THREE NEW CLERODANE-TYPE DITERPENOIDS, CLERODININ C, CLERODININ D AND CLERODIOL, FROM CLERODENDRON BRACHYANTHUM SCHAUER

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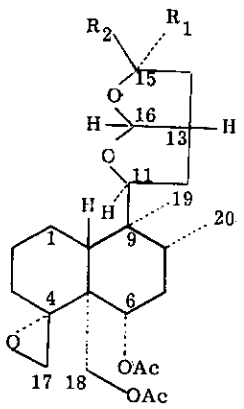
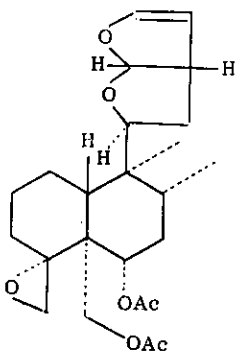
Abstract — Clerodinin C, clerodinin D and clerodiol, three new clerodane-type diterpenoids have been isolated from the hexane extract of the leaves of Clerodendron brachyanthum Schauer. Their structures have been elucidated by means of spectroscopic and chemical evidence.

Many interesting chemical components including clerodane-type diterpenes, alkaloids, triterpenes, phytosterols, flavanes and their glycosides[1] were extensively studies from the species of Clerodendron. In view of the potential use of species of Clerodendron as a native drug (served as antihypotension, diuretic and anthelmintic etc.)[1a,1b,2] and our interests in clerodane-type diterpenes, the chemical studies on the hexane extract of the leaves of Glerodendron brachyanthum Schauer (Verbenaceae) were performed in our laboratory. We have elucidated two new clerodane-type diterpenes, clerodinin A (1a) and clerodinin B (1b) and three known crystals, clerodin (2), stigmasta-5,22-25-triene-3β-ol, and 3-epi-glutinol[3]. In the same extract[3], three other unknown crystals, namely, clerodinin C (1c), clerodinin D (1d) and clerodiol (3), were also isolated. In this paper we describe our study of the other remained three new structures of clerodinin C (1c), clerodinin D (1d) and clerodiol (3).

The first of the new diterpenoids, clerodinin C (1c), had a molecular formula $C_{26}H_{40}O_8$ on the basis of elementary analysis and mass spectra (M^+ at m/z 480) and its ir spectrum showed absorption bands at 3055 (oxirane ring), 1735, 1725, 1255, and 1025 (ester groups) cm^{-1}. The nmr spectrum (Table 1) of clerodinin C (1c) exhibited signals for two acetate groups [^1H nmr ($CDCl_3$) 1.91 and 2.06]. It also showed the following signals due to protons on carbon atoms bearing oxygen atoms: ^1H nmr ($CDCl_3$) 4.65 (1H, dd, 11.0, 4.6 Hz, H-6), 4.34 and 4.86 (AB system, J = 12.1 Hz, H-18), 2.17 (1H, d, J = 3.9 Hz, H-17), and 2.95 (1H, dd, J = 3.9, 2.3 Hz, H-17)[4]. In addition, characteristic signals of a tertiary methyl group (δ 0.90) and of a secondary



1a R_1=H, R_2=OMe
b R_1=OMe, R_2=H
c R_1=H, R_2=OEt
d R_1=OEt, R_1=H



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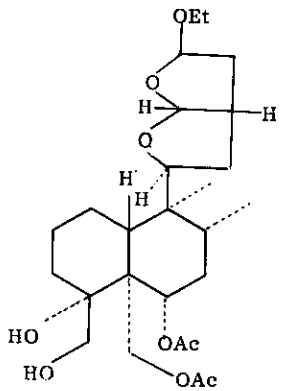

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Table 1. ^{1}H nmr data (δ-values) for clerodinin C (1c), clerodinin D (1d) and clerodiol (3) (300 MHz, $CDCl_3$, TMS as internal standard)

H	1c	1d	3
6	4.65 dd	4.66 dd	4.97 dd
	(11.0, 4.6)*	(11.1, 4.6)	(10.0, 4.8)
11	4.39 dd	4.00 dd	4.40 dd
	(11.3, 5.4)	(11.9, 4.5)	(10.3, 5.9)
13	2.78 m	2.98 m	2.82 m
15	5.04 d (5.7)	5.20 d (4.7)	5.06 d (5.3)
16	5.74 d (5.4)	5.69 d (5.4)	5.74 d (5.4)
17	2.17 d (3.9)	2.18 d (4.0)	3.89, 4.00
	2.95 dd	2.96 dd	d (11.2)
	(3.9, 2.3)	(4.0, 2.3)	
18	4.34, 4.86	4.35, 4.86	4.63, 4.99
	d (12.1)	d (12.1)	d (10.0)
19	0.90 s	0.93 s	0.99 s
20	0.85 d (6.4)	0.84 d (6.4)	0.89 d (6.3)
CH_3CO-	1.91 s	1.91 s	1.99 s
CH_3CO-	2.06 s	2.06 s	2.08 s
CH_3CH_2O-	1.14 t (6.8)	1.15 t (6.9)	1.15 t (6.9)
CH_3CH_2O-	3.39, 3.70 m	3.41, 3.70 m	3.41, 3.71 m

*Figures in parentheses are coupling constants in Hz.

methyl group (δ 0.85, d, J = 6.4 Hz) were also observed. Likewise, the presence of a hexahydrofuranofuran system was inferred from an acetal proton signal at ^{1}H nmr ($CDCl_3$) 5.74 (d, J = 5.4 Hz), and of signals at 5.04 (d, J = 5.7 Hz), 4.39 (dd, J = 11.3, 5.4 Hz), and 2.78 (m), attributable, respectively, to H-16, H-15, H-11, and H-13. The last resonance and the appearance of signals at ^{1}H nmr ($CDCl_3$) 1.14 (3H, t, J = 6.8 Hz) and 3.39 and 3.70 (each 1H, m) suggested the substitution of an H-15 proton for an ethoxy group. Furthermore, the occurrence of this C-15 substituted hexahydrofuranofuran moiety was confirmed by the presence in its mass spectrum of significant ions at 157 and 111[1f]. The ^{1}H nmr spectrum of clerodinin C (1c) is similar to clerodin (2) except an ethyl acetal in replacement of enol ether.

The second compound, clerodinin D (1d), had a molecular formula $C_{26}H_{40}O_8$ on the basis of elementary analysis and mass spectrum [M^+ at m/z 480] and its ir spectrum revealed the presence of esters (1735, 1725, and 1250 cm^{-1}) and an oxirane ring (3065 cm^{-1}). The mass, ir and ^{1}H nmr (Table 1) spectra of (1c) and (1d) are very similar. The ^{1}H nmr spectrum of clerodinin D (1d) revealed it containing two acetate groups [1.91 and 2.06], one tertiary methyl group [0.93], one secondary methyl group [0.84 (d, J = 6.4 Hz)], an ethoxy group [1.15 (3H, t, J = 6.9 Hz) and 3.41 and 3.70 (each 1H, m)], and 1,2-disubstituted oxirane ring [2.18 (d, J = 4.0 Hz) and 2.96 (dd, J = 4.0, 2.3 Hz)]. The other signals (coupling constant and chemical shift) in (1d) are similar to those in (1c). Protons, H-11, H-13 and H-15, in (1d) and (1c) exhibited slightly different chemical shift only. Clerodinin D (1d) also expressed same mass spectrum of clerodinin C (1c) with different relative intensity only. Of course the significant ions at 157 and 111[1f] are present, it revealed the presence of C-15 substituted hexahydrofuranofuran moiety. From the above evidence, clerodinin D (1d) must be a C-15 epimer of clerodinin C (1c). ^{13}C Nmr spectra (Table 2) of (1c) and (1d) also supported the assigned structures.

Clerodin (2) is very sensitive to acidic conditions, therefore the chemical correlation among clerodin (2), clerodinin C (1c) and clerodinin D (1d) must be achieved under weak acidic conditions. Dissolution in ethanol with equal volume of acetic acid as catalyst at ambient temperature converted clerodin (2) into corresponding (1c) and (1d) smoothly. The products (1c) and (1d) were obtained with 2:1 ratio in high yield. Finally, the assignment of the ethoxy group at C-15 in (1c) is located at β-orientation. Because the ethanol molecule approaches from the sterically less hindered β-side, thus giving predominantly (1c). Therefore, the ethoxy group at C-15 in (1d) must be located at α-orientation.

The third new crystal, clerodiol (3), had a molecular formula $C_{26}H_{42}O_9$ on the basis of elementary analysis and mass spectrum (M^+ at m/z 498), and its ir spectrum revealed the presence of hydroxy group (3450 cm^{-1}) and esters (1735, 1725, and 1245 cm^{-1}), and no oxirane ring absorption bands.

Table 2. ^{13}C Nmr data (δ-values) for clerodinin C (1c), clerodinin D (1b) and clerodiol (3) (300 MHz, $CDCl_3$, TMS as internal standard), a: assignment could be exchanged.

C	1c	1d	3
1	22.1 t	22.1 t	21.9 t
2	25.0 t	25.1 t	22.4 t
3	39.5 t	39.5 t	39.2 t
4	65.0 s	65.0 s	74.2 s
5	45.5 s	45.4 s	45.4 s
6	72.0 d	72.0 d	74.5 d
7	33.4 t[a]	33.5 t[a]	33.5 t[a]
8	36.1 d	36.0 d	35.8 d
9	40.1 s	40.0 s	40.8 s
10	48.3 d	48.2 d	48.5 d
11	83.5 d	83.2 d	83.6 d
12	32.5 t[a]	32.4 t[a]	32.2 t[a]
13	40.7 d	40.6 d	40.0 d
14	32.7 t[a]	32.7 t[a]	32.7 t[a]
15	103.8 d	104.7 d	103.9 d
16	109.1 d	109.1 d	109.0 d
17	48.4 t	48.4 t	62.7 t
18	61.8 t	61.7 t	63.0 t
19	16.4 q	16.3 q	16.9 q
20	14.1 q	14.0 q	14.9 q
$\underline{C}H_3CO-$	21.1 q	21.1 q	21.1 q
$\underline{C}H_3CO-$	21.1 q	21.1 q	21.3 q
$CH_3\underline{C}O-$	170.0 s	170.0 s	169.9 s
$CH_3\underline{C}O-$	170.9 s	170.9 s	170.1 s
$\underline{C}H_3CH_2O-$	15.0 q	16.3 q	15.0 q
$CH_3\underline{C}H_2O-$	63.0 t	62.9 t	63.1 t

Assignment established by EDPT method.

As summarized in Table 1, the ^{1}H nmr spectra of clerodiol (3) showed containing one tertiary methyl, one secondary methyl and two acetate residues. The nmr spectrum showed two AB quartets, a primary carbinol methylene group at δ 3.89 and 4.00 (each 1H, d, J = 11.2 Hz, H-17), and a acetoxy methylene group at 4.63 and 4.99 (each 1H, d, J = 10.0 Hz, H-18), and a double doublet at δ 4.97 (J = 10.0, 4.8 Hz). The presence of a hexahydrofuranofuran was shown by the following data. A double doublet at δ 4.40 (J = 10.3, 5.9 Hz), a multiplet at 2.82, a doublet at 5.06 (J = 5.3 Hz), and a doublet at 5.74 (J = 5.4 Hz) were assigned to C-11, C-13, C-15, and C-16 protons, respectively, commonly observed in the compounds containing C-15 substituted hexahydrofuranofuran rings. The last absorption and the appearance of signals at ^{1}H nmr ($CDCl_3$) 1.15 (3H, t, J = 6.9 Hz) and 3.41 and 3.71 (each 1H, m) suggested the substitution of a H-15 proton for an ethoxy group. The significant ions, m/z at 157 and 111, are also present in the mass spectrum of clerodiol (3). The assignment of structure of clerodiol (3) also confirmed by its ^{13}C nmr spectrum (Table 2). By the comparison of chemical shift of H-11, H-13, H-15, and H-16 in (1c), (1d) and (3), the configuration of ethoxy group at C-15 in (3) must be same as that in (1c). The only difference in structures between (1c) and (3) is that (3) possessed an α-glycol in replacement of 1,2-disubstituted oxirane in (1c). The clerodiol (3) and methanesulfonyl chloride in dry pyridine was heated at 60°C for 5h, clerodinin C (1c) was isolated after purification. The result confirmed the structure of clerodiol C (3) as another new clerodane-type diterpenoid.

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 polarimenter at room temperature. Ir spectra were recorded on a JASCO A-102 spectrophotometer. ^{1}H and ^{13}C nmr spectra run on a Bruker 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). EI-ms and uv spectra were taken on a JEOL-JMS-100 spectrometer and Hitachi RMS-4 spectrophotometer, respectively.

Extraction and Isolation

The air dried leaves of Clerodendron brachyanthum Schauer (0.68 kg), harvested in Taipei, were extracted with hexane (8 ℓ) three times (one hour for every time under reflux). 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 gradient) to give clerodinins A (1a) (78 mg) and B (1b) (30 mg). The residue (3.4 g) from the filtrate was chromatographed on silica gel with the binary solvent

system (hexane + ethyl acetate) to give clerodinin A (1a) (30 mg), clerodinin B (1b) (25 mg), and three known compounds, clerodin (2) (95 mg), stigmasts-5,22,25-trien-3β-ol (35 mg), and 3-epi-glutinol (40 mg) together with three products named clerodinin C (30 mg), clerodinin D (35 mg), and clerodiol (30 mg)[3].

Clerodinin C (1c); mp 147-149°C; $[\alpha]_D^{25°}$ + 30.0° (c 1.00 in $CHCl_3$); ir (KBr) (ν_{cm}-1) 3055, 1735, 1725, 1255, 1100, 1025, 1000, 965, 855, and 635; ms m/z (%) 480 (51), 435 (21), 375 (23), 315 (46), 297 (58), 157 (13), 111 (54), and 43 (100); Anal. Calcd for $C_{26}H_{40}O_8$: C, 64.98; H, 8.39. Found C, 64.81; H, 8.45.

Clerodinin D (1d); mp 163-165°C; $[\alpha]_D^{23°}$ - 31.5° (c 1.00 in $CHCl_3$); ir (KBr) (ν_{cm}-1) 3060, 1735, 1725, 1250, 1105, 1025, 1000, 970, 860, and 640; ms m/z (%) 480 (49), 435 (35), 375 (30), 315 (40), 297 (55), 157 (15), 111 (60) and 43 (100); Anal. Calcd for $C_{26}H_{40}O_8$: C, 64.98, H, 8.39. Found C, 64.66; H. 8.29.

Clerodiol (3); mp 153-155°C: $[\alpha]_D^{23°}$ + 140.0° (c 1.00 in $CHCl_3$); ir (KBr) (ν_{cm}-1) 3450, 1735, 1725, 1245, 995, 100, 970 and 730; ms m/z (%) 498 (1), 470 (6), 452 (5), 350 (27), 240 (77), 165 (67), 157 (91), 111 (58), 107 (100) and 55 (90); Anal. Calcd for $C_{26}H_{42}O_9$: C, 62.63; H, 8.49. Found C, 62.35; H, 8.52.

Preparation of Clerodinin C (1c) and Clerodinin D (1d) from Clerodin (2)

To a solution of clerodin (2) (30 mg) in 0.5 ml of ethanol, 0.5 ml of glacial acetic acid was added dropwisely. The mixture was left standing at room temperature overnight. The reaction mixture was poured into excess water and precipitates appeared. The combined precipitates and ether extract of filtrate was purified by silica gel chromatography (ethyl acetate: hexane = 1:2 solvent system as eluent) to give clerodinin C (1c) (16 mg) and clerodinin D (1d) (8 mg).

Coversion of Clerodiol (3) to Clerodinin C (1c)

Methanesulfonyl chloride (100 mg) was added to a solution of clerodilol (3) (15 mg) in 1 ml of dry pyridine, and the reaction mixture was heated of 55°-60°C for 5 h. Then it was poured into excess ice water and extracted with ether. The combined ether extracts were washed with 3 N HCl, 5 % $NaHCO_3$ and finally with water. The product, after removal of solvent, was purified by silica gel chromatography (ethyl acetate: hexane = 1:2 solvent system as eluent) to give clerodinin C (1c) (6 mg).

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