Two New Clerodane-Type Diterpenoids, Clerodinins A and B, from *Clerodendron brachyanthum* SCHAUER

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Two neoclerodane diterpenoids, clerodinins A and B, together with three known compounds, clerodin, stigmasta-5,22,25-trien-3β-ol and 3-*epi*-glutinol, have been isolated from the hexane extract of the leaves of *Clerodendron brachyanthum*, and their structures determined.

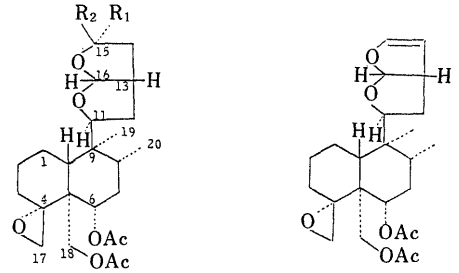
Keywords *Clerodendron brachyanthum*; Verbenaceae; clerodane-type diterpenoid; clerodinin A; clerodinin B

Extensive chemical studies of the constituents of the species of *Clerodendron* (*C.*) have been reported,[1] and many interesting components including clerodane-type diterpenoids, alkaloids, triterpenoids, phytosterols, flavonoids, and their glycosides have been isolated. In connection with our interest in clerodane-type diterpenoids and in view of the potential use of species of *Clerodendron* as a crude drug (antihypotensive, diuretic, anthelmintic, *etc.*),[1a,b,2] chemical studies on *C. brachyanthum* SCHAUER (Verbenaceae), which has been transplanted to Taiwan from the Phillippines, were undertaken in our laboratory. This report deals with the chemical constituents of the hexane extract of its leaves.

The air-dried leaves of *C. brachyanthum* were repeatedly extracted with hexane. The hexane extract was evaporated *in vacuo* to give precipitates and the mother liquid. After repeated purification on silica gel with a hexane–ethyl acetate solvent system, two new clerodane-type diterpenoids, clerodinins A (**1a**) and B (**1b**), and three known compounds, clerodin (**2**),[1c] stigamasta-5,22,25-trien-3β-ol (**3**),[1g] and 3-*epi*-glutinol (**4a**),[3] were isolated together with three unidentified products. Clerodin (**2**) and stigmasta-5,22,25-trien-3β-ol (**3**) were identified from their physical data. 3-*epi*-Glutinol (**4a**) has been prepared[3] by lithium aluminum hydride reduction of alnusenone (glut-5(6)-en-3-one), and it has been isolated from *Euphorbia cyparissias*.[4]

The spectral data of 3-*epi*-glutinol (**4a**) have not been reported. The identification of 3-*epi*-glutinol (**4a**) was achieved by nuclear magnetic resonance (NMR) spectral assignment in addition to comparison of the melting points of its acetate (**4b**) (mp 234—236 °C) and its oxidative product, glut-5(6)-en-3-one (mp 237—240 °C) with those reported in the literature.[4]

The infrared (IR) spectrum of clerodinin A (**1a**), $C_{25}H_{38}O_8$, showed the presence of ester groups (1735, 1725, and 1255 cm^{-1}) and an oxirane ring (3030 cm^{-1}). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum (Table I) of clerodinin A (**1a**) showed signals of two acetate groups (δ 1.91 and 2.07). It also exhibited the following signals due to protons on carbon atoms bearing oxygen atoms: δ 4.66 (1H, dd, J=10.9, 4.7 Hz, H-6), 4.87 and 4.35 (AB system, J=12.1 Hz, H-18), 2.96 (1H, dd, J=3.9, 2.3 Hz, H-17)[5] and 2.18 (1H, d, J=3.9 Hz, H-17). In addition, the characteristic signals of a tertiary methyl group (δ 0.90) and of a secondary methyl group (δ 0.85, d, J=6.4 Hz) were also observed. Likewise, the presence of a



1a : R_1=H, R_2=OMe
1b : R_1=OMe, R_2=H
1c : R_1+R_2=O
1d : R_1+R_2=H+OH

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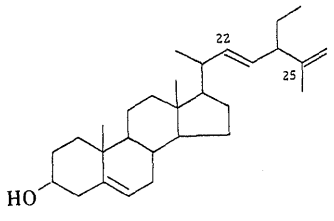

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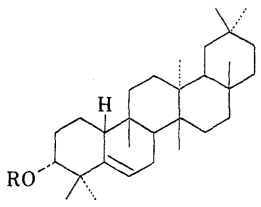

4a : R=H
4b : R=Ac

TABLE I. ^{1}H-NMR Data (δ-Values) for Clerodinin A (**1a**), Clerodinin B (**1b**) and Clerodin (**2**)

H	1a	1b	2
6	4.66 dd (10.9, 4.7)	4.65 dd (11.3, 4.2)	4.64 dd (11.5, 4.3)
11	4.34 dd (11.1, 4.3)	3.98 dd (11.8, 4.4)	3.98 dd (11.5, 4.7)
13	2.77 m	2.95 m	3.52 m
14			4.76 t (2.4)
15	4.94 d (5.6)	5.07 d (4.8)	6.42 t (2.4)
16	5.76 d (5.4)	5.67 d (5.4)	5.96 d (6.2)
17	2.18 d (3.9)	2.16 d (4.1)	2.17 d (4.1)
17	2.96 dd (3.9, 2.3)	2.94 dd (4.1, 2.3)	2.94 dd (4.1, 2.3)
18	4.35, 4.87 d (12.1)	4.35, 4.86 d (12.2)	4.33, 4.85 d (12.1)
19	0.90 s	0.93 s	0.92 s
20	0.85 d (6.4)	0.83 d (6.3)	0.79 d (6.3)
CH_3CO-	1.91 s	1.91 s	1.90 s
CH_3CO-	2.07 s	2.07 s	2.06 s
CH_3O-	3.29 s	3.29 s	

300 MHz, $CDCl_3$, TMS as an internal standard. Figures in parentheses are coupling constants in Hz.

TABLE II. ^{13}C-NMR Data (δ-Values) for Clerodinin A (**1a**), Clerodinin B (**1b**) and Clerodin (**2**)

C	1a	1b	2
1	22.1 t	22.2 t	22.1 t
2	25.0 t	24.9 t	24.9 t
3	39.5 t	39.1 t	39.5 t
4	65.0 s	65.0 s	65.0 t
5	45.5 s	45.6 s	45.5 t
6	72.1 d	71.9 d	71.9 d
7	33.5 t[a)]	33.4 t[a)]	33.3 t[a)]
8	35.9 d	36.1 d	36.1 d
9	40.0 s	40.1 s	40.0 s
10	48.3 d	49.4 d	48.3 d
11	83.2 d	83.5 d	84.5 d
12	32.6 t[a)]	32.7 t[a)]	32.6 t[a)]
13	40.5 d	40.5 d	45.9 d
14	32.7 t[a)]	32.6 t[a)]	107.6 d
15	104.7 d	104.9 d	146.8 d
16	109.2 d	107.2 d	101.8 d
17	48.5 t	49.5 t	48.6 t
18	61.8 t	61.7 t	61.6 t
19	16.3 q	16.3 q	16.3 q
20	14.0 q	13.9 q	14.0 q
$\underline{C}H_3CO$-	21.2 q	21.1 q	21.1 q
$\underline{C}H_3CO$-	21.1 q	21.1 q	21.1 q
$CH_2\underline{C}O$-	170.0 s	170.0 s	170.0 s
$CH_3\underline{C}O$-	170.8 s	170.8 s	170.8 s
CH_3O-	54.4 q	54.6 q	

300 MHz, $CDCl_3$, TMS as an internal standard. Assignments established by off-resonance and DEPT methods. *a)* Assignments may be interchanged. s, singlet; d, doublet; t, triplet; q, quartet.

hexahydrofuranofuran system was inferred from the signals at δ 5.76 (d, J=5.4 Hz), 4.94 (d, J=5.6 Hz), 4.34 (dd, J=11.1, 4.3 Hz) and 2.77 (m), attributable to H-16, H-15, H-11, and H-13, respectively. The signal at δ 3.29 (3H, s) suggested the substitution of a methoxy group at C-15. Furthermore, the occurrence of this C-15 substituted hexahydrofuranofuran moiety was confirmed by the presence of significant peaks at m/z 143, 111, 81 and 69[1f)] in the mass spectrum (MS). ^{1}H-NMR spectrum of clerodinin A (**1a**) is similar to that of clerodin (**2**) except for a methyl acetal in place of an enol ether. The structure of **1a** was further confirmed by carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy (Table II): the signals of C-15 and the methoxy group appeared at δ 104.7 and 54.5, respectively.

Clerodinin B (**1b**), $C_{25}H_{38}O_8$, revealed the presence of esters (1735, 1720 and 1230 cm^{-1}) and an oxirane ring (3080 cm^{-1}). The MS, IR and ^{1}H-NMR (Table I) spectra of **1b** were very similar to those of **1a**. Clerodinin B (**1b**) showed ^{1}H-NMR signals due to one secondary methyl group [δ 0.83 (d, J=6.3 Hz)], one tertiary methyl group (δ 0.93), two acetate groups (δ 1.91 and 2.07), and one methoxy group (δ 3.29). It also showed the following signals due to protons on carbon atoms bearing oxygen atoms: δ 2.16 (1H, d, J=4.1 Hz, H-17), 2.94 (1H, dd, J=4.1, 2.3 Hz, H-17),[5)] 3.98 (1H, dd, J=11.8, 4.4 Hz, H-11), 4.35 and 4.86 (each 1H, AB system, J=12.2 Hz, H-18), 4.65 (1H, dd, J=11.3, 4.2 Hz, H-6), 5.07 (1H, d, J=4.8 Hz, H-15) and 5.67 (1H, d, J=5.4 Hz, H-16). The signal of H-13 appeared at δ 2.95 (1H, m). The significant peaks in the MS at m/z 143, 111, 81 and 69[1f)] also confirmed the presence of a C-15 substituted (with a methoxyl group) hexahydrofuranofuran moiety. The structure of **1b** was further confirmed by its ^{13}C-NMR (Table II) spectrum: the signals of C-15 and the methoxy group appeared at δ 104.9 and 54.6, respectively. From the above evidence, clerodinin A (**1a**) must be a C-15 epimer of clerodinin B (**1b**). The chemical correlation between clerodinin A and clerodinin B and determination of the relative configuration of the C-15 methoxyl group in clerodinins A and B were achieved as follows.

Clerodinin A (**1a**) was oxidized with chromic acid in the solution of aqueous acetic acid to give a γ-lactone (**1c**) (mp 192—193 °C, ν_{max}: 1785 cm^{-1}), which was identical with the oxidation product of clerodinin hemiacetal (**1d**).[1c)] It was believed that the first step involved the conversion of the methoxyl group in **1a** to the hydroxyl group in **1d** before oxidation. Clerodinin B (**1b**) also yielded a γ-lactone (**1c**) under similar oxidation conditions. This result provides further evidence that clerodinins A and B are C-15 epimers. Clerodin (**2**) is very sensitive to acidic conditions, and therefore the preparation of **1a** and **1b** from clerodin must be achieved under weakly acidic conditions. Dissolution of **2** in methanol with an equal volume of acetic acid as a catalyst at ambient temperature smoothly gave **1a** and **1b** in a 2:1 ratio in high yield. Finally, the methoxyl group at C-15 in **1a** must have β-orientation, since the methanol molecule should approach from the sterically less hindered β-side, thus giving predominantly **1a**. Therefore, the methoxyl group at C-15 in **1b** is presumed to have α-orientation.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter at room temperature. IR spectra were recorded on a JASCO A-102 spectrometer. ^{1}H- and ^{13}C-NMR spectra were run on a Brucker AM 300 at 300 MHz in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ-values and coupling constants (J) are given in hertz (Hz). Electron impact-mass spectrum (EI-MS) was taken on a JEOL-JMS-100.

Extraction and Isolation The air dried leaves of *C. brachyanthum* SCHAUER (0.68 kg), harvested in Taipei, were extracted with hexane (8 l) three times. The combined extracts were evaporated *in vacuo* to about 500 ml to yield the filtrate and a precipitate, which was filtered off. The precipitate (535 mg) was purified on a silica gel column with a binary solvent system (hexane+ethyl acetate) to give clerodinins A (**1a**, 78 mg) and B (**1b**, 30 mg). The residue from the filtrate (3.4 g) was chromatographed on silica gel with the binary solvent system (hexane+ethyl acetate) to give clerodinin A (**1a**), clerodinin B (**1b**), and three known compounds, clerodin (**2**) (95 mg), stigmasta-5,22,25-trien-3β-ol (**3**) (35 mg), and 3-*epi*-glutinol (**4a**) (40 mg) together with three unidentified products named clerodinin C, clerodinin D, and clerodiol.

Clerodinin A (1a) mp 158—160 °C, $[\alpha]_D^{25}$ +28.8° (c=1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3030, 1735, 1725, 1365, 1255, 1085, 1000, 965, 885, 635. MS m/z (%): 466 (2, M$^+$), 315 (25), 297 (28), 253 (5), 236 (20), 173 (19), 143 (88), 111 (100), 81 (4), 69 (4), 43 (32). ^{1}H-NMR: Table I. ^{13}C-NMR: Table II. *Anal.* Calcd for $C_{25}H_{38}O_8$: C, 64.36; H, 8.21. Found: C, 64.98; H, 8.27.

Clerodinin B (1b) mp 198—200 °C, $[\alpha]_D^{25}$ −30.5° (c=1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3080, 1735, 1720, 1365, 1265, 1253, 1235, 1020, 985, 865, 635. MS m/z (%): 466 (3, M$^+$), 315 (45), 297 (47), 253 (5), 173 (8), 143 (48), 111 (100), 81 (4), 69 (6), 43 (57). ^{1}H-NMR: Table I. ^{13}C-NMR: Table II. *Anal.* Calcd for $C_{25}H_{38}O_8$: C, 64.36; H, 8.21. Found: C, 64.01; H, 8.21.

Clerodin (2)[1c)] mp 168—169 °C, $[\alpha]_D^{25}$ −110° (c=1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3080, 1735, 1725, 1615, 1360, 1250, 1240, 1080, 1015, 1005, 855, 735, 635. MS m/z (%): 434 (3, M$^+$), 319 (19), 203 (60), 182 (61), 173 (77), 159 (58), 145 (58), 119 (58), 111 (87), 81 (56), 69 (55), 55 (73), 43 (100). ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

Stigmasta-5,22,25-trien-3β-ol (3)[1g)] mp 151—152.5 °C, $[\alpha]_D^{25}$ −54° (c=1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3480, 3040, 1645, 1380, 1370, 1060, 1050, 960, 885. MS m/z (%): 410 (32, M$^+$), 381 (57), 363 (66), 271 (66), 159 (70), 109 (110), 95 (90), 81 (83). ^{1}H-NMR ($CDCl_3$) δ: 0.65, 0.98, and 1.65 (each 3H,

s), 0.80 (3H, d, $J=7.5$ Hz), 0.98 (3H, d, $J=6.5$ Hz), 2.39 (1H, q, $J=7.5$ Hz, H-24), 3.49 (1H, m, H-3), 4.67 (2H, br s, H-26), 5.15 (1H, dd, $J=15.2$, 7.5 Hz, H-22), 5.21 (1H, dd, $J=12.2$, 7.5 Hz, H-23), 5.32 (1H, d, $J=6.6$ Hz, H-6).

3-*epi*-Glutinol (4a)[4] mp 204—206 °C, $[\alpha]_D^{25}$ +96° ($c=1.0$, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3480, 3080, 1385, 1370, 1360, 1090, 1035. MS *m/z* (%): 426 (3, M^+), 259 (52), 134 (81), 119 (88), 95 (100), 81 (67), 69 (90), 55 (85). ^{1}H-NMR ($CDCl_3$) δ: 0.82, 0.93, 0.96, 0.98, 1.02, 1.07, 1.14, and 1.23 (each 3H, s), 3.44 (1H, br s, $W_{1/2}=6$ Hz, H-3), and 5.61 (1H, d, $J=5.9$ Hz, H-6).

Acetylation of 3-*epi*-Glutinol (4a) When treated by a usual method, 3-*epi*-glutinol (**4a**) (10 mg) gave glut-5(6)-en-3α-yl acetate: mp 233—236 °C.[4] IR ν_{max}^{KBr} cm^{-1}: 3080, 1735, 1385, 1365, 1285, 1240, 1075, 825. ^{1}H-NMR ($CDCl_3$) δ: 0.82, 0.93, 0.96, 0.98, 1.02, 1.05, 1.08, 1.14, and 1.99 (each 3H, s), 4.67 (1H, t, $J=2.5$ Hz, H-3), 5.52 (1H, d, $J=6.0$ Hz, H-6).

Preparation of the γ-Lactone (1c) from Clerodinin A (1a) and Clerodinin B (1b) A solution of CrO_3 (30 mg) in 0.5 ml of glacial acetic acid containing 0.5 ml of water was added dropwise to a solution of clerodinin A (**1a**) (15 mg) in 0.2 ml of glacial acetic acid. The mixture was allowed to stand at room temperature for 6 h. The reaction mixture was treated by a usual method to give the γ-lactone (**1c**) (mp 192—193 °C, IR ν_{max}^{KBr} cm^{-1}: 1785)[1c] (10 mg). Clerodinin B (**1b**) (15 mg) gave the same product **1c** (10 mg) under the same oxidation conditions.

Conversion of Clerodin (2) to Clerodinin A (1a) and Clerodinin B (1b) Clerodin (**2**) (30 mg) was dissolved in 0.5 ml of methanol containing 0.5 ml of glacial acetic acid and kept at room temperature overnight. The reaction mixture was treated by a usual method to give clerodinin A (16 mg) and clerodinin B (8 mg) after being purified by silica gel chromatography.

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