3-(3"-methoxy-4"-hydroxy-benzyl)-2R,3R-γ-butyrolactone], salicifoline (2) [Z-2(3'-methoxy-4'-hydroxybenzylidene)-3-(3"-methoxy-4"-hydroxy benzyl)-3R-γ-butyrolactone] and isosalicifoline (3) [E-2(3'-methoxy-4'-hydroxybenzylidene)-3-(3"-methoxy-4"-hydroxy-hydroxybenzyl)-3R-γ-butyrolactone].

The leaves of *Bupleurum salicifolium* also yielded a C_{17} polyacetylene with antibiotic activity[7], the triterpene betulin, the coumarins: 6,7,8-trimethoxy-coumarin[8], herniarin[9], scopoletin[10], scoparone[11], limettin[12] and the known lignans[2-5,13-14] : bursehernin, matairesinol dimethyl ether, kaerophyllin, guayadequiol, pluviatolide, guamaroline, buplerol, matairesinol, epipinoresinol, (-)-arctigenin, (-)-nortrachelogenin, thujaplicatin methyl ether and guayarol. Some of these lignans have significant nematostatic activity[15], anti HIV[16] and inhibitory activity against cyclic adenosine monophosphate phosphodiesterase[17].

Compound **1** was isolated as an amorphous solid. It showed negative optical activity, a [M^+] at m/z 374 and molecular formula $C_{20}H_{22}O_7$. Its IR spectrum revealed the presence of hydroxy groups (3543 cm^{-1}), a lactone ring (1779.4 cm^{-1}) and an aromatic nucleus (1615, 1463 cm^{-1}). UV absorption bands appeared at 276 and 224 nm. The ^{1}H NMR spectrum of **1** (Table 1) contained a singlet at δ 3.88 corresponding to two equivalent OMe groups, two broad singlets at δ 5.56 and δ 5.62 due to phenolic OH, a group of signals attributable to six benzene protons characteristic of two 1,3,4-trisubstituted aromatic rings, and a singlet, 2H, at δ 2.97 characteristic of the methylene group at C-5 in dibenzyl-γ-butyrolactone type lignans with an OH group at C-2 with the same *cis* disposition as H-3[3]. The mass spectra of **1** displayed a base peak at m/z 137 typical of 3-methoxy-4-hydroxy-benzyl groups and another peak at m/z 164 ($C_{10}H_{12}O_2$, 137 +C_2H_3), which

confirmed the presence of a proton on C-3, and the position of an OH group on C-2.

The physical and spectroscopic data of **1** were identical to a subproduct obtained by Khamlach *et al*[18] in the enantioselective synthesis of (-)-nortrachelogenin. This compound was named (-)-epinortrachelogenin and its absolute configuration was established as 2R,3R. It has now been isolated for the first time as a natural product. It is only the second natural dibenzyl-γ-butyrolactone known to have an OH group at C-2 with the same *cis* disposition as H-3. **1** formed a triacetate when treated with excess Ac_2O in the presence of pyridine and DMAP.



(1) (2) (3)

Figure 1.

Salicifoline (**2**) was isolated as an oil with negative optical activity ($[\alpha]_D^{20}$=-61.3°), [M+] m/z 356 and molecular formula $C_{20}H_{20}O_6$. It exhibited UV absorption at 336, 288, 228 nm characteristic of lignans containing a dibenzyl butyrolactone skeleton with a double bond at the 2,5-position in the γ-butyrolactone ring[19-21]. The IR spectrum showed characteristic signals for an α,β unsaturated γ-lactone (1735 cm^{-1}), olefinic double bond (1648 cm^{-1}), aromatic nucleus (1593, 1492, 1470 cm^{-1}) and hydroxy groups (3546 cm^{-1}). Its ^1H NMR spectra (Table 1) had the characteristics of 2-benzylidene-3-benzyl-γ-butyrolactone-type Z isomer[2], notably a multiplet centred at δ 3.30 corresponding to H-3, two double doublets centred at δ 4.34 (H-4β) and δ 4.13 (H-4α) and a doublet at δ 8.23 attributed to H-2' which was very close to the carbonyl group. The ^1H NMR spectrum also had signals for two methoxy groups at δ 3.94 and δ 3.82, two phenolic OH at δ 5.96 and δ 5.56, and signals for six benzene protons. The mass spectra had a fragment at m/z 137 as its base peak, which is characteristic of either a guayacyl (3-methoxy-4-hydroxy-benzyl group) or an isoguayacyl (3-hydroxy-4-methoxy-benzyl) group and another peak at m/z 164 (137 + C_2H_5). The isoguayacyl group is very unusual in naturally occurring lignans but is present in guamarol, isoguamarol, guamaroline and chasnarolide[5,6] isolated from the roots of *B. salicifolium*. ROESY experiments on **2** (Fig 2) unequivocally established the regiosubstitution of both aromatic rings and the presence of two guayacyl groups in the molecule of **2**. The carbon assignments (Table 1) are based on ^{13}C-^1H, HMBC (Table 2) and DEPT experiments.

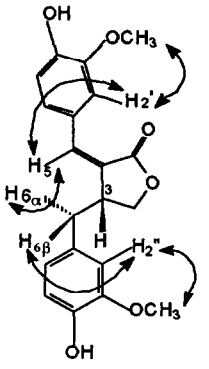


Figure 2
Roesy Experiment on 2

The ROESY of **2** indicated *cis-trans* isomerization. This conversion may possibly be catalyzed by the usual trace of hydrocloric acid in $CDCl_3$, as the spectrum was run after the compound had been in solution for 48h. After this time the conversion of product **2** was 38.5% and both isomers could be separated by

Table 1 : ^1H NMR (200 MHz) and ^{13}C NMR (50 MHz) Data (δ, $CDCl_3$) of 1, 2 and 3.

H	1	2	3	C	1	2	3
3	2.82 m	3.30 m	3.84 m	1	177.89	174.64	176.00
4α	4.18 dd	4.13 dd	4.28 d	2	75.77	124.10	125.90
	(9.2,7.8)	(9.0, 3.5)	(4.2)	3	48.10	44.58	39.80
4β	4.18 dd	4.34 dd	4.28 d	4	69.40	70.04	69.73
	(9.2, 7.8)	(9.0, 7.3)	(4.2)	5	38.26	141.12	137.37
5	2.96 s	6.65 d	7.52 d	6	32.06	40.89	37.59
		(1.8)	(1.9)				
6α	3.13 dd	2.86 m	3.06 dd	1'	129.59	126.37	126.80
	(13.1, 3.6)		(14.4, 4.3)	2'	110.73	113.05	112.75
6β	2.65 dd	2.86 m	2.63 dd	3'	146.81	145.99	146.79
	(13.2,11.4)		(10.0, 4.5)	4'	145.26	147.50	147.74
2'	6.77 br s	8.23 d	7.03 d	5'	114.58	114.52	115.02
		(1.9)	(1.9)	6'	123.36	126.59	125.90
5'	6.89 d	6.87 d	7.00 d				
	(8.0)	(8.2)	(8.3)	1"	124.69	129.58	129.80
6'	6.70	7.02 dd	7.21 dd	2"	112.85	111.81	111.74
	overlapping	(8.3, 1.9)	(8.3, 1.9)	3"	146.50	146.47	146.77
2"	6.70 br s	6.55 d	6.64 d	4"	144.51	144.46	144.84
		(1.5)	(1.9)	5"	114.39	113.77	114.77
5"	6.86 d	6.87 d	6.87 d	6"	121.09	121.84	124.03
	(7.8)	(8.2)	(8.0)	OMe	55.94	56.01	56.09
6"	6.70 dd	6.71 dd	6.70 dd	OMe	55.99	55.90	55.98
	(7.8, 1.9)	(8.0, 1.8)	(7.0, 2.0)				
OMe	3.88 s	3.94 s	3.92 s				
	3.88 s	3.82 s	3.82 s				
OH	5.56 s	5.96 s	5.92 s				
	5.62 s	5.96 s	5.53 s				

Table 2: Three Bond ^1H-^{13}C Couplings in Compound 2

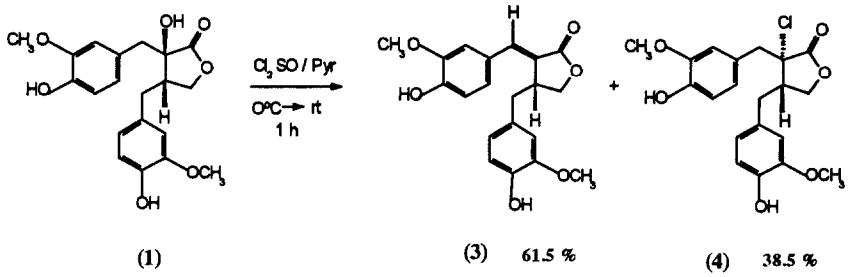
Irradiated Protons	Observed Carbons
H-5	C-3, C-2', C-6'
H-6	C-3*, C-4, C-1"*, C-2", C-6"
H-2'	C-5, C-3'*, C-4', C-6'
H-5'	C-1', C-3', C-4'*, C-6'*
H-6'	C-1'*, C-2', C-4', C-5'*
H-2"	C-4", C-6"
H-5"	C-1", C-3", C-4"*, C-6"*
H-6"	C-6, C-2", C-4"

***Two bond coupling enhancement observed.**

preparative TLC. To avoid this isomerization, the sample was prepared at the same time that the HMBC and HMQC experiments were made and no transformation was observed.

Isosalicifoline (**3**) was isolated as an oil. It showed a [M^+] at m/z 356, molecular formula $C_{20}H_{20}O_6$, negative optical activity and an IR spectrum with absorption bands for conjugated carbonyl groups (1742 cm^{-1}) and OH groups (3538 cm^{-1}). The ^1H NMR spectra of **3** (Table1) were similar to those of **2**, the main difference being the presence of a doublet at δ 4.28 (H-4) and a doublet at δ 7.52 due to the olefinic hydrogen H-5. The mass spectra of **3** had the same fragments as that of **2**. The foregoing data all indicated that **3** possesses the same structure as **2** except for the stereochemistry of the double bond. The physical and spectroscopic data of **3** were identical to the product analysed when **2** had been isomerized as described above.

Isosalicifoline (**3**) was obtained as one of several products from the reaction of (-)-epinortrachelogenin (**1**) with $SOCl_2$ and pyridine (Scheme 1). This reaction established the absolute configuration of **3** as 3R (and also that incidentally that of **2** as well). This is to be expected on biogenetic grounds as all benzylidene-benzyl-γ-butyrolactone type lignans isolated from *B. salicifolium* have had this stereochemistry at C-3[2-5]. Formation of the Z isomer (**2**) was not in evidence. The chlorine atom present in derivative **4** was introduced by inversion of the configuration at C-2, as can be deduced from the ^1H NMR ($CDCl_3$) data, in accordance with the results obtained by Ikahama *et al*[22].



Scheme 1

Benzylidene-benzyl-γ-butyrolactone type lignans are few in number and most have piperonyl groups (3,4-dioxymethylene benzyl), veratryl groups (3,4-dimethoxy benzyl) or syringanyl groups (3,5-dimethoxy, 4-hydroxy benzyl). Salicifoline (**2**) and isosalicifoline (**3**) are the first known natural benzylidene benzyl-γ-butyrolactones to have two guayacyl groups.

Currently, experiments are under way to obtain greater amounts of these compounds for some of their biological activities to be evaluated as many natural products with an unsaturated lactone ring are notable for their physiological properties with cytotoxic, antibiotic, phytotoxic, fungicidal and other activities[23-25].

To obtain a rough idea about the geometry and energy of these molecules, an analysis was made using Molecular Mechanics Calculations. After multiple minimizations of **1** it was concluded than there were two main conformers **A** and **B** in relation to the lactone ring (Fig 3). The signs obtained for the dihedral angles agree with those given in Toromanoff's Table[24] for these types of conformer.

When the minimizations of **1** took place via conformer **A** an average value $J_{3\text{-}4\alpha}$=1.4 Hz was obtained and when through conformer **B**, the average was $J_{3\text{-}4\alpha}$=11.1 Hz. **1** showed an experimental $J_{3\text{-}4\alpha}$=9.0 Hz, and it is reasonable to assume that this experimental value is the average of the coupled constants of **B** and **A**. The experimental value $J_{3\text{-}4\alpha}$ agrees with the following ratio **B:A** 4:1. Moreover, the benzyl groups on C-2 and C-3

are predominantly equatorial in disposition while the OH group on C-2 is axial. With regard to conformational rotamers around the carbons C2-C5 and C3-C6, the structures with minimal energy were **1-A'** (28.97 Kcal/mol), **1-B'** (30.83 Kcal/mol) and **1-C'** (31.50 Kcal/mol) (Fig 4). The corresponding populations were 83.4%, 16.5% and 1.4% , respectively.

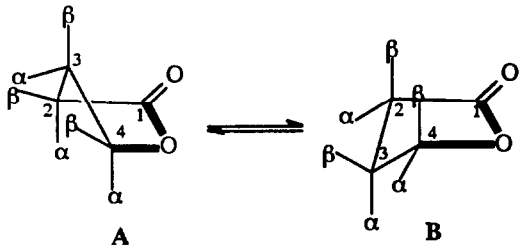
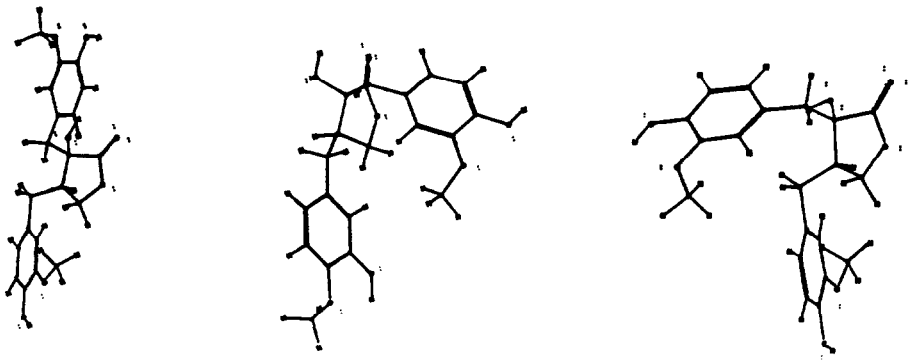


Figure 3.



1-A' 83.4%
(28.97 Kcal/mol)

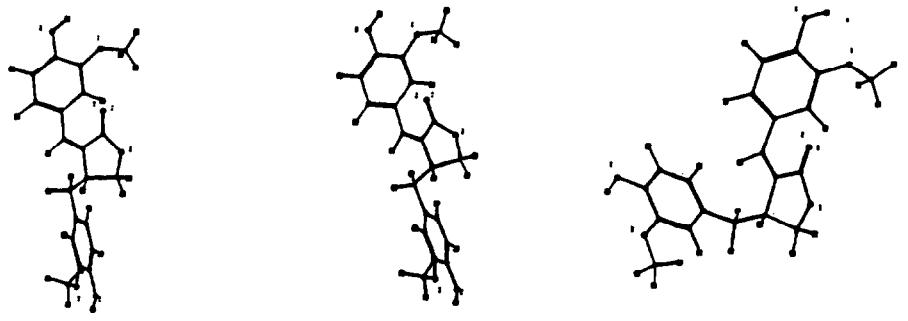
1-B' 16.5%
(30.83 Kcal/mol)

1-C' 1.4%
(31.50 Kcal/mol)

Figure 4

The minimization of **2** via conformer **A** yielded an average $J_{3\text{-}4\alpha}$=1.57 Hz while that via conformer **B** produced $J_{3\text{-}4\alpha}$=11.57 Hz. **2** displayed an experimental $J_{3\text{-}4\alpha}$=3.5 Hz, and therefore the main conformer should be **A** and the benzyl group on C-3 predominantly equatorial . The rotamers of minimum energy proved to be **2-A'** (33.46 Kcal/mol), **2-B'** (33.97 Kcal/mol) and **2-C'** (34.12 Kcal/mol) (Fig 5) and the populations were 57.5%, 24.0% and 18.5%, respectively.

With compound **3** the results were similar to those obtained with **2**. Conformer **A** is more notable (**A:B**, 7:3) because **3** had an experimental $J_{3\text{-}4\alpha}$=4.0 Hz; $J_{3\text{-}4\alpha}$=1.31 Hz and $J_{3\text{-}4\alpha}$=11.45 Hz were obtained for conformers **A** and **B**, respectively. The rotamer of minimal energy around the carbon C3-C6 was **3-A'** (31.09 Kcal/mol) (Fig 6). This disposition has been also observed under X-ray in compounds with similar structure[18,25]. It is energetically more favourable than other disposition such as **3-B'** (Fig 6) but several ^{1}H NMR studies have showed a NOE effect between H4β and H2' and H6' and it is possible than these types of molecule could adopt a **3-B'** disposition when in solution.

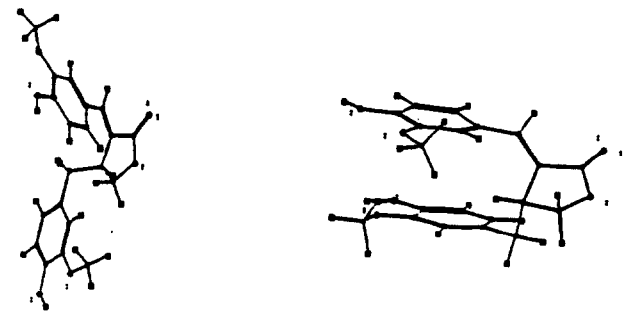


2-A' 57.5%
(33.46 Kcal/mol)

2-B' 24.0%
(33.97 Kcal/mol)

2-C' 18.5%
(34.12 Kcal/mol)

Figure 5



3-A'
(31.09 Kcal/mol)

3-B'
(33.97 Kcal/mol)

Figure 6

EXPERIMENTAL

IR spectra were taken on a PE 681 spectrophotometer and ^{1}H and ^{13}C NMR on a Bruker W-200SY at 200 and 50 MHz, respectively, with TMS as internal reference. The HMBC and HMQC were run on a Bruker at 400 MHz. Optical rotations were measured on a PE 550-SE. MS were recorded on a VG Micromass ZAB-2F and a Hewlett Packard 5995. UV spectra were collected on a Perkin Elmer model 550-SE. Schleicher-Schüll F-100/LS 254 and prep. TLC 1510/LS 254 foils were used for TLC while silica gel (0.2-0.63 mm) and sephadex LH-20 were used for CC. The Molecular Mechanics PC Model Program (version 3.3) was used in the Molecular Mechanics Calculations.

Isolation. Leaves (3.2 Kg) of well-grown specimens of *B. salicifolium* gathered in the Barranco Rio Badajoz de Güimar, Tenerife were extracted with EtOH. This extract was treated with H_2O, then n-hexane and then

C_6H_6 to afford a dark residue (112.8 g) which was repeatedly chromatographed on silica gel using mixtures of n-hex: EtOAc of increasing polarity as eluants and on Sephadex LH-20 eluting with n-hex: $CHCl_3$: MeOH (2:2:1) yielding the following compounds: the new lignans salicifoline (**2**) (10 mg), isosalicifoline (**3**) (8 mg) and (-)-epinortrachelogenin (**1**) (10 mg), betulin (400 mg), 6,7,8-trimethoxy coumarin (6 mg), bursehernin (89 mg), a C_{17} polyacetylene (150 mg), kaerophyllin (15.9 mg), matairesinol dimethyl ether (515.8 mg), guayadequiol (9.3 mg), herniarin (6.7 mg), pluviatolide (2.6 mg), guamaroline (5 mg), p-hydroxy-phenethyl-alcohol (6 mg), buplerol (43 mg), matairesinol (110.8 mg), epipinoresinol (23.8 mg), matairesinol dimethyl ether (515.8 mg), scopoletin (10.3 mg), (-)-arctigenin (17.9 mg), scoparone (3 mg), limettin (3.4 mg), (-)-nortrachelogenin (67.3 mg), guayarol (10 mg), thujaplicatin methyl ether (7.8 mg).

(-)-Epinortrachelogenin (**1**). White amorphous solid, m.p. 171-172ºC; $[\alpha]_D^{20}$=-5º (c=0.5, $CHCl_3$); $[\alpha]_D^{20}$=-9º (c=0.2, EtOH), λ_{max}^{EtOH}: 276, 224 nm; ν_{max} (film) cm^{-1}: 3543.1, 3021.5, 2938.1, 2923.4, 1779.4, 1615.4, 1558.4, 1514.0, 1463.9, 1373.2, 1269.2, 1036.0; EIMS m/z (%): 374 (M^+) (7%), 164 (4%), 137 (100%); HREIMS: calculated for $C_{20}H_{22}O_7$, 374.14438; found 374.14259; calculated for $C_{10}H_{12}O_2$,164.08373; found 164.08433 ; calculated for $C_8H_9O_2$, 137.06025; found 137.06035; ^1H NMR: (see Table 1); ^{13}C NMR:(see Table 1).

(-)-Epinortrachelogenin Acetate. Ac_2O (0.3 ml) and a small amount of DMAP (4-dimethylaminopyridine) were added to a solution of **1** in pyridine (2 drops). The reaction mixture was left at room temperature (19ºC) for 24h and diluted with H_2O/ice, forming an insoluble precipitate in the H_2O. The precipitate was washed with a solution of $NaHCO_3$ and H_2O successively, leaving a residue (4.7 mg) of (-)-epinortrachelogenin acetate. ^1H NMR (200 MHz) ($CDCl_3$) δ: 2.05 (s, 3H, CH_3COO), 2.31 (s, 6H, 2 CH_3COOPh), 2.80 (m, 1H, H-6α), 3.00 (m, 1H, H-6β), 3.09 (d, J=14.4 Hz, 1H, H-5α), 3.31 (d, J=14.4 Hz, 1H, H-5β), 3.60-3.80 (m, 2H, H-3+H-4α), 3.82 (s, 6H, OMe), 4.10-4.30 (m, 1H, H-4β), 6.69 (dd, 1H, H-6"), 6.70 (d, J=1.9 Hz, H-2"), 6.86 (dd, J=8.0, 1.9 Hz, 1H, H-6'), 6.89 (broad s, 1H, H-2'), 6.98 (d, J=8.6 Hz, 2H, H-5'+H-5").

Reaction of 1 with $SOCl_2$. 10 mg of **1** were treated at 0ºC with previously distilled $SOCl_2$ (3 drops) and with an excess of pyridine. The resultant mixture was left at room temperature for 1h and then it was treated with H_2O and extracted several times with Et_2O, the ethereal phase was washed with 5% HCl, saturated solution of $NaHCO_3$ and finally with brine. The organic phase obtained was dried with anhydrous $MgSO_4$ and after the solvent had been removed, gave an oily residue the TLC of which showed the presence of two products. The one with greater Rf was predominant as was confirmed by ^1H NMR ($CDCl_3$) showing a mixture of two compounds in the ratio 61.5:38.5. The resultant residue was chromatographed in preparative TLC using EtOAc / $CHCl_3$ (1/1). The product with lower Rf showed identical Rf in tlc, ^1H NMR ($CDCl_3$) data and optical activity as isosalicifoline (**3**). The product with greater Rf (3.0 mg) was identified as 2S-chloro-2-(3'-methoxy-4'-hydroxy-benzyl)-3S-(3"-methoxy-4"-hydroxy-benzyl)-γ-butyrolactone (**4**). ^1H NMR (200 MHz) ($CDCl_3$): 2.61-2.73 (m, 2H, H-6), 2.85-3.00 (m, 1H, H-3), 3.14 (d, J=14.1 Hz, 1H, H-5α), 3.64 (d, J=14.1 Hz, 1H,H-5β), 3.83 (s, 3H, OMe), 3.88 (s, 3H, OMe), 3.92-4.05 (m, 2H, H-4), 5.55 (s, 1H, OH), 5.61 (s, 1H, OH), 6.56 (d, J=1.8 Hz, H-2"), 6.61 (dd, J=8.0, 2.0 Hz, 2H, H-6' + H-6"), 6.71 (d, J=2.0 Hz, 1H, H-2'), 6.84 (d, J=7.9 Hz, 2H, H-5'+H-5"); EIMS m/z (%): 394 [M^+] (7), 149 (100), 137 (87); HREIMS: calculated for $C_{20}H_{21}O_6Cl$,394.0997; found 394.0999

Salicifoline (**2**).Yellow oil; $[\alpha]_D^{20}$=-61.3º (c=0.08, $CHCl_3$); λ_{max}^{EtOH} nm 336, 288, 228; ν_{max} (film) cm^{-1} 3546.2, 1735.3, 1690.0, 1663.0, 1593.0, 1492.0, 1470.0, 1270.0, 1185.3, 1122.1; EIMS m/z (%) 356 (M^+) (32%), 219 (2), 192 (1), 176 (4), 164 (5), 149 (12), 137 (100); HREIMS calculated for $C_{20}H_{20}O_6$, 356.12599;

found 356.12590; calculated for $C_{10}H_{12}O_2$, 164.07590; found 164.07588; ^1H NMR (see Table 1); ^{13}C NMR (see Table 1).

Isosalicifoline **(3).** Yellow oil; $[\alpha]_D^{20}$=-26.5º (c=0.07, $CHCl_3$); λ_{max}^{EtOH} nm 336, 290, 234; ν_{max} (film) cm^{-1} 3537.6, 3020.3, 2927.0, 2853.9, 1741.6, 1644.4, 1605.0, 1595.0, 1514.8, 1463.9, 1453.9, 1272.0, 1123.1; EIMS m/z (%): 356 (M^+) (37), 193 (7), 192 (2), 176 (11), 164 (3), 163 (5), 149 (24), 137 (100); HREIMS: calculated for $C_{20}H_{20}O_6$, 356.1254; found 356.1254; calculated for $C_{10}H_9O_4$, 193.0563; found 193.0560; calculated for $C_{10}H_8O_4$, 192.0586; found 192.0584 ; calculated for $C_{10}H_{12}O_3$,176.0847; found 176.0555 ; calculated for $C_{10}H_{12}O_2$, 164.0786; found 164.0797; calculated for $C_{10}H_{11}O_2$, 163.0759; found 163.0729; calculated for $C_9H_9O_2$, 149.0598; found 149.0596; calculated for $C_8H_9O_2$, 137.0641; found 137.0638. ^1H NMR (see Table 1); ^{13}C NMR (see Table 1).

REFERENCES.

1-. González A.G.,Estévez-Reyes R.,Mato C. *J. Nat. Prod.* 1989. **52(5)**, 1139.
2-. González A.G.,Estévez-Reyes R.,Mato C.and Estévez-Braun A. *Phytochemistry.* 1990. **9(2)**, 675.
3-. González A.G.,Estévez-Reyes R.,Mato C.and Estévez-Braun A. *Phytochemistry.* 1990. **29(6)**, 1981.
4-. González A.G. ,Estévez-Reyes R.,Estévez-Braun A. *J. Chem Res.* 1990. 220.
5-. Estévez-Reyes R.,Estévez-Braun A. and González A.G. *Phytochemistry.*1992. **31(8)**, 2841.
6-. Estévez-Reyes R.,Estévez-Braun A. and González A.G. *J Nat Prod.* 1993. **56(7)**, 1177.
7-. Estévez-Braun A.,Estévez-Reyes R.,Moujir L.,Ravelo A.G. and González. A. *Nat.Prod. Lett.* 1993, In press.
8-. González A.G.,Estévez-Reyes R.,Jaraiz I., *Anales de Química.* 1972, **68(4)**, 415.
9-. Estévez-Reyes R.,González A.G.,Rodriguez-Luis F., *Anales de Química.* 1966, 775-779.
10-. González A.G.,Estévez-Reyes R.,Herrera- Velazquez R., *Anales de Química.*1975, 437-440.
11-. González A.G.,Estévez-Reyes R.,Arencibia J.B.,Pérez J.P.*Anales de Química.*1974, **70(3)**, 286-288.
12-. Estévez-Reyes R.,González A.G., *Phytochemistry.*1970, **9**, 833-840.
13-. Nishibe S.,Hisada S.,Inagaki I., *Chem. Pharm. Bull.* 1973, **21**, 1108.
14-. Nishibe S.,Hisada S.,Inagaki I., *Chem. Pharm. Bull.*1971, **19**, 866.
15-. González-Pérez J.A.,Estévez-Braun A.,Estévez-Reyes R.,Ravelo A.G., *J.Chem. Ecol.* Ms 174.In press
16-. Eich E.,Schulz J.,Trumm S.,Sarm P.S.,Maidhof A.,Herz H.,Schröder H.C.,Müller W.E., *Planta Med.* 1990, **56**, 506.
17-. Cassady J.M. and Douros, J.D.Anticancer Agents Based on Natural Product Models.1980. New York. Academic Press.
18-. Khamlach K.,Dhal R.,Brown E. *Tetrahedron Letters.* 1989, **30(17)**, 2221-2224.
19-. Chatterjee A.,Das B., Prangué T. *Phytochemistry.* 1981. **20**, 2047.
20-. Schrecker A.W.,Hartwell J.L. *J. Am. Chem. Soc.* 1954. **76**, 4896.
21-. Burden R.S.,Crombie L.,Whiting D.A. *J. Chem. Soc.* 1969. 693.
22-. Ikahama M.,Uno H., Ishikawa F. *Chem. Pharm. Bull.* 1969. **12**, 267.
23-. Rao Y.S., *Chem. Rev.* 1975, **76**, 625.
24-. Evidente A.,Randazzo G. and Ballio A. *J. Nat. Prod.* 1986, **49**, 593.
25-. Myin S.H.,Cortes D.,Lurens A. and Hocquecilla R. *Phytochemistry.* 1991, **30**, 3335.
26-. Toromanoff E.. *Tetrahedron.* 1980. **36**, 2809.
27-. Shieh H.L.,Cordell G.A.,Lankin D.C.,Lotter H. . *J. Org. Chem.* 1990. **55**, 5139.

ACKNOWLEDGMENTS.

We are indebted to the Gobierno Autónomo Canario (Project Nº 11/ 08-03-90), CYCIT (Project SAF 92-1028) and Colegio Libre Emerito for financial help. AEB has received a grant from the Ministerio de Educación y Ciencia (Spain).

(*Received in UK* 20 *January* 1994; *revised* 23 *February* 1994; *accepted* 25 *February* 1994)