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CIS-CLERODANE-TYPE DITERPENE LACTONES FROM *EPHEMERANTHA COMATA*

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Key Word Index—*Ephemerantha comata*; Orchidaceae; *cis*-clerodane; diterpene; glucoside; butenolide.

Abstract—Two new *cis*-clerodane-type diterpene lactones named ephemeric acid and ephemeroside have been isolated from *Ephemerantha comata* Hunt & Summerh (Orchidaceae) and their structures have been characterized on the basis of spectroscopic evidence.

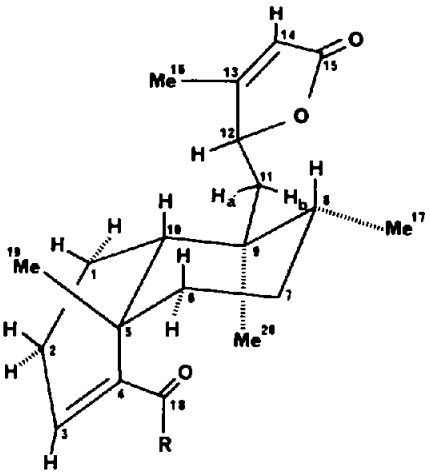
INTRODUCTION

In the course of studies on medicinal resources of plants of the genus *Dendrobium*, we investigated the constituents of *Ephemerantha comata* Hunt & Summerh (Japanese name: Kusukusu-Sekkoku) which once belonged to the *Dendrobium* genus but now belongs to the genus *Ephemerantha* [1]. Here we wish to report the isolation and structural characterization of two diterpenes, ephemeric acid (**1**) and ephemeroside (**2**).

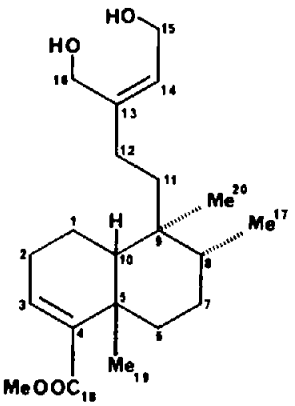
RESULTS AND DISCUSSION

The ethyl acetate soluble fraction of methanolic extract of the fresh whole plants was chromatographed on a silica gel column using a gradient solution of chloroform and methanol as eluent to give two fractions. The less polar fraction was further separated by silica gel preparative TLC using a 3:1 mixture of chloroform and ethyl acetate to afford ephemeric acid (**1**) in a 0.0075% yield from the fresh plants. The more polar fraction was separated by medium pressure liquid chromatography (MPLC) and preparative TLC, successively, using silica gel with a mixed solution of chloroform and methanol (85:15) as mobile phase to give ephemeroside (**2**) in 0.016% yield.

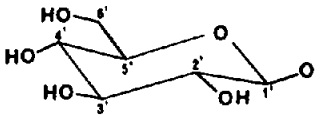
Ephemeric acid (**1**) $[\alpha]_D - 75.0°$ ($CHCl_3$; *c* 0.56) seems to be a diterpene from the HRMS [*m/z* 232.1972 $(C_{20}H_{28}O_4)^+$] and ^{13}C NMR spectrum (Table 1). **1** has an α,β-unsaturated carboxylic acid group [ν3000 (br) and 1665 cm^{-1}; δ_H 6.83 (1H, *t*, J = 3.66 Hz); δ_C 141.6 (*d*), 137.7 (*s*) and 169.5 (*s*)] and gave the corresponding methyl ester (**3**) [ν 1710 cm^{-1}; δ_H 3.72 (3H, *s*) and 6.52 (1H, *t*, J = 3.90 Hz); δ_C 51.4 (*q*), 138.2 (*d*), 138.6 (*s*) and 168.4 (*s*)] on treatment with diazomethane. A combination of ^1H–^1H and ^{13}C–^1H COSY experiments suggested the presence of the partial structure –C(=)CH–CH_2–CH_2–CH(–)– in **1**, which was also supported by the ^1H NMR spectra with the aid of double resonance experiments. Moreover, **1** exhibited two singlet and one doublet methyl signals at δ0.84 (*s*), 1.28 (*s*) and 0.81 (*d*) in its ^1H NMR spectrum. These spectral feature are consistent with a clerodane-type carbon skeleton, a bicyclic ring system [2]. This was further confirmed by the comparison of ^{13}C NMR spectra of ephemeric acid (**1**), methyl ephemerate (**3**) and methyl floridiolate (**4**) [3], which were quite similar to one another except for the δ values at C-12–C-16. In addition, ^{13}C NMR spectrum of **1** has the particularly low field signal [(δ_C 33.2 (*q*) (C-19)] due to the angular methyl group as seen in that of **4** δ_C 33.6 (*q*)) [2, 4], indicating that **1** should have a *cis* fused clerodane-type carbon skeleton. This assignment was further confirmed by the NOE experiments of **1** as described later. The remaining moiety ($C_5H_5O_2$) including C-12–C-16 attached to C-11 of ephemeric acid (**1**) was characterized on the basis of the following spectral data. IR absorption bands at 1755 and 1635 cm^{-1}, coupled with ^1H and ^{13}C NMR data [δ_H 2.0 (3H), 4.91 (1H) and 5.82 (1H); δ_C 14.0 (*q*), 81.9 (*d*), 116.8 (*d*), 169.5 (*s*) and 173.2 (*s*)], were assignable to a β-methyl butenolide group. The stereochemistry of ephemeric acid (**1**) was established by the NOESY experiments. A crossed peak between H-19 (δ_H 1.28) and H-10 (δ_H 1.62) shows the *cis* relationship between Me group at C-5 and H-10. The stereochemistry of Me group at C-8 was established by observation of a crossed peak between H-8 (δ_H 1.90) and H-10. No crossed peak between two Me groups at C-9 (δ_H 0.84) and C-5, between Me group at C-9 and H-8, and between Me group at C-9 and H-10 was observed whereas crossed peaks between the Me group at C-9 and H-2 (δ_H 2.41) and between the Me group at C-9 and H-3 (δ_H 6.83) were observed. These findings indicate the *trans* relationships between the two Me groups at C-9 and C-5, and between Me group at C-8 and H-10, respectively. The *cis* configuration between C-12 and C-15 was confirmed by the observation of a crossed peak between Me group at C-13 and H-14. Finally, the stereochemistry of C-12 was determined as follows. A crossed peak between H-10 and H-12 (δ_H 4.91) was very clearly observed. Crossed peaks



1 R= HO



4



2 R=

3 R= MeO

between Me at C-13 and Hα-1 (δ_H 1.80), and between Me group at C-13 and H-11a uncoupled with H-12 (the dihedral angle between H-11a and H-12 was *ca* 90° on a Dreiding model) were also observed. These data indicated that ephemeric acid had the stereochemistry as shown below. The appearance of the Hα-6 signal in unusually low field (δ_H 2.75) seems to be due to the anisotropy effect of the carboxylic acid and supports the stereochemistry of **1**. From above results, the structure of ephemeric acid can be represented as **1** (5*S*, 8*R*, 9*S*, 10*R*, 12*R*) or its antipode.

Ephemeroside (**2**) ($[\alpha]_D$ − 43.2° (MeOH; *c* 1.0) seems to be a β-D-glucoside of ephemeric acid (**1**) from the ^{13}C NMR spectra of both **1** and **2** (Table 1) together with SIMS [*m/z* 495 $(C_{26}H_{28}O_9 + H)^+$ and 517 $(C_{26}H_{28}O_9 + Na)^+$] in **2**. When treated with hydrogen chloride in methanol, erephemeroside was readily converted into (−)-ephemeric acid (**1**) and a mixture of methyl-α-D-glucopyranoside and its β-isomer. The mixture was subjected to acetylation, separation and then identification with an authentic sample of the corresponding acetate. On treating with diazomethane, ephemeroside gave no corresponding methyl ester but was completely recovered.

These chemical data indicate that the structure of ephemeroside is 17-β-D-glucopyranosyl ester of ephemeric aid (**2**) [δ_H 5.06 (1H, *d*, *J* = 9.77 Hz (H-1'), δ_C 95.1 (*d*) (C-1')].

In most of clerodane type diterpenes, the stereochemistry between C-12 and C-15 has the *trans* configuration as in **4**. The clerodane-type diterpenes having the *cis* configuration at that position are very rare and all of them are *trans*-clerodane [5–7]. Ephemeric acid (**1**) and ephemeroside (**2**) are the first *cis*-clerodanes having a butenolide which adopts the *cis* configuration between C-12 and C-15.

EXPERIMENTAL

Mp were uncorr. ^{1}H and ^{13}C NMR spectra were taken at 400 and 25 MHz respectively. Chemical shifts are given in ppm from TMS as an int. standard. Coupling constants are given in Hz.

Extraction and isolation—Fresh whole plants of *Ephemerantha comata* Hunt & Summerh (200 g), which were collected in Taiwan (R.O.C.), were immersed in MeOH (800 ml) at room temp. for 6 days and then filtered. The filtrates were concd under red. pres. to leave a greenish brown residue (7.2 g) which was partitioned between H_2O and AcOEt. The EtOAc layer was dried over Na_2SO_4 and then concd under red. pres. to leave a greenish-brown oil (2.7 g) which was directly chromatographed on silica gel (Merck 7734, 20 g) and eluted with a gradient of $CHCl_3$ and MeOH to afford two fractions [R_f 0.4 and 0.1, respectively, on silica gel (Merck 5715), $CHCl_3$–MeOH (10:1)]. The first fraction (0.5 g) was separated by preparative TLC (Merck 13895 and 5744) using a soln of $CHCl_3$–EtOAc (3:1) and then purified by recrystallization from $CHCl_3$–hexane to give ephemeric acid (**1**) (15 mg) as colourless needles. The second fraction (0.75 g) was subjected to MPLC (Merck Lobar column Si60, size A) and prep. TLC (Merck 5744), successively, using a soln of $CHCl_3$–MeOH (85:15) to give ephemeroside (**2**) (32 mg) as viscous liquid.

Ephemeric acid (**1**). Mp 183–185°; $[\alpha]_D$ − 75.0° ($CHCl_3$; *c* 0.56); HRMS *m/z*: Found 332.1972 $[M^+]$ ($C_{20}H_{28}O_4$ requires 332.1985); IR ν^{KBr} cm^{-1}: 3000 *br*, 1755, 1665, 1635; ^{1}H NMR see Table 2; ^{13}C NMR: see Table 1.

Ephemeroside (**2**). $[\alpha]_D$ − 43.2° (MeOH; *c* 1.0); SIMS *m/z*: 495 $(M + H)^+$, 517 $(M + Na)^+$; ^{1}H NMR (acetone-d_6): δ0.79 (3H, *d*, *J* = 6.59 Hz), 0.85 (3H, *s*), 1.29 (3H, *s*), 2.09 (3H, *d*, *J* = 1.46 Hz),

Table 1. ^{13}C NMR spectral data of ephemeric acid (1), ephemeroside (2) and methyl floridiolate (4)

Position	1	2	4 [3]
1	17.1 *t*	17.6 *t*	16.8 *t*
2	24.2 *t*	24.7 *t*	24.0 *t*
3	141.6 *d*	141.2 *d*	138.9 *d*
4	137.7 *s*	138.6 *s*	138.6 *s*
5	40.5 *s*	41.5 *s*	40.2 *s*
6	36.5 *t*	37.3 *t*	37.8 *t*
7	28.7 *t*	29.4 *t*	28.5 *t*
8	38.9 *d*	39.5 *d*	36.9 *d*
9	36.5 *s*	37.3 *s*	36.3 *s*
10	46.1 *d*	46.5 *d*	45.3 *d*
11	40.7 *t*	41.3 *t*	36.8 *t*
12	81.9 *d*	82.3 *d*	28.9 *t*
13	169.5 *s*	171.5 *s*	144.6 *s*
14	116.8 *d*	116.7 *d*	126.0 *d*
15	173.2 *s*	173.2 *s*	58.3 *t**
16	14.0 *q*	14.0 *q*	60.0 *t**
17	16.4 *q*	16.9 *q*	15.9 *q*
18	169.5 *s*	166.4 *s*	168.7 *s*
19	33.2 *q*	33.6 *q*	33.6 *q*
20	17.5 *q*	17.8 *q*	18.0 *q*
OMe	–	–	51.3 *q*
1′		95.1 *d*	
2′		73.7 *d*	
3′		78.1*d**	
4′		71.3 *d*	
5′		78.2 *d**	
6′		62.5 *t*	

*Values can be interchanged.

The solvents were $CDCl_3$ in 1 and 4, and acetone-d_6 in 2.

Table 2. ^1H NMR spectral data of ephemeric acid (1) in $CDCl_3$

Position	(ppm)	J (Hz)
1-α	1.80 *m*	
1-β	2.12 *m*	
2-α	2.41 *m*	
2-β	2.31 *m*	
3	6.83 *t*	3.66
4	–	
5	–	
6-α	2.75 *dt*	11.47, 3.18
6-β	1.20 *m*	
7-α	1.14 *m*	
7-β	1.34 *m*	
8-β	1.90 *m*	
9	–	
10-β	1.62 *d*	6.35
11-a	1.46 *dd*	10.25, 15.87
11-b	1.93 *d*	15.87
12	4.91 *d*	10.25
13	–	
14	5.82 *q*	1.46
15	– *q*	1.46
16	2.09 *d*	1.46
17	0.81 *d*	6.59
18	–	
19	1.28 *s*	
20	0.84 *s*	

Assignments were based on the ^1H–^1H and ^{13}C–^1H COSY.

5.06 (1H, *d*, *J* = 10.01 Hz), 5.59 (1H, *d*, *J* = 8.30 Hz), 5.80 (1H, *q*, *J* = 1.46 Hz), 6.70 (1H, *t*, *J* = 3.79 Hz); ^{13}C NMR: see Table 1.

Reaction of **1** *with diazomethane.* A soln of ephemeric acid (1) (7 mg) in $CHCl_3$ (1 ml) was treated with excess CH_2N_2 in ether (10 ml) at room temp. for 30 min. and then concd under red. pres. to leave a colourless viscous liquid. The product was purified by prep. TLC (Merck 5744) using $CHCl_3$ to afford the corresponding methyl ester (3) (7 mg), HRMS *m/z*: Found 346.2139 [M^+] ($C_{21}H_{30}O_4$ requires 346.2143); IR ν^{film} cm^{-1}: 1760, 1710; ^1H NMR ($CDCl_3$): δ0.80 (3H, *d*, *J* = 6.59 Hz), 0.83 (3H, *s*), 1.27 (3H, *s*), 2.08 (3H, *d*, *J* = 1.46 Hz), 3.72 (3H, *s*), 4.90 (1H, *d*, *J* = 10.50 Hz), 5.80 (1H, *q*, *J* = 1.46 Hz), 6.52 (1H, *t*, *J* = 3.90 Hz); ^{13}C NMR ($CDCl_3$): δ17.1 (*t*), 23.9 (*t*), 138.2 (*d*), 138.6 (*s*), 40.7 (*s*), 36.7 (*t*), 28.6 (*t*), 38.8 (*d*), 36.5 (*s*), 45.9 (*d*), 40.5 (*t*), 81.8 (*d*), 169.4 (*s*), 116.7 (*d*), 173.1 (*s*), 14.0 (*q*), 16.4 (*q*), 168.4 (*s*), 33.3 (*q*), 17.4 (*q*), 51.4 (*q*).

Reaction of **2** *with hydrogen chloride in methanol.* 2 (20 mg) was dissolved in MeOH (5 ml) containing HCl and then heated under reflux for 5 hr. After evaporation of the solvent under red. pres. the residue was subjected to partition between $CHCl_3$ and H_2O. The organic layer was dried over Na_2SO_4 and then concd to afford ephemeric acid (1) (10 mg) ($[\alpha]_D$ − 71.6°). The H_2O layer was successively concd under red. pres. acetylated with Ac_2O (0.3 ml) and pyridine (0.3 ml) and separated by prep. TLC (Merck 5715) using $CHCl_3$–ether–hexane (1:1:1) to afford an acetate (3 mg) ($[\alpha]_D$ + 66°, $CHCl_3$), which was identical with an authentic sample of methyl-α-D-glucopyranoside tetraacetate $[\alpha]_D$ + 100.3° ($CHCl_3$; *c* 2.0) derived from D-glucose.

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