One Alkenylphenol and Steroids from the Aquatic Plant *Monochoria vaginalis*

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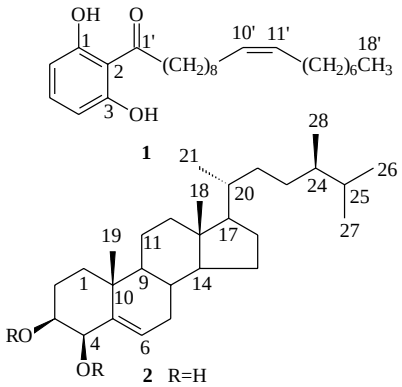
Two new compounds have been isolated from the whole plant of *Monochoria vaginalis* and characterized as: (10*Z*)-1-(2,6-dihydroxyphenyl)octadec-10-en-1-one (**1**) (20*R*,24*R*)-campest-5-ene-3β,4β-diol (**2**) together with nine known ones. The structures of these compounds were elucidated on the basis of spectral data and chemical evidence.

Keywords: *Monochoria vaginalis*; Pontederiaceae; Phenol; Sterol; Ozonolysis.

INTRODUCTION

The aquatic plant *Monochoria vaginalis* (BURM. f.) PRESL (Pontederiaceae) is widely distributed in the south of Taiwan and used for the treatment of asthma and fever.[1] Recently we reported the isolation and structural elucidation of two new cerebrosides and one new acylglycosyl sterol from *M. vaginalis*.[2] As a continuing study, a new alkenylphenol, (10*Z*)-1-(2,6-dihydroxyphenyl)octadec-10-en-1-one (**1**) and a new sterol, (20*R*,24*R*)-campest-5-ene-3β,4β-diol (**2**) together with nine known compounds, 24-methylenelophenol (**3**),[3-4] 4α-methyl-5α-ergosta-7,24(28)-diene-3β,4β-diol (**4**),[5] stigmast-4-en-3-one (**5**),[6] 6β-hydroxystigmast-4-ene-3-one (**6**),[6] cyclolauden-3β-ol (**7**),[7-8] vomifoliol (**8**),[9] dehydrovomifoliol (**9**),[10] 3-oxo-α-ionol (**10**),[11] and (-)-loliolide (**11**)[12] have been isolated and characterized by spectral and chemical evidence.



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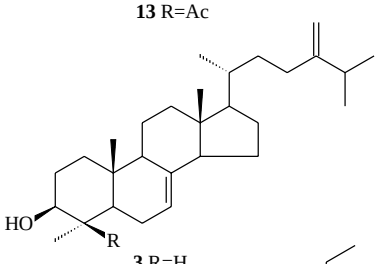
2 R=H
13 R=Ac



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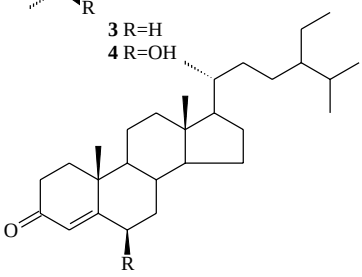
4 R=OH



5 R=H
6 R=OH

RESULTS AND DISCUSSION

The molecular formula of compound **1** was determined to be $C_{24}H_{38}O_3$ by high-resolution HR-EIMS. The IR spectrum revealed hydroxy (3310 cm^{-1}), chelating conjugated ketone (1635 cm^{-1}) and aromatic (1590, 1520 cm^{-1}) absorptions. The UV spectrum exhibited absorption (λ_{max}) in methanol at 223, 269 (sh) and 342 (sh) nm. The ^{1}H-NMR spectrum

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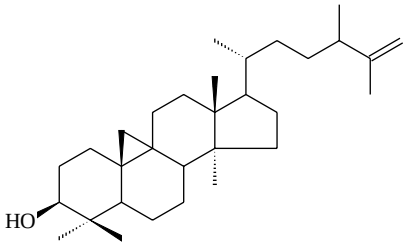

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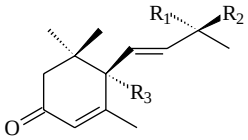
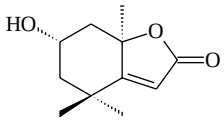


8 R_1=R_3=OH, R_2=H
9 R_1, R_2=O, R_3=OH
10 R_1=OH, R_2=R_3=H



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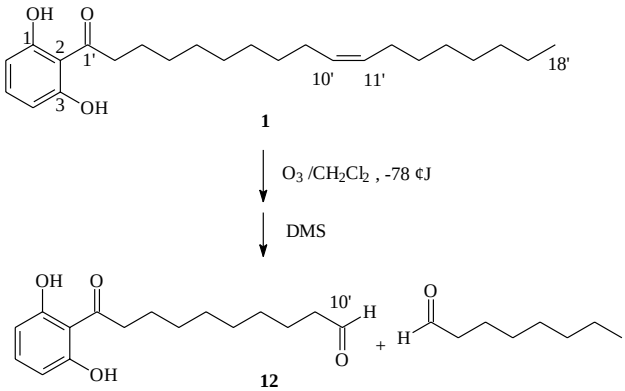
showed AM_2 type aromatic protons at δ 6.39 (2H, d, *J* = 8.0 Hz, H-4, 6) and 7.22 (1H, t, *J* = 8.0 Hz, H-5). The 8.0 Hz coupling constant indicated that three protons were adjacent to each other. Irradiation at δ 6.39 collapsed the triplet at δ 7.22 to a singlet. The other peaks in the ^{1}H-NMR spectrum showed a terminal methyl group at δ 0.86 (3H, t, *J* = 8.8 Hz, H-18′), an alkyl chain at δ 1.28 $(CH_2)_n$, a methylene group at δ 3.12 (2H, t, *J* = 7.4 Hz, H-2′) and a disubstituted double bond at δ 5.35 (2H, m, HC=CH). The ^{13}C-NMR spectrum of **1** contained a carbonyl group signal at δ 207.8 (C-1′), 1,2,3-trisubstituted aromatic signals at δ 108.5 (C-4, 6), 111.0 (C-2), 135.6 (C-5), 161.2 (C-1, 3), and a disubstituted olefinic signal at δ 130.0 (HC=CH). These NMR data suggested compound **1** possesses one carbonyl group, two hydroxyl groups as well as a disubstituted double bond in the C_{18} side chain.[13,14] The presence of a base fragmentation peak in HR-EIMS spectrum at *m/z* 137.0243 (Calcd for $C_7H_5O_3$; 137.0239) suggested the existence of a dihydroxybenzoyl moiety in **1**. The side chain olefin adopting a *Z* configuration could be supported by CH_2 peaks at δ 27.5, 27.7 (C-9′, 12′) in ^{13}C-NMR spectrum since the chemical shift values of allylic methylene were around δ 27 and 33 for *cis* and *trans* olefine, respectively.[15] Ozonolysis of **1** produced an aldehyde (**12**) (Fig. 1), which indicated that the double bond of the side chain was located between C-10′ and C-11′. From the above evidence, the structure of **1** was deduced as (10*Z*)-1-(2,6-dihydroxyphenyl)octadec-10-en-1-one.

The sterol **2** was isolated as a diacetyl derivative **13** which showed the pseudo-molecular peak at *m/z* 440.3635 [M^+-HOAc, $C_{30}H_{48}O_2$] by HR-EIMS. The diacetyl groups were revealed by the NMR signals (Table 1) at δ_H 2.05, 2.11 and δ_C 21.1, 21.5, 170.1, 170.3. The ^{1}H-NMR spectrum showed six methyl signals at δ 0.67 (3H, s, H_3-18), 0.78 (3H, d, *J* = 6.8 Hz, H_3-28), 0.80 and 0.85 (each 3H, d, *J* = 6.7 Hz, H_3-26, -27), 0.91 (3H, d, *J* = 6.5 Hz, H_3-21) and 1.13 (3H, s, H_3-19). Two acetoxyl methine signals at δ 4.74 (1H, ddd, *J* = 12.1, 4.4, 3.1Hz, H-3) and 5.50 (1H, d, *J* = 3.1 Hz, H-4) should have 3*S*, 4*R* configurations by the *J* values at 12.1 and 3.1 Hz, respectively. Additionally, the ^{1}H-NMR spectrum showed one olefinic signal at δ 5.82 (1H, dd, *J* = 5.1, 2.9 Hz, H-6). The above data and ^{13}C-NMR spectrum were almost identical with the authentic 3β,4β-diacetoxystigmast-5-ene.[16] The EI-MS spectrum signals at *m/z* 312 (M^+-HOAc-C_9H_{19}-H) and 271 (M^+-2HOAc-C_9H_{19}) revealed the presence of a saturated C_9 side chain. Furthermore, by comparing the chemical shift of H-21, H-26, H-27 and H-28 methyl signals suggested 20*R*,24*R* configurations.[3,5] Thus the structure of **2** was elucidated as (20*R*,24*R*)-campest-5-ene-3β,4β-diol.

EXPERIMENTAL SECTION

General Methods

EIMS spectra were taken with a JMS-HX-100 instrument and HR-EIMS were recorded on a JEOL LMS-SX 102 system. UV spectra were recorded on a Perkin Elmer Lambda 5 UV/VIS spectrophotometer. IR spectra were taken on a JASCO FT-IR-110 infrared spectrophotometer. Optical rotations were measured on a JASCO DIP-360 digital polarimeter.^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AM-400 NMR and Bruker DMX-600 NMR spectrometers. Column chromatography was performed using sil-



1 **12**

Fig. 1. Ozonolysis of compound **1**.

Table 1. ^{1}H- and ^{13}C-NMR Data of Compound **13**

Position	^{13}C	^{1}H	Position	^{13}C	^{1}H
1	36.8		16	28.2	
2	22.5		17	56.0	
3	72.9	4.74 (ddd, 12.1, 4.4, 3.1)	18	11.8	0.67 (s)
4	76.2	5.50 (d, 3.1)	19	20.2*	1.13 (s)
5	138.2		20	36.1	
6	131.7	5.82 (dd, 5.1, 2.9)	21	18.7	0.91 (d, 6.5)
7	32.0		22	33.7	
8	31.9		23	29.4	
9	50.2		24	38.8	
10	36.1		25	31.6	
11	25.5		26	20.4*	0.80 (*d*, 6.7)
12	39.6		27	20.5*	0.85 (*d*, 6.7)
13	42.3		28	15.4	0.78 (*d*, 6.8)
14	56.8		OAc	21.1; 170.1	2.08 (*s*)
15	24.2		OAc	21.5; 170.3	2.01 (*s*)

400 MHz (^{1}H) and 100 MHz (^{13}C), $CDCl_3$
*Assignments may be interchanged

ica gel (230-400 mesh, Merck) and charcoal activity (chromatography, Wako). Thin-layer chromatography (TLC) was conducted on precoated Kiesel gel 60 F_{254} plates (0.25 mm, Merck). Spots were located by ultraviolet illumination and by spraying the ferric chloride reagent or 10% sulfuric acid following by heating. MPLC was carried out on a Buchi MPLC system (pump, Buchi 688; detector, KAUER).

Plant Material

The dry whole plant (4.3 Kg) of *M. vaginalis* was collected from the south of Taiwan in August 1998. A voucher specimen was deposited at the Department of Chemical Engineering, Ta-Hwa Institute of Technology, Hsinchu, Taiwan.

Extraction and Separation

Dried whole plants of *M. vaginalis* were extracted with methanol (five times, each time 20 L) under reflux conditions for 4-6 hr. The methanolic layer was chromatographed on a charcoal column, eluting with MeOH, MeOH-CH_2Cl_2 (7:3) and CH_2Cl_2 to afford three fractions. Each fraction was concentrated to give brownish viscous residue. The CH_2Cl_2 layer (120 g) was chromatographed on a silica gel column (hexane-ethyl acetate gradient) to give six fractions (Frs 1-6). Fr. 3 was further separated by MPLC using silica gel columns (25% ethyl acetate/*n*-hexane) and prep. TLC (silica gel, 5% MeOH/CH_2Cl_2) to afford **1, 5** and **7**. Fr. 5 was separated by a combination of MPLC (silica gel, 50% ethyl acetate/*n*-hexane) and prep. TLC (silica gel, 10% MeOH/CH_2Cl_2) to afford **2-4**, **6**, **8-11**. Yields: compound **1** (39 mg), **2** (3 mg), **3** (18 mg), **4** (23 mg), **5** (52 mg), **6** (26 mg), **7** (16 mg), **8** (22 mg), **9** (2 mg), **10** (3 mg)**, 11** (2 mg).

(*Z*)-1-(2,6-Dihydroxyphenyl)-octadec-10-en-1-one (1)

A colorless gum, HR-EIMS *m/z*: 374.2831 (M^+, Calcd for $C_{24}H_{38}O_3$; 374.2821), *m/z*: 137.0243 (Calcd for $C_7H_5O_3$; 137.0239). EI-MS *m/z* (rel. int.): 374 (M^+, 20), 330 (19), 189 (18), 165 (73), 152 (76), 137 (100), 123 (14). IR (neat) ν_{max} cm^{-1}: 3310, 2950, 1635, 1590, 1520, 1240, 1040, 720. UV (MeOH) λ_{max} nm (ε): 342 (3000), 269 (11900), 223 (13800). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.86 (3H, t, J = 7.8 Hz, H-18′), 1.28 (H-4′ to H-8′; H-13′ to H-17′, br s), 1.62 (2H, m, H-3′), 2.02 (4H, m, H-9′, 12′), 3.12 (2H, t, J = 7.4 Hz, H-2′), 5.35 (center of AB system of H-10′ and H-11′, $J_{10,11}$~10 Hz), 6.39 (2H, d, J = 8.0 Hz, H-4, 6), 7.22 (1H, t, J = 8.0 Hz, H-5), 9.60 (br s, OH). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 14.1 (C-18′), 27.5, 27.7 (C-9′, 12′), 22.3, 22.7, 29.2, 29.4, 33.3 (C-3′ to C-8′; C-13′ to C-17′), 44.8 (C-2′), 108.5 (C-4, 6), 111.0 (C-2), 130.0 (C-10′, 11′), 135.6 (C-5), 161.2 (C-1, 3), 207.8 (C-1′). Ozonolysis of **1**: Compound **1** (5 mg) was dissolved and stirred in dichloromethane 5 mL at -78 °C (acetone-dry ice), and then ozone gas was blown in. The resulting solution was treated with DMS (Dimethyl sulfide, 3 mL). After being stirred for 12 hr, the homogeneous mixture was concentrated and then partitioned by ether/H_2O. The ether extract was purified by prep-TLC to give **12**. Compound **12**: A colorless gum, HR-MS *m/z*: 278.1534 (M^+, Calcd for $C_{16}H_{22}O_4$). EI-MS *m/z* (rel. int.): 278 (M^+, 8), 277(3), 137 (10), 149 (19), 87 (70), 74 (80), 18 (100). IR (neat) ν_{max} cm^{-1}: 3350, 2950,

2850, 2710, 1720, 1620, 1590, 1460.

(20*R*,24*R*)-3β,4β-Diacetoxycampest-5-ene (13)

Amorphous gum. $[\alpha]_D^{22}$ -45.5 (*c* = 0.1, $CHCl_3$). HR-MS *m/z*: 440.3635 [(M-HOAc)$^+$, Calcd for $C_{30}H_{48}O_2$; 440.3654]. EI-MS *m/z* (rel. int.): 440 (9), 399 (32), 398 (100), 396 (19), 312 (5), 271 (4). IR (neat) ν_{max} cm^{-1}: 2900, 2850, 1740, 1650, 1220, 1060. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: Table 1. ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: Table 1.

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