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VERRUCOSANE DITERPENE FROM THE LIVERWORT *PLAGIOCHILA STEPHENSONIANA*

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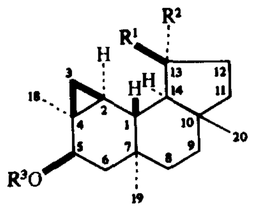
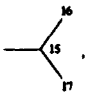
Key Word Index—*Plagiochila stephensoniana*; Jungermanniales; Hepaticae; (+)-13-*epi*-neoverrucosan-5β-ol; verrucosane-type diterpene; X-ray analysis.

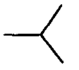
Abstract—From the non-pungent liverwort *Plagiochila stephensoniana*, a new verrucosane-type diterpenoid has been isolated along with 3-methoxy-4′-hydroxybibenzyl and the sesquiterpenoid spathulenol. The structure of the new diterpene, (+)-13-*epi*-neoverrucosan-5β-ol, as elucidated by extensive 2D NMR spectroscopy was confirmed and its absolute configuration established by X-ray analysis of the *p*-bromobenzoyl derivative.

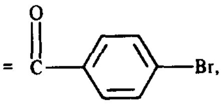
INTRODUCTION

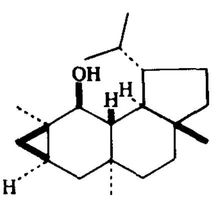
Although a number of verrucosane-type diterpenoids with a novel fused 3,6,6,5-tetracyclic carbon skeleton are known, they occur only in the liverworts *Mylia verrucosa* [1–4], *Gryrothyra underwoodiana* [5] and *Scapania bolanderi* [6]. In the course of our continuing search for biologically actives substances [7, 8] in *Plagiochila* species, we reinvestigated the chemical constituents of non-pungent *Plagiochila stephensoniana* [9] indigenous to New Zealand, and succeeded in isolating a new verrucosane diterpene (**1**) along with the previously known 3-methoxy-4′-hydroxybibenzyl (**5**) [9] and spathulenol (**6**) [8]. In this paper we wish to report on the structure including the absolute configuration of the new diterpene as established by 2D NMR methods and X-ray analysis of its *p*-bromobenzoyl derivative.



1 R^1 = , $R^2 = R^3 = H$

2 R^2 =  , $R^1 = R^3 = H$

3 R^1 =  , R^3 = , $R^2 = H$



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RESULTS AND DISCUSSION

A combination of column chromatography on silica gel and Sephadex LH-20 of an ether extract of *P. stephensoniana* led to the isolation of a new diterpene (**1**), along with the known 3-methoxy-4′-hydroxybibenzyl (**5**) [9] and spathulenol (**6**) [8].

Compound **1** had the molecular formula $C_{20}H_{34}O$ (M^+ 290.2602). Its IR, ^1H and ^{13}C NMR spectra indicated the presence of one secondary hydroxyl group (3600 cm^{-1}; δ_C 71.37), three tertiary methyl groups [δ_H 0.80, 0.83 and 1.21 (each *s*)], an isopropyl group [1378 and 1385 cm^{-1}; δ_H 0.86 and 0.93 (each *d*, $J = 6.6$ Hz)] and a cyclopropane ring (3060 cm^{-1}; δ_H 0.31 and 0.61), but did not show the presence of any carbonyl and unsaturated groups. This suggested that **1** was a saturated tetracyclic diterpene. The 2D ^1H–^1H COSY spectrum was examined to clarify the connectivity of each proton in **1**. The signals at δ_H 0.31 and 0.61 assignable to the methylene protons in the cyclopropane ring, showed coupling with the signals at δ_H 0.80 (*ddd*, $J = 8.0$, 4.8, 4.5 Hz), which were further coupled with the lower field signal at δ_H 1.24 (*dd*, $J = 13.2$, 4.5 Hz). One (δ_H 0.31) of the

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methylene protons on the cyclopropane ring showed long-range coupling with the methyl signal at δ_H 1.21 suggesting the methyl group was located at the carbon (C-4) next to the cyclopropane methylene group (C-3). The isopropyl methyl signals had cross peaks with the two-proton multiplet signals which appeared at δ_H 2.11, which were found to be connected with different carbons (δ_C 44.91 and 29.76) from the ^{1}H–^{13}C COSY spectrum. One of them, assigned to H-13, revealed a coupling to a double doublet signal at δ_H 1.61 (*dd*, $J = 13.2$, 9.5 Hz), which was further coupled with the signal at δ_H 1.24. These results led to a successive connectivity of $CH_2(3)–CH(2)–CH(1)–CH(14)–CH(13)–CH(15)–(Me)_2$, and also indicated a *trans*-diaxial relationship between H-1 and H-14 (large J value of 13 Hz). In addition, the carbinyl proton at δ_H 4.03 (*dd*, $J = 10.7$, 7.3 Hz) was coupled with the two geminal protons at δ_H 0.71 (*dd*, $J = 12.5$, 10.7 Hz) and 1.70 (*dd*, $J = 12.5$, 7.3 Hz), one (δ0.71) of which showed long-range coupling with the methyl signal at δ_H 0.80, and thereby a partial structure $HCOH(5)–CH_2(6)–C(7)–Me$ was suggested. It was further possible to correlate four methylene groups as □-CH_2(δ35.25)–CH_2(δ36.63)-□ and □-CH_2(δ40.90)–CH_2(δ25.34)-, by careful examination of both the ^{13}C–^{1}H COSY and the ^{1}H–^{1}H COSY spectra, although these protons were only slightly resolved to each other. Moreover, examination of the ^{13}C–^{1}H long-range COSY spectrum of **1** resulted in complete assignments of equivocal carbon signals (Table 1), and substantiated the connectivities of the partial structures discussed above for **1**. These evidences led to the conclusion that the plain structure for **1** corresponded to neoverrucosan-5-ol already reported by Matsuo *et al* [9]. The IR and ^{1}H NMR data for **1**, however, were not in agreement with those of (−)-neoverrucosan-5β-ol (**2**), and also its specific rotation had a positive sign opposite to that of **2**. These inconsistencies indicated that the structure of **1** was in part stereochemically different from that of **2**.

Table 1. ^{13}C NMR data for compounds **1** and **3** (100.16 MHz for **1**, 22.25 MHz for **3**, $CDCl_3$, TMS as int. standard)

C	1	3
1	45.78	45.8
2	27.47	27.3
3	20.22	20.1
4	23.32	21.9
5	71.37	76.4
6	47.03	43.1
7	37.52	37.7
8	35.25	35.2
9	36.63	36.6
10	42.45	42.4
11	40.90	40.9
12	25.34	25.4
13	29.76	29.8
14	50.55	50.6
15	44.91	45.0
16	21.29*	21.3†
17	23.87*	23.9†
18	25.62	25.7
19	17.06	17.0
20	20.33	20.3
1′		165.9
2′		127.7
3′		131.6
4′		131.1
5′		146.3

*,†May be interchangeable.

In order to establish the stereostructure including the absolute configuration of **1**, X-ray measurement of the *p*-bromobenzoate (**3**) was carried out. The ORTEP drawing of **3** is shown in Fig. 1 and the cnnfiguration of the isopropyl group was established to be β epimeric at C-13 in **2**. The absolute chemistry of the molecule was determined by Bijovet's anomalous dispersion method based on the observed and calculated structure factors of 18 Friedel pairs. The absolute configuration of **3**, which is shown in Fig. 1, matches that of neoverrucosan-5β-ol (**2**) [10].

All the previously known verrucosane (**4**) and neoverrucosane diterpenoids contain an α-isopropyl group at C-13 and this type of diterpene bearing a β-oriented one has not been recorded before the isolation of (+)-13-*epi*-neoverrucosan-5β-ol (**1**) described in this paper [8]. From the biosynthetic point of view, however, the presence of epimeric congener on a terminal isopropyl group is not rare in natural products since its stereochemistry relies upon the conformation adopted by GG pp on cyclization [11, 12]. *P. stephensoniana* is chemically a very isolated species among the Plagiochlilaceae since it produces a large amount of bibenzyl derivative [9], and the present *epi*-neoverrucosane diterpenoid has not been found in any *Plagiochila* species so far examined [8, 13, 14].

EXPERIMENTAL

Mps: uncorr; ^{1}H NMR (400 and 90 MHz) and ^{13}C NMR (100.16 and 22.25 MHz): $CDCl_3$, TMS as int. standard; CC: silica gel (Merck, 70–230 mesh and Wakogel C-300); TLC: precoated silica gel plates F_{254} (Merck, 0.25 mm). Spots were visualized by 30% H_2SO_4 followed by heating. The X-ray measurement was carried out by a Syntex R3 four-circle differactometer.

Plant material. The liverwort, *Plagiochila stephensoniana* Mitten. was collected in New Zealand in November 1986 and identified by Dr E. O. Campbell and Y. A. A voucher specimen has been deposited at the Herbarium of the Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and isolation. Air-dried and powdered whole plants (202.06 g) were extracted at room temp. for two months. The resultant Et_2O extract was evapd *in vacuo* to give a crude extract (3.43 g), which was separated into seven fractions by CC on silica gel: fr. 1 (438 mg) (*n*-hexane, 100%); fr. 2 (87 mg) (EtOAc–*n*-hexane, 1:19); fr. 3 (634 mg) (EtOAc–*n*-hexane, 1:9); fr. 4 (70 mg) (EtOAc–*n*-hexane, 1:4); fr. 5 (1.5 g) (EtOAc–*n*-hexane, 3:7); fr. 6 (199 mg) (EtOAc–*n*-hexane, 1:1); fr. 7 (63 mg) (EtOAc, 100%).

Spathulenol (**6**) (17 mg) was obtained from fr. 4 by CC on silica gel ($CHCl_3$–EtOAc, 19:1). Fr. 5 was further separated into four fractions by Sephadex LH-20 ($CHCl_3$–MeOH, 1:1). The fourth fraction gave 3-methoxy-4′-hydroxybibenzyl (**5**) (1.10 g), and the second fraction (175 mg) was purified by repeated chromatography on silica gel (C_6H_6–EtOAc, 19:1 → EtOAc, 100%, and then $CHCl_3$–EtOAc, 19:1) to afford 13-*epi*-neoverrucosan-5β-ol (**1**) (55 mg) as a colourless prism (from *n*-hexane), mp 151–153.5°; $[\alpha]_D$ 45.5 (*c* 0.8; $CHCl_3$); MS *m/z* (rel.

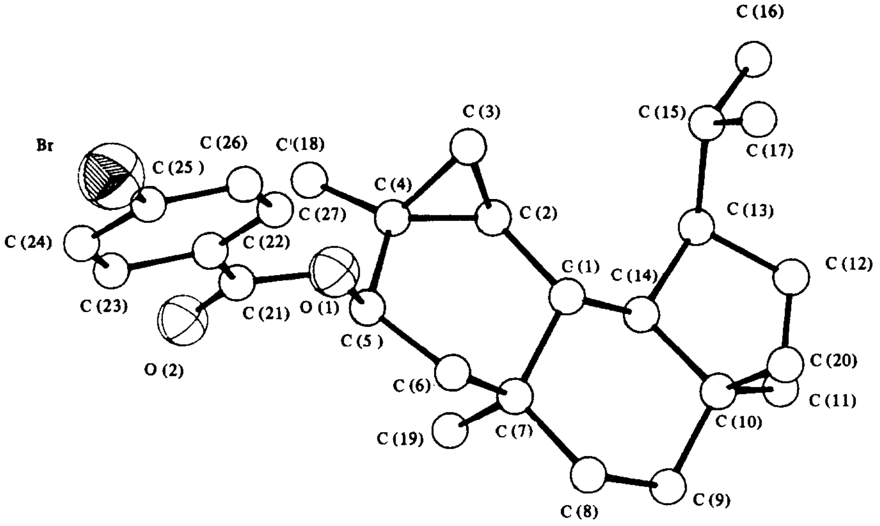

Fig. 1. ORTEP drawing of the molecular structure of compound 3.

int.): 290.2602 $[M]^+$ (27) (calc. 290.2610 for $C_{20}H_{34}O$), 275 (34) $[M-14]^+$, 257 (13) $[M-15-18]^+$, 247 (39) $[M-43]^+$, 229 (38), 161 (46); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 3060 (cyclopropane ring), 1378, 1385; ^{1}H NMR (400 MHz, $CDCl_3$): δ0.31 (1H, *dd*, $J=4.8$, 4.6 Hz, H-3β), 0.61 (1H, *dd*, $J=8.0$, 4.6 Hz, H-3α), 0.71 (1H, *dd*, $J=12.5$, 10.7 Hz, H-6β), 0.80 (3H, *s*, Me-19), 0.80 (1H, *ddd*, $J=8.0$, 4.8, 4.5 Hz, H-2), 0.83 (3H, *s*, Me-20), 0.86 and 0.93 (each 3H, *d*, $J=6.6$ Hz, Me-16 and -17), 1.21 (3H, s, Me-18), 1.24 (1H, *dd*, $J=13.2$, 4.5 Hz, H-1), 1.61 (1H, *dd*, $J=13.2$, 9.5 Hz, H-14), 1.70 (1H, *dd*, $J=12.5$, 7.3 Hz, H-6α), 2.11 (2H, *m*, H-13 and -15), 4.03 (1H, *dd*, $J=10.7$, 7.3 Hz, H-5); ^{13}C NMR: see Table 1.

p-Bromobenzoylation of 1. To a soln of 1 (20.1 mg) in C_5H_5N (3 ml) was added *p*-bromobenzoyl chloride (30 mg) in one portion, and then the reaction mixture was allowed to stand at room temp. for 10 hr. Usual work-up afforded the *p*-bromobenzoate 3 (23 mg) as a crystal; mp 124–125°; $[\alpha]_D$ 47.2 ($CHCl_3$; *c* 1.15); ^{1}H NMR (90 MHz, $CDCl_3$): δ0.84 (3H, *s*), 0.81 and 0.95 (each 3H, *d*, $J=6.3$ Hz), 0.93 (3H, *s*), 1.19 (3H, *s*), 5.53 (1H, *dd*, $J=10.3$, 7.5 Hz), 7.56 (2H, *d*, $J=8.6$ Hz), 7.92 (2H, *d*, $J=8.6$ Hz); ^{13}C NMR: see Table 1.

X-ray analysis of 3. A crystal of 3 was grown in MeOH under slow evapn at room temp. Crystal data: $C_{27}H_{37}O_2Br$, monoclinic, $P2_1$, $a=6.311(2)$, $b=19.543(9)$, $c=9.970(4)$ Å, $\beta=97.53(3)°$, $Dx=1.25$ g/cm^3 and μ (MoKα) $=18.0$ cm^{-1}. The cell dimensions and intensities were measured on a four-circle diffraction with ω-scan mode for 2θ less than 45°. A total of 1653 independent reflections were collected, among which 1462 reflections $[I > 1.96\sigma(I)]$ were stored as observed. The structure was solved by the heavy atom method. All the hydrogen atoms except for 7 atoms connected with methylene carbons were located in a difference map computed after block diagonal least-squares anisotropic refinement of non-hydrogen atoms. The final refinement cycle gave $R=0.042$. The absolute configuration of the molecule was determined by Bijovet's anomalous-dispersion method based on the observed and calculated structure factors of 18 Friedel pairs. The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Center, University Chemical Laboratory.

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