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The Stereochemistry of Thuriferic and Epithuriferic Acids

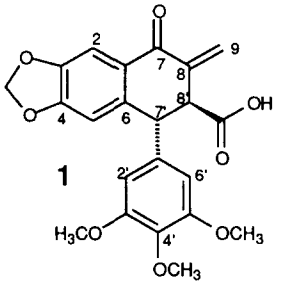
José L. López-Pérez*, Esther del Olmo, Beatriz de Pascual-Teresa, Marta Merino and Arturo San Feliciano

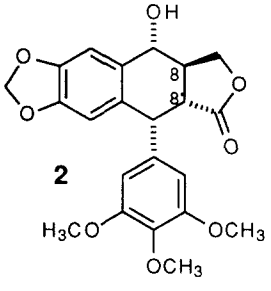
Departamento de Química Orgánica y Farmacéutica. Facultad de Farmacia. Universidad de Salamanca. E-37007 Salamanca. Spain.

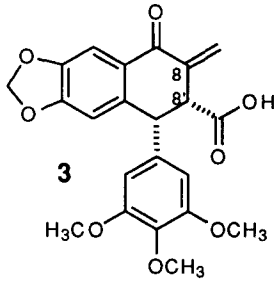
Abstract.- A new lignan, epithuriferic acid has been prepared from isopicropodophyllone. Its stereochemistry has been established on the basis of spectral, chemical and molecular modelling findings and compared to that of its 8′ epimer thuriferic acid, isolated from the *Juniperus thurifera* leaves.

Introduction.

Thuriferic acid (**1**), a cyclolignan isolated from *Juniperus thurifera* leaves[1] presents, instead of the characteristic γ-lactone of podophyllotoxin (**2**) and related cyclolignans, a free carboxylic group and a α,β-unsaturated ketone. This moiety could account for the alkylating and electrophilic properties towards proteins and nucleic acids[2].





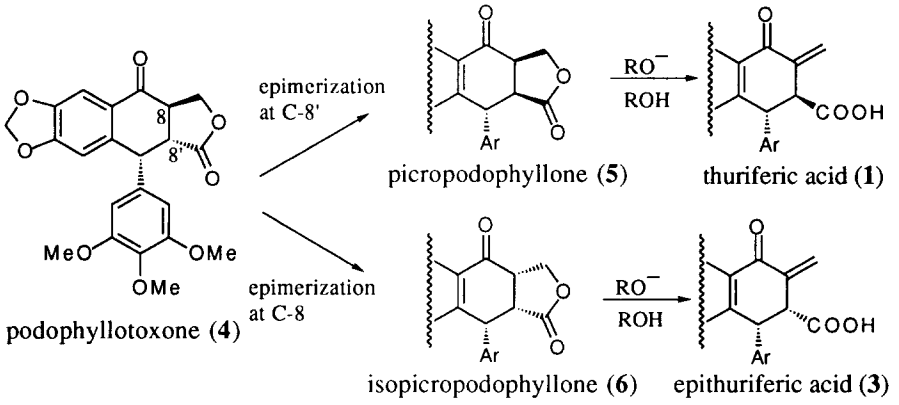


The stereochemistry of the natural compound **1** was established by means of an array of spectroscopic techniques, in particular NMR[1]. Further, Höfert and Matusch reported the preparation of its 8′-epimer **3**[3], while actually, the product obtained was **1**, as was demonstrated by us through chemical, spectroscopic and molecular modelling studies[4,5]. In addition, thuriferic acid has been synthesized by Ogiku, T. *et al*[6] Consequently we decided to prepare epithuriferic acid for two main reasons. First, to clarify completely the stereochemical aspects related to the position 8′ in both compounds and second to evaluate its antineoplastic activity, since podophyllotoxin (**2**) as antitumoral agent, shows a potency one hundred times higher that its 8′-epimer picropodophyllin and a similar increase could be expected in the bioactivity of epithuriferic acid (**3**) with respect to that of thuriferic acid (**1**)[2].

Results and Discussion

The possibility of preparing thuriferic acid (**1**), either from podophyllotoxone (**4**) or picropodophyllone (**5**), implies, in the first case, an epimerization at C-8′ before the opening of the lactone conducting to picropodophyllone (**5**) which is thermodynamically more stable[7].

The presence of two chiral epimerizable centres in position α to the ketonic and lactonic carbonyls, allows two structural possibilities for the reaction product after treating the strained *trans* lactone **4** with bases. Indeed, the epimerization at C-8 will conduct to isopicropodophyllone (**6**), whereas, the epimerization at 8′ will conduct to picropodophyllone (**5**). These two lactones, in turn, will conduct to epithuriferic acid (**3**) and thuriferic acid (**1**) respectively (Scheme I). The former transformation was proposed by Höfert and Matusch[3], who assumed that the greatest acidity of the H-8 proton, in position alfa to the ketone, in comparison with that of H-8′, alfa to the lactonic carboxyl group, was sufficient to favour the preferable abstraction of the first. However, this possibility has been discarded by means of theoretical as well as experimental evidence[5] because from the stereochemical point of view, the accessibility of bases to the α-oriented proton H-8 is much more hindered due to the presence of the alfa oriented trimethoxyphenyl group. Consequently, the epimerization at 8′, would take place, thus reassuring the previously published results.



Scheme I.

The preparation of the epithuriferic acid (**3**) from podophyllotoxone (**4**), will not then be feasible, by the basic treatment and it would imply the formation of the *cis* lactonic cyclolignan of the iso series **6**, which would not be able to experiment a double epimerization to give place to the contrary *cis* picrolactone **5**.

Isopicropodophyllone (**6**) can be easily obtained from the podophyllotoxone (**4**), which, in its turn, is prepared by oxidation of podophyllotoxin (**2**)[8]. In effect, whereas podophyllotoxone (**4**) evolves towards picropodophyllone (**5**) in the presence of bases[6], in an acidic medium, the carbon which epimerises is C-8, conducting to a balanced mixture of podophyllotoxone/ isopicropodophyllone. Spectroscopical data for isopicropodophyllone (**6**) coincides with that reported in the literature[9]. Later treatment in basic soft conditions (KOH/MeOH 1%) of **6** leads to a mixture of the reaction from which, junaphtoic acid[10,11] and epithuriferic acid (**3**) are isolated. Epithuriferic acid shows ^{1}H and ^{13}C NMR spectra very similar to those of thuriferic acid (**1**)[1] (table I and II; NMR data of thuriferic acid are included in the tables for comparison). Nevertheless, some differences are observed, the deshielding of the olefinic proton 9a (5,78

ppm instead of 5.39) and the deshielding of the protons 2' and 6' of the trimethoxyphenyl ring (6.38 ppm instead of 6.22 ppm), along with the coupling constant between protons 7' and 8' of 5.2 Hz in place of 3.4 Hz of the thuriferic acid. These facts can be easily explained and are in agreement with the change of configuration at 8'. The coupling constant between protons 7' and 8', 5.2 Hz, did not allow us to establish clearly the conformational disposition of that part of the molecule. In consequence, additional studies of nOe difference and molecular modelling were carried out.

A Montecarlo random conformational search[12,13], allowed us to find two main conformers for this compound: A-1 with the trimethoxyphenyl ring in a pseudoaxial disposition and a pseudoequatorial carboxylic group, and A-2, with these moieties just in the opposite disposition. The orientation of the exocyclic methylene is another important difference between both conformations (fig. 1). Relative stabilities were determined by both Molecular Mechanics and Semiempirical methods (MNDO, AM1 and PM3)[14] for each conformer. Steric energies and heats of formation are summarized in Table III. Both conformations show a very similar stability. By AM1 semiempirical method, A-2 is slightly more stable whereas, using COSMIC force field calculation[15], A-1 results the more stable (Table III).

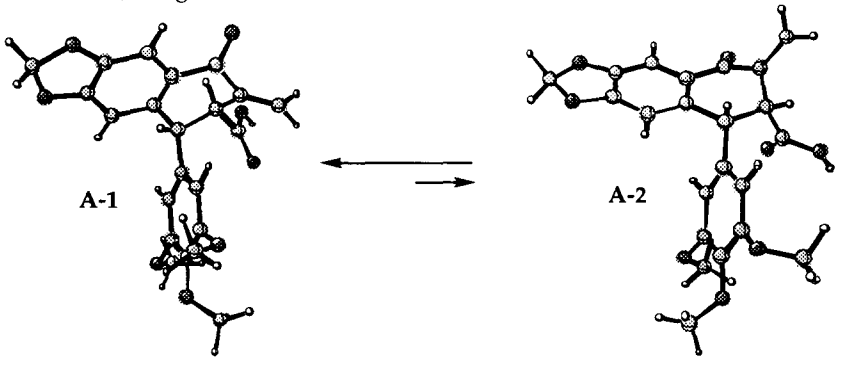


Fig. 1. The two main conformers of epithuriferic acid

Dihedral angles between the benzylic proton H-7' and the proton geminal to the carboxylic acid H-8' have been measured and the coupling constants for these hydrogens theoretically calculated for each conformer[16](Table III: 4.9 Hz for the conformation A-1 and 8.3 Hz for the conformation A-2). The experimental value for this coupling constant, 5.2 Hz is in-between the calculated values for both conformers and closer to that of A-1.

Table III. Calculated Coupling Constants and Heats of Formation and Steric energy of epithuriferic acid.

Conformer	Dihedral Angle $H_{7'}$-$H_{8'}$ (°)	$J_{H_{7'}-H_{8'}}$ (Hz)	ΔH MNDO (Kcal/mol)	ΔH AM1 (Kcal/mol)	ΔH PM3 (Kcal/mol)	COSMIC (Kcal/mol)
A-1	48	4,9	-230,1	-216.19	-220.51	+24.86
A-2	30	8,3	-232,8	-216.29	-221.72	+25.03

The nOe differences observed are summarized in table IV, together with the interprotonic distances measured in the global minimum conformations after optimization by MNDO. The observation of a nOe on H-7' when {H-5} is irradiated, and viceversa, will indicate

a conformational disposition A-1 for epithuriferic acid. However, the observation of another nOe difference between the H-8', geminal to the carboxylic group, and the olefinic proton H-9a, could indicate rather an A-2 conformation, because the distance between both protons on the conformation A-1 (3.5Å) is too large to allow a nOe difference effect.

Table IV. Interprotonic distances in the main conformers of epithuriferic acid obtained by MOPAC and Noe effects experimentally observed (*** =strong; *=weak).

Conformer	Interprotonic distances (Å)				
	5-7'	7'-(2',6')	7'-8'	8'-(2',6')	8'-9a
A-1'	2.4	2.3	2.4	4.1	3.5
A-2	3.1	2.3	2,4	3.0	2.4
noe observed	**	***	***	-	*

In consequence, nOe difference experiments, as well as the coupling constant (5.2 Hz) between the H-7' and H-8', seem to indicate the existence of an equilibrium of populations between the two main conformers of epithuriferic acid, with a relatively larger amount of the conformer A-1, which shows a coupling constant closest to the experimental value. This fact has been further reinforced by the observation, that when the ^{1}H NMR spectrum is carried out at low temperature (-50 °C), a diminution of the coupling constant between H-7' and H-8' up to 4.9 Hz is observed as well as a light shielding of the signals corresponding to the protons H-2, H-2' and H-6' and mainly of the signal corresponding to H-8' (0.15 ppm) making this spectrum more similar to that of thuriferic acid, which contains the trimethoxyphenyl group in a pseudoaxial disposition.

In order to establish what chiral centre is epimerized when 7-ketolignans of this group are treated with bases, ^{1}H NMR spectra have been run in deuterated methanol to determine, in this way, what proton is interchanged with deuterium in each case. When picropodophyllone (**5**)

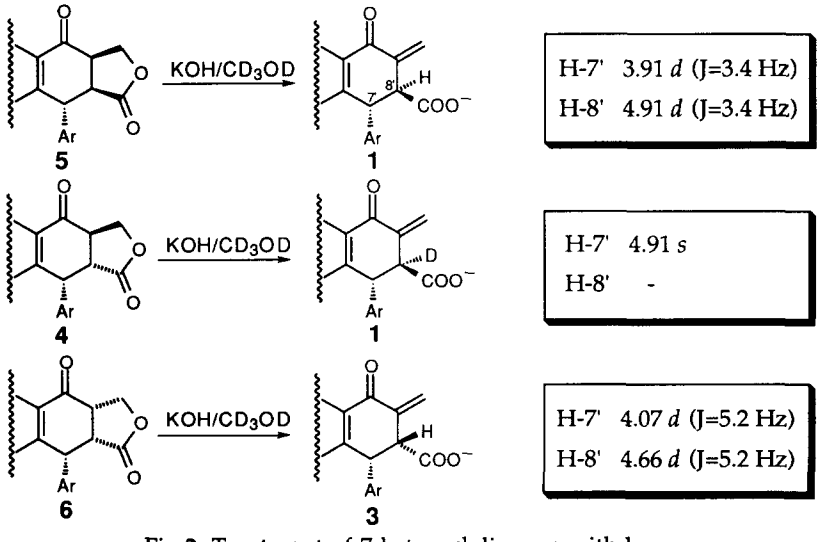


Fig 2. Treatment of 7-ketocyclolignans with bases.

or isopicropodophyllone (**6**) are treated with KOH/CD_3OD, potassium salts of thuriferic acid or epithuriferic acid are respectively obtained. In both cases, the benzylic proton H-7′ resounds as a doublet, indicating that no exchange with deuterium has occurred. In consequence, the stereochemistry at C-8′ is maintained with respect to the original cyclolignan **5**. On the other hand, when podophyllotoxone (**4**) is treated in the same conditions, thuriferic acid is obtained, but the signal corresponding to the H-8′ has disappeared, and then the benzylic proton H-7′ is now a singlet. This fact indicates that the proton geminal to the carboxylic acid, H-8′ has been interchanged by deuterium, also demonstrating that an inversion of the configuration at C-8′ has occurred.

By treating epithuriferic acid (**3**) with a dry stream of HCl, 9-chloro-8,9-dihydro-epithuriferic acid (**8**) was obtained. This substance shows important spectroscopic differences with respect to 9-chloro-8,9-dihydro-thuriferic acid (**7**) (tables I and II) obtained by the same treatment from thuriferic acid, suggesting not only a different configuration at C-8′, but also a different conformation. In effect, in the ^1H NMR spectrum of **8** appears one double doublet ($J_{8'-7'}$=5.7 Hz; $J_{8'-8}$=10.5 Hz) centered at 3.75 ppm corresponding to the H-8′ geminal to the carboxylic group. The coupling constant $J_{8'-8}$=10.5 Hz can only be in agreement with an anti-diaxial arrangement of both protons; consequently, the carboxyl group must be in a α-equatorial disposition; whereas the coupling constant $J_{8'-7'}$=5.7 Hz indicates an equatorial disposition of the $H_{7'}$ and hence the trimethoxyphenyl moiety must be in a pseudoaxial disposition. This confirms that, the 9-chloro-8,9-dihydro-epithuriferic acid (**8**) shows a conformation sufficiently

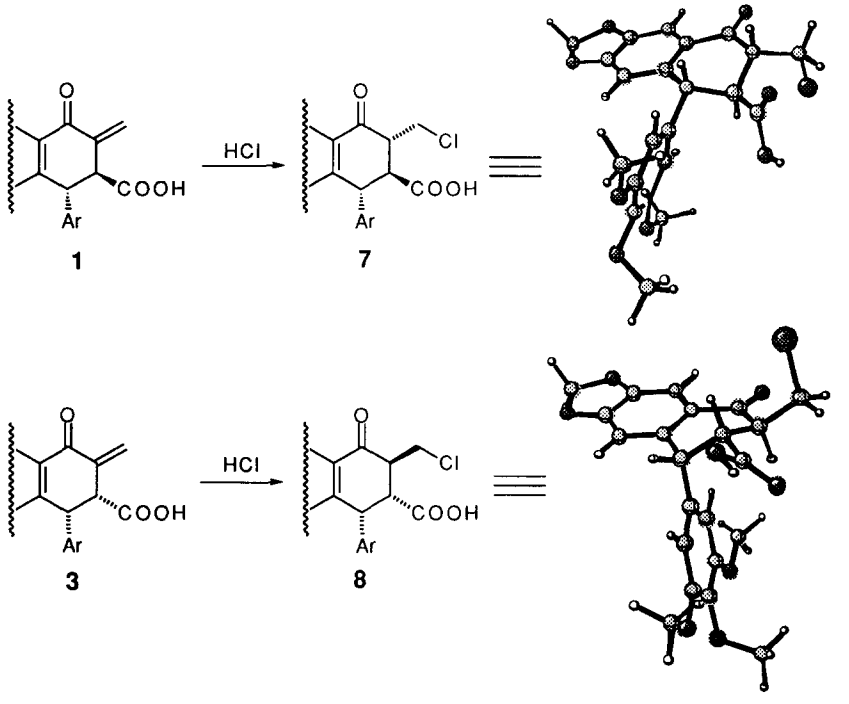


Figure 3. 9-Chlorine derivatives of 8,9-dihydro-thuriferic and 8,9-dihydro-epithuriferic acids

different than that of 9-chloro-8,9-dihydro-thuriferic acid (**7**). Molecular modelling studies of both compounds show an unique global minimum conformation for each one of these substances.

Table I. ^{1}H NMR data for compounds **1, 3, 7 and 8**

H	1	3	7	8
2	7.57 s	7.60 s	7.54 s	7.45 s
5	6.55 s	6.53 s	6.24 s	6.19 s
8	--	--	3.23 dt (11.9; 2.9)	3.15 dt (10.5 ;4.1)
9a	5.39 sa	5.78 sa	4.41 dd (11.3: 2.9)	4.37 dd(12.1 ; 4.1)
9b	6.37 sa	6.44 sa	3.74 dd (11.3; 3.2)	3.40 t (11.0)
10	6.02 s	6.03 s	6.00 s	6.01 s
2', 6'	6.22 s	6.38 s	6.42 s	6.43 s
7'	4.63 d (3.4)	4.53 d (5.2)	4.34 d (11.6)	4.56 d (5.7)
8'	3.90 d (3.4)	4.07 d (5.2)	3.60 dd (11.6;10.8)	3.75 dd (10.5; 5.7)
10', 12'	3.79 s	3.72 s	3.81 s	3.84 s
11'	3.73 s	3.88 s	3.87 s	3.84 s

($CDCl_3$, δ values in ppm, J in Hz, TMS int. std)

Table II. ^{13}C NMR data for compounds **1, 3, 7 and 8**

C	1	3	7	8
1	126.99	127.70	126.75	127.34
2	106.23	107.08	106.45	106.02
3	147.64	147.93	147.62	145.27
4	152.73	152.84	152.95	153.51
5	108.55	108.33	108.57	108.96
6	139.55	140.35	141.66	143.18
7	184.07	184.07	191.15	191.61
8	137.90	138.97	50.58	51.28
9	126.00	125.32	41.90	42.82
10	101.75	102.07	102.12	103.14
1'	136.69	134.57	135.62	137.10
2',6'	105.35	107.08	106.89	108.89
3',5"	152.86	153.53	153.79	154.53
4'	136.69	138.27	130.80	138.62
7'	47.66	48.79	48.54	49.27
8'	54.86	53.27	50.82	51.57
9'	174.55	174.60	174.64	172.99
10',12'	55.75	56.37	56.48	56.54
11'	60.30	60.87	60.95	60.51

($CDCl_3$, δ in ppm, TMS int. std)

In summary, the structures of thuriferic and epithuriferic acids have been definitively established. These compounds and the corresponding chlorinated derivatives show different conformations, which could be of interest in order to obtain relevant information for the structure-antitumoral activity relationships.

Experimental Part

Chemistry

GENERAL EXPERIMENTAL PROCEDURES.- Melting points were determined in silicone bath and are uncorrected. Ir spectra were performed in $CHCl_3$ solution. NMR spectra were recorded at 200/50 MHz ($^1H/^{13}C$) in $CDCl_3$ solution. Chemical shifts (δ) are given in ppm, referred to internal TMS, and coupling constants (*J*) in Hz. Mass spectra (EI) were recorded under ionization energy of 70 eV. Column chromatography was performed over silica gel (0.063-0.2 mm). Flash chromatographies, with 3-85 mL/min flow rates, over silica gel (0.040-0.063 mm). TLC was performed on precoated silica gel polyester plates (0.25 mm thickness) with fluorescent indicator UV_{254}. A solution of 10% phosphomolybdic acid in EtOH or 10% H_2SO_4 in EtOH were used for visualization, after heating at 110°C. PLC was developed on SiF_{254} plates.

Epithuriferic acid (**3**). Compound **6** (200 mg) was treated with 5 ml of 1% KOH/MeOH. The mixture was left 10 minutes at room temperature yielding, after usual work and flash chromatography on Si gel, 50 mg of junaphtoic acid and 110 mg of **3**. Colourless crystals m. p.=174-176°C. $[\alpha]^{23}(\lambda)$=-120.2°(589), -126.0°(578), -151.0°(546), -254.5°(436) (c=0.4; $CHCl_3$). IR: 3200-2800, 2780, 1715, 1680, 1600, 1500, 1490, 1260, 1130, 1040, 1010, 940. ^1H NMR (table I). ^{13}C NMR (table II). Spectral properties of junaphtoic acid are identical to those described in reference 10.

Potassium salt of thuriferic acid from podophyllotoxone. Compound **4** (10 mg) was treated with 0.5 ml of 1% KOH/CD_3OD, in the tube of NMR. After 5 min, the salt of thuriferic acid was formed. ^1H nmr (CD_3OD) δ (ppm): 3.89 (s, 9H, OMe), 4.91 (s, H-7'), 5.36 (d, J=2.0 Hz, H-9a), 6.20 (s, O-CH_2-O), 6.27 (d, J=2.0, H-9b), 6.57 (s, H-2', H-6'), 6.76 (s, H-5), 7.67 (s, H-2).

Potassium salt of thuriferic acid from picropodophyllone. By the same procedure described before from 10mg of **5**, the salt of thuriferic acid was obtained. ^1H nmr (CD_3OD) δ (ppm): 3.89 (s, 9H, OMe), 3.91 (d, J=3.4 Hz, H-8') 4.91 (d, J=3.4 Hz, H-7'), 5.36 (d, J=2.0 Hz, H-9a), 6.20 (s, O-CH_2-O), 6.27 (d, J=2.0, H-9b), 6.57 (s, H-2', H-6'), 6.76 (s, H-5), 7.67 (s, H-2).

Potassium salt of epithuriferic acid. In the same procedure as described before, 12 mg of **6** were treated with 0.5 ml of KOH/CD_3OD, obtaining the salt of epithuriferic acid. ^1H nmr (CD_3OD) δ (ppm): 3.92 (s, 3H, OMe), 3.95 (s, 6H, OMe), 4.07 (d, J=5.2 Hz, H-8') 4.66 (d, J=5.2 Hz H-7'), 5.88 (d, J=1.8 Hz, H-9a), 6.16 (s, O-CH_2-O), 6.35 (d, J=1.8, H-9b), 6.61 (s, H-5), 6.82 (s, H-2', H-6'),7.64 (s, H-2).

9-chloro-8,9-dihydro-thuriferic acid (**7**). By treatment of **1** (30 mg in 6 ml of CH_2Cl_2) with a dry stream of HCl for 30 minutes, 33 mg of **7** were obtained, a colourless oil with spectral properties identical to those described for this compound[1]

9-chloro-8,9-dihydro-epithuriferic acid (**8**). By the same method described for **7**, 20 mg of **8** were obtained from 35 mg of **3** after flash chromatography. Colorless crystals m. p.=120-122°C. IR: 3100-2900, 1710, 1680, 1600, 1500, 1490, 1340, 1260, 1140, 1050, 1010, 940. ^1H NMR (table I). ^{13}C NMR (table II).

Molecular Modelling. Calculations were performed on a Silicon Graphics Indigo computer. Compounds **3, 7,** and **8** were built using facilities of Macromodel[17] v.4. Conformational analysis of every compound was performed by a Monte Carlo random search. All freely rotating bonds were searched with MM2 minimization. Full geometry optimization of the several low-energy conformations of every compound were performed using Stewart's MNDO, AM1 and PM3. Hamiltonian in MOPAC 6.0.

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