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SESQUITERPENOIDS FROM *EUPHORBIA WANGII*

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Key Word Index—*Euphorbia wangii*; Euphorbiaceae; sesquiterpenoids; caryophyllene derivatives.

Abstract—Four sesquiterpenes, structurally related to caryophyllene, cyclocaryophylla-4-en-8-ol, euphanginol, 14-hydroxy-4β,5α-epoxy-4,5-dihydrocaryophyllene and clovandiol, were isolated from whole plants of *Euphorbia wangii*. Their structures were elucidated by spectroscopic methods including 2D NMR techniques and by some chemical transformations. One of them is a new compound. This is the first investigation on sesquiterpenoids from the genus *Euphorbia*. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The genus *Euphorbia* is known to contain a variety of irritant diterpenoids [1, 2], but no sesquiterpenoids have been isolated from this genus. We have previously reported on the isolation and structural determination of some new diterpenoids from *Euphorbia* species, which are indigenous in western China and are used in Chinese folk medicine as antitumour remedies [3–6]. We now report on the isolation and structural elucidation of one new caryophyllene derivative, euphanginol (**2**), together with three known sesquiterpenes, cyclocaryophylla-4-en-8-ol (**1**), 14-hydroxy-4β,5α-epoxy-4,5-dihydrocaryophyllene (**3**) and clovandiol (**4**), which often coexist in the same plant as *ent*-kaurane diterpenoids [7], from *E. wangii* Oudejans.

RESULTS AND DISCUSSION

The acetone extract of the air-dried whole plants of *E. wangii* was decolourized with activated charcoal in the usual fashion, and the decolourized filtrate was concentrated to yield a syrup, which was subjected to column chromatography on silica gel (200–300 mesh) to afford compounds **1**–**4**.

The known clovandiol (**4**) had identical IR, EI-mass spectrometry, ^{1}H NMR and ^{13}C NMR spectra with those published in the literature [8, 9]. Compound (**3**), on the basis of its ^{1}H NMR, ^{13}C NMR (DEPT), ^{1}H-^{1}H COSY, ^{1}H-^{1}H NOESY, and ^{1}H-^{13}C COSY data, was shown to be 14-hydroxy-4β,5α-epoxy-4,5-dihydrocaryophyllene, whose EI-mass spectrometry, IR and ^{1}H NMR spectra were consistent with the data in ref. [10]. However, the ^{13}C resonances of **3** were completely assigned by 2D NMR techniques (^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY) and the configuration of the 4β,5α-epoxy group was confirmed by the cross peaks between H-14 (δ 3.89 and 3.21) and Me-13 (δ 1.02), and between H-5 (δ 2.93) and H-1 (δ 1.90) in the ^{1}H-^{1}H NOESY.

Compound **1** gave an IR absorption band for a hydroxyl group (3364 cm^{-1}). Its EI mass spectrum showed an $[M]^+$ at m/z 220 and $[M-H_2O]^+$ at m/z 202. HR mass spectrometry gave a molecular formula of $C_{15}H_{24}O$($[M]^+$, m/z = 220.1830). A combination of ^{13}C NMR and DEPT spectra indicated the presence of 15 carbons, two methyls, seven methylenes, two methines, two quaternary carbons (an oxygenated at δ 75.1) and a trisubstituted double bond (δ 126.1 and 138.1). The ^{1}H NMR spectrum of **1** displayed a trisubstituted double bond signal at δ 5.39 (1H, *ddd*, J = 2.7, 8.0, and 8.0 Hz). The ^{1}H NMR (δ 0.98, 6H) and ^{13}C NMR (δ 33.3 *s*, 30.0 *q*, and 21.8 *q*) spectra of **1** were compared with those of **3** and indicated the presence of a *gem*-dimethyl cyclobutane ring in **1**. From these data, **1** had to be a tricyclic sesquiterpene structurally related to caryophyllene [11–14]. Further 2D NMR experiments (^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY) showed the partial structures of **1**, which were separated by quaternary carbons i.e. —CH_2—CH—CH—CH—CH_2, —C=CH—CH_2—CH_2— and —CH_2—CH_2—. By attachment of the partial structures, compound **1** was established as cyclocaryophylla-4-en-8-ol, previously prepared by Uchida by the oxidation of β-caryophyllene (**5**) with lead tetraacetate [15]. In a previous paper [16], it was

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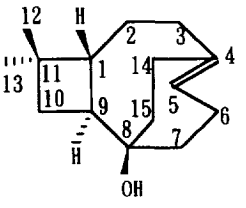

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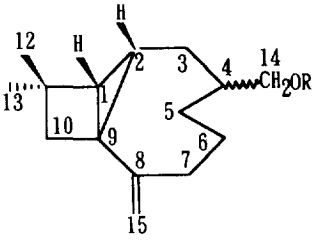

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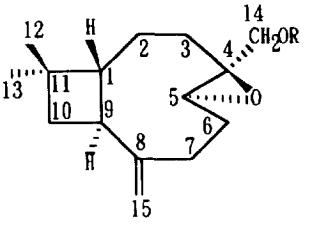
2a R=Ac



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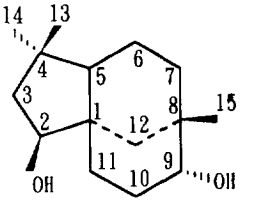

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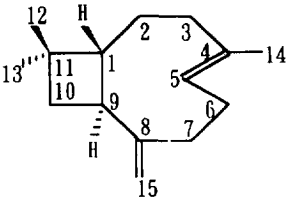

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reported that **1** is also present in clove oil based on a comparison of its GC-mass spectrometry with an authentic synthetic sample. However, the characterization of compound **1** as a natural product by NMR spectra is reported here for the first time. In addition, its ^1H and ^{13}C NMR spectral chemical shifts were unambiguously assigned by 2D NMR techniques (^1H-^1H COSY and ^1H-^{13}C COSY), and this corrected several erroneous assignments of ^{13}C resonances in the literature [15].

Compound **2** displayed IR absorption bands for a hydroxyl group (3335 cm^{-1}), and an exocyclic methylene group (3069, 1642, and 885 cm^{-1}). The EI mass spectrum exhibited an $[M]^+$ at *m/z* 220, and the HR mass spectrum showed a molecular ion peak at *m/z* 220.1822, which agreed with a molecular formula $C_{15}H_{24}O$ and four degrees of unsaturation. The ^1H NMR spectrum of **2** revealed the presence of two tertiary methyl signals (δ 0.95 and 1.00), exocyclic methylene signals (δ 4.51 and 4.57, each 1H, *m*), and a hydroxyl proton (δ 3.48, exchanged by D_2O), as well as two hydroxymethylene proton signals (δ 3.41 and 3.59, each 1H, *m*). The ^{13}C NMR and DEPT spectra showed 15 carbons including two methyls, six methylenes (an oxygenated at δ 62.8), three methines, two quaternary carbons, and an exomethylene (δ 108.2 and 152.1). Taking into account the above data and the required four degrees of unsaturation, compound **2** had to be a tricyclic sesquiterpene alcohol. In addition, the presence of a *gem*-dimethyl cyclobutane ring was suggested by comparison of the ^1H and ^{13}C NMR of **2** with those of **1** and **3**. 2D NMR experiments (^1H-^1H COSY and ^1H-^{13}C COSY) showed two main fragments in **2**, separated by quaternary carbons, —CH—CH—CH_2—CH(CH_2OH)—CH_2—CH_2—CH_2—C=CH_2— and —CH_2—. A ^1H-^{13}C COLOC NMR experiment gave cross peaks between C-8 (δ 152.1) and H-7 (δ 1.85 and 1.48), between C-9 (δ 54.3) and H-1 (δ 2.20), H-2 (δ 1.85), between C-11 (δ 42.5) and H-12 (δ 0.95) and H-13 (δ 1.00), and H-10 (δ 1.29, and 1.85), and between C-1 (δ 61.6) and H-12 (δ 0.95), and H-13 (δ 1.00), so that the tricyclic sesquiterpene structure of **2** contained a four-, three- and eight-membered ring system with the exocyclic methylene occurring between C-15 and C-8 together with a hydroxyl group at C-14. The quaternary signal of C-

Table 1. ^{1}H NMR spectral data of compounds **1**–**4***,†

H	**3**‡	**3a**‡	**1**§	**2**‡	*2a*‡	**4**§
1	1.90 *t* (9.6)	1.96 *t* (9.9)	1.61 *ddd* (10.2)	2.20 *d* (4.4)	2.22 *d* (4.5)	—
2	1.56 *m*	1.57 *m*	1.51 *m*	1.85 *m*	1.94 *m*	3.78 *dd* (5.8, 10.4)
2′	1.56 *m*	1.57 *m*	1.37 *m*	—	—	—
3	2.53 *dt* (3.5, 12.8)	2.36 *m*	2.08 *dd* (7.8, 11.6)	1.60 *m*	1.62 *m*	1.60–1.71 *m*
3′	0.70 *dt* (5.5, 12.8)	0.81 *ddd* (4.5, 7.2, 8.6)	1.76 *ddd* (7.8, 11.6, 11.6)	1.46 *m*	1.48 *m*	1.63–1.71 *m*
4				1.79 *m*	1.88 *m*	
5	2.93 *dd* (4.3, 10.8)	3.01 *dd* (4.4, 10.9)	5.39 *ddd* (2.7, 8.0, 8.0)	2.32 *m*	2.33 *brdd* (2.6, 7.1)	—
6	2.17 *m*	2.18 *m*	2.40 *m*	1.56 *m*	1.54 *m*	—
6′	1.31 *m*	1.31 *m*	1.90 *m*	1.56 *m*	1.54 *m*	1.10–1.60 *m*
7	2.33 *m*	2.30 *m*	2.21 *ddd* (4.4, 13.5, 13.5)	1.87 *m*	1.88 *m*	
7′	2.08 *m*	2.09 *m*	1.43 *m*	1.48 *m*	1.49 *m*	—
9	2.72 *q* (9.6)	2.80 *q* (9.6)	1.89 *m*			2.30 *brs*
10	1.63 *m*	1.63 *m*	1.54 *m*	1.83 *d* (11.4)	1.91 *d* (11.5)	2.02 *m*
11	—	—	1.39 *m*	1.29 *d* (11.4)	1.29 *d* (11.5)	2.02 *m*
12	0.99 *s*	1.03 *s*	0.98 *s*	0.95 *s*	0.95 *s*	1.10–1.60 *m*
13	1.02 *s*	1.06 *s*	0.98 *s*	1.00 *s*	1.00 *s*	0.84 *s*
14	3.89 *d* (12.2)	4.54 *d* (12.1)	2.41 *m*	3.59 *m*	4.02 *dd* (7.4, 10.9)	0.94 *s*
14′	3.21 *dd* (1.2, 12.2)	3.56 *dd* (1.2, 12.2)	1.96 *m*	3.41 *m*	3.59 *dd* (7.4, 10.9)	
15	4.98 *d* (1.9)	5.85 *d* (1.2)	2.27 *ddd* (8.8, 13.1, 13.1)	4.57 *m*	4.58 *m*	1.02 *s*
15′	4.82 *d* (1.9)	5.01 *br s*	1.44	4.51	4.52 *m*	—
OH	3.71	—		3.48	—	—
OAc	—	2.04 *s*	—		1.98 *s*	—

* *J* (Hz) in parentheses.
† Assignments from 2D-COSY experiments.
‡ $(CD_3)_2CO$.
§ $CDCl_3$.

Table 2. ^{13}C NMR spectral data of compounds **1–4***

C	3†	3a†	1‡	2†	2a†	4‡
1	50.6 *d*	49.6 *d*	47.8 *d*	61.6 *d*	61.7 *d*	44.2 *s*
2	27.1 *t*	27.0 *t*	33.5 *t*	58.0 *d*	58.0 *d*	80.8 *d*
3	35.9 *t*	34.1 *t*	36.4 *t*	26.6 *t*	26.7 *t*	47.5 *t*
4	62.6 *s*	60.1 *s*	138.1 *s*	51.7 *d*	48.1 *d*	34.7 *s*
5	63.6 *d*	63.6 *d*	126.1 *d*	32.8 *t*	32.9 *t*	50.5 *d*
6	30.7 *t*	31.1 *t*	23.6 *t*	33.5 *t*	33.6 *t*	26.0 *t*
7	29.7 *t*	29.6 *t*	35.4 *t*	30.0 *t*	30.0 *t*	33.1 *t*
8	152.6 *s*	152.6 *s*	75.1 *s*	152.1 *s*	151.8 *s*	37.1 *s*
9	49.2 *d*	49.6 *d*	52.0 *d*	54.3 *s*	54.3 *s*	75.1 *d*
10	40.1 *t*	40.4 *t*	26.2 *t*	46.3 *t*	46.0 *t*	26.4 *t*
11	34.6 *s*	35.0 *s*	33.3 *s*	42.5 *s*	42.4 *s*	20.6 *t*
12	29.9 *q*	29.9 *q*	30.0 *q*	26.9 *q*	26.9 *q*	35.5 *t*
13	21.8 *q*	21.7 *q*	21.8 *q*	26.1 *q*	26.2 *q*	28.3 *q*
14	62.0 *t*	64.6 *t*	24.5 *t*	62.8 *t*	65.3 *t*	31.4 *q*
15	113.0 *t*	113.7 *t*	37.6 *t*	108.2 *t*	108.5 *t*	25.4*q*
OAc		170.8 *s*				170.9 *s*
		20.6 *q*				20.8 *q*

* Assignments from DEPT, ^1H-^{13}C COSY, and ^1H-^{13}C COLOC data.
† $(CD_3)_2CO$.
‡ $CDCl_3$.

9 (δ_C 54.3), the doublet proton signal of H-1 (δ_H 2.20, $J = 4.4$ Hz), and the ABq proton signals of H-10 (δ_H 1.83, 1.29, $J = 11.4$ Hz) in the NMR spectra of **2**, compared with those of **1**, **3** and **5**, further revealed that the cyclopropanyl ring was formed by cyclization between C-9 and C-2 of **5**. The location of attachment of the hydroxyl group at C-14 was also confirmed by the shift effects of the acetylation product **2a**. A β-orientation of H-2 was assigned by the cross peaks between H-1 (δ 2.20) and H-12 (δ 0.95), and between H-2 (δ 1.95) and H-12 (δ 0.95) in the ^1H-^1H NOESY spectrum. However, the relative configuration of H-4 could not be confirmed due to the absence of cross peaks in the ^1H-^1H NOESY spectrum. As a result, compound **2** was confirmed as a new tricylic sesquiterpene alcohol, named euphanginol, which is structurally related to caryophyllene [12, 15].

EXPERIMENTAL

General. Mps uncorr.; IR: KBr, Nicolet 170-SX; ^1H NMR (400.13 MHz) and ^{13}C NMR (100.62 MHz): Bruker AM 400 FT-NMR, TMS as int. standard and $CDCl_3$ as solvent; HR-MS and EI-MS: VG ZAB-HS, direct inlet, 70 eV.

Plant material. Euphorbia wangii Oudejans was collected in Zouqu County Gansu Province, China, in September 1990 and identified by Associate Prof. Zhi-Li Zhao. A voucher specimen (no. 9046) was deposited at the Herbarium in the Department of Pharmacy, Lanzhou Medical College.

Extraction and isolation. Air-dried and powdered whole plants of *E. wangii* (10 kg) were repeatedly extracted (×4) with Me_2CO at room temp. The combined extracts were evapd to give a concd soln, which was decolourized with activated charcoal in the usual fashion. The decolourized filtrate was concd to yield a syrup (374 g). The syrup was absorbed on silica gel and chromatographed (CC), eluting with petrol (60–90°), Et_2O, EtOAc and Me_2CO, successively. The Et_2O eluate (82 g) was subjected to CC on silica gel and eluted with a gradient of cyclohexane and EtOAc to give 7 frs. Fr. 4 was purified by silica gel CC with CH_2Cl_2–Et_2O (8:1) to give **1** (17 mg) and **2** (23 mg); fr. 5 was purified by silica gel CC with petrol–Me_2CO (4:1) to give **3** (90 mg); and fr. 6 was purified by silica gel CC with petrol–Me_2CO (2:1) to give the known sesquiterpene **4** (35 mg).

Cyclocaryophylla-4-en-8-ol (**1**). Needles, mp 148–149°; $[\alpha]_D^{24.5}$ +166.9° ($CHCl_3$, *c* 0.88). IR ν_{max} cm^{-1}: 3364, 2954, 2852, 1454, 1360, 1274, 1191, 1116, 1088, 1046, 990, 923, 886, 852; EIMS *m/z* (rel. int.): 220 $[M]^+$ (2), 202 $[M\text{-}H_2O]^+$ (23), 187 $[M\text{-}H_2O\text{-}Me]^+$ (21), 159 (30), 146 (32), 131 (33), 119 (22), 105 (33), 91 (49), 81 (54), 67 (37), 55 (48), 41 (100); HRMS *m/z* 220.1830, $C_{15}H_{24}O$ requires: 220.1827; ^1H NMR and ^{13}C NMR: Tables 1 and 2.

Euphanginol (**2**). Gum, $[\alpha]_D^{14}$ +20.12° (Me_2CO, *c* 0.60). IR ν_{max} cm^{-1}: 3335, 3068, 2938, 2871, 1642, 1467, 1452, 1382, 1363, 1068, 1030, 885; EIMS *m/z* (rel. int.): 220 $[M]^+$ (58), 205 $[M\text{-}Me]^+$ (22), 202 $[M\text{-}H_2O]^+$ (14), 189 (66), 177 (44), 159 (57), 145 (41), 131(45), 119 (48), 105 (100), 91 (100), 79 (57), 69 (74), 55 (39); HRMS *m/z* 220.1822, $C_{15}H_{24}O$ requires: 220.1827); ^1H NMR and ^{13}C NMR: Tables 1 and 2.

Acetylation of euphanginol. **2** (33 mg) was dissolved in Ac_2O–pyridine (1:1). The soln was left overnight at room temp. and worked up in the usual way to yield

2a (25 mg). Gum; IR ν_{max} cm^{-1}: 3068, 2942, 2870, 1742, 1643, 1455, 1387, 1366, 1236, 1034, 970, 885; EIMS *m/z* (rel. int.): 262 $[M]^+$ (22), 219 $[M\text{-}Ac]^+$ (3), 202 $[M\text{-}HOAc]^+$ (95), 187 (18), 175 (14), 159 (100), 145 (22), 133 (36), 119 (47), 105 (46), 91 (72), 69 (55), 55 (30), 43 (100); ^1H NMR and ^{13}C NMR: Tables 1 and 2.

14-*Hydroxy*-4β,5α-*epoxy*-4,5-*dihydrocaryophyllene* (**3**). Needles, mp 40–42°; IR ν_{max} cm^{-1}: 3428, 3069, 2947, 2864, 1631, 1452, 1367, 1277, 1158, 1110, 1046, 1015, 947, 869 cm^{-1}; EIMS *m/z* (rel. int.): 237 $[M+1]^+$ (12), 219 $[M+1\text{-}H_2O]^+$ (52), 201 $[M+1\text{-}2H_2O]^+$ (100), 187 (20), 175 (25), 159 (30), 145 (44), 133 (32), 131 (33), 121 (52), 93 (85), 81 (52), 69 (45), 55 (34); ^1H NMR and ^{13}C NMR: Tables 1 and 2.

Acetylation of 14-*hydroxy*-4β,5α-*epoxy*-4,5-*dihydrocaryophyllene*. **3** (57 mg) was dissolved in Ac_2O–pyridine (1:1). The soln was left overnight at room temp. and worked up in the usual way to yield **3a** (55 mg). Gum; IR ν_{max} cm^{-1}: 3064, 2948, 2866, 1740, 1630, 1452, 1390, 1368, 1234, 1162, 1117, 1034, 950, 908, 871; EIMS *m/z* (rel. int.): 279 $[M+1]^+$ (3), 253 (5), 219 $[M+1\text{-}HOAc]^+$ (11), 201 (15), 185 (7), 175 (13), 149 (17), 136 (22), 119 (32), 108 (55), 93 (82), 79 (70), 69 (48), 55 (46), 43 (100); ^1H NMR and ^{13}C NMR: Tables 1 and 2.

Clovandiol (**4**). Needles, mp 152–153°. IR ν_{max} cm^{-1}: 3423, 3315, 2949, 2860, 1463, 1353, 1080, 1040, 994, 963; EIMS *m/z* (rel. int.): 238 $[M]^+$ (14), 220 (31), 205 (15), 182 (37), 164 (100), 150 (30), 135 (38), 123 (26), 107 (36), 93 (36), 81 (34), 69 (25), 55 (33), 41 (67); ^1H NMR and ^{13}C NMR: Tables 1 and 2.

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