Clerodane Diterpenes from *Tinospora rumphii*

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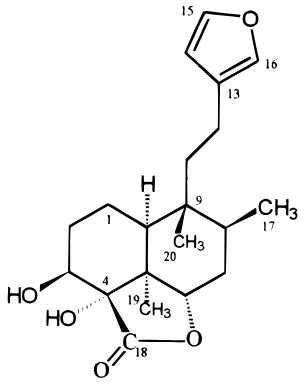
Two new diterpenes (**1** and **2**) were obtained from the leaves of *Tinospora rumphii*, along with the known compounds tinotufolin D and vitexilactone. The structures of compounds **1** and **2** were established on the basis of spectroscopic studies.

Tinospora rumphii Boerl. (syn. *T. crispa*; Menispermaceae) is widely distributed throughout the Philippines. Aqueous plant extracts are prescribed in the treatment of stomach trouble, indigestion, diarrhea, and topical ulcers.[1,2] The leaves in powdered form are used against fever. A preparation with coconut oil is an effective cure for rheumatism and also for flatulence among children. A decoction of the stem is reputed to be an excellent remedy for itches and cancerous wounds. The plant is also used as an antimalarial.[1,3]

Previous studies on the genus *Tinospora* have led to the isolation of clerodane-type furanoid diterpenes and glucosides.[4−13] We now report the isolation of two new diterpenes (**1** and **2**) together with the known diterpenes tinotufolin D[13] and vitexilactone,[14] from the $CHCl_3$ extract of *T. rumphii* leaves. The structures of **1** and **2** were elucidated by extensive NMR, IR, UV, and mass spectrometry analysis after separation by Si gel chromatography.



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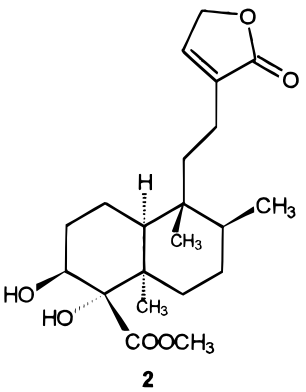

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Table 1. ^{13}C NMR (δ Values) Data of Compounds **1** and **2** in $CDCl_3$[a]

position	**1**	**2**
1	19.0	20.7
2	28.2	27.9
3	71.6	72.2
4	81.0	81.3
5	45.5	41.5
6	75.9	25.6
7	29.2	26.2
8	37.1	36.5
9	39.8	39.2
10	42.2	42.3
11	45.5	41.5
12	19.7	20.8
13	125.1	136.8
14	110.8	144.3
15	142.8	70.1
16	138.5	174.4
17	18.7	18.0
18	181.0	175.6
19	17.8	25.3
20	22.8	23.1
OCH_3		52.8

[a] Run at 100 MHz; multiplicities determined by a J_{mod} ^{13}C experiment.

To determine the number of attached protons, a *J*-modulated spin−echo spectrum for X-nuclei coupled to ^1H was obtained. The J_{mod} ^{13}C NMR spectral data of **1** and **2** (Table 1) indicated that both compounds have the same basic diterpene skeleton, with **2** possessing an additional sp^3 carbon resonance assigned to a methyl ester. The ^{13}C and ^1H NMR (Table 2) spectral data further indicated that **1** contains a β-substituted furan ring, whereas in **2** this functional group is replaced by a butenolide moiety. Three methyl proton signals, two as singlets and one as a doublet, were also common to both compounds.

High-resolution EIMS of **1** gave a molecular ion at *m*/*z* 348.1937, with the calculated value for $C_{20}H_{28}O_5$ being 348.1937. Thus, **1** has an index of hydrogen deficiency of 7. The UV spectrum showed a λ_{max} at 207 nm, supporting the presence of a furan ring.[8,14−17] The ^1H NMR spectrum of **1** (Table 2) indicated resonances for two carbinyl protons at δ 4.00 (1H, br s) and 5.00 (1H, dd, J = 9.2, 4.0 Hz), consistent with the presence of one or more hydroxyl groups (IR ν_{max} 3450, 1129, and 1208 cm^{-1}) and a lactone (IR ν_{max} 1754 and 1258 cm^{-1}), respectively. The COSY NMR spectrum of **1** indicated four isolated spin systems, one of which was a β-substituted furan [δ 6.25 (H-16), 7.35 (H-15), and 7.2 (H-14)]. The remaining three spin systems

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Table 2. ^{1}H NMR (δ Values) Spectral Data and HMBC Correlations of Compounds **1** and **2** in $CDCl_3$[a]

position	**1**	HMBC (**1**)	**2**	HMBC (**2**)
1	1.52 m, 1.90 m	2H-2, H-10	1.55 m, 1.90 m	
2	1.80 m, 1.85 m	2H-1	1.80 m, 1.90 m	
3	4.00 br s		3.83 br s	
4		H-6, H-19		H-19
5		H-6, H-10, H-19		H-7a, H-19
6	5.00 dd (9.2, 4.0)	2H-7, H-19	1.10 m, 2.50 m	H-19
7	1.85 m, 2.10 m	H-6, H-8, H-17	1.35 m, 1.80 m	H-6a, H-17
8	2.05 m	2H-7, H-10, H-11, H-17, H-20	1.60 m	H-17, H-20
9		2H-7, H-8, H-11, H-17, H-20		H-17, H-20
10	1.85 m	2H-1, 2H-2, H-19, H-20	1.75 m	2H-1, H-8, H-11, H-19, H-20
11	1.50 m, 1.87 m	2H-12, H-10, H-20	1.45 m, 1.95 m	H-12, H-20
12	2.40 (2H) m	2H-11	2.35 (2H) m	H-11, H-14
13		H-12, H-14, H-15		H-12, H-14, H-15
14	6.25 dd (1.2, 0.6)	H-12, H-16	7.06 br s	H-12, H-15
15	7.35 t (1.2)	H-14, H-16	4.76 br s	H-14
16	7.20 br s	H-12, H-14, H-15		H-14, H-15
17	1.10 d (5.3)	2H-7, H-8	1.01 d (7.2)	H-8
18				H-3, OCH_3
19	1.25 s	H-6, H-10	1.20 s	H-6
20	0.95 s	H-10, 2H-11	0.93 s	
OCH_3			3.85 s	

[a] Run at 400 MHz; *J* values in Hz.

were as follows: the proton at δ 1.52 (H-1a) was coupled to the hydrogen at δ 1.90 (H-1b) and the methylene protons at δ 1.80 (H-2a) and 1.85 (H-2b), which in turn were coupled to the carbinyl proton at δ 4.00 (H-3). The methylene protons at δ 1.85 (H-7a) and 2.10 (H-7b) were coupled to a carbinyl proton at δ 5.00 (H-6) and the methine proton at δ 2.05 (H-8). On the other hand, the methylene protons at δ 1.50 (H-11a) and 1.87 (H-11b) showed cross-peaks with the two-proton multiplet at δ 2.40 (2H-12). The lactone ring was indicated by a carbonyl resonance at δ 181.0. The ^{1}H and ^{13}C NMR assignments for **1** were determined from their HMQC correlations, and all the connectivities were verified by an inverse long-range heteronuclear experiment optimized for $J = 10$ Hz (Table 2). All long-range correlations observed were consistent with the proposed structure for compound **1**.

The relative stereochemistry of **1** was determined on the basis of NOESY experiments, which indicated key through-space correlations for the proposed framework. The carbinyl proton at δ 4.00 (H-3) was found to be close in space to the hydroxyl protons at δ 2.75 and 3.05. Thus, the carbinyl proton (br s) was assigned to the equatorial position and the hydroxyl protons were assigned to the axial positions. The Me group (δ 1.25, H-19) attached to C-5 was determined as being close in space to the methine proton at δ 1.85 (H-10). Thus, they were found to lie on the same side of **1,** indicating equatorial and axial orientations, respectively. The carbinyl proton at δ 5.00 (H-6) was found to be close to the C-8 Me protons at δ 1.10 (H-17) and the protons at δ 1.90 (H-1b) and 1.85 (H-7a). This indicated that H-6 ($J = 9.2$, 4.0 Hz) and the C-8 Me group are in axial positions. The C-9 Me protons at δ 0.95 (H-20) were close to the C-8 Me protons at δ 1.10 (H-17) and the proton at δ 1.50 (H-1a). This indicates that the C-9 Me group is equatorial. This stereochemistry is supported by comparison with the spectral data for tinotufolin D,[13] which was also isolated from the *T. rumphii* extract and which could result by dehydration of **1**. Thus, compound **1** was established as (2aβ,3α,5aβ,6β,7α,8aα)-6-[2-(3-furanyl)ethyl]-2a,3,4,5,5a,6,7,8,8a,8b-decahydro-2a,3-dihydroxy-6,7,8b-trimethyl-2H-naphtho[1,8-*bc*]furan-2-one.

The structure of **2** was supported by the HREIMS, which gave an $[M - H_2O]^+$ ion at *m*/*z* 362.2082; the calculated value for $C_{21}H_{30}O_5$ is *m*/*z* 362.2093. Comparison of the ^{1}H and ^{13}C NMR spectral data of **1** and **2** (Tables 1 and 2) indicated the presence of a butenolide function[18] [δ 7.06 (1H) and 4.76 (2H)] in **2** instead of a furan ring. The appearance of a methoxyl group at δ 3.85 (3H, s) in **2** and the absence of a carbinyl proton at ca. δ 5.0 in **1** indicated that the lactone in **1** was changed into a methyl ester in **2**. The presence of two carbonyls of the butenolide and ester were apparent from the ^{13}C NMR resonances at δ 174.4 and 175.6 and the IR maxima at 1748, 1712, 1255, and 1229 cm^{-1}. The presence of a butenolide was supported by the UV spectrum, which showed a λ_{max} at 211 nm.[18] The hydroxyl groups in **1** were retained (IR ν_{max} 3450 cm^{-1}). This was supported by the NMR spectral data of **2** (Tables 1 and 2), which retained the carbinyl proton at δ 3.85 and the carbinyl carbons at δ 72.2 and 81.3.

The ^{1}H and ^{13}C NMR assignments for **2** were verified by HMQC and HMBC experiments (Table 2), and the relative stereochemistry of **2** was established in a manner similar to **1**. The carbinyl proton appeared at δ 3.85 (H-3) as a broad singlet, and thus it was assigned to the equatorial position, with the C-3 and C-4 hydroxyls in axial positions. On the basis of the NOESY experiment, the ester Me was determined as being close in space to the C-5 Me protons, with the latter in turn close to H-10. The C-8 and C-9 Me protons remain close in space. Thus, **2** has the same relative stereochemistry as **1** and tinotufolin D,[13] and its structure was established as methyl(1α,4aα,5α,6β,8aα)-5-[2-(3-furan-3-ene-2-one)ethyl]-1,2,3,4,4a,5,6,7,8,8a-decahydro-1,2-dihydroxy-1-naphthalenecarboxylate.

Experimental Section

General Experimental Procedures. Optical rotations were measured on an Optical Activity Ltd. automatic polarimeter. IR spectra were recorded on a Perkin-Elmer 1600 Fourier Transform IR spectrometer and UV spectra on a HP 8452A diode array spectrometer. NMR spectra were recorded on a Bruker Avance 400 in $CDCl_3$ at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The high- and low-resolution EIMS were recorded on a Micromass AutoSpec mass spectrometer. Column chromatography was performed with silica gel 60 (70−230 mesh); TLC was performed with plastic-backed plates coated with silica gel F_{254}; plates were visualized by spraying with vanillin−H_2SO_4 and warming.

Plant Material. The sample was collected from Antipolo, Rizal, Philippines, in November 1997. It was identified as *Tinospora rumphii* Boerl (Menispermaceae) by Mercedita

Bangis at the Philippine National Museum, and a voucher specimen DLSUCD # 035 is kept at the Chemistry Department of De La Salle University.

Extraction and Isolation. Air-dried leaves of *Tinospora rumphii* (700 g) were ground in an osterizer, then extracted with $CHCl_3$ (2.8 L) at room temperature for 2 days. The mixture was filtered and the filtrate concentrated in vacuo to afford a crude extract (50 g). This extract was dissolved in EtOH (900 mL), then placed on an ice bath. To the solution was added 4% aqueous $Pb(OAc)_2$ (900 mL) to precipitate the more polar components.[19] The mixture was then filtered and the filtrate concentrated in vacuo until a mixture of water and an oily residue remained. The concentrate was extracted with $CHCl_3$, and the extract was dried with anhydrous Na_2SO_4, then filtered. The filtrate was concentrated in vacuo to afford the treated extract (10 g), which was fractionated by gravity column chromatography using increasing proportions of acetone in chloroform (10% increments) as eluents. The 10−20% acetone in chloroform fractions were rechromatographed (2×) in 15% ethyl acetate in petroleum ether to afford tinotufolin D[13] (12.5 mg). The 30−50% acetone in chloroform fractions were rechromatographed (3×) in dichloromethane−diethyl ether−acetonitrile (9:0.5:0.5) to afford two colorless oils [**1** (5 mg) and **2** (15.5 mg)] and crystalline vitexilactone[14] (20 mg).

Compound 1: colorless oil; $[\alpha]_D^{30}$ −32° (*c* 0.05, EtOH); IR (KBr) ν_{max} 3450 (br, OH), 1754 (lactone), 1258, 1208, 1129; UV (EtOH) λ_{max} 207 nm (ϵ 3560); ^{1}H NMR and ^{13}C NMR data are listed in Tables 1 and 2; EIMS *m/z* 348 $[M]^+$ (14), 254 (66), 218 (34) 173 (21), 191 (66), 149 (100), 121 (58), 110 (73); HREIMS *m/z* 348.1937 $[M]^+$ ($C_{20}H_{28}O_5$ requires 348.1937).

Compound 2: colorless oil; $[\alpha]_D^{30}$ +96° (*c* 0.02, EtOH); IR (KBr) ν_{max} 3450 (br, OH), 1748 (lactone), 1712 (ester), 1255, 1229; UV (EtOH) λ_{max} 211 nm (ϵ 3740); ^{1}H NMR and ^{13}C NMR data are listed in Tables 1 and 2; EIMS *m/z* 362 $[M]^+$ (15), 325 (48), 309 (71) 267 (33), 251 (86), 247 (100), 235 (57), 123 (54); HREIMS *m/z* 362.2082 $[M - H_2O]^+$ ($C_{21}H_{30}O_5$ requires 362.2093).

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