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AN AROMADENDRANE-TYPE SESQUITERPENOID FROM THE LIVERWORT *CALYPOGEIA AZUREA*

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Key Word Index—*Calypogeia azurea*; Hepaticae; aromadendrane; β-gurjunene; sesquiterpene; azulene.

Abstract—A new aromadendrane-type sesquiterpenoid, 1,2-dehydro-3-oxo-β-gurjunene, together with known 1,4-dimethyl-azulene, has been isolated from the liverwort *Calypogeia azurea* grown in the field. The structure of 1,2-dehydro-3-oxo-β-gurjunene was established by spectroscopic methods, including X-ray analysis that provided its relative stereochemistry.

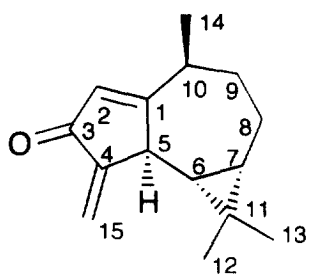
INTRODUCTION

Liverworts are known to be a rich source of sesquiterpenoids and diterpenoids, including new structural types [1–7]. Several of these display various biological activities [1]. For example, 4-methylazulene-1-carboxylic acid produced by suspension cells of *Calypogeia azurea* Stotler et Crotz showed anti-inflammatory and anti-ulcer activity [8]. *Calypogeia* species (Calypogeiaceae) are rich sources of azulenes and indenes [1, 9]. Meuche and Huneck reported the first isolation and structure elucidation of azulene derivatives from *C. azurea* [10]. We have re-investigated *C. azurea* and now report the structure of the newly isolated aromadendrane-type sesquiterpenoid 1,2-dehydro-3-oxo-β-gurjunene (**1**) from *C. azurea* grown in the field.

RESULTS AND DISCUSSION

The ethyl ether extract of *Calypogeia azurea* was chromatographed on silica gel and further purified by HPLC on an Si-60 column to give a new sesquiterpenoid **1**, together with the known 1,4-dimethylazulene (**2**).

1,2-dehydro-3-oxo-β-gurjunene (**1**) (5.7 mg from 109.8 g air-dried gametophytes) was obtained as colourless crystals (mp 90.0–91.5°), $[\alpha]_D^{23}$ +3.33. The molecular formula was determined by HR-EIMS as $C_{15}H_{20}O$ (*m/z* 216.1504; calculated for 216.1514). The UV absorption at 255 nm and IR absorptions at 1692 and 1658 cm^{-1}, together with a ^{13}C peak resonating at δ 196.31, indicated the presence of an α,β-unsaturated carbonyl group. The ^1H NMR spectrum displayed two singlet methyls (δ 1.06 and 1.23), one doublet methyl (δ 1.26, J = 6.4 Hz), three olefinic protons (δ 5.20, 5.97 and 6.10) and eight other protons. The presence of a cyclopropane ring was suggested by the ^1H peaks resonating at δ 0.76 (*dddd*, $J_{H\text{-}6,H\text{-}7}$ = 9.3 Hz, H-7) and 0.40 (*t*, $J_{H\text{-}5,H\text{-}6}$ = 9.3 Hz, H-6). The combined ^1H and ^{13}C NMR assignments with DEPT, PFG-DQFCOSY, PFG-HMQC, PFG-HMBC and selec-



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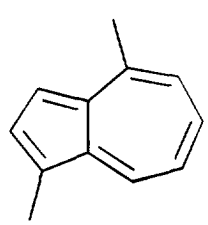

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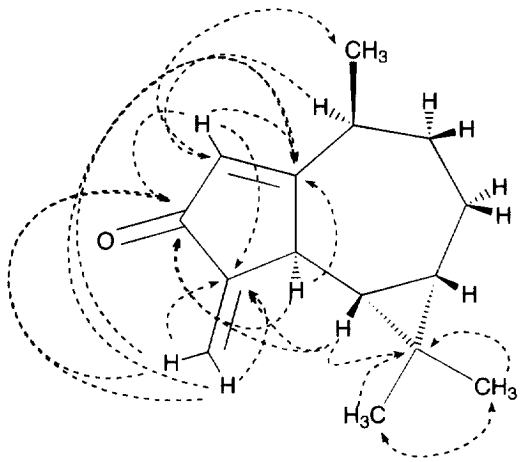

Fig. 1. Important HMBC correlations of **1**.

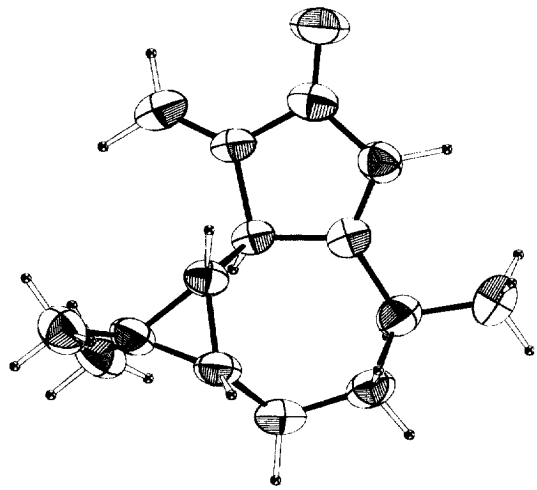

Fig. 3. An ORTEP drawing of **1**.

tive PFG-ROESY experiments revealed the complete structure of **1**. A partial structure, >CHCH-$CHCH_2CH_2CH(CH_3)$- unit in the cycloheptane ring was confirmed by ^{1}H-^{1}H COSY, ^{1}H-^{1}H DQF COSY and HMQC. A > C=CH_2 moiety was indicated by ^{1}H signals at δ 5.12 (*d*, J = 1.0 Hz), and 5.97 (*s*), and ^{13}C signals at δ 114.24 (CH_2) and 149.26 (C). The ^{1}H-^{1}H long-range couplings between H-5 and methylene protons at C-15 indicated that a C-4=C-15H_2 unit is attached to C-5. Segments as described above were assembled by HMBC connectivities (Fig. 1), that are in agreement with an aromadendrane skeleton. NOESY and ROESY experiments performed on **1** established the relative configurations according to Fig. 2. From NOEs between H-5 and H-10, between H-5 and Me-12, between H-6 and Me-13, between H-7 and Me-13, between H-7 and H-9β, and between H-8α and H-10, the inspection of a molecular model required the proposed configuration with the protons at C-5 and C-10 in the axial α-position. This structure was finally proven by a single crystal X-ray analysis of **1** (Fig. 3).

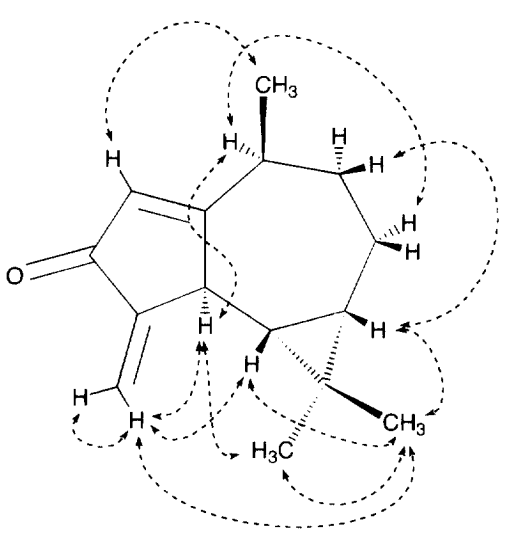

Fig. 2. NOE and ROE interactions of **1**.

Meuche and Huneck [10] reported the first isolation of 1,4-dimethylazulene in the blue oil bodies of *C. azurea*. Re-investigations of *C. azurea* revealed the presence of a number of azulenes [8, 11]. The sesquiterpenes anastreptene, calamenene and *ent*-bicyclogermacrene, were also isolated from *C. azurea* [1]. Nakagawara *et al.* [8] proposed that bicyclogermacrene and anastreptene are plausible biosynthetic precursors of azulenes in suspension cells of *C. azurea*. 1,2-Dehydro-3-oxo-β-gurjunene (**1**) may be also biogenetically related to azulenes.

EXPERIMENTAL

General. NMR measurements were performed on JEOL JNM-A600 with a pulsed field gradient (PFG) unit spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solns at 600 and 150 MHz, and the chemical shifts are given relative to the TMS peak at 0.00 ppm. 2D DQF-COSY, HMQC, HMBC, and selective 1D-PFG-ROESY experiments were recorded using standard pulse sequence with z-axis PFG. The usual pulse sequence were used in NOESY and DEPT experiments. IR: KBr pellet, UV and optical rotations: EtOH and $CHCl_3$, respectively. CD was measured in MeOH.

Plant material. Calypogeia azurea (109.8 g, DW) was collected at Rakko mountain (altitude 500 m), Hokkaido, Japan, in September 1995 and was identified by Dr Furuki. A voucher specimen is deposited at the Department of Bioresource Science, Obihiro University of Agriculture and Veterinary Medicine.

Extraction and isolation. Powdered dry plant material was extracted with Et_2O ($\times$ 3). The combined Et_2O soln. was evapd to dryness under red. pres. The residue (569.6 mg) was separated into 9 frs by vacuum liquid chromatography (VLC) on silica gel (5.4 $\times$ 2.0 cm i.d., *n*-hexane–EtOAc stepwise gradient). Sepn of

fr. 1 (*n*-hexane, 65.0 mg) by HPLC (*n*-hexane–EtOAc, 19:1) yielded **2** (9.1 mg), and sepn of fr. 2 (*n*-hexane–EtOAc; 19:1, 156.2 mg) by HPLC (*n*-hexane–EtOAc. 9:1) yielded **1** (5.7 mg).

1,2-*Dehydro*-3-*oxo*-β-*gurjunene* (**1**). Needles (from *n*-hexane–EtOAc), mp 90.0–91.5°; $[\alpha]_D$ +3.33 (*c* 0.240, $CHCl_3$); CD (MeOH *c* 0.053); $\Delta\varepsilon_{204}$ −0.47, $\Delta\varepsilon_{221.5}$ +2.52, $\Delta\varepsilon_{284}$ −1.62, $\Delta\varepsilon_{336}$ +0.14; HR-EIMS; $[M]^+$ 216.1504 (called for $C_{15}H_{20}O$ 216.1514) EIMS *m/z* (rel. int.): 216 (49), 201 (45), 188 (12), 173 (50), 160 (46), 145 (40), 122, (40), 120 (50), 95 (100), 91 (53), 67 (35), 55 (31), 41 (46); UV λ_{max} nm (log ε): 255 (3.93); IR ν_{max} cm^{-1}: 1692, 1658, 1592, 1458, 1388, 1275, 1150, 940, 892, 866 and 675; ^1H and ^{13}C NMR: see Table 1.

X-ray crystal analysis of **1**. Crystal data: $C_{15}H_{20}O$, orthorhombic $P2_12_12_1$, $a = 8.4634$ (3), $b = 10.0911$ (6), $c = 15.6503$ (8) Å^3, $z = 4$, $Dc = 1.075$ g cm^{-1}, λ (CuKα) = 1.54184 Å, $\mu = 4.7$ cm^{-1}, F (000) = 472, $R = 0.074$ for 1162 unique reflections with $|F_o| > 2\sigma(|F_o|)$. The structure was solved by the direct method using MULTAN 78. Hydrogen atoms were calculated assuming ideal geometry.

Table 1. ^1H and ^{13}C NMR spectral data of compound **1** (600 MHz, $CDCl_3$)

C		H		
1	184.18			
2	128.89		6.10	*t*, *J* = 1.0 Hz
3	196.31			
4	149.26			
5	44.94		3.06	*d*, *J* = 9.3 Hz
6	31.57		0.40	*t*, *J* = 9.3 Hz
7	29.39		0.76	*dddd*, *J* = 5.9, 6.4, 9.3, 11.7 Hz
8	24.36	α	2.09	*m*, *J* = 6.4, 13.7 Hz
		β	1.21	*m*, *J* = 11.7, 13.7 Hz
9	35.50	α	1.45	*dd*, *J* = 11.8, 12.7 Hz
		β	1.95	*m*, *J* = 5.9, 12.7 Hz
10	40.47		2.34	*ddq*, *J* = 5.9, 6.4, 11.8 Hz
11	20.51			
12	16.35		1.23	*s*
13	28.50		1.06	*s*
14	19.79		1.26	*d*, *J* = 6.4 Hz
15	114.24	a	5.20	*d*, *J* = 1.0 Hz
		b	5.97	*s*

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