Sesquiterpenes from *Baeckea frutescens*

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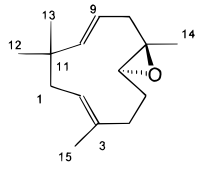
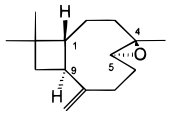
The aerial parts of *Baeckea frutescens* yielded three sesquiterpenes (**1**−**3**) belonging to the humulane, caryophyllane, and clovane classes, including the new sesquiterpene (−)-clovane-2,9-diol (**3**). Circumstantial evidence is presented that these classes are biogenetically related.

Baeckea frutescens L. (syn. *Baeckea chinensis* Gaertner; Myrtaceae) is widespread in Hong Kong and is used in traditional Chinese medicine for treating rheumatism and snake bites.[1] There has been one previous phytochemical study of *B. frutescens*, which reported three phloroglucinols.[2]

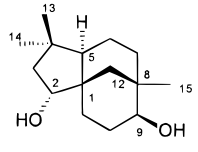
Column chromatography followed by HPLC of a CH_2Cl_2 extract of the aerial parts of *B. frutescens* yielded three sesquiterpenes (**1**−**3**), which were identified largely on the basis of 2D-NMR spectroscopy. Analysis of the results of the PFG−HSQC, the PFG−HMBC, and the $^1H-^1H$ COSY (proton−proton couplings) NMR spectra (500 MHz) established the structure of **1** and also allowed determination of chemical shifts for all carbons and protons in the molecule. Compound **1** has been isolated previously as a constituent of *Zingiber zerumbet* and named humulene epoxide II;[3−6] our spectra agreed well with ^{13}C- and partial ^1H-NMR data reported for humulene epoxide II in the literature.[3,7,8] Full ^{13}C- and ^1H-NMR assignments for **1** are presented in Table 1 for the first time. The optical rotation of humulene epoxide II from nature is variously reported as −25°, −31°, −10° and −43°,[4,5,8,9] whereas **1** from *Baeckea frutescens* had zero optical rotation and must therefore be a racemic mixture of *trans* epoxides.



1



2



3

The ^{13}C-NMR signals (125 MHz) for the C-12- and C-13 methyl groups in **1** showed strong line broadening relative to other peaks. This broadening was progressively reduced as the temperature was raised, both resonances attaining a normal line-shape at 60 °C in C_6H_6-d_6. Such behavior would be consistent with a degree of conformational rigidity in the portion of the 11-membered ring next to the *gem*-dimethyl group, relative to the rest of the molecule, which is overcome at higher temperatures. In support of this, correlations in the NOESY spectrum clearly demonstrated that there is a preferred conformation for the two double bonds flanking the C-12 and C-13 methyl groups (as shown in Scheme 1) at room temperature (NOESY gave no clear indication of a conformational preference for the other half of the ring). Observation of this conformational preference is interesting because the two double bonds are almost parallel, and the alkene protons at the C-2 and C-10 positions project towards different faces of the molecule. This same conformation has been proposed to account for rearrangements of humulene epoxide II (**1**) in superacid and has been suggested to be the most stable conformer from molecular modeling studies.[10] Our own molecular modeling calculations (using the MM2* force field of Macromodel[11]) failed to show a clear conformational preference for **1**: the minimized energies for the five lowest energy conformers were within 10 kJ mol^{-1} of each other. Conformations calculated for the second and third lowest energy states incorporated the partial structures shown for conformers **a** and **b** in Scheme 1.

The observed conformation for **1** would naturally yield *trans*-caryophyllanes (possibly via an intramolecular ene reaction), which are often found in association with humulanes. In support of this possibility, we have isolated two other sesquiterpenes (**2** and **3**) from the same extract whose structures are consistent with such cyclization of (±)-humulene epoxide II.

Compound **2** was identified as the known compound caryophyllene-4β,5α-epoxide (1*R*,4*R*,5*R*,9*S*) from its NMR spectra (300 MHz) and optical rotation.[12−16] Our assignments of the proton and carbon resonances for **2** agreed with those reported in the literature.[17] Compound **2** can clearly be formed from **1** by direct cyclization of the 6*R*,7*R* form of **1**, as postulated above (Scheme 2).

Compound **3** was identified as the novel compound (−)-clovane-2,9-diol (1*R*,2*R*,5*R*,8*S*,9*S*) in the following manner. Accurate mass spectrometry of **3** demonstrated the molecular formula $C_{15}H_{26}O_2$. IR spectroscopy showed a strong band at 3383 cm^{-1} indicative of hydroxyl group functionality(ies). The ^{13}C/DEPT-NMR spectrum (125 MHz) indicated 15 carbons with 24 directly attached protons and analysis of the chemical shifts suggested the presence of two oxygenated carbons (δ_C 80.9 CH, 75.1 CH) and the absence of double bonds. Consequently, **3** was established as a tricyclic compound with two hydroxyl groups. The complete structure for **3** and assignments for all proton and carbon resonances were deduced from analysis of 2D-NMR spectra (500 MHz) (high-resolution spectra were required because of the overlap of several proton chemical shifts), and the relative stereochemistry of **3** was deduced from correlations observed in the NOESY spectrum (Table 2). In fact, both the ^1H- and ^{13}C-NMR spectra for **3** agreed well with those reported for clovane-2,9-diol (1*S*,2*S*,5*S*,8*R*,9*R*).[12,13,18] The optical rotation for **3** from *B. frutescens* ($[\alpha]_D$ −3.5°) was of opposite sign to that

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Table 1. NMR Data for Compound **1**

position	δ_C	multiplicity[a]	δ_H	HMBC correlation from δ_H to δ_C	$^1H-^1H$ COSY correlation (δ)
1	40.2	CH_2	1.99	131.9, 125.7, 36.5, 29.0	4.99, 1.87
			1.87	131.9, 125.7	4.99, 1.99
2	125.7	CH	4.99	36.6, 15.1	1.99, 1.87
3	131.9	C			
4	36.6	CH_2	2.20	131.9, 125.7, 24.8	2.10, 2.14
			2.10	131.9, 125.7, 24.8	2.20
5	24.8	CH_2	2.14	62.0	2.20, 1.37
			1.37	63.2, 36.6	2.52, 2.14
6	62.0	CH	2.52	24.8	1.37
7	63.2	C			
8	42.5	CH_2	2.57	143.1, 122.1, 63.2, 17.2	5.29, 1.65
			1.65	143.1, 122.1, 63.2, 17.2	5.92, 2.57
9	122.1	CH	5.29	143.1, 63.2, 42.6, 36.5	5.16, 2.57, 1.65
10	143.1	CH	5.16	122.1, 42.6, 36.5, 29.0, 25.6	5.29
11	36.5	C			
12	29.0	CH_3	1.09	143.1, 40.2, 36.5, 25.6	
13	25.6	CH_3	1.11	143.1, 40.2, 36.5, 29.0	
14	17.2	CH_3	1.31	63.2, 42.6	
15	15.1	CH_3	1.57	131.9, 125.7, 36.6	

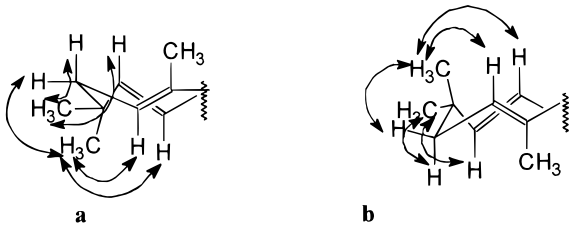
[a] Values were determined from the DEPT spectrum.

Table 2. NMR Data for Compound **3**

position	δ_C	multiplicity[a]	δ_H	HMBC correlation from δ_H to δ_C	$^1H-^1H$ COSY correlation (δ)	NOESY correlation (δ)
1	44.2	C				
2	80.9	CH	3.79	47.6, 35.5, 26.4	1.72, 1.51	1.72, 1.56, 1.51, 0.91, 0.86
3	47.6	CH_2	1.72	80.9, 50.5, 44.2, 37.2, 25.4	3.79, 1.51	3.79, 1.51, 0.86
			1.51	80.9, 44.2, 37.2, 31.5, 25.4	3.79, 1.72	3.79, 1.72, 1.03
4	37.2	C				
5	50.5	CH	1.43	44.2, 37.2, 35.5, 31.5, 26.4, 25.4, 20.7		2.00, 1.03
6	20.7	CH_2	1.42	50.5, 34.7, 33.1	1.32, 1.12	1.12
			1.32	50.5, 37.2, 33.1	1.42, 1.39, 1.12	0.86
7	33.1	CH_2	1.39	75.1, 50.5, 34.7, 28.3, 20.7	1.32, 1.12	0.96
			1.12	75.1, 34.7, 28.3, 20.7	1.42, 1.39, 1.32	3.33, 1.42
8	34.7	C				
9	75.1	CH	3.33	35.5, 26.4	2.00, 1.64	2.00, 1.64, 1.12, 0.96
10	26.0	CH_2	2.00	26.4	3.33, 1.68, 1.64, 1.08	3.33, 1.64, 1.43
			1.64	75.1, 44.2, 34.7	3.33, 2.00, 1.68, 1.08	3.33, 2.00
11	26.4	CH_2	1.68	50.5, 44.2, 35.5, 26.0	2.00, 1.64, 1.08	1.08
			1.08	35.5	2.00, 1.68, 1.64	1.68
12	35.5	CH_2	1.56	75.1, 50.5, 44.2, 34.7, 33.1, 28.3, 26.4	0.91	3.79, 0.91
			0.91	80.9, 75.1, 44.2, 34.7, 28.3, 26.4	1.56	3.79, 1.56
13	25.4	CH_3	0.86	50.5, 47.6, 37.2, 31.5		3.79, 1.72, 1.32, 1.03
14	31.5	CH_3	1.03	50.5, 47.6, 37.2, 25.4		1.51, 1.43, 0.86
15	28.3	CH_3	0.96	75.1, 35.5, 34.7, 33.1		3.33, 1.39

[a] Values were determined from the DEPT spectrum.

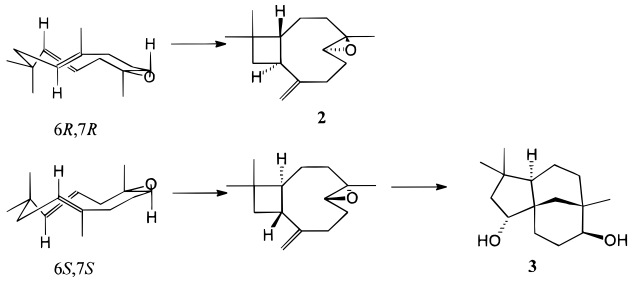
Scheme 1. Preferred Conformations for **1** (**a** and/or **b**) As Deduced from NOESY Correlations (Indicated by Double-Headed Arrows)



reported in the literature (variously given as +3.2° or +5° from both natural and synthetic sources,[13,19] (+)-clovane-2,9-diol (1*S*,2*S*,5*S*,8*R*,9*R*) prepared by ourselves from **2** (commercially available) according to a literature procedure[19] gave $[\alpha]_D$ +3.8° (*c* 4.5, $CHCl_3$). It thus seems likely that **3** is the mirror image of the compound reported in the literature: we propose the name (−)-clovane-2,9-diol, associated with the absolute stereochemistry (1*R*,2*R*,5*R*,8*S*,9*S*).

Compound **3** can be derived from the 6*S*,7*S* form of **1** by cyclization to caryophyllene oxide

Scheme 2. Proposed Conversion of **1** (Racemic) into **2** and **3**



(1*S*,4*S*,5*S*,9*R*) followed by subsequent rearrangement to the clovane skeleton (Scheme 2). (+)-Clovane-2,9-diol (1*S*,2*S*,5*S*,8*R*,9*R*) has been reported to arise from **2** both naturally and synthetically by just such a rearrangement.[13, 19]

Experimental Section

General Experimental Procedures. FTIR spectra were recorded in CCl_4 on a Shimadzu FTIR-8201 PC

instrument. All NMR experiments were performed on a Bruker DPX 300 or a DRX 500 instrument with $CDCl_3$ as solvent. PFG−HSQC and PFG−HMBC spectra were normally recorded with 2048 data points in F_2 and 128 data points in F_1, while high-resolution experiments had 8192 data points in F_2 and 1024 data points in F_1. MS were recorded in EI mode (70 eV) on a Finnigan-MAT 95 MS spectrometer. Column chromatography was made on Si gel Merck (60−200 μm). TLC plates were developed using *p*-anisaldehyde. HPLC separations were performed using a PREP-SIL 20 mm × 25 cm column, flow rate 8 mL/min, with refractive index detection.

Plant Material. *Baeckea frutescens* was collected in November 1995, during flowering at Plover Cove Country Park, New Territories, Hong Kong. A voucher specimen is deposited in the University of Hong Kong Herbarium (GDBROWN 96/1).

Extraction and Isolation. The aerial parts of *B. frutescens* (577 g) were ground to a fine powder under liquid N_2 and immediately extracted with CH_2Cl_2 in a Soxhlet apparatus (8 h). The organic extract was then dried and evaporated under vacuum to yield a dark green oil (8.85 g; 1.5% w/w), which was separated chromatographically as follows: **1** (15 mg) by column chromatography (R_f 0.25 in 6% EtOAc/hexane; staining violet) followed by HPLC (t_R 15.9 min in 6% EtOAc/hexane); **2** (15 mg) by column chromatography (R_f 0.25 in 6% EtOAc/hexane; staining yellow) followed by HPLC (t_R 16.4 min in 6% EtOAc/hexane); **3** (7 mg) by column chromatography (R_f 0.12 in 50% EtOAc/hexane; staining pink) followed by HPLC (t_R 23.9 min in 50% EtOAc/hexane).

(±)-Humulene epoxide II (1): obtained as a colorless oil; $[\alpha]_D$ 0.0° (*c* 0.64, $CHCl_3$,); IR ν max 2961, 2930, 2870, 1690, 1460, 1448, 1387, 1074 cm^{-1} ;^{1}H NMR (500 MHz) δ 5.29 (1H, ddd, J = 15.8, 10.2, 5.1 Hz, H-9), 5.16 (1H, d, J = 15.8 Hz, H-10), 4.99 (1H, dd, J = 9.5, 5.2 Hz, H-2), 2.57 (1H, dd, J = 12.2, 5.1 Hz, H-8), 2.52 (1H, dd, J = 10.2, 3.9 Hz, H-6), 1.99 (1H, dd, J = 13.7, 9.2 Hz, H-1), 1.87 (1H, dd, J = 13.7, 5.2 Hz, H-1), 1.65 (1H, dd, J = 12.2, 10.2 Hz, H-8), 1.57 (3H, s, H-15), 1.31 (3H, s, H-14), 1.11 (3H, s, H-13); 1.09 (3H, s, H-12); ^{13}C-NMR data, see Table 1; EIMS *m/z* [M^+] 220.1822 (Δ +0.5 mmu for $C_{15}H_{24}O$) (2), 138 (36), 133 (30), 111 (30), 109 (100), 107 (57).

Caryophyllene 4β,5α-Epoxide (2). Data were in agreement with published values.[12−18]

(−)-Clovane-2,9-diol (3): obtained as a white solid; mp 147−149 °C; $[\alpha]_D$ −3.5° (*c* 0.16, $CHCl_3$); IR ν max 3383, 2952, 2930, 2868, 1463, 1074 cm^{-1}; ^{1}H NMR (500 MHz) δ 3.79 (1H, dd, J = 10.0, 6.0 Hz, H-2), 3.33 (1H, s, H-9), 1.03 (3H, d, J = 2.0 Hz, H-14), 0.96 (3H, s, H-15), 0.86 (3H, s, H-13); ^{13}C-NMR data, see Table 2; EIMS *m/z* [M^+] 238.1929 (Δ +0.3 mmu for $C_{15}H_{26}O_2$) (25), 220 (57), 182 (49), 179 (42), 164 (100), 161 (32), 123 (18).

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