Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron 62 (2006) 2370–2379
Tetrahedron

Correct structures of Diels–Alder adducts from the natural cyclolignan thuriferic acid and its 8-epimer

José L. López-Pérez,[a,*] Andrés Abad,[a,b] Esther del Olmo[a] and Arturo San Feliciano[a]

[a]*Departamento de Química Farmacéutica, Facultad de Farmacia, Campus Unamuno, Universidad de Salamanca, 37007 Salamanca, Spain*
[b]*Departamento de Química, Facultad de Ciencias, Universidad de Los Andes, Mérida, Venezuela*

Received 10 October 2005; revised 23 November 2005; accepted 28 November 2005

Available online 20 December 2005

Abstract—A detailed analysis of one- and two-dimensional ^{1}H and ^{13}C NMR data for the *endo* and the *exo* adducts, obtained by Diels–Alder reaction of thuriferic and epithuriferic acids with cyclopentadiene is described. The unequivocal spectral data assignment of the *endo* and *exo* structures was complemented with molecular modelling studies and confirmed through X-ray diffraction studies.

1. Introduction

Thuriferic acid **1**, a lignan isolated from *Juniperus thurifera L*[1] and its 8′-epimer, epithuriferic acid **2**[2] (Fig. 1), are two non-lactonic cyclolignans, related to podophyllotoxin **3**. Podophyllotoxin is a well-known naturally occurring lignan endowed with potent cytotoxicity, acting as a potent inhibitor of microtubule assembly.[3] In spite of its initial use as an anticancer drug, human clinical trials were soon abandoned due to its toxicity. An extensive semi-synthetic

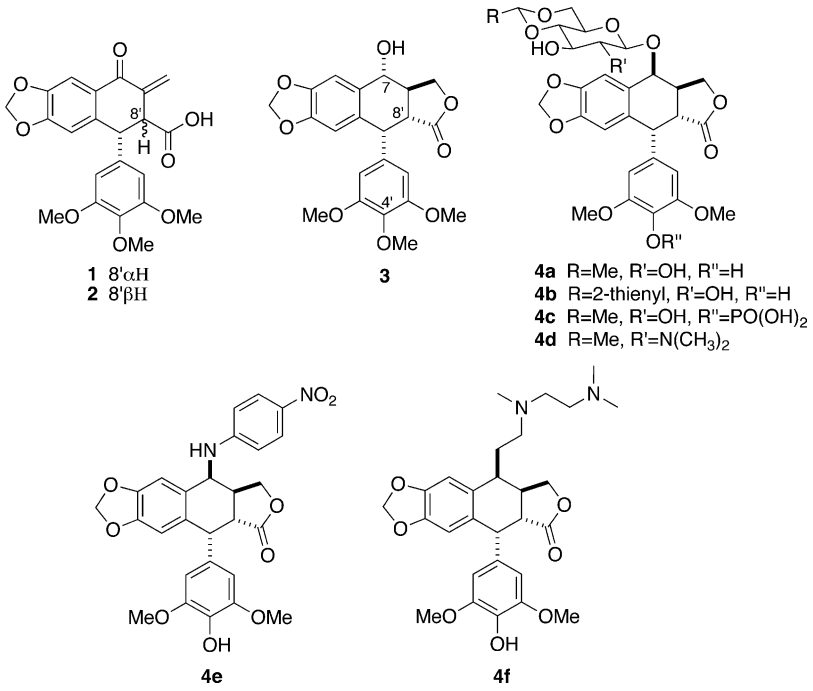

Figure 1. Compounds **1**, **2** and **3**, lignans in the market and some of those under clinical trials.

Keywords: Lignans; Podophyllotoxin; Thuriferic acid; Epithuriferic acid; Diels–Alder.
* Corresponding author. Tel.: +34 923 294528; fax: +34 923 294515; e-mail: lopez@usal.es

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.11.068

programme at Sandoz resulted in the development of etoposide **4a** and teniposide **4b**, two glycoside derivatives of 4′-demethylepipodophyllotoxin,[4] which did not interact significantly with tubulin, but caused extensive DNA breaking, as a consequence of their interaction with DNA–topoisomerase II. Etoposide is currently one of the most prescribed anticancer drugs, with good clinical prognosis against several types of cancer.[5] Continuous efforts have led to the synthesis of new related compounds, displaying decreased toxicity and side effects, metabolic inactivation, drug resistance, and increased water solubility. Etopophos **4c** is the etoposide phosphate designed to overcome the limitations associated with the poor solubility of etoposide.[6] NK611[7] **4d**, GL331 **4e** and TOP-53 **4f** (Fig. 1) are three related derivatives, which are currently under clinical trials.[8] GL331 presents a promising potential in the treatment of gastric carcinoma, colon cancer and non-small cell lung cancer[9] and it is more potent than etoposide.[10] NK611 can be administered orally.[11] TOP-53 is active against neoplasms resistant to etoposide.[12] Other related compounds have shown antiviral[13] and immunosuppressive activities.[14] Podophyllotoxin itself is actually prescribed for removing condiloma and other venereal warts.

The structures of thuriferic and epithuriferic acids are well established[15] and reconfirmed by total synthesis.[16] Their α,β-unsaturated ketone fragment has attracted our attention, because it may act as a dienophile and undergo cycloaddition reactions, which could lead to novel structures with enhanced bioactivity. Previously, we have prepared diverse norbornenecarboxylate esters of podophyllotoxin and its epimers and diastereoisomers through Diels–Alder cycloaddition, by treating the dienophilic acrylates of these cyclolignans with cyclopentadiene.[17] Some of the resulting adducts showed a one-fold increase in their cytotoxicity when compared to that of the natural product **3**.

Presently, we have studied the Diels–Alder cyclocondensations of thuriferic and epithuriferic acids with cyclopentadiene, that afford complex mixtures containing not only the expected *endo*/*exo* adducts, but also other structurally indeterminate compounds. The structures of these adducts have been established on the basis of 2D NMR spectral data, modelling studies and X-ray diffraction data. Their antineoplastic cytotoxicities have been evaluated and the results will be published elsewhere.

In a paper published by Höfert and Matusch[18] two adducts, obtained by cycloaddition of thuriferic acid methyl ester with cyclopentadiene, were reported. These authors proposed for thuriferic acid **1** the erroneous opposite configuration at the C-8′ position. As a consequence, the configuration of the corresponding position in the cycloadducts was also erroneous. Besides, the authors did not justify satisfactorily the configuration of the new stereocenter C-8, generated in the course of the reaction. This induced us to carry out the same cycloaddition and to extend the study to epithuriferic acid, in order to clarify the configuration of the adducts at C-8 and C-8′. We used the free acids instead the methyl esters, to avoid additional steric hindrance and to facilitate the formation of other minor stereoisomers.

2. Results and discussion

The starting podophyllotoxin **3** was isolated from commercial *podophyllum* resin and transformed into thuriferic **1** and epithuriferic acids **2** through reported procedures[1,2] (Fig. 2). These acids contain an α,β-unsaturated ketone fragment, which may undergo the cycloaddition reaction. Therefore, lignans **1** and **2** were treated with cyclopentadiene in order to obtain the corresponding adducts. Cyclopentadiene was prepared by cracking of dicyclopentadiene and used immediately after its preparation. The best results were obtained when dicyclopentadiene was dropped slowly over hot paraffin, with stirring at temperatures under 240 °C. Cyclopentadiene distilled at 40–42 °C. Initially, the reactions were performed at −18 °C, in absence of a catalyst, and needed about 4 weeks to go to completion in the case of thuriferic acid (α and β faces hindered by the trimethoxyphenyl and the carboxyl groups, respectively) and 3 days in that of epithuriferic acid. When $AlCl_3$ was added as catalyst, thuriferic acid needed only 4 days for completion, while the *endo*/*exo* ratio was practically maintained. For epithuriferic acid, the reaction time was not substantially modified, but the *endo*/*exo* ratio changed significantly due to the influence of the catalyst on the

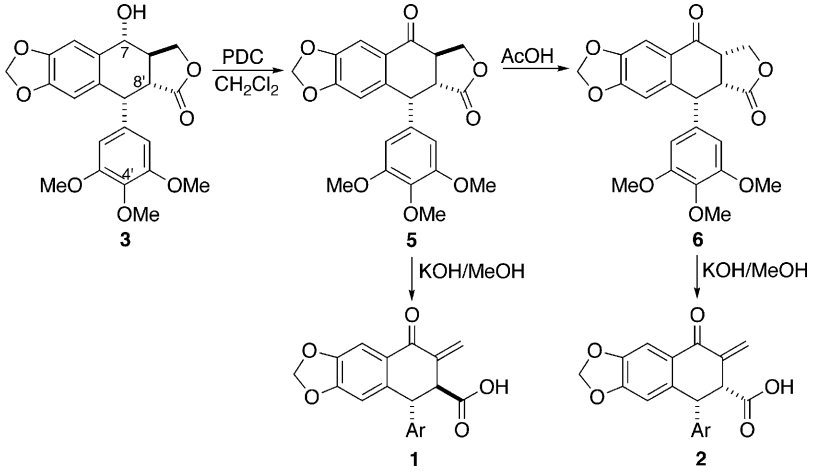

Figure 2. Preparation of thuriferic and epithuriferic acids from podophyllotoxin.

transition state conformation,[19] leading to a decrease in the proportion of the *exo* attack. In fact, in absence of catalyst, the *endo*/*exo* ratio was 1:5 whereas in its presence was 1:3. Analysis of the reaction mixtures by TLC, after treating the crude reaction product with diazomethane, showed that the four possible diastereoisomeric norbornene adducts from thuriferic acid (**1**β-*en*, **1**α-*ex*, **1**α-*en*, and **1**β-*ex*) (Fig. 3) were formed. On the other hand, in the case of epithuriferic acid only two adducts (**2**β-*ex*, **2**β-*en*) were detected (Fig. 6). The α/β codes used here to denominate the adducts, indicate the attacked face of the lignan considered as the substrate and the *en*/*ex* codes represent the *endo*/*exo* orientation of the approach. The determination of the diastereomeric excess of the four Diels–Alder reactions was performed by HPLC analysis (Figs. 3 and 6). The identification of the methyl esters of the resulting adducts was achieved through the analysis of ^{1}H, ^{13}C NMR and 2D NMR spectroscopic data. The ^{1}H and ^{13}C chemical shifts of these compounds were fully assigned using COSY, HMQC, and HMBC NMR correlations. Apart from the cross-peak correlations corresponding to the lignan fragment in the HMBC spectra, some other diagnostic connectivities were observed between the bicycloheptene and the lignan fragments. Indeed, correlations between the C-8 and the protons H-7′, H-8′, H-8e and H-9 were observed for all the adducts. Besides, the signal of H-8a correlated with those of carbons 8b, 8c, 8d, 8e and 9. All the correlations allowed the unambiguous assignment of the ^{1}H and ^{13}C NMR spectral signals. Nevertheless, the chemical shift differences observed for the norbornene signals of each adduct were not sufficient to establish unambiguously the configuration of the C-8a and C-8d stereocenters generated during the cyclocondensation. Similarly, the analysis of the possible influence of the lignan fragment did not allow the satisfactory comparison of chemical shifts of the adducts with those of related norbornenes reported in the literature.[20] Because of this, we carried out some molecular modelling studies for the methyl esters of the adducts. A conformational search[21] for each methylated adduct from thuriferic and epithuriferic acids allowed us to find two main conformers for each adduct. In the case of methylated adducts from thuriferic acid (Fig. 4), the trimethoxyphenyl ring and the methoxycarbonyl groups are placed in a pseudodiaxial disposition in one conformer, and in a pseudodiequatorial disposition in the other. However, in the case of methylated adducts from epithuriferic acid, one of these moieties is oriented pseudoaxially and the other pseudoequatorially, alternatively, for both conformers of each adduct. All of the compounds were later subjected to ab initio calculations at the HF/6-31G* level.[22] The results of calculations appear in Figures 4 and 7 and are in excellent agreement with those found experimentally by NMR. The use of ROESY correlations and the distance values derived from theoretical models (Tables 1 and 2) enabled the definitive stereochemical determinations.

In the adduct **1**α-*ex*, the ROE observed between H-8′ and H-8b indicated that this compound came through the *exo* approach. Besides, other ROE between H-8′ and H-9a clearly indicated that the attack of the cyclopentadiene took place from the alpha face of thuriferic acid. This finding was corroborated by the observed broadening of the two singlets corresponding to the aromatic protons (6.30 ppm) and to the two symmetric methoxyls (3.77 ppm) of the trimethoxyphenyl group, thus indicating the existence of a rotational restriction due to steric hindrance provoked by their close proximity to the olefinic proton H-8b of the norbornene fragment. Additionally, the ^{1}H NMR spectrum of the adduct **1**α-*ex* showed a coupling constant H7′–H8′ of 1.5 Hz, indicating a 1,2-pseudodiaxial arrangement of those trimethoxyphenyl and methoxycarbonyl groups,[2] in agreement with theoretical results (see conformation of **1**α-*ex*-*ax* in Fig. 4). Finally, the structure was confirmed unambiguously by X-ray diffraction (Fig. 5). Interestingly, the conformation of **1**α-*ex* in the crystal resulted different from that deduced by NMR in solution, showing a trans-pseudodiequatorial disposition for both trimethoxyphenyl and methoxycarbonyl residues (conformation **1**α-*ex*-*eq*). Nevertheless, this X-ray conformation could not explain neither the coupling constant H7′-H8′ (1.5 Hz) nor the ROE

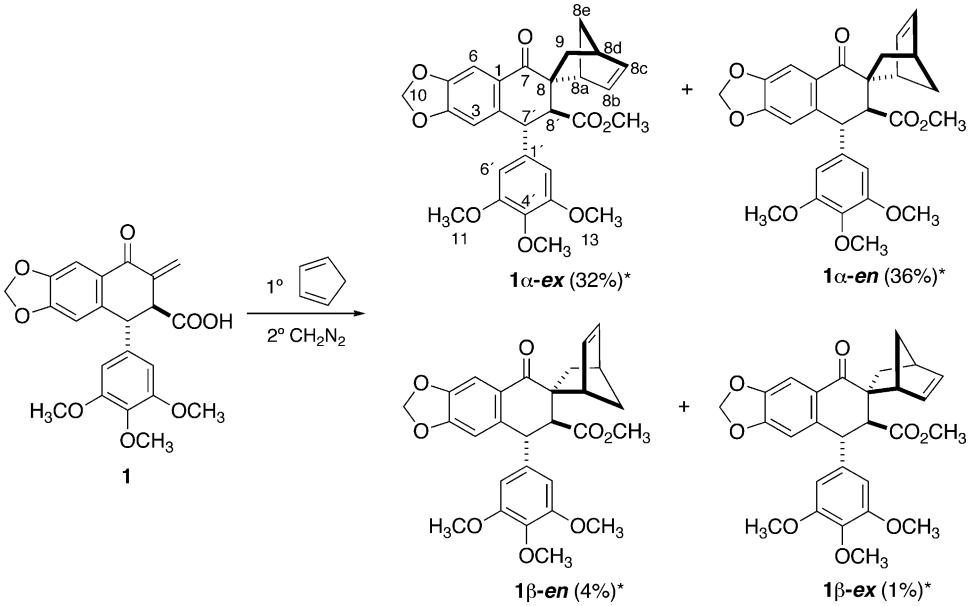

Figure 3. Adducts from thuriferic acid **1**. (*) Yields from HPLC analysis.

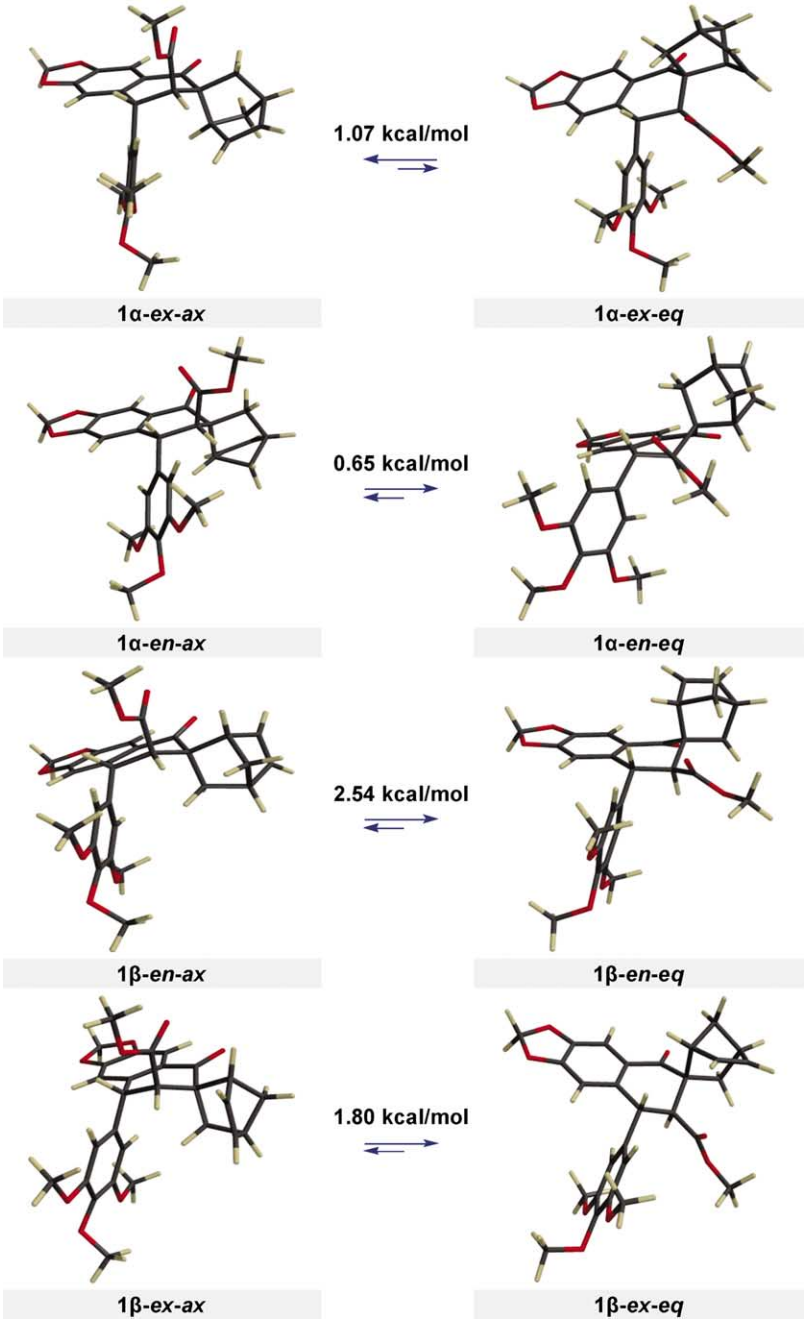

Figure 4. Theoretical model of the four adducts from thuriferic acid **1** and cyclopentadiene and the energies (kcal/mol) obtained by ab initio method at HF/6-31G* level. The energy differences are expressed in kcal/mol.

Table 1. Significant interprotonic distances (Å) in theoretical models for the two main conformers of each adduct from thuriferic acid

Conformer/H		**8′–8b**	**8′–8e$_a$**	**8′–8e$_b$**	**7′–8a**	**8′–9a**	**8′–9b**
1α-*ex*	*ax*	**2.75**[a]	4.81	4.61	4.24	**2.57**[a]	3.73
	eq	3.64	4.55	5.07	4.99	3.71	4.17
1α-*en*	*ax*	5.04	**2.12**[a]	3.77	3.99	**2.53**	3.73
	eq	6.61	3.73	4.73	4.79	3.70	4.15
1β-*en*	*ax*	5.06	**2.17**	3.74	4.77	**2.52**	3.74
	eq	5.26	3.90	5.20	**2.31**[a]	**2.70**[a]	3.23
1β-*ex*[b]	*ax*	3.09	4.79	4.62	5.00	**2.51**	3.71
	eq	4.51	4.67	5.53	**2.60**	4.53	5.06

In bold: calculated distances, for which a ROE could be expected.
[a] Experimentally observed ROEs.
[b] Experiment not performed for this compound.

Table 2. Significant interprotonic distances (Å) in theoretical models for the two main conformers of each possible adduct from epithuriferic acid

Conformer/H		7′–8a	7′–8b	8′–8b	8′–8e	8′–9a	8′–9b
2α-*ex*[a]	*ax*	4.21	4.62	5.53	5.53	2.75	3.14
	eq	4.91	4.89	**2.64**	4.72	**2.42**	3.69
2α-*en*[a]	*ax*	3.98	6.06	5.31	3.71	**2.42**	3.15
	eq	4.74	6.01	5.00	**2.04**	**2.44**	3.72
2β-*en*	*ax*	4.64	6.47	4.45	3.18	3.68	4.27
	eq	**2.06**[b]	4.49	**2.93**	**2.05**	3.75	**2.50**[b]
2β-*ex*	*ax*	4.88	4.99	**2.94**	4.49	4.27	3.70
	eq	**2.32**[b]	**2.73**[b]	4.57	4.57	3.74	**2.49**

In bold: calculated distances, for which a ROE could be expected.
[a] Compound not found.
[b] Experimentally observed ROEs.

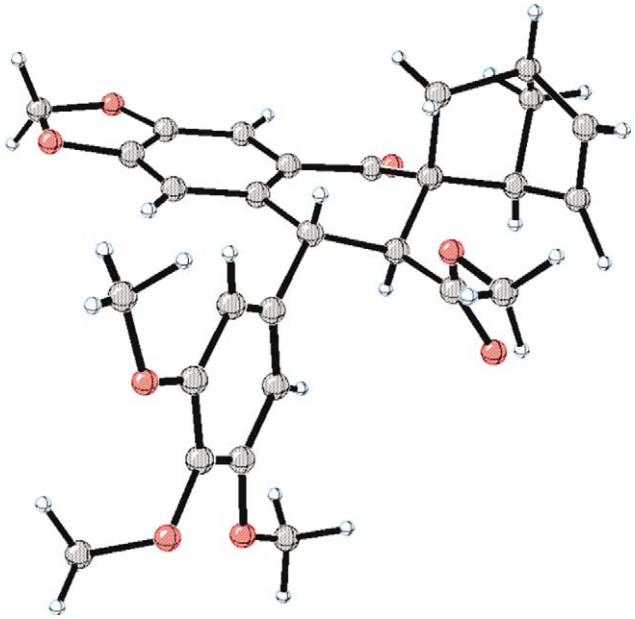

Figure 5. Diagram showing the crystal structure of compound **1**α-*ex*-*eq*.

observed between the H-8′ and H-8 signals, because in the ordinarily used NMR experimental conditions, the distance between these two protons (3.6 Å, in the crystal structure) would be too large for the ROE being observed, thus indicating a conformational change with the change of aggregation state. This change can be justified because the energy difference between both conformations is small (0.6 Kcal/mol) (Fig. 4). The spectral data of this adduct are identical to those described by Höfert and Matusch[18] for the adduct formed by the beta-*endo* approach of cyclopentadiene to epithuriferic acid **2**. In consequence, C-8 and C-8′ have the opposite configurations to those published by these authors. In addition, the HMBC NMR correlations allowed us to reassign some previous incorrect assingments.

The ^{1}H NMR spectrum of adduct **1**β-*en* shows a coupling constant between the H-7′ and H-8′ of 10.8 Hz, indicating a pseudodiequatorial arrangement for those trimethoxyphenyl and carboxyl groups.[2] The ROE observed between H-7′ and H-8a indicated that this adduct came through the approach of the cyclopentadiene from the beta face of the lignan. Besides, another important ROE was observed between H-8′ and one of the hydrogen atoms of the methylene 9, indicating the *endo* orientation of the approach. The comparison of data derived from this ROE, along with the distances measured on the theoretical models, proved that this compound is compatible only with the structure **1**β-*en*. All this data are in complete agreement with the energy values found in the theoretical calculations, demonstrating a greater stability for the pseudodiequatorial conformation of this **1**β-*en* adduct.

The ^{1}H NMR spectrum of the **1**α-*en* adduct shows a coupling constant between the H-7′ and H-8′ of 3.5 Hz, slightly larger than that of the **1**α-*ex* adduct, that could be explained by the smaller energy difference between the two mayor conformers of this adduct, in comparison with that of the **1**α-*ex* adduct (Fig. 4). The ROE observed between H-8′ and H-8e indicated that this adduct came from the *endo* approach of the cyclopentadiene as in the case of compound **1**β-*en*; in consequence, this must be the alpha-*endo* adduct. Spectral data of this adduct were almost identical to those described by Höfert and Matusch[18] for the adduct formed by the beta-*exo* approach to epithuriferic acid **2**. Finally, the **1**β-*ex* structure must correspond to the beta-*exo* adduct. Its ^{1}H NMR spectrum shows a coupling constant of 6.0 Hz between H-7′ and H-8′, a value in-between that of the adducts **1**α-*ex* and **1**β-*en*, indicating similar populations of both extreme conformers, due to the small energy difference existing between them.

As in the case of thuriferic acid, in the molecular modelling studies on the methylated adducts from epithuriferic acid, two main conformers were found for every adduct (Figs. 6 and 7). Nevertheless, the energy differences between the pairs of conformers were greater in this case, over 7–9 Kcal/mol. This difference means that the conformational equilibrium is displaced almost completely to the preferred conformation, in which the trimethoxyphenyl group is placed in a pseudoequatorial arrangement forcing the methoxycarbonyl group to adopt a pseudoaxial disposition. The theoretical coupling constant between H-7′ and H-8′ in the lower energy conformations, over 6 Hz, is close to the experimental values (6.2 Hz in **2**β-*ex* and 5.6 Hz in **2**β-*en*).

In the two adducts obtained from epithuriferic acid, **2**β-*ex* and **2**β-*en*, the strong ROE correlation observed between the benzylic proton H-7′ and the olefinic proton H-8a of the norbornene, clearly indicates that both compounds came from the beta approach of cyclopentadiene to the lignan (Table 2). Additionally, in the **2**β-*ex* adduct, another ROE between H-7′ and the olefinic H-8b indicated the *exo* aproach. This adduct was the major reaction product, in

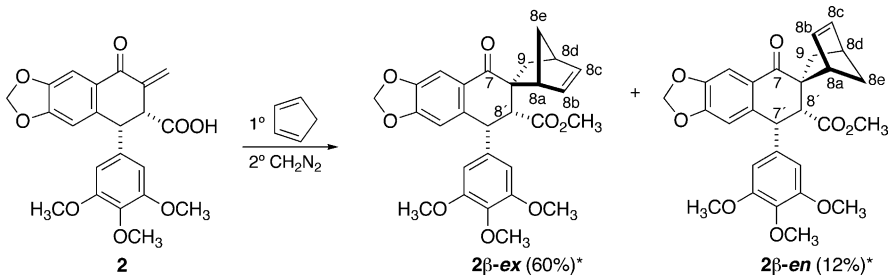

Figure 6. Adducts from epithuriferic acid **2**. (*) Yields from HPLC analysis.

contrast with the case of thuriferic acid cycloaddition. This results clearly indicate that the steric hindrance of the methoxycarbonyl plays a determinant role on regulating the reaction pathway and the configuration of the resulting adducts.

In the ROE experiment of the **2**β-*en* adduct, one additional correlation between H-8′ and one of the methylenic protons H-9 was observed, indicating that this adduct comes through the *endo* approach from the beta face of the lignan.

To resume, thuriferic acid, with both alpha and beta faces highly hindered for the diene attack, reacts very slowly, but gives all the four possible stereoisomeric adducts, with predominance of those resulting from the alpha approach,

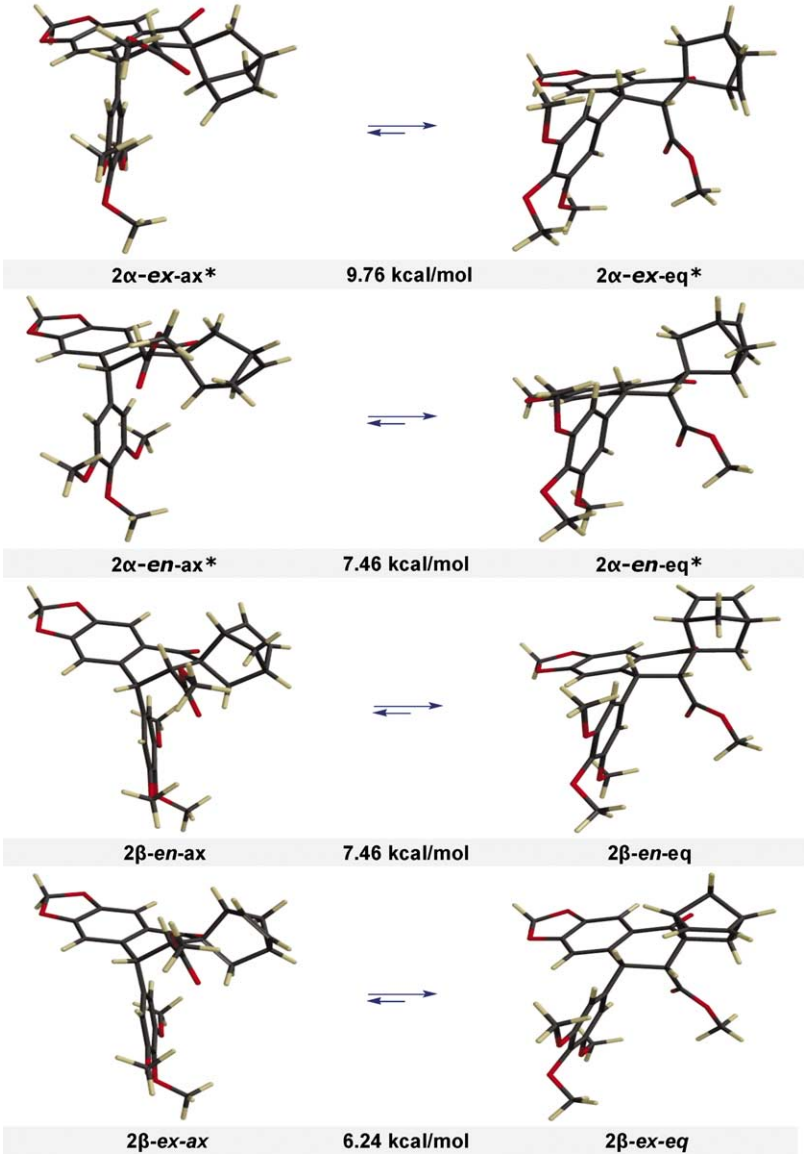

Figure 7. Theoretical model of the four possible adducts formed from epthuriferic acid **2** and cyclopentadiene obtained by ab initio method at HF/6-31G* level. The energy differences are expressed in kcal/mol. *Not formed in the cycloaddition conditions.

independently of the presence or absence of the catalyst. On the other hand, in the case of epithuriferic acid, with a much less hindered beta face, reacts faster and only the two adducts resulting from the beta approach of the diene were detected. Nevertheless, it must be considered that the beta adducts of thuriferic acid were always formed in a very low proportion (1–4% within all the experiments performed) and their scarcity, probably, could be the reason for which, they were not detected previously in the research of Höfert and Matusch.[18]

In the thuriferic acid cycloaddition, the major alpha adduct and the major beta adduct, resulted from an *endo* approach, in agreement with the frontier molecular orbitals theory. On the contrary, in the epithuriferic acid cycloaddition, the relative stability of its conformers governed the approach and the major product was the *exo* adduct, as it is proven by the broadening of the trimethoxyphenyl NMR signals as stated above. All these results are in agreement with the conformational study on both epimers, previously reported by us.[15]

3. Experimental

3.1. General methods

Purification and drying according to accepted general procedures.[23] If not otherwise stated, commercially available solvents of the highest purity were used. Melting points were determined on a Büchi 510-K melting point apparatus and are uncorrected. Optical rotations were recorded on a Perkin Elmer 241 polarimeter in chloroform solution. IR spectra were recorded in a Nicolet Impact 410 spectrophotometer. NMR spectra were measured using Bruker AC 200 (200 MHz) and Bruker DRX 400 (400 MHz) instruments. The chemical shifts are in δ values (ppm) relative to the internal standard TMS. Reported as: chemical shift (multiplicity, coupling constant, assignment). Reported assignments were determined with the help of COSY, HMQC, HMBC, and NOESY 2D-Spectra. For EIMS and HRFABMS analysis, a VG-TS250 mass spectrometer (70 eV) was used. X-ray diffraction data were collected on a four-circle Seifert XRD 3003 SC diffractometer (CuF_α, $\lambda=1.5418$ Å), graphite monochromator, room temperature, ω–2θ scans. Scattering factors for neutral atoms and anomalous dispersion correction for C and O were taken from 'International Tables for X ray Crystallography'.[24] Full matrix least-squares refinement with anisotropic thermal parameters for non-H atoms was carried out by minimizing $w(F_o^2-F_c^2)^2$. Refinement on F^2 for all reflections, weighted R factors (R_w), and all goodness of fit S are based on F^2, while conventional R factors (R) are based on F; R factors based on F^2 are statistically about twice as large those based on F, and R factors based on all data will be even larger. All calculations were performed using CRYSOM[25] software for data collection, XRAY80[26] for data reduction, SHELXTLTM[27] to resolve and refine the structure and to prepare figures for publication. Silica gel 60 (Merck, 230–400 mesh) was used for flash chromatography; precoated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm) were used for TLC analysis.

3.2. HPLC

HPLC analyses were carried out using a Waters Delta 600 with a Chromolith RP-18e 100–4.6 column. Wavelength 220–400 nm; column temperature 30 °C; injection volume 50 ml; acetonitrile and water buffered at pH 2.6 served as solvents.

3.3. Sources of precursors and synthesis of compounds

3.3.1. Podophyllotoxin (3). The title compound was obtained in pure form by recrystalization from the ethyl acetate extract of *Podophyllum emodi* resin.

3.3.2. Podophyllotoxone (5). Compound **3** (1.5 g) in 35 ml of dry CH_2Cl_2 was treated with 1.5 g of PDC. The suspension was stirred for 3 h at room temperature. Usual work up afforded after flash chromatography (CH_2Cl_2/EtOAc 1:1) 1.26 g (84%) of **5**. Spectroscopic and physical data were identical to those reported.[28]

3.3.3. Isopicropodophyllone (6). Compound **5** (400 mg) in 28 ml of acetic acid were refluxed for 1 h. After addition of H_2O, extraction with EtOAc and usual work up, the reaction crude was chromatographied (CH_2Cl_2/EtOAc 95:5) yielding 280 mg (70%) of **6** and 88 mg of **5**. Spectroscopic and physical data of **6** were identical to those reported.[28]

3.3.4. Thuriferic acid (1). Compound **5** (700 mg) was treated with 15 ml of 1% KOH/MeOH. The mixture was left 30 min at room temperature yielding, after usual work up and flash chromatography on Si gel, 670 mg of **1**. Mp = 92–96 °C (Et_2O). Spectroscopic and physical data were identical to those reported.[2]

3.3.5. Epithuriferic acid (2). Compound **6** (200 mg) was treated with 5 ml of 1% KOH/MeOH. The mixture was left 10 min at room temperature yielding, after usual work up and flash chromatography on Si gel, 50 mg of junaphtoic acid[29] and 110 mg of **2**. Mp = 92–96 °C (Et_2O). Spectroscopic and physical data were identical to those reported.[2]

3.3.6. Adducts of Diels–Alder reaction from 1. To a solution of thuriferic acid (600 mg, 1.42 mmol) in anhydrous CH_2Cl_2 (30 mL) at −18 °C under nitrogen atmosphere, freshly distilled cyclopentadiene (0.2 mL, 2.7 mmol) was added dropwise. After 27 days, HPLC control showed that the reaction was over. The reaction mixture was concentrated in vacuo followed by dilution with EtOAc (20 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (100 mL), dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. After methylation of the crude reaction product with diazomethane in ethereal solution, purification was carried out by flash chromatography eluting with *n*-hexane–ethyl acetate (2/1.2) to give the corresponding adducts **1**β-*en* (15 mg; hexane/AcOEt 2:1) (2.1%), **1**α-*ex* (135 mg, hexane/AcOEt 1:1) (18.9%), **1**α-*en* (145 mg; hexane/AcOEt 1:1.5) (20.3%), and **1**β-*ex* (4 mg; hexane/AcOEt 1:2) (0.6%).

3.3.6.1. Adduct (1β-*en*). Mp 146–149 °C (white solid); $[\alpha]^{22}$ −163 (Na 589), −170 (Hg 578), −217 (546) (*c* 1%, $CDCl_3$); IR (film) γ_{max}: 3025, 2960, 2840, 1733, 1680,

1617, 1588, 1506, 1480, 1463, 1245, 1127, 1037, 1008, 935 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.40 (d, J= 8.0 Hz, H-8ea), 1.50 (d, J=8.0 Hz, H-8eb), 1.70 (dd, J= 3.6, 12.0 Hz, H-9a), 2.86 (s, H-8d), 3.00 (dd, J=2.4, 12.0 Hz, H-9b), 3.19 (s, H-8a), 3.46 (d, J=10.8 Hz, H-8′), 3.57 (s, COOMe), 3.77 (s, H-11, H-13), 3.83 (s, H-12), 4.84 (d, J=10.8 Hz, H-7′), 5.60 (dd, J=3.0, 5.5 Hz, H-8b), 5.98 (dd, J=1.0, 4.8 Hz, H-10), 6.25 (dd, J=3.0, 5.6 Hz, H-8c), 6.30 (s, H-2′, H-6′), 6.32 (s, H-3), 7.22 (s, H-6). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 31.00 (C-9), 42.26 (C-8d), 46.51 (C-8a), 46.78 (C-7′), 48.42 (C-8e), 51.80 (COOMe), 55.98 (C-8′), 56.13 (C-8), 56.13 (C-11, C-13), 60.79 (C-12), 101.64 (C-10), 105.71 (C-6), 106.36 (C-2′, C-6′), 108.67 (C-3), 127.82 (C-1), 132.91 (C-8b), 137.01 (C-4′), 139.01 (C-1′), 139.54 (C-2), 139.68 (C-8c), 147.16 (C-4), 151.95 (C-5), 153.16 (C-3′, C-5′), 172.23 (C-9′), 197.23 (C-7). HRFABMS *m/z* 492.1792 (calcd For $C_{28}H_{28}O_8$, 492.1784).

3.3.6.2. Adduct (1α-*ex*). Mp 150–152 °C (colourless crystals); $[\alpha]^{22}$ +54 (Na 589), +59 (Hg 578), +62 (546), +106 (436) (*c* 1%, $CDCl_3$); IR (film) γ_{max}: 3057, 2958, 2836, 1734, 1680, 1617, 1589, 1505, 1481, 1463, 1246, 1126, 1037, 1009, 935 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.60 (dd, J=3.0, 6.0 Hz, H-9a), 1.10 (d, J=8.5 Hz, H-8ea), 1.70 (d, J=8.6 Hz, H-8eb), 2.70 (s, H-8d), 2.74 (d, J=3.8 Hz, H-8a), 2.80 (dd, J=4.0, 8.0 Hz, H-9b), 3.08 (d, J=1.5 Hz, H-8′), 3.59 (s, COOMe), 3.77–3.82 (br s, H-11, H-13), 3.83 (s, H-12), 4.46 (d, J=1.5 Hz, H-7′), 4.60 (dd, J=3.0, 4.0 Hz, H-8b), 5.96 (d, J=1.1 Hz, H-10), 5.96 (dd, J=3.0, 4.0 Hz, H-8c), 6.30–6.45 (br s, H-2′, H-6′), 6.51 (s, H-3), 7.62 (s, H-6). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 35.45 (C-9), 41.34 (C-8a), 46.00 (C-8e), 46.18 (C-7′), 50.99 (C-8d), 52.03 (COOMe), 53.44 (C-8), 56.42 (C-11, C-13), 58.07 (C-8′), 60.97 (C-12), 101.70 (C-10), 106.75 (C-3), 107.08 (C-2′, C-6′), 109.33 (C-6), 127.94 (C-1), 135.34 (C-2), 135.59 (C-8b), 137.29 (C-4′), 138.92 (C-1′), 139.12 (C-8c), 147.69 (C-4), 151.63 (C-5), 153.03 (C-3′, C-5′), 174.43 (C-9′), 197.01 (C-7). HRFABMS *m/z* 492.1786 (calcd for $C_{28}H_{28}O_8$, 492.1784).

3.3.6.3. Adduct (1α-*en*). Mp 156–158 °C (white solid); $[\alpha]^{22}$ +106 (Na 589), +109 (Hg 578), +126 (546), +236 (436) (*c* 1%, $CDCl_3$); IR (film) γ_{max}: 3059, 2953, 1734, 1681, 1617, 1589, 1505, 1480, 1463, 1246, 1128, 1037, 1009, 935 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.30 (d, J=8.8 Hz, H-8e-b), 1.50 (dd, J=3.0, 12.0 Hz, H-9a), 1.70 (d, J=8.5 Hz, H-8e-a), 1.98 (d, J=12 Hz, H-9b), 2.70 (s, H-8d), 2.90 (s, H-8a), 3.54 (d, J=3.5 Hz, H-8′), 3.60 (s, COOMe), 3.77 (s, H-11, H-13), 3.83 (s, H-12), 4.54 (d, J= 3.5 Hz, H-7′), 5.70 (dd, J=2.8, 5.0 Hz, H-8b), 5.9 (dd, J= 3.4 Hz, H-10), 6.13 (dd, J=3.0, 5.0 Hz, H-8c), 6.41 (s, H-2′, H-6′), 6.48 (s, H-3), 7.39 (s, H-6). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 35.65 (C-9), 42.77 (C-8d), 46.18 (C-7′), 47.33 (C-8e), 48.50 (C-8a), 52.07 (COOMe), 54.58 (C-8), 56.42 (C-11, C-13), 58.06 (C-8′), 60.97 (C-12), 101.65 (C-10), 106.31 (C-2′, C-6′), 106.38 (C-6), 109.30 (C-3), 128.55 (C-1), 133.40 (C-8b), 135.90 (C-2), 137.10 (C-4′), 137.76 (C-8c), 139.79 (C-1′), 147.43 (C-4), 151.40 (C-5), 153.21 (C-3′, C-5′), 173.91 (C-9′), 196.48 (C-7). HRFABMS *m/z* 492.1788 (calcd for $C_{28}H_{28}O_8$, 492.1784).

3.3.6.4. Adduct (1β-*ex*). (Amorphous white solid); IR (film) γ_{max}: 3061, 2954, 1736, 1680, 1614, 1588, 1503, 1478, 1460, 1245, 1128, 1036, 1009, 937 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 2.73 (m, H-8a), 3.06 (d, J=6.0 Hz, H-8′), 3.58 (s, H-14), 3.74 (s, H-11, H-13), 3.82 (s, H-12), 4.49 (d, J=6.0 Hz, H-7′), 5.62 (sa, H-10), 5.97 (dd, J=3.0, 5.0 Hz, H-8b), 6.24 (dd, J=3.0, 5.0 Hz, H-8c), 6.31 (s, H-2′, H-6′), 6.41 (s, H-3), 7.53 (s, H-6). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 35.50 (C-9), 42.56 (C-8d), 46.53 (C-8a), 46.78 (C-7′), 48.11 (C-8e), 51.8 (C-14), 51.86 (C-8), 56.12 (C-11 13), 56.12 (C-8′), 60.83 (C-12), 101.70 (C-10), 105.74 (C-6), 106.29 (C-2′ 6′), 108.97 (C-3), 127.79 (C-1), 134.70 (C-8b), 136.94 (C-4′), 139.0 (C-1′), 139.29 (C-8c), 139.3 (C-2), 147.49 (C-4), 151.82 (C-5), 153.19 (C-3′ 5′), 173.15 (C-9′), 198.91 (C-7). HRFABMS *m/z* 492.1788 (calcd for $C_{28}H_{28}O_8$, 492.1784).

3.3.7. Adducts of Diels–Alder reaction from 1 in presence of $AlCl_3$. To a solution of thuriferic acid (50 mg, 0.12 mmol) in anhydrous CH_2Cl_2 (10 mL) at −78 °C under nitrogen atmosphere, 5 mg of $AlCl_3$ and freshly cracked distilled ciclopentadiene (0.1 mL, 1.3 mmol) was added dropwise. After 4 days the reaction was over and HPLC analysis showed that the reaction products were the same in a similar proportion to that observed without catalyst.

3.3.8. Adducts of Diels–Alder reaction from 2. To a solution of epithuriferic acid (200 mg, 0.49 mmol) in anhydrous CH_2Cl_2 (20 mL) at −18 °C under nitrogen atmosphere, freshly distilled cyclopentadiene (0.1 mL, 1.3 mmol) was added dropwise. After 3 days, HPLC showed that the reaction was over. The reaction mixture was concentrated in vacuo and diluted with EtOAc (20 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (100 mL), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure. The reaction crude was trated with diazomethane and then chromatograpied eluting with hexane/ethyl acetate to give the adducts **2**β-*ex* (98 mg; hexane/AcOEt 2:1) (41.2%) and **2**β-*en* (7 mg; hexane/AcOEt 1:2) (3.0%).

3.3.8.1. Adduct (2β-*ex*). (Yellow oil); $[\alpha]^{22}$ +106 (Na 589), +109 (Hg 578), +126 (546), +236 (436) (*c* 1%, $CDCl_3$); IR (film) γ_{max}: 2970, 2945, 2839, 1731, 1675, 1616, 1590, 1504, 1481, 1461, 1423, 1253, 1126, 1037, 1007, 935, 756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.20 (dd, J=3.6, 12.0 Hz, H-9b), 1.60 (dd, J=1.0, 8.7 Hz, H-8eb), 1.80 (dd, J=1.0, 8.7 Hz, H-8ea), 2.30 (dd, J=2.5, 12.0 Hz, H-9b), 2.96 (br s, H-8d), 3.13 (br s, H-8a), 3.32 (s, COOMe), 3.36 (d, J=6.2 Hz, H-8′), 3.60–3.85 (br s, H-11, H-13), 3.85 (s, H-12), 4.80 (d, J=6.2 Hz, H-7′), 5.6 (dd, J= 2.9, 5.8 Hz, H-8b), 5.99 (d, J=1.82 Hz, H-10), 6.20–6.40 (br s, H-2′, H-6′), 6.30 (dd, J=2.6, 5.5 Hz, H-8c), 6.43 (s, H-3), 7.44 (s, H-6). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 32.39 (C-9), 42.55 (C-8d), 45.23 (C-8e), 46.86 (C-7′), 49.52 (C-8a), 51.25 (COOMe), 56.17 (C-11, C-13), 56.84 (C-8), 59.14 (C-8′), 60.81 (C-12), 101.57 (C-10), 106.42 (C-3), 108.46 (C-6), 128.24 (C-1), 132.53 (C-8b), 135.96 (C-2), 137.17 (C-1′), 137.39 (C-4′), 141.06 (C-8c), 147.43 (C-5), 151.39 (C-4), 153.32 (C-3′, C-5′), 173.22 (C-9′), 196.84 (C-7), 106.42 br s (C-2′, C-6′). HRFABMS *m/z* 492.1779 (calcd for $C_{28}H_{28}O_8$, 492.1784).

3.3.8.2. Adduct (2β-*en*). (Yellow oil); $[\alpha]^{22}$ +106 (Na 589), +109 (Hg 578), +126 (546), +236 (436) (*c* 1%,

$CDCl_3$); IR (film) γ_{max}: 2984, 2949, 2841, 1732, 1676, 1616, 1590, 1504, 1478, 1462, 1419, 1250, 1127, 1037, 1008, 935, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.61 (dd, $J=2.0$, 11.6 Hz, H-9b), 1.34 (dd, $J=2.0$, 8.4 Hz, H-8ea), 1.68 (d, $J=8.8$ Hz, H-8eb), 2.84 (d, $J=3.6$, 12.0 Hz, H-9b), 2.90 (ba, H-8a), 2.93 (d, $J=5.6$ Hz, H-8′), 2.95 (br s, H-8d), 3.31 (s, COOMe), 3.40–3.84 (br s, H-11, H-13), 3.86 (s, H-12), 4.50 (d, $J=5.6$ Hz, H-7′), 5.70 (dd, $J=2.8$, 5.6 Hz, H-8b), 5.99 (dd, $J=1.2$, 7.0 Hz, H-10), 6.30–6.42 (br s, H-2′, H-6′), 6.40 (dd, $J=2.8$, 5.6 Hz, H-8c), 6.42 (s, H-3), 7.60 (s, H-6). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 33.74 (C-9), 43.37 (C-8d), 46.2 (C-8a), 47.87 (C-8e), 47.89 (C-7′), 51.45 (COOMe), 55.35 (C-8), 56.23 (C-11, C-13), 59.98 (C-8′), 60.9 (C-12), 101.66 (C-10), 105.99 (C-3), 108.72 (C-6), 129.08 (C-1), 131.17 (C-8b), 136.76 (C-2), 137.35 (C-1′), 137.35 (C-4′), 139.26 (C-8c), 147.2 (C-5), 151.46 (C-4), 153.33 (C-3′, C-5′), 173.11 (C-9′), 195.67 (C-7), 105.99 br s (C-2′, C-6′). HRFABMS *m/z* 492.1779 (calcd for $C_{28}H_{28}O_8$, 492.1784).

3.3.9. Adducts of Diels–Alder reaction from 2 in presence of $AlCl_3$. To a solution of epithuriferic acid (50 mg, 0.12 mmol) in anhydrous CH_2Cl_2 (10 mL) at −78 °C under nitrogen atmosphere, 5 mg of $AlCl_3$ and freshly cracked distilled ciclopentadiene (0.1 mL, 1.3 mmol) was added dropwise. After 2 days the reaction was over and HPLC analysis showed a rate between **2**β-*ex*/**2**β-*en* of 3:1.

3.4. X-ray analysis of compound 1α-*ex*

Compound **1**α-*ex*, $C_{28}H_{28}O_8$. Crystal dimensions 0.30×0.40×0.70 nm; crystallizes in monoclinic space group $P2_1$, with $Z=2$, and unit cell parameters, $a=6.7220(13)$ Å, $b=16.706(3)$ Å, $c=11.047(2)$ Å, $\langle=90°$, $\beta=106.51°$ (3), $\gamma=90°$. The unit cell parameters were determined by least squares refinement on the 2θ values of 25 strong well centred reflections in the range 4.17–60.00°. The structure of $C_{28}H_{28}O_8$ was resolved by direct methods and refined in the space group $P2_1$. Resulting absolute structure parameter: 0.09(28). Full crystallographic details have been deposited at the Cambridge Crystallographic Data Centre No. CCDC 286044.

3.5. Molecular modelling

Calculations were performed initially on a Silicon Graphics Indigo computer. Compounds were built using Macromodel V.4.[21] Conformational analysis was performed by a Monte Carlo random search. All freely rotating bonds were searched with MM2[30] minimization to a gradient of less than 0.001 Kcal/mol. Full geometry optimization of the two main conformers of each compound was performed using a molecular orbital ab initio method at the Hartree–Fock level of theory with the 6-31G* basis set using the SPARTAN 04′ Macintosh program distributed by Wavefunction Inc.

Acknowledgements

Financial support came from MCyT (SAF2001-0037-C04-04) and FIS (RIS-G03-173). A.A. thanks the University of Los Andes, Merida (Venezuela) for a fellowship. Colaborative research performed under the auspices of 'Programa Iberoamericano CYTED. Subprograma X'.

References and notes

1. San Feliciano, A.; López, J. L.; Medarde, M.; Miguel del Corral, J. M.; Pascual-Teresa, B.; Puebla, P. *Tetrahedron* **1988**, *44*, 7255–7260.
2. López-Pérez, J. L.; del Olmo, E.; de Pascual-Teresa, B.; Merino, M.; San Feliciano, A. *Tetrahedron* **1996**, *52*, 4903–4910.
3. Jardine, I. Podophyllotoxins. In *Anticancer Agents Based on Natural Product Models*, *Vol. 16*; Academic: New York, 1980; p 319.
4. Stahelin, H.; von Wartburg, A. *Prog. Drug. Res.* **1989**, *33*, 169–266. Stahelin, H. F.; von Wartburg, A. *Cancer Res.* **1991**, *51*, 5–15.
5. VanVliet, D. S.; Tachibana, Y.; Bastow, K. F.; Huang, E. S.; Lee, K. H. *J. Med. Chem.* **2001**, *44*, 1422–1428.
6. Schacter, L. *Semin. Oncol.* **1996**, *23*, 1–7.
7. Rassmann, I.; Thodtmann, R.; Mross, M.; Huttmann, A.; Berdel, W. E.; Manegold, C.; Fiebig, H. H.; Kaeser-Frohlich, A.; Burk, K.; Hanauske, A. R. *Invest. New Drugs* **1998**, *16*, 319–324.
8. Chang, H.; Shyu, K. G.; Lee, C. C.; Tsai, S. C.; Wang, B. W.; Hsien Lee, Y.; Lin, S. *Biochem. Biophys. Res. Commun.* **2003**, *302*, 95–100.
9. Zhu, X. K.; Guan, J.; Tachibana, Y.; Bastow, K. F.; Cho, S. J.; Cheng, H. H.; Cheng, Y. C.; Gurwith, M.; Lee, K. H. *J. Med. Chem.* **1999**, *42*, 2441–2446.
10. Huang, T. S.; Lee, C. C.; Chao, Y.; Shu, C. H.; Chen, L. T.; Chen, L. L.; Chen, M. H.; Yuan, C. C.; Whang-Peng, J. *Pharm. Res.* **1999**, *16*, 997–1002.
11. Rassmann, I.; Schrodel, H.; Schilling, T.; Zucchetti, M.; Kaeser-Frohlich, A.; Rastetter, J.; Hanauske, A. R. *Invest. New Drugs* **1996**, *14*, 379–386.
12. Utsugi, T.; Shibata, J.; Sugimoto, Y.; Aoyagi, K.; Wierzba, K.; Kobunai, T.; Terada, T.; Oh-hara, T.; Tsuruo, T.; Yamada, Y. *Cancer Res.* **1996**, *56*, 2809–2814.
13. Zhu, X. K.; Guan, J.; Xiao, Z.; Cosentino, L. M.; Lee, K. H. *Bioorg. Med. Chem.* **2004**, *12*, 4267–4273.
14. Gordaliza, M.; Faircloth, G. T.; Castro, M. A.; Miguel del Corral, J. M.; Lopez-Vazquez, M. L.; San Feliciano, A. *J. Med. Chem.* **1996**, *39*, 2865–2868.
15. López-Pérez, J. L.; del Olmo, E.; de Pascual-Teresa, B.; Merino, M.; Martín, S.; San Feliciano, A. *Tetrahedron* **1995**, *51*, 6343–6348.
16. Pohmakotr, M.; Komutkul, T.; Tuchinda, P.; Prabpai, S.; Kongsaeree, P.; Reutrakul, V. *Tetrahedron* **2005**, *61*, 5311–5321.
17. López-Pérez, J. L.; del Olmo, E.; de Pascual-Teresa, B.; Abad, A.; San Feliciano, A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1283–1286.
18. Höfert, P. H.; Matusch, R. *Helv. Chim. Acta* **1994**, *77*, 771–777.
19. Winkler, J. D.; Kim, S.; Condroski, K. R.; Asensio, A.; Houk, K. N. *J. Org. Chem.* **1994**, *59*, 6879.
20. Breitmaier, E.; Woelter, W. *Carbon-13 NMR Spectroscopy. High-Resolution Methods and Applications in Organic Chemistry*, 3rd ed.; VCH: Weinheim, Germany, 1987.

21. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440–467.
22. *Spartan '04 Macintosh*, Wavefunction, Inc.
23. Hünig, S.; Märkl, G.; Sauer, J. *Einführung in Die Apparativen Methoden in Der Organischen Chemie*, 2nd ed.; Würzburg: Regensburg, 1994. Author Collective Organikum, 17th ed.; VEB Deutscher Verlag der Wissenschaften: Berlin, 1988.
24. Brown, P. J. In Wilson, A., Ed.; International Tables for Crystallography; Kluwer-Academic: Dordrecht, 1992; Vol. C.
25. Martínez-Ripoll M, Cano FH. *An Interactive Program for Operating Rich, Seifert Single-Crystal Four-Circle Diffractometers*; Institute of Physical Chemistry, Rocasolano, C.S.I.C., Serrano, 19, Madrid, Spain, 1996.
26. Stewart J. M., Kundell F. A., Baldwin J. C. *The X-ray80 System*; Computer Science Centre, University of Maryland: College Park, MD, USA, 1990.
27. *Siemens SHELXTLTM,* version 5.0; Siemens Analytical X-ray Instruments Inc.: Madison, WI 53719, 1995.
28. Miguel del Corral, J. M.; Gordaliza, M.; López, J. L.; del Olmo, E.; Castro, M. A.; Morales, L.; López, M. L. *Helv. Chim. Acta* **1995**, *78*, 1793–1796.
29. San Feliciano, A.; Miguel del Corral, J. M.; Gordaliza, M.; Castro, A. *An. Quim.* **1992**, *88*, 256–259.
30. Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127–8134.