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X-Ray Analysis of Volubilide, a New Decacyclic Diels–Alder C20–C30 Adduct from *Hyppocratea volubilis* L.

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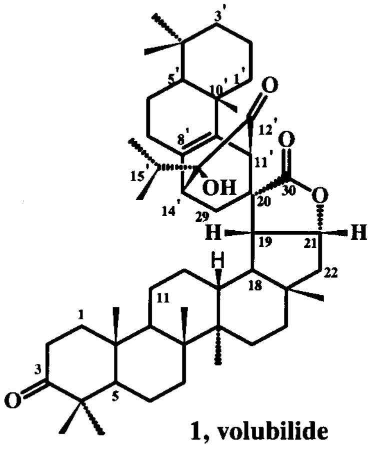
Abstract—A new Diels–Alder adduct, **1,** has been isolated from *Hyppocratea volubilis* L. and its structure determined by means of ^{1}H and ^{13}C NMR spectroscopic studies including H-C heteronuclear correlation (HETCOR), and long-range correlation with inverse detection (HMBC). The absolute configuration was determined by X-ray analysis. © 2000 Elsevier Science Ltd. All rights reserved.

The climbing plant *Hyppocratea volubilis* L. (Celastraceae) is distributed throughout the humid eastern region of Paraguay and investigation of the chemical composition of its roots resulted in the isolation (Fig. 1) of a new decacyclic Diels–Alder C20–C30 adduct, **1**, with a structure based on two monomer units from abietane and lupane di/triterpene skeletons.

From 1991 onwards, Farnsworth and co-workers have isolated several cytotoxic lupanes with a 30–21α olide[1] system and other related compounds[2] from *Kokoona ochracea* (Elm.) Merrill (Celastraceae). More recently, lactones[3] with farnesyl protein transferase inhibitory activity were obtained from *Lophopetalum wallichii* Kurz (Celastraceae). As part of our study of bioactive American Celastraceae, this paper describes the structural elucidation of a hitherto unknown metabolite, **1**, a decacyclic C20–C30 Diels–Alder adduct derived from the dienophile, triterpene ochraceolide A[1] **2** and a diene, diterpene **3**, never isolated as a monomer, but present in the dimer maytenone[4] (**4**). Celastraceae species biosynthesize triterpene dimers[5,6] and trimers,[7] possibly by hetero Diels–Alder reactions[8] although the formation of the 1–4 dioxane system found in all these products may be due to a radical coupling process as postulated for other kinds of natural products.[9] Previous data[5–7] plus those reported here would seem to indicate the probable presence of enzymatic systems with homo and/or hetero Dielsalderase activity.[10]

Repeated chromatography on silica gel and Sephadex LH-20 afforded a crystalline product. HREIMS, ^{13}C, ^{1}H NMR and X-ray data[11] gave the formula $C_{50}H_{74}O_5$. The MS showed the molecular ion at *m/z* 754 and fragments corresponding to the two units C20 and C30. These data, together with the number of methyl groups and unsaturations indicated that the product consisted of two terpene units. Analysis of the ^{13}C NMR data and HMBC and HMQC experiments identified the C-30 unit as a lupane derivative with a 30–21α lactone group having no signals for the 20(29) double bond found in ochraceolide A (**2**). This suggests that the 20(29) bond could be involved in the junction with the diterpene unit which contained a



1, volubilide

Figure 1.

Keywords: Celastraceae; decacyclic; terpene; crystalline structure.

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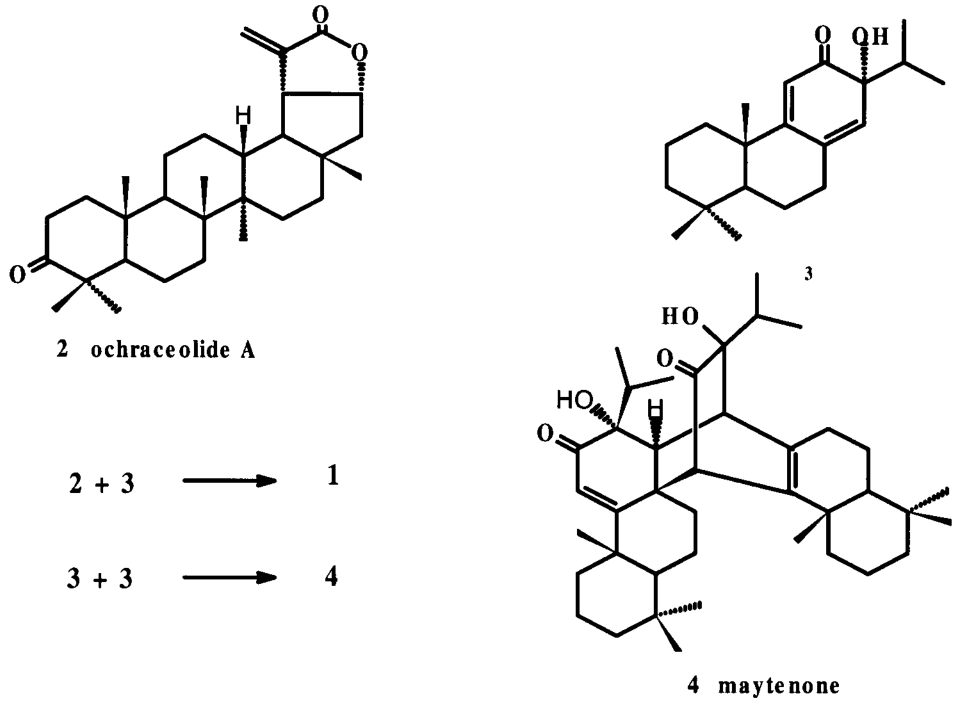

Figure 2.

ketone, a tertiary alcohol and a cyclohexene ring, pointing to a possible Diels–Alder reaction between a diterpene diene and ochraceolide A as a dienophile.[12]

We recently isolated maytenone[4] (**4**) from *Chrossopetalum rhacoma* (Celastraceae), and its X-ray data was obtained for the first time. These, together with new ^{1}H and ^{13}C NMR spectral assignments surprisingly[13] showed the base diterpene unit which acts as diene and dienophile in maytenone to be the same as that found in **1**, according to ^{1}H and ^{13}C NMR, MS and HMBC and HMQC experiments. The most significant MS findings were the fragment for $M^{+}-18$, and the signals at *m*/*z* 452, and at *m*/*z* 302 coming from a retro Diels–Alder process, that correspond to ochraceolide A and diterpene **3** unit respectively. The last is a maytenone precursor, that was never isolated as a natural product (Fig. 2).

As triterpenes are only found as chiral species in an enantiomeric series, the absolute configuration of the decacyclic adduct, **1**, is 5*R*, 8*R*, 9*R*, 10*R*, 13*R*, 14*R*, 17*R*, 18*S*, 19*R*, 20*R*, 22*S*, 5′*S*, 8′*R*, 9′*S*, 10′*S*, 11′*R*, 13′*R*, 14′*R*. We have called **1** volubilide in view of its plant origin.

The crystalline structure shows vertical molecular packing, with solvent of crystallization (toluene) filling apolar holes left in the packing. Neither hydrogen bonds nor pockets or polar zones have been found in the packing. This can be explained as the polar groups are ordered (stacked one over the other always with apolar parts of the molecules in between) in a similar way as the molecules in the columnar packing. In formal terms, the structure contains parallel associations of two of the adduct molecules with axial symmetry in the A, B, C, D and E rings of the triterpene and the A ring of the diterpene unit.[11]

Table 1 sets out the ^{13}C NMR data. The ORTEP figure shows the crystalline structure of the compound (Fig. 3).

These results once again suggest that Celastraceae species possess enzymatic systems catalyzing Diels–Alder reac-

Table 1. ^{13}C NMR data of compound **1** (data are based on ^{1}H–^{13}C 2-dimensional and DEPT experiments)

	Unit C30	Unit C20
1	39.6	48.0
2	33.9	18.7
3	217.9	18.4
4	47.2	33.1
5	54.6	48.2
6	19.5	23.2
7	33.6	34.0
8	41.1	136.0
9	49.3	134.8
10	36.7	36.3
11	21.7	45.3
12	26.5	213.4
13	37.9	78.7
14	43.1	55.2
15	41.3	34.0
16	34.9	16.9
17	43.6	17.3
18	49.5	20.9
19	50.3	32.8
20	54.8	21.4
21	81.1	
22	29.6	
23	26.6	
24	21.0	
25	16.0	
26	15.9	
27	14.4	
28	20.0	
29	18.4	
30	177.7	

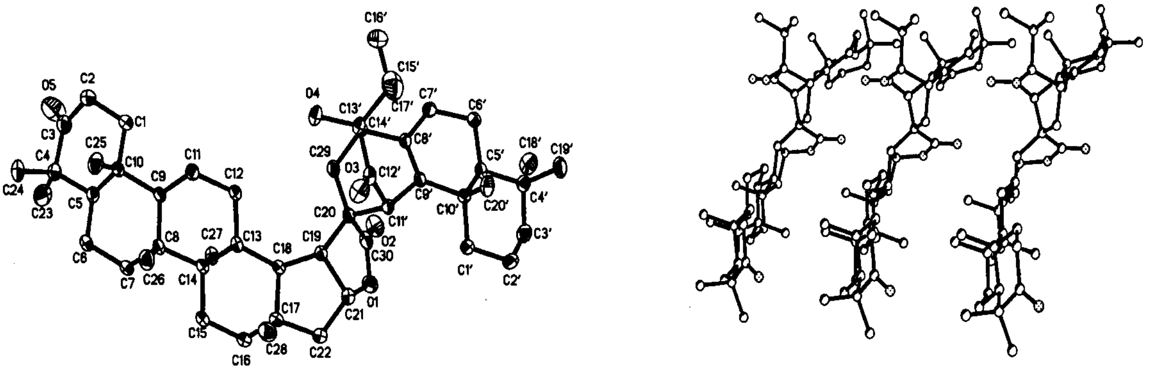

Figure 3. ORTEP view of volubilide (1) with 50% probability ellipsoids and linear disposition observable due to column packing. Hydrogen atoms were omitted for clarity.

Table 2. Crystal data and structure[11] refinement for **1**

Empirical formula	$C_{50}H_{74}O_5{\cdot}C_7H_8$	F (000)	928
Formula weight	847.23	Standard intensity decay	0.50%
Temperature	293 (2) K	PSI scan absorption correction	Max: 1.000000, min: 0.763563
Wavelength	0.71073 Å	Theta range for data collection	1.61 to 28.28 deg
Crystal system	Monoclinic	Index ranges	$-9\leq h\leq 9$, $-28\leq k\leq 23$, $-21\leq l\leq 13$
Space group	P 2, (1)	Reflections collected	13373
Unit cell dimensions	a=7.1846 (1) Å	Independent reflections	10809
	b=21.3104 (3) Å	Refinement method	Full-matrix least-squares on F^2
	c=15.8545 (3) Å	Data/parameters	10809/571
	β=97.691 (1) deg	Goodness-of fit on F^2	1.033
Volume	2405.59 (7) Å^3	Final R indices [$I>2\sigma(I)$]	$R1$=0.0508, w$R2$=0.1283
Z	2		
Density (calculated)	1170 Mg/m^3	R indices (all data)	$R1$=0.0649, w$R2$=0.1415
Absorption correction	SADABS[16] (empirical)	Largest diff. Peak and hole	0.338 and −0.240 e.Å^{-3}

tions. In this way, Johnson et al. attempted to synthesize maytenone and its analogues via Diels–Alder reactions but did not succeed.[14]

Plant Materials

The root bark of *Hippocratea volubilis* (2.7 kg) was collected at Cerro Mbatovi (6-02-98) Paraguari Department, Paraguay. A voucher specimen is filed with the Herbarium of the Facultad de Ciencias Químicas of the UNA Leg I. Basualdo & N. Soria 6790. The plant material was extracted with 1:1 *n*-hexane:diethyl ether in a Soxhlet, yielding 20 g of extract. The extract was chromatographed on Sephadex LH-20 using *n*-hexane–chloroform–methanol (2:1:1) as eluent, followed by flash chromatography on silica gel with gradient of *n*-hexane–ethyl acetate yielding a crystalline product **1** (500 mg), on fractions 5, 6 and 7.

X-Ray Crystallographic Study[11]

A summary of the crystal data and data collection parameters for compound **1** is given in Table 2. A crystal of approximate dimensions of 0.25×0.30×0.40 mm was mounted on a SIEMENS SMART CCD diffractometer equipped with a graphite monochromator and measured at room temperature. Using MoKα radiation and the ω-2θ mode, 10809 independent reflections were collected. The structure was solved by direct methods and refined by full-matrix least-squares. All calculations were done with the program SHELX-97.[15] Hydrogen atoms were introduced in calculated positions and refined in riding mode. Thermal vibrations for non H-atoms was assumed to be anisotropic.[11]

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11. Crystallographic data (excluding structure factors) for the estructure reported in this paper have been deposited at the Cambridge Crystallographic Data Center as suplementary publication n° CCDC 136027. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax +44 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

12. Physical and spectral data for compound **1:** $[\alpha]_D^{25}=+8.42$ (*c* 1.01, $CHCl_3$), mp: 174°C; IR: 3515; 2940; 2360; 1764; 1709; 1461; 1387; 1177; 756. ^1H NMR (δ, $CDCL_3$): 5.0 (1H, m, H-21); 1.04 (3H, s, H-23); 0.87 (3H, s, H-24); 1.04 (3H, s, H-25); 0.90 (3H, s, H-26); 0.86 (3H, s, H-27); 0.77 (3H, s, H-28); 2.1 (1H, t, H-5′); 2.7 (1H, bs, H-11′); 2.9 (1H, s, H-14′); 1.7 (1H, m, H-15′); 0.81 (3H, d, J=6.8 Hz, H-16′); 0.94 (3H, d, J=6.8 Hz, H-17′); 0.77 (3H, s, H-18′); 0.79 (3H, s, H-19′); 0.99 (3H, s, H-20′); 2.41 (6H, m); 2.13 (2H, m); 1.33 (22H, m). HRMS: calculated for $C_{50}H_{74}O_5$ $[M^+]$, 754.55270, found, 754, 55363.

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