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13-*EPI*-NEO- AND 13-*EPI*-HOMOVERRUCOSANE DITERPENOIDS FROM THE LIVERWORT *SCHISTOCHILA NOBILIS**

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Key Word Index—*Schistochila nobilis*; Jungermanniales, Hepaticae, 13-*epi*-neoverrucosan-5β-ol, 13-*epi*-homoverrucosan-5β-ol, diterpenoids; gymnomitrene; sesquiterpenoid, chemosystematics

Abstract—13-*epi*-Homoverrucosan-5β-ol, a novel diterpene alcohol with a 7,6,5-fused carbon skeleton, was isolated from the New Zealand liverwort, *Schistochila nobilis*, together with the previously known 13-*epi*-neoverrucosan-5β-ol and the sesquiterpene hydrocarbon, gymnomitrene (= β-barbatene). The stereostructure of the new diterpenoid was elucidated by NMR spectra and chemical transformation of 13-*epi*-neoverrucosan-5β-ol by dilute acid

INTRODUCTION

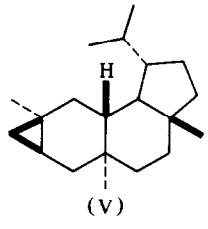
The Schistochilaceae (Hepaticae) contain four genera; *Pleurocladopsis*, *Schistochila*, *Paraschistochila* and *Pachschistochila* and there are 54 species in non-tropical area [2] In temperate and subantarctic Australasia, some 32 species are known [2] Recently, we reported that the major components of the New Zealand liverwort *Schistochila appendiculata* are long chain alkyl phenols [3]

More recently, two neoverrucosane-type diterpenoids, neoverrucosan-5β-ol (**1**) and homoverrucosan-5β-ol (**2**) were isolated from the Taiwanese *S. rigidula* [4]. *Schistochila nobilis* is one of the beautiful liverworts and it is only distributed in New Zealand. As part of a chemosystematic study of the Schistochilaceae, we analysed the lipophilic components of *S. nobilis* and isolated a new diterpene alcohol, 13-*epi*-homoverrucosan-5β-ol (**4**), along with the known 13-*epi*-neoverrucosan-5β-ol (**3**) [5] and the sesquiterpene hydrocarbon, gymnomitrene (= β-barbatene) (**5**) [6] In this paper, we wish to report the chemical structure of the new diterpenoid and to discuss the chemosystematics of *S. nobilis*.

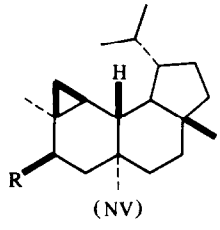
RESULTS AND DISCUSSION

A combination of silica gel and high pressure liquid chromatographies (HPLC) of the ether extract of *S nobilis* resulted in the isolation of two diterpenoids (**3** and

*Part 28 in the series of 'Chemosystematics of Bryophytes' For Part 27, see ref [1]

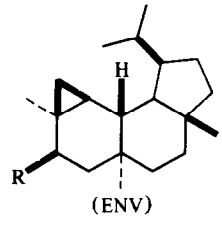


(V)



(NV)

1 R = OH



(ENV)

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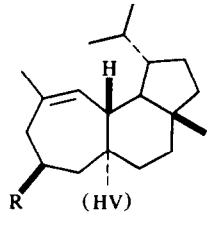
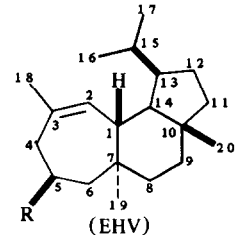


(HV)

2 R = OH



(EHV)

4 R = OH

4) and gymnomitrene (=β-barbatene) (5) [6]. The spectral and physical data of 3 were consistent with those of 13-*epi*-neoverrucosan-5β-ol which was recently isolated from the New Zealand *Plagiochila stephensoniana* and its absolute stereochemistry was established by X-ray crystallographic analysis of the *p*-bromobenzoate of 3 [5] The molecular formula of 4 was confirmed as $C_{20}H_{34}O$ by high resolution mass spectrometry The spectral data (see Experimental) of 4 indicated the presence of a secondary hydroxyl group, two tertiary, an isopropyl and a vinylic methyl groups and a vinylic proton The mass, ^1H- and ^{13}C NMR spectra were very similar to those of (+)-homoverrucosan-5β-ol (2) obtained from 1 by acid treatment [7, 8], except for the melting point and the value of specific optical rotation, indicating that 4 might be the stereoisomer of 2 This assumption was further supported by the following evidence Compound 3 dissolved in acetone was treated with dilute sulphuric acid to give a ring expanded product whose spectral and physical data were identical to those of the natural diterpene alcohol (4) Thus, the structure of the new diterpene alcohol was established to be 13-*epi*-homoverrucosan-5β-ol (4) The diterpene (4) is not an artifact since the presence of 4 in the crude extract was confirmed by TLC and GC-MS and compound 3 in *n*-hexane and ethyl acetate was not converted into 4 in the presence of silica gel even under reflux

The distribution of verrucosane-, neoverrucosane-, homoverrucosane-, 13-*epi*-neoverrucosane- and 13-*epi*-homoverrucosane- type diterpenoids in the Hepaticae is shown in Table 1 These diterpenoids, which have not been found in any higher plants, are distributed only in morphologically quite different genera, the *Schistochila* [4], *Plagiochila* [5], *Mylia* [6–13], *Gyrothyra* [14] and *Scapania* [15]

13-*epi*-Neoverrucosan-5β-ol (3) has been isolated from *Plagiochila stephensoniana* collected in New Zealand [5] This species is morphologically absolutely different from *S nobilis* and the two species grow in completely different places and do not intermingle with each other *S appendiculata* biosynthesizes the long chain alkyl phenols as major components and no terpenoids have been detected even by GC-MS [3] On the other hand, no long-chain alkyl phenols have been found in *S nobilis* Thus, there is no chemical affinity between the two species The present results support the classification of the two species in the *Schistochila* genus The former species has been classified as the subgenus, *Schistochila*, the section *Schistochila* and the latter one as the subgenus *Chaetoschistochila*, section *Volantes* [2]. It is also noteworthy that *S nobilis* produces 13-*epi*-neoverrucosane- and 13-*epi*-homoverrucosane diterpenoids and *S rigidula* elaborates their 13-epimers [4]

EXPERIMENTAL

Mps uncorr The solvents used for the spectral measurements were TMS-$CDCl_3$ [^1H NMR (400 MHz), ^{13}C NMR (100 MHz)] and $CHCl_3$ ($[\alpha]_D$) TLC, GC and GC-MS were carried out as previously reported [16] Prep HPLC was performed on a Dovelosil (20 ϕ × 25 cm) column using *n*-hexane–EtOAc (4 1) as eluant, flow rate, 9 0 ml/min

Plant material Schistochila nobilis (Hook) Trev was collected in Stewart Island, New Zealand, in November 1986 and identified by Dr E O Campbell The voucher specimen was deposited in the Herbarium, Institute of Pharmacognosy, Tokushima Bunri University

Extraction and isolation The fresh *S nobilis* was dried for 5 days and ground mechanically The powder (58 3 g) was extracted with Et_2O for 10 days to obtain a green oil (3 42 g) The crude extract was chromatographed on silica gel using *n*-hexane–EtOAc gradient to provide four fractions From Fr 1 (*n*-hexane 100%) pure gymnomitrene (5) [6] (80 mg) was obtained Fr 2 (*n*-hexane–EtOAc 4 1) gave diterpene mixtures which were further purified by HPLC to give 13-*epi*-neoverrucosan-5β-ol (3) (220 mg) and 13-*epi*-homoverrucosan-5β-ol (4) (56 mg) The spectral data and physical constants (mp and $[\alpha]_D$) of the former compound and its *p*-bromobenzoate were identical to those of 3 isolated from *Plagiochila stephensoniana* and its *p*-bromobenzoate [5] Compound 4 mp 123–124°, $[\alpha]_D$ +47 1 (*c* 0 55), IR ν_{max}^{KBr} cm^{-1} 3400, 1466, 1031, ^1H NMR δ 0 79, 0.81 (each 3H, *d*, *J* = 6 6 Hz, H-16, H-17), 0 84 (3H, *s*, H-19), 0 86 (3H, *s*, H-20), 1 15 (1H, *m*, H-11), 1 19 (1H, *ddd*, *J* = 12 5, 3 2, 3 2 Hz, H-9β), 1 30 (1H, *ddd*, *J* = 12 5, 12 5, 3 2 Hz, H-8β), 1 39 (1H, *ddd*, *J* = 12.5, 3 2, 3 2 Hz, H-8α), 1 47 (1H, *ddd*, *J* = 10 7, 10 7, 2 2 Hz, H-6β), 1 52 (1H, *m*, H-11), 1 53 (1H, *m*, H-12), 1 57 (1H, *m*, H-14), 1 51 (1H, *ddd*, *J* = 12.5, 12 5, 3 2 Hz, H-9α), 1 72 (1H, *m*, H-12), 1 78 (3H, *s*, H-18), 1 82 (1H, *qd*, *J* = 6 6, 6 6 Hz, H-15), 1 90 (1H, *ddd*, *J* = 10 7, 2 2, 2 2 Hz, H-6α), 2 01 (1H, *m*, H-13), 2 06 (1H, *ddd*, *J* = 10 7, 2 2, 2 2 Hz, H-4α), 2 40 (1H, *dd*, *J* = 13 2, 5 6 Hz, H-1), 2 49 (1H, *ddd*, *J* = 10 7, 10 7, 2 2 Hz, H-4β), 3 65 (1H, *dddd*, *J* = 10 7, 10 7, 2 2, 2 2 Hz, H-5), 5 28 (1H, *d*, *J* = 5 6 Hz, H-2), ^{13}C NMR δ 19.4 (C-19), 19 9 (C-20), 21 4 (C-16 or C-17), 23 7 (C-16 or C-17), 25 1 (C-

Table 1 Distribution of verrucosane (V)-, neoverrucosane (NV)-, homoverrucosane (HV)-, 13-*epi*-neoverrucosane (ENV)- and 13-*epi*-homoverrucosane (EHV)-type diterpenoids in the Hepaticae

Species	Family	Diterpenoids V	NV	HV	ENV	EHV
*Schistochila appendiculata** [3]	Schistochilaceae					
S nobilis	Schistochilaceae				+	+
S rigidula [4]	Schistochilaceae		+	+		
Plagiochila stephensoniana [5]	Plagiochilaceae				+	
Mylia anomala [12, 13]	Jungermanniaceae	+				
M taylorii [12]	Jungermanniaceae	+				
M verrucosa [7–11]	Jungermanniaceae	+	+			
Gyrothyra underwoodiana [14]	Gyrothyraceae	+				
Scapania bolanderi [15]	Scapaniaceae	+	+			

*Terpenoids have not been detected even by GC/MS

12), 25 6 (C-18), 29 8 (C-15), 37 0 (C-8), 37 6 (C-7), 38.8 (C-9), 40.7 (C-11), 40.8 (C-10), 41 1 (C-1), 42.9 (C-4), 44.6 (C-13), 51.6 (C-14), 58.7 (C-6), 65 5 (C-5), 130.9 (C-3), 132.5 (C-3) The assignments of ^1H and ^{13}C NMR signals were confirmed by spin decoupling, difference NOE, ^1H–^1H, ^{13}C–^1H and ^{13}C–^1H long range 2D-COSY NMR spectral data HRMS $[M]^+$ (found: 290.2604, calc. for $C_{20}H_{34}O$ 290 2609), EIMS *m/z* (rel. int) 290 $[M]^+$ (40), 229 (73), 191 (100), 121 (49), 107 (65), 95 (67), 81 (68), 69 (67), 55 (60), 41 (70) Fr 3 (*n*-hexane–EtOAc 1 1) (430 mg) and Fr 4 (EtOAc 100%) (170 mg) contained complex acidic diterpenoids which remain to be identified

Acid treatment of **3** To compound **3** (10 mg) in Me_2CO (4 ml) was added 0 5 N H_2SO_4 (1 ml) and the reaction mixture was refluxed with stirring for 2 5 hr Work-up as usual gave a homoallyl alcohol (5 ml) whose physical constants and spectral data were identical to those of the natural 13-*epi*-homo-verrucosan-5β-ol (**4**)

Treatment of **3** *with* SiO_2 Silica gel (14 g) was added to compound **3** (14 mg) in *n*-hexane–EtOAc (4 1, 100 ml) and the mixture refluxed at 70–80° for 12 hr. The solvent was evaporated, after filtration to remove the silica gel, to recover the starting material (**3**)

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