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UNAMBIGUOUS CONFIGURATIONAL AND CONFORMATIONAL DETERMINATION OF THURIFERIC ACID

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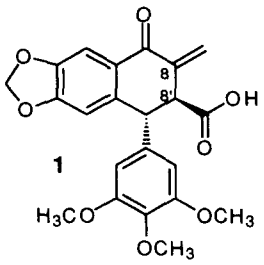
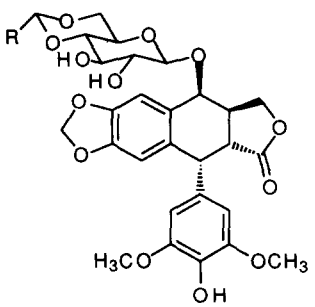
Abstract.- The stereochemistry of thuriferic acid has been checked on the basis of spectral, chemical and molecular modelling findings. Under basic catalysis, the carbon alfa to the lactone carbonyl group in podophyllotoxone must be inverted instead of the carbon alfa to the ketone group, before the β-elimination, to give thuriferic acid.

Introduction

Thuriferic acid (**1**) is a cyclolignan isolated from *Juniperus thurifera* leaves. Its structure was established by means of an array of spectroscopic techniques, in particular NMR[1]. In addition to this new compound, podophyllotoxin (**4**) and other related cyclolignans[2,3] were isolated. These substances have attracted considerable attention due to their biological properties. In fact, some semisynthetic derivatives of podophyllotoxin, like etoposide (**2**) and teniposide (**3**) are now used in the treatment of several human tumors[4,5] and others are under clinical trials.





2 R=CH_3

3 R=

It is important to point out like structural feature of thuriferic acid, the presence of an α,β-unsaturated carbonyl group, which constitutes the main difference with the rest of cyclolignans from the Podophyllotoxin family. This moiety could account for the alkylating and electrophilic properties towards proteins and nucleic acids. This compound was requested by the National Institute of Cancer in Bethesda (USA) where essays on 51 cell lines of different kinds of cancer were carried out, showing a general cytotoxic effect for all kind of tumors, with a medium-low activity level and a low selectivity[6].

In a recent work by Höfert and Matusch[7], the preparation of the 8′ epimer of thuriferic acid from podophyllotoxone (**6**) was reported. Its optical rotation was not described. However, its spectroscopic data is in complete agreement with that reported by us either for the natural or the synthetic thuriferic acid obtained from picropodophyllone (**7**). Therefore, some queries about the stereochemistry of the molecule have emerged.

The aim of the present work is to ascertain definitively the stereochemistry and the spatial arrangement of this molecule. Some chemical transformation and molecular modelling studies, including molecular mechanics and semiempirical methods, have been carried out with this purpose.

Results and Discussion

With the purpose of checking the stereochemistry of this compound, molecular modelling studies have been undertaken. A Montecarlo random conformational search[8,9], allowed us to find two main conformers for each C-8' epimer of thuriferic acid. Other conformers with slight energetic variations have also been found. Relative stabilities for each pair of conformers were determined by MNDO[10], and the calculated heats of formation are summarized in Table I. Dihedral angles between the benzylic proton 7' and the proton geminal to the carboxylic acid have been measured and the coupling constants for these hydrogens have been theoretically calculated for each conformer[11](Table I). From this study a greater stability have been deduced for the β configuration of the carboxylic acid, since both **B** conformers have

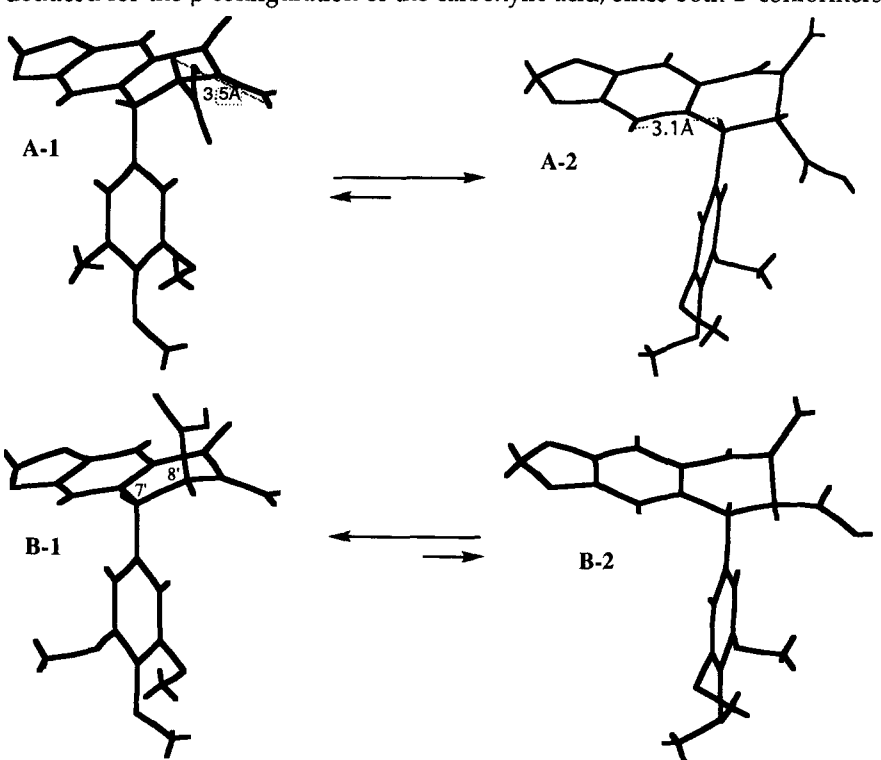


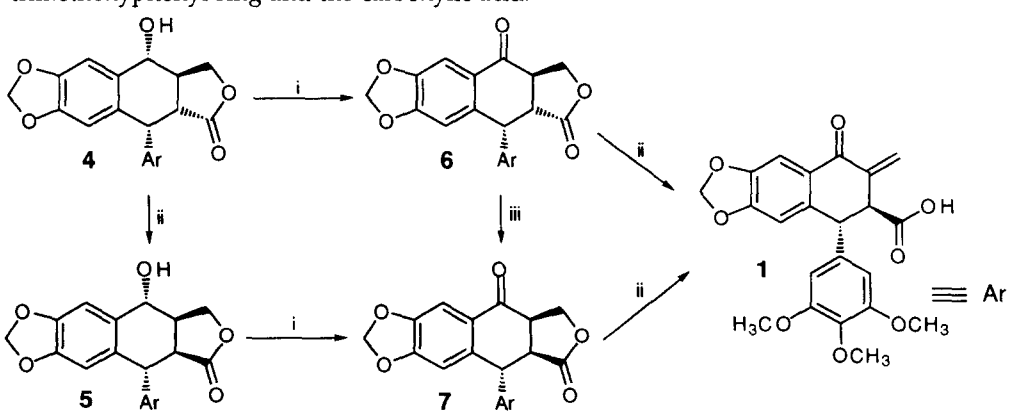
Figure 1. Two main conformers for each C-8' epimer of thuriferic acid

a heat of formation about 3 Kcal/mol lower than the two **A** conformers; on the other hand, the calculated coupling for **A** conformers are always larger than the experimental value (3.4 Hz) while, in the case of conformers **B**, the experimental value is in-between the calculated values for both conformers. The presence of population equilibrium between both **B** conformers, with a relatively larger amount of the conformer **B-1** which has a lower energy, and the coupling constant closest to the experimental value, seems to be the most reasonable assumption. In addition, the calculated interprotonic distances for **B** conformers are in agreement with the NOE effects experimentally observed, whilst they were in disagreement with values for conformers **A** (Figure 1).

Conformer	Dihedral Angle $H_{7'}$-$H_{8'}$ (º)	$J_{H_{7'}-H_{8'}}$ (Hz)	ΔH (Kcal/mol)
A-1	48	4,9	-230,1
A-2	30	8,3	-232,8
B-1	77	1,9	-235,4
B-2	16	11,8	-233,7

Table I. Theoretical Coupling Constants and Heats of Formation of conformers for each C-8' epimer of thuriferic acid.

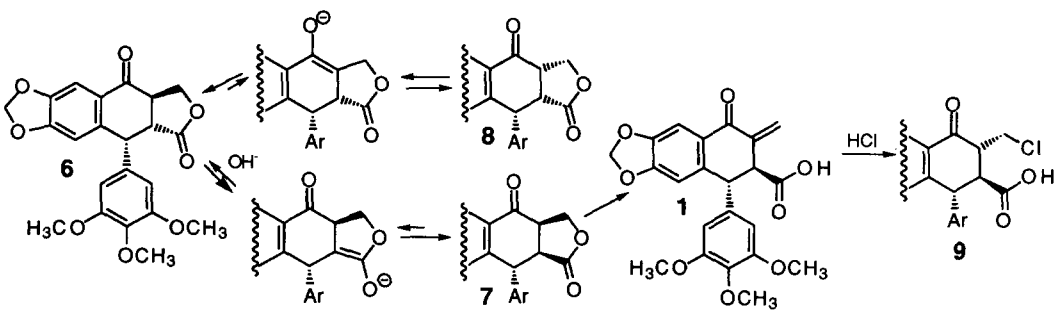
To confirm these results and establish unambiguously the stereochemistry of this compound, we undertook the preparation of thuriferic acid using as starting materials both 8' epimers, podophyllotoxin (**4**) and picropodophyllin (**5**), compounds with a perfectly established structure[12] (Scheme 1). After oxidation with CrO_3 in pyridine, the corresponding ketones podophyllotoxone (**6**) and picropodophyllone (**7**) were obtained almost quantitatively. Each one, was then treated with KOH/MeOH, both leading to the same product thuriferic acid (**1**), described above. Under basic catalysis, the carbon alfa to the lactone carbonyl group of podophyllotoxone (as indeed in all known podophyllotoxin analogues) should be readily inverted[13], followed by β-elimination to give thuriferic acid with a *trans* disposition between the trimethoxyphenyl ring and the carboxylic acid.



i) CrO_3/pyr; ii)KOH/MeOH 5%; iii)$NaHCO_3/H_2O$/MeOH 1:9.

Scheme 1. Preparation of thuriferic acid from podophyllotoxin and picropodophyllin

Podophyllotoxone (**6**), picropodophyllone (**7**) and isopicropodophyllone (**8**) were also calculated and their lowest energy conformers were taken into account. Podophyllotoxone, contains a *trans*-fused lactone ring leading to a strained and rigid molecule (ΔH=-236.4 Kcal/mol). Under basic catalysis, H-8 and H-8′ could be abstracted, giving by reprotonation either isopicropodophyllone (**8**) (ΔH=-242.5 Kcal/mol) or the most stable isomer picropodophyllone (**7**) (ΔH=-245.1 Kcal/mol). Furthermore, in podophyllotoxone, H-8 in *alfa* disposition presents a greater steric hindrance than H-8′ in *beta* disposition. This fact can be corroborated by comparing the atomic accessible surface area for both hydrogens defined by the locus of a rolling sphere probe of the radius of OH^- group (7.3 square Å for H-8′ and 5.70 for H-8)[14]. In consequence, H-8′ could be abstracted more easily than H-8, leading to picropodophyllone (**7**), rather than isopicropodophyllone (**8**). In conclusion, the basic media produces the epimerization of podophyllotoxone to the thermodynamically more stable and kinetically easier to obtain picropodophyllone, before the opening of the lacton ring to the thuriferic acid. Höfert and Matusch[8], in their work, assumed just the opposite. In fact, the compound assigned in their paper for isopodophyllotoxone (in reality should be isopicropodophyllone) shows identical spectroscopic data to picropodophyllone[1,15].



Scheme 2

These results have been demonstrated by using weaker basic conditions, which led to the epimerization of the podophyllotoxone (**6**) in 8′ before the β-elimination. In effect, by treating podophyllotoxone with $MeOH/H_2O$ saturated with $NaHCO_3$, a mixture of picropodophyllone/thuriferic acid 80/20 was obtained. Besides it has been reconfirmed because podophyllotoxone (**6**) treated with 5% KOH/CD_3OD, afforded thuriferic acid (potassium salt) showing in its ^1H NMR spectrum a singlet at δ 4.91 ppm corresponding to the H-7′, whereas the signal of H-8′ is not observed at all. This indicates that enolization took place in the lactone carbonyl, and in consequence, a Deuterium atom entered at the position 8′ before the β-elimination.

Finally, by treating thuriferic acid with a dry stream of HCl, 9-chloro-8,9-dihydrothuriferic acid (**9**) was obtained. This substance shows spectroscopic differences with respect to compound **7**, suggesting the existence of a more fixed and different conformational arrangement. The coupling constants $J_{H_{7'}\text{-}H_{8'}}$=11.9 and $J_{H_8\text{-}H_{8'}}$=11.3 Hz in compound **9** [1] can only be in agreement with a antidiaxial arrangement of H-8′ in relation with H-7′ and H-8 and hence the carboxyl group must be in a β-equatorial disposition.

After these results, the structure of thuriferic acid is definitively established, as well as the course of the reaction produced by basic treatment of podophyllotoxone.

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 $CHCl_3$ 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_2SO4 in EtOH were used for visualization, after heating at 110°C. PLC was developed on SiF_{254} plates.

Podophyllotoxin (**4**). 100g of Podophyllin, resin from *Podophylum emodi* (Berberidaceae) were extracted with EtOAc and then fractionated with 4% aq. NaOH. The neutral part was crystallized in benzene and afforded 18 g of **4**. M. p.=183-185°C.

Picropodophyllin (**5**). Compound **4** (400 mg) in 25 ml of 5% KOH in MeOH was stirred for 4 hours at room temperature. After neutralization and extraction with EtOAc, 394 mg of **5** were obtained. M.p.=232-234°C (MeOH). Spectral properties identical to those described in ref. 12.

Podophyllotoxone (**6**) 400 mg of **4** in 15 ml of pyridine were treated with 250 mg of CrO_3 in 5 ml of pyridine. The suspension was stirred overnight at room temperature; usual work up afforded after flash chromatography, 350 mg of **6**. M. p 190-192°C (MeOH). Spectral properties were identical to those described for this product[15].

Picropodophyllone (**7**). Using the same method described for podophyllotoxone, 162 mg of **7** were obtained from 200 mg of **5**. Spectral and physical properties are identical to those reported in reference 15 for this product.

Thuriferic acid (**1**). **6** (110 mg) was treated with 5 ml of 5% KOH/MeOH. The mixture was left 30 minutes at room temperature yielding, after usual work and flash chromatography on Si gel, 90 mg of **1**. M. p.=92-96°C(Et_2O). Physical data were reported in a previous work[1]. ^1H nmr ($CDCl_3$) d (ppm): 3.73 (s, 3H, OMe), 3.79 (s, 6H, OMe), 3.90 (d, J=3.4 Hz, H-8'), 4.63 (d, J=3.4 Hz, H-7'), 5.39 (sb, H-9a), 6.02 (s, O-CH_2-O), 6.22 (s, H-2', H-6'), 6.37 (sb, H-9b), 6.55 (s, H-5), 7.57 (s, H-2).

From 150 mg of compound **7**, treated with 7 ml of 5% KOH/MeOH by the same procedure described above, an identical product **1** was obtained, with the same yielding.

Compound **6** (150 mg) was treated with 5 ml of MeOH/H_2O saturated with $NaHCO_3$ (95/5). The mixture was left overnight at room temperature; usual work up afforded after flash chromatography on Si gel, 115 mg of **7** and 30 mg of **1**.

Potassium salt of thuriferic acid. To 20 mg of **6** in 0.5 ml of CD_3OD, 0.1 mg of KOH were added. After 10 min, the salt of thuriferic acid was obtained. ^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).

9-chloro-8,9-dihydrothuriferic acid (**10**). 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 **10** were obtained, a colorless oil with spectral properties identical to those described for this compound[1].

Molecular Modelling. Calculations were performed on a Silicon Graphics Indigo computer. Compounds **5, 6, 7, 8** were built using facilities of Macromodel[16] v.4. Conformational analysis of each 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 andPM3. Hamiltonian in MOPAC 6.0. Heats of formation given were obtained by MNDO. The result obtained by AM1 and PM3 are in agreement with that.

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