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THURIFERIC ACID. A NOVEL LIGNAN TYPE FROM *JUNIPERUS THURIFERA* L

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Abstract - The isolation and structural determination of a novel type of lignan, thuriferic acid, obtained from the hexane extract of the leaves of *Juniperus thurifera* L (Cupressaceae) are described. Its structure was confirmed by synthesis and the signals of its ^{1}H and ^{13}C NMR spectra were assigned unequivocally by two-dimensional techniques.

INTRODUCTION

Furthering our research into the chemical composition of the leaves of *Juniperus thurifera* (Cupressaceae), in the present work we report on the isolation, structural determination and semisynthesis of a new lignan, thuriferic acid, with different structural characteristics to those described previously. In earlier works components of different types obtained from the leaves of this species were described: phenylpropane derivatives[1], coumarins[2], monoterpenoids[3], sesquiterpenoids and diterpenoids[4] and a large number of lignans[5] of the monoepoxylignan, bisepoxylignan, butanolide and cyclolignan types. Two dimensional studies NMR have been carried out on some of these lignans[6,7].

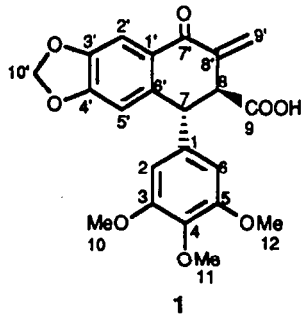
RESULTS AND DISCUSSION

In the present work, an oil, as the most polar substance, was isolated without heat treatment from the insoluble fraction of the hexane extract of the leaves of the plant. Its mass spectrum showed a molecular peak at M^+ 412 corresponding to the formula of $C_{22}H_{20}O_8$. Its IR spectrum shows bands of a carboxyl group (3300-2400, 1710 cm^{-1}), an α,β-unsaturated ketone (1680 cm^{-1}), aromatic rings (1600, 1500, 1490 cm^{-1}) and aryl-alkyl ethers (1260, 1130, 1050 cm^{-1}). According to its ^{1}H NMR spectrum, with signals of three methoxyls (two of them chemically identical), a methylenedioxy group and of aromatic rings, the molecule must contain 3,4,5-trimethoxyphenyl and 3,4-methylenedioxyphenyl groupings. Apart from the signals corresponding to these, its ^{13}C NMR spectrum shows others belonging to the carboxylic group (174.55 ppm), the conjugated ketone (184.07 ppm), two methines (47.66 and 54.86 ppm) and a methylidene group (126.00 ppm).

These data show that the substance is a cyclolignan that, unlike the others found in this plant, does not show an absorption band characteristic of γ-lactone (1770 cm^{-1}), but rather one characteristic of free carboxylic acid (1710cm^{-1}).

These findings support the proposal of structure 1 for this substance. The presence of the carbonyl group at 7' is deduced from the chemical shift of the aromatic proton at 2' (7.57 ppm) compared with

what is offered in the literature for picropodophyllotoxon and podophyllotoxon[8], and the configuration of carbon atom 8 is deduced from the coupling constant of its proton with H_7 (3.1 Hz)[9].



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Attempts to lactonize the substance to transform it into picropodophyllotoxon 4, by treatment with acids or bases did not yield the expected product. Treatment with dry HCl afforded the corresponding chloro derivative 2 which gave the Me ester 2a on treatment with CH_2N_2.

TABLE I. ^{1}H NMR SPECTRAL DATA FOR COMPOUNDS 1-5a (200.0 MHz)

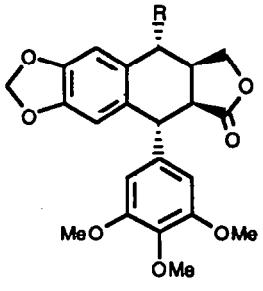
H	1	2	2a	5	5a
2, 6	6.22*s*	6.42*s*	6.40*s*	6.40*s*	6.38*s*
7	4.63*d* (3.1)	4.34*d* (11.6)	4.33*d* (11.3)	4.30*d* (11.3)	4.30*d* (11.6)
8	3.90*d* (3.1)	3.60*dd*(11.5, 10.8)	3.58*dd* (11.5, 10.8)	3.45*dd*(11.9, 11.3)	3.45*dd* (12.3, 11.6)
2'	7.57*s*	7.54 s	7.54*s*	7.47*s*	7.48*s*
5'	6.55*s*	6.24*s*	6.28*s*	6.20*s*	6.24*s*
8'	-	3.23*dt* (11.9, 2,9)	3.26*ddd*(12.1, 3.1, 2.9)	2.98*ddd*(11.9,4.9, 3.1)	3.03*ddd*(12.3, 4.8, 3.2)
9'	5.39*sa* 6.37*sa*	4.41*dd* (11.3, 2.9) 3.74*dd* (11.3, 3.2)	4.40*dd*(11.3, 2.9) 3.72*dd* (11.3, 3.2)	3.96*dd* (9.8, 4.9) 3.65*dd* (9.8, 3.1)	4.00*dd* (9.8, 4.8) 3.59*dd* (9.8, 3.2)
10'	6.02*s*	6.00*s*	6.00*s*	5.97*s*	5.98*d* (1.3)
10, 12	3.73*s*	3.81*s*	3.83*s*	3.78*s*	3.82*s*
11	3.79*s*	3.87*s*	3.88*s*	3.84*s*	3.86*s*
OMe	-	-	-	3.22*s*	3.31*s*
COOMe	-	-	3.49 s	-	3.47*s*

$CDCl_3$, TMS as internal standard, δ, ppm.

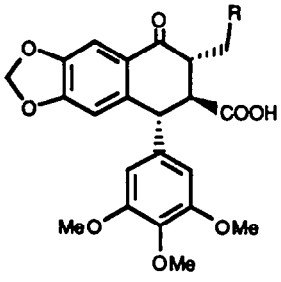
In view of the impossibility of transforming the compound into picropodophyllotoxon, its synthesis from picropodophyllotoxin 3, also isolated from the plant[5], was carried out. Oxidation of 3 with CrO_3 in pyridine[10] afforded almost quantitatively 4 whose spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR) are in concordance with picropodophyllotoxon[8,11]. Treatment of 4, with 10% KOH/MeOH afforded the natural product 1 apart from some side products among which another substance was isolated whose spectroscopic properties (Table I and II) point to the absence of the methylidene group which changes to a methoxy-methyl group and hence the substance has the structure 5 which gave the Me esther 5a on treatement with CH_2N_2. The latter was not obtained when 4 was treated with *ter* BuOK/*tert*-BuOH instead of KOH/MeOH, obtaining only 1.

Other differences between products 1 and 5 such as the coupling constant of methine 7, the shielding of protons 2' and 5', and the deshielding of aryl protons of the trimethoxyphenyl grouping suggest the existence of a different conformation for each substance. The coupling constant $J_{H_7\text{-}H_8}$=11.3

Hz in compound **5** points to a 1,2-diaxial arrangement of both protons and hence both the trimethoxyphenyl grouping and the carboxyl group would be in an equatorial disposition. By contrast, in substance **1** the coupling constant between H_7 and H_8 is 3.7 Hz and the remaining variations observed in its spectra point to a 1,2-diaxial arrangement of the trimethoxyphenyl and carboxyl groups which, furthermore, would account for the impossibility of lactonizing substance **1**.



3 R = OH
4 R = O



2 R = Cl
5 R = OMe

TABLE II. ^{13}C NMR SPECTRAL DATA FOR COMPOUNDS 1-5a (50.3 MHz)

*C	1	2	2a	4	5	5a
1	136.69	135.62	135.91	137.32	136.03	136.35
2	105.35	106.89	106.85	104.79	106.72	106.84
3	152.86	153.79	153.71	153.58	153.55	153.57
4	136.69	138.07	138.13	137.88	137.73	137.86
5	152.86	153.79	153.71	153.58	153.55	153.57
6	105.35	106.89	106.85	104.79	106.72	106.84
7	47.66	48.54	48.91	43.27	48.48	49.89
8	54.86	50.82	51.64	46.34	51.43	51.62
9	174.55	174.64	172.58	175.32	176.66	172.97
10	55.75	56.48	56.46	56.04	56.28	56.41
11	60.30	60.95	60.85	60.49	60.81	60.69
12	55.75	56.48	56.46	56.04	56.28	56.41
1'	126.99	126.75	126.88	127.07	126.64	127.06
2'	106.23	106.45	106.47	105.76	106.24	106.27
3'	147.64	147.62	147.60	148.22	147.44	147.44
4'	152.73	152.95	152.85	153.58	152.66	152.59
5'	108.55	108.57	108.53	109.18	108.45	108.46
6'	139.55	141.66	141.70	139.45	141.75	141.64
7'	184.07	191.15	191.38	193.27	193.01	193.28
8'	137.90	50.58	50.64	43.11	49.70	49.89
9'	126.00	41.90	41.76	70.24	69.69	70.10
10'	101.75	102.12	102.06	102.02	101.90	101.67
OMe					59.07	59.24
COOMe			51.91			51.97

$CDCl_3$, TMS as internal standard, δ, ppm
* numbering in this table is that used in ref.[12]

These deductions were confirmed by some NOE-difference experiments. Indeed, in **1** NOE was observed on H_8, $H_{2,6}$ and $H_{5'}$ when {H_7} was irradiated while in **5** NOE was only observed on the aromatic protons upon irradiation of {H_7}.

TABLA III. 2-D HETERONUCLEAR CORRELATIONS DATA FOR 1

$\delta^{13}C$ δ(type)	Attached H[O]	long-range[◆] coupled H	Atom No.
47.66(CH)	4.63(d)	2, 6, 5'	7
54.86(CH)	3.90(d)	9'a	8
55.75(CH_3)	3.73(s)		10, 12
60.30(CH_3)	3.79(s)		11
101.75(CH_2)	6.02(s)		10'
105.35(CH)	6.22(s)		2, 6
106.23(CH)	7.57(s)		2'
108.55(CH)	6.55(s)		5'
126.00(CH_2)	5.39(s) 6.37(s)		9'
126.99(C)		7, 5'	1'
136.89(C)		11, 7, 2,6	1, 4
137.90(C)		8, 7, 9'b	8'
139.55(C)		8, 7, 2'	6'
147.64(C)		10', 5', 2'	3'
152.73(C)		10', 5', 2'	4'
152.86(C)		10, 12, 2, 6	3, 5
174.55(CO)		8, 7	9
184.07(CO)		9'a, 9'b, 2'	7'

$CDCl_3$, TMS as internal standard, δ ppm (multiplicity)
[O] From observations on its direct H-C 2D-spectrum
[◆] From observations on its indirect H-C 2D-spectrum

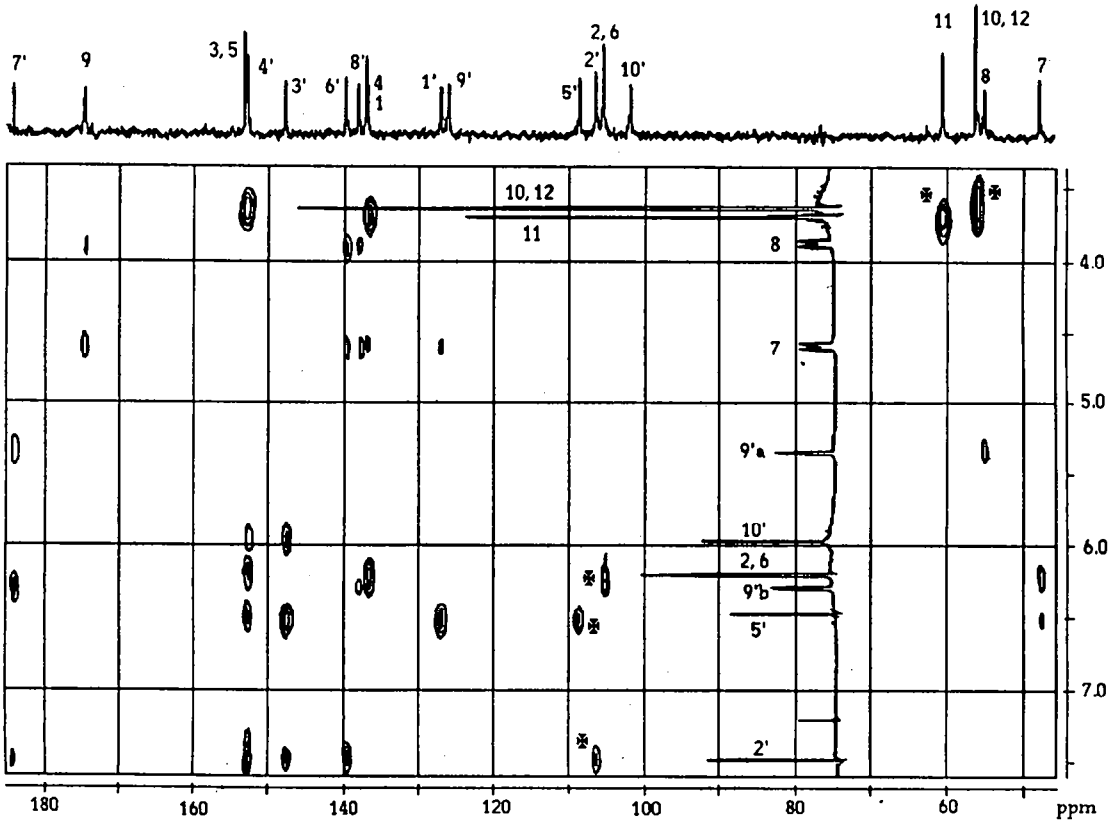
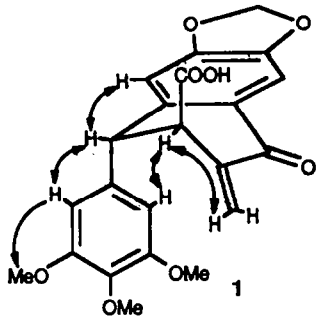


Figure 1. Long-range and one-bond (⌘) H/C correlations for **1**, with its ^1H and ^{13}C spectra



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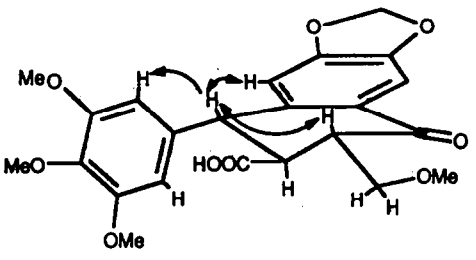

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Conformers of 1 and 5 and observed NOEs

Regarding the assignment of the signals in the ^{13}C NMR spectrum of thuriferic acid, it is clear that the aryl carbons 2 and 6, methoxyls 10 and 12 and their supporting carbons 3 and 5, which are signals with double intensity at 105.35, at 55.75, and at 152.86 ppm respectively, belong to the 3,4,5-trimethoxyphenyl grouping. Other signals can be assigned by means of DEPT spectra; this is the case of the two methylenes, one of them doubly oxygenated (101.75 ppm) corresponding to the methylenedioxy grouping, and the other olefinic (126.00 ppm), that can be assigned to 9'. Unequivocal assignment of the other signals, aliphatic methines and aromatic non-protonated carbon atoms, was performed using direct and indirect proton-carbon two-dimensional heteronuclear correlations (fig. 1) obtained from a synthetic sample, identical to the natural compound. A description of the latter is offered in the experimental part. Apart from assignment of all proton and carbon signals these experiments allowed us to confirm the structure proposed for this new lignan (Table III)

EXPERIMENTAL

General experimental procedures. Mps were determined in capillaries and are uncorrected. Optical rotations were measured in $CHCl_3$. UV spectra were recorded in EtOH; λ_{max} values are expressed in nm. IR spectra were obtained in $CHCl_3$. $\tilde{\nu}$max values are expressed in cm^{-1}. ^1H NMR (200.13 MHz) and ^{13}C NMR (50.3 MHz) spectra were measured in $CDCl_3$ with TMS as internal standard on a Bruker WP 200 SY spectrometer. δ values are expressed in ppm. EIMS were obtained at 70 ev. *m/z* values followed by relative abundance (%) are stated. Flash chromatography was run on silica gel (Merck Nº 9385)

Plant material, extraction and isolation . The plant material was collected in September at Prádena (Segovia, Spain). Voucher specimens are deposited in the Botany Department, Faculty of Biology, Salamanca. (register number SALA No. 7193).

Juniperus thurifera leaves (15 kg) were extracted with hexane, and the resulting solution was cooled at 0° overnight to give 615 g (34.0 % over whole extract) of insoluble fraction, which was successively defatted with MeOH and a saturated solution of urea in MeOH. 130g of the resulting product was chromatographed over Si gel with CH_2Cl_2/MeOH mixtures of increasing polarity, yielding, among other substances[1-6]:

Thuriferic Acid **1**(150mg) eluted with CH_2Cl_2/MeOH(98:2), colourless oil $[\alpha]^{23}(\lambda)$=-179.6°(589), -180.7° (578), -230.5°(546), -622.8°(436). UV: 205(ε=35200), 235(ε=16000), 273(ε=4900), 326(ε=4600). EIMS: 412(36), 368(14), 367(38), 277(6), 199(11), 181(16), 168(22), 153(23), 139(19), 113(17), 85(66), 83(100). IR: 3300-2400, 2780, 1710, 1680, 1600, 1500, 1490, 1260, 1130, 1050, 1010, 940. ^1H NMR (table I). ^{13}C NMR (table II).

50 mg of **1** in 5 ml of CH_2Cl_2, were treated with dry HCl for 30 minutes to afford 52 mg of **2**, colourless oil. IR: 3300-2450, 3040, 2780, 1710, 1680, 1620, 1595, 1500, 1485, 1250, 1130, 1040, 1000, 940, 910. ^1H NMR (table I). ^{13}C NMR (table II).

2a, colourless oil, $[\alpha]^{23}(\lambda)$=-35.5°(589), -37.2°(578), -43.1°(546), -84.8°(436). UV: 203(ε=42.100), 236(ε=20900),278(ε=6500), 325(ε=6800). IR: 3020, 2820, 1725, .1680, 1620, 1595, 1510, 1485, 1240, 1130, 1040, 1010, 940, 890, 850. ^{1}H NMR(table I). ^{13}C NMR (table II).

***Picropodophyllotoxin* 3**, colourless crystals mp 232-34°C(MeOH). $[\alpha]^{23}(\lambda)$= +136.0° (589), +136.0°(578), +143.6°(546), +160.5°(436). Spectral properties identical to those described[5]. Compound **3** (400 mg) in pyridine (12 ml) was added to 400 mg of CrO_3 in pyridine (8 ml) and stirred at room temperature. After 3h, it gave **4** (390 mg).

***Picropodophyllotoxon* 4**, colourless oil. $[\alpha]^{23}(\lambda)$=-121.7°(589), 124.8°(578), -150.8°(546), -400.4°(436). UV: 207(ε=46900), 240(ε=21400), 281(ε=7100), 325(ε=6700). IR: 3040, 2840, 1780, 1670, 1620, 1595, 1505, 1485, 1260, 1130, 1110, 1040, 1020, 1005, 940, 895. ^{1}H NMR: 3.28(1H, d, J=5.2, H_8), 3.29(1H, m, $H_{8'}$), 3.71(3H, s, H_{11}), 3.75(6H, s, H_{10}, H_{12}) 4.31(1H, dd, J=9.2, 5.3, H_{9a}), 4.67(1H, d, J=5.2, H_7), 4.71(1H, d, J=9.2, H_{9b}), 6.00(2H, s, $H_{10'}$), 6.21(2H, s, H_2 and H_6), 6.65(1H, s, $H_{5'}$), 7,45(1H, s, $H_{2'}$). ^{13}C NMR(table II). **4** (60 mg) was treated with 1 ml KOH/MeOH of 10% for 30 minutes at room temperature. This crude material was extracted and flash chromatographed on Si gel, yielding **1** (20 mg) and **5**(10 mg). **4** (300 mg) was treated with *tert* BuOK/*tert* BuOH/ H_2O (4/95/5) for 10 minutes at room temperature yielding pure **1** (295 mg).

5 is a colourless oil. $[\alpha]^{23}(\lambda)$=-33.4°(589), -34.7°(578), -37.4°(546). UV: 207(ε=32200), 236(ε=15900), 277(ε=5000), 327(ε=4800). IR: 3400-2420,3060, 2840, 1710, 1675, 1620, 1595, 1505, 1230, 1130, 1040, 1005, 940, 910, 850. ^{1}H NMR(table I), ^{13}C NMR (table II). **5a** is a colourless oil, IR: 1740, 1680, 1620, 1600, 1505, 1480, 1250, 1130, 1110, 1040, 1005, 940. ^{1}H NMR (table I), ^{13}C NMR (table II).

2D-NMR EXPERIMENTS.- *Heteronuclear ^{1}H-^{13}C correlations (200/50.3 MHz).* The pulse sequence XHDEPT.AU from the Bruker DISnmr 85 program library was used. 124 FIDs of 192 scans each with 1 sec recycle delay and incrementing t_1 from 3 μsec to 130 msec, were acquired on a 0.5 M solution of **1** at 32°C. Spectral widths (SW_2 and SW_1, respectively) were selected covering the range from 32 to 133 ppm in F_2 and the 0.3 to 2.8 ppm range in F_1. Polarization transfer was tuned for a value of $^1J_{C,H}$=135 Hz. After sine-bell filtration in both domains with one degree of zero-filling in F_1, a matrix of 256/1024 data points with digital resolution (DR)= 9.9 Hz/pt in F_2 and 5.2 Hz/pt in F_1 was obtained (figure 1).

The long-range ^{1}H/^{13}C correlation was performed on the same sample. SW_2 covered the range from 28 to 193 ppm and SW_1 from 1.2 to 9.5 ppm with the F_1 carrier suitably placed to avoid folding of signals. Number of scans per FID was 1280. Response was tuned for $^nJ_{C,H}$=7.8 Hz. DR was 16.3 Hz/pt in F_2 and 6.5 Hz/pt in F_1 (Table III).

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