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EPI-NEOVERRUCOSANE- AND *EPI*-HOMOVERRUCOSANE-TYPE DITERPENOIDS FROM *FOSSOMBRONIA ALASKANA**

CAROLA GRAMMES, GUNTHER BURKHARDT, MICHAEL VEITH,† VOLKER HUCH† and HANS BECKER‡

Pharmakognosie und Analytische Phytochemie der Universität des Saarlandes, FR 12.3, 66041 Saarbrücken, Germany; † Institut für Anorganische Chemie, Universität des Saarlandes, FR. 11.2, 66041 Saarbrücken, Germany

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Key Word Index—*Fossombronia alaskana*; Fossombroniaceae; Metzgeriales; Hepaticae; *in vitro* culture; chemotaxonomy; isolation and structure elucidation; tricyclic and tetracyclic diterpenes; X-ray analysis; *epi*-neoverrucosanes; *epi*-homoverrucosanes.

Abstract—*Fossombronia alaskana*, a rare arctic liverwort, was axenically cultured. Phytochemical investigation of the gametophytes afforded five new *epi*-neoverrucosane-type diterpenoids (5-oxo-*epi*-neoverrucosane, 13-hydroxy-5-oxo-*epi*-neoverrucosane, 8α-acetoxy-13-hydroxy-5-oxo-*epi*-neoverrucosane, 8α,16-diacetoxy-13-hydroxy-5-oxo-*epi*-neoverrucosane and 8α,13-dihydroxy-5-oxo-*epi*-neoverrucosane) together with the previously known 5β-hydroxy-*epi*-neoverrucosane. The overall number of natural products with the *epi*-neoverrucosane skeleton is now seven. In addition, the new homoverrucosane-type diterpene 5,18-dihydroxy-*epi*-homoverrucosane was isolated. The structures were elucidated by X-ray crystallography (5-oxo-*epi*-neoverrucosane), two-dimensional NMR spectroscopy and chemical degradation, resulting in some unsaturated *epi*-neoverrucosane structures. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

In 1974, a new species of *Fossombronia* from the northern part of Alaska was described by Steere and Inoue [1]. Since then, *F. alaskana* has been reported from a second location in Western Greenland [2]. Due to its geographical occurrence and the small size of the gametophytes, phytochemical investigation of this rare species required axenically cultured plant material.

Recent investigations on the lipophilic compounds from *in vitro* cultured liverworts resulted in the isolation of numerous new natural products [3, 4]. In particular, a wide variety of terpenoid structures with unusual carbon skeletons were reported. Structures with the 3,6,6,5-tetracyclic framework of verrucosanes were first detected in the liverwort *Mylia verrucosa* [5]. Later, some modified structures were isolated from different species, and based on the structural relationships, these were named neoverrucosanes and homoverrucosanes (Fig. 1). Neoverrucosanes differ in the position of the three-membered ring; homoverrucosanes, however, showed ring expansion to a tricyclic system. All these isomers, as well as their 13-*epi*-derivatives, may exist. Phytochemical investigation of *F. alaskana* considerably extended the number of this outstanding group of diterpenes; a review of the work undertaken is given in Table 1. Here, we present the recent results of our research on the chemical constituents of *Fossombronia* species [6].

RESULTS AND DISCUSSION

In vitro cultured plant material of *F. alaskana* was air dried and extracted with ether. Chromatographic separation by HPLC, VLC on silica gel and column chromatography on Sephadex LH 20 gave five new *epi*-neoverrucosane-type diterpenoids (**1–5**), together with the previously known 5β-hydroxy-*epi*-neoverrucosane (**6**). In addition, a new tricyclic diterpenoid was isolated as a minor component.

5-Oxo-*epi*-neoverrucosane (**1**) was assigned the molecular formula $C_{20}H_{32}O$ (electron impact (EI): MS, $[M]^+$ *m/z* 288) and ^{13}C distortionless enhancement by polarization transfer (DEPT). The ^{13} CNMR and DEPT spectra clearly exhibited 20 carbon resonances assignable to five methyl groups, six methylene groups, five methine groups and three quaternary carbon atoms, together with a carbonyl resonance at δ 211.3 (IR 1683 cm^{-1}), indicating a tetracyclic diterpene framework.

The ^{1}H NMR spectrum confirmed the presence of

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‡ Author to whom correspondence should be addressed.

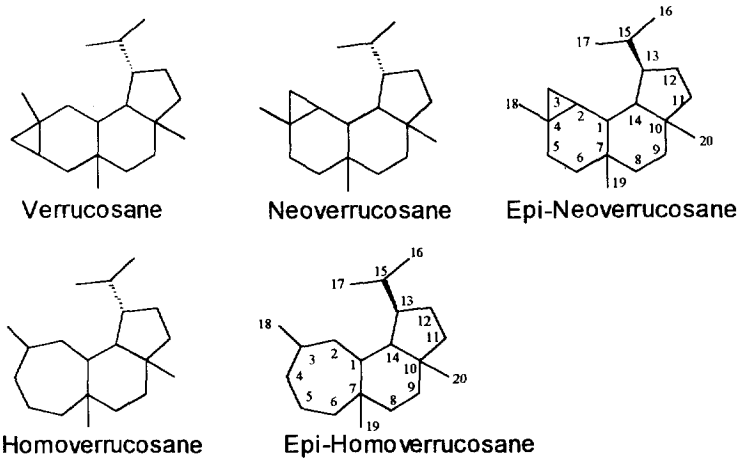


Fig. 1. Verrucosane-type skeleton. The numbering of the carbon atoms in the structure elucidation are the same as those used in this figure.

five methyl groups: two singlets at δ 0.82 and 0.87 (H-19 and H-20), a third singlet at δ 1.22 indicating an angular methyl group of a cyclopropane moiety (H-18), and two doublets at δ 0.87 and 0.94 (H-16 and H-17) forming part of an isopropyl moiety, together with the septet at δ 2.07 (H-15). These signal patterns led to the tentative assignment of a neoverrucosane-type diterpene.

In general, resonances of cyclopropyl protons (H-3) are observed at shift values between δ 0.3 and 0.8. The absence of these characteristics high-field resonances may result from the anisotropic influence of a carbonyl function (^{13}C NMR: δ 211.3) located nearby. However, these protons can be observed in NMR experiments utilizing an anisotropic solvent. Measurement in benzene-d_6 revealed protons at δ 0.58 and 0.81 (H-3a and H-3b). The characteristic doublet resonances of an AB spin system (^{1}H NMR: δ 1.94 and 2.02, J = 15.3 Hz) could be assigned to the two H-6 ketovicinal protons.

Configurational analysis of the isopropyl moiety at C-13 should allow the structural determination of neoverrucosanes (H-13β) and *epi*-neoverrucosanes (H-13α). However, due to poorly resolved ^{1}H NMR spectra with strongly interfering signals, it was not feasible to obtain further information about the stereochemistry at C-13 by nuclear Overhauser effect (NOE) or two-dimensional (2D) homonuclear chemical shift correlation spectroscopy (COSY) experiments.

In order to establish the correct stereostructure of the molecule, X-ray measurement of the crystal was

Table 1. Taxonomic distribution of natural compounds with verrucosane (V) neoverrucosanes (NV) and homoverrucosanes (HV) carbon skeletons and their 13-*epi*-derivatives in plants

		Hepaticae						
Order	Family	Species	V	NV	epi-NV	HV	epi-HV	Refs
Jungermanniales	Jungermanniaceae	*Mylia anomala*	*					17
		M. taylorii	*					18
		M. verrucosa	*	*				5, 9, 19, 20
	Lophocoleaceae	*Heteroscyphus planus*			*			21
	Scapaniaceae	*Scapania bolanderi*	*	*				22
	Gyrothyraceae	*Gyrothyra underwoodiana*	*					23
	Schistochilaceae	*Schistochila acuminata*		*		*		24
		S. rigidula		*		*		25
		S. nobilis			*		*	25
	Plagiochilaceae	*Plagiochila cristata*					*	26
		P. stephensoniana			*			8
Metzgeriales	Fossombroniaceae	*Fossombronia alaskana*			*		*	14

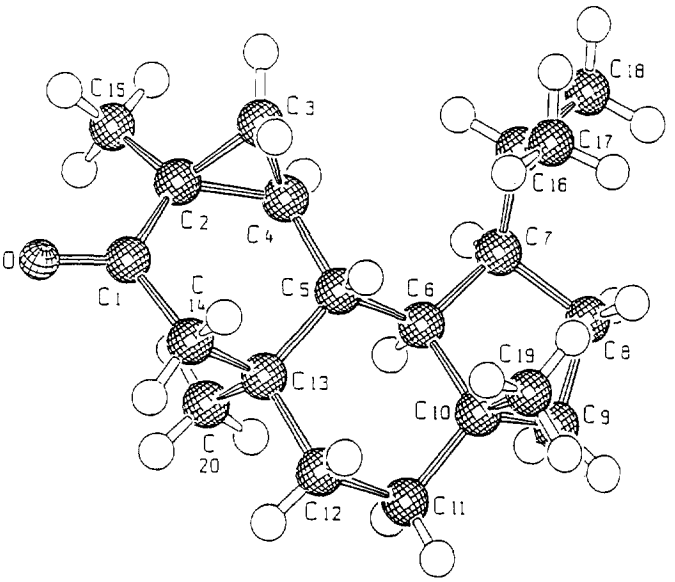


Fig. 2. Schakal plot of 5-oxo-*epi*-neoverrucosane (**1**) (selected bond lengths (Å) and angles (°): C1–0 1.222 (7), C_1–C_2 1.480; C_1–C_{14} 1.501(9); C2–C1-C14 117.4(5)). (Numbering is different from Fig. 1.)

necessary. The resulting relative configuration (Fig. 2) revealed the molecule as 5-oxo-*epi*-neoverrucosane (**1**).

13-Hydroxy-5-oxo-*epi*-neoverrucosane (**2**) had similar NMR spectra to compound **1** and this led us to assume that **2** was a second verrucosane-type diterpene which contained a keto group and a tertiary alcohol as functional groups.

By analogy to compound **1**, the carbonyl (^{13}C NMR δ 211.4, IR 1678 cm^{-1}) could be assigned at C-5, thus inducing the characteristic coupling pattern of the ketovicinal AB spin system of H-6 protons and the striking downfield shift of the two cyclopropyl protons H-3. Evaluating the correlations from a H,H COSY in benzene-d_6 gave an unambiguous coupling sequence (from H-3 to H-14), thus again proving a neoverrucosane type skeleton. Measurement of HETCOR and COLOC sequences enabled us to assign all carbon and proton resonances in the molecule, and finally revealed the tertiary alcohol at C-13. The relative stereochemistry of this compound and the following ones was deduced by biogenetic considerations and its chemical relationship to compound **1**.

The ^{1}H NMR and ^{13}C NMR signal patterns of 8α-Acetoxy-13-hydroxy-5-oxo-*epi*-neoverrucosane **3** are very similar to those of compound **1**, except for the presence of two additional oxygen functions in the molecule. The carbonyl resonance at δ 170.4 in conjunction with the oxygenated methine signal (^{1}H NMR δ 4.71, ^{13}C NMR δ 75.0) could be assigned to an acetoxy group on C-8 by HETCOR and COLOC sequences. NOE experiments (Fig. 3) revealed the stereochemistry of the acetoxy group to be C-8α. Further examination of the 2D NMR spectra placed the remaining alcohol group δ 84.6 at C-13, corresponding to the substitution pattern of compound **2**.

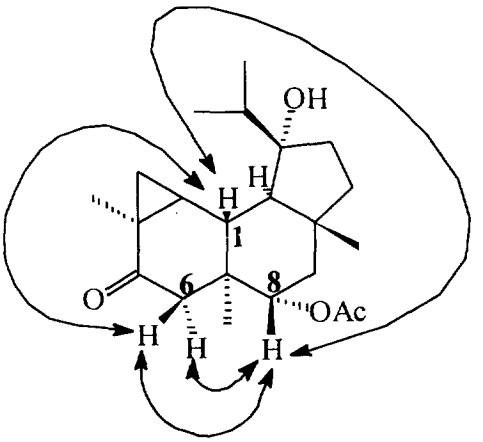


Fig. 3. NOE of 8-acetoxy-13-hydroxy-5-oxo-*epi*-neoverrucosane (**3**).

8α,13-Dihydroxy-5-oxo-*epi*-neoverrucosane (**4**) gave rise to NMR data which were virtually identical to those of compound **3**, with the notable exception of the presence of two alcoholic functions instead of an alcoholic and an acetoxy group. Based on compound **3**, the secondary alcohol could be assigned at C-8α and the tertiary one at C-13.

8α,16-Diacetoxy-13-hydroxy-5-oxo-*epi*-neoverrucosane (**5**) was assigned the molecular formula $C_{24}O_{36}O_6$ (EI-MS, $[M]^+$ *m/z* 420). The carbonyl groups at δ 170.9 and 170.4 together with two methyl groups at δ 21.1 and 20.9 could be assigned as acetoxy groups. With regard to the values of C-7 to C-9, analysis of the ^{13}C NMR spectra of compounds **2**–**4** placed the secondary acetoxy function at C-8 (Table 2).

The remaining two oxygen functions, representing

Table 2. ^{13}C NMR data for *epi*-neoverrucosanes (100 MHz, $CDCl_3$)

C	1	2	3	4	5	6
1	45.1[a]	46.4	44.8	44.8	45.5	45.9
2	30.2[b]	28.8	27.7	28.0	29.0	27.6
3	29.4	29.0	28.7	28.8	29.8	20.3
4	29.9	28.9	28.9	28.9	27.6	23.4
5	211.3	211.4	210.1	210.7	209.7	71.4
6	53.6	53.8	49.6	49.9	49.4	47.0
7	41.8[c]	41.9	45.3	46.7	44.9	37.5
8	35.3	34.9[b]	75.0	73.2	74.8	35.3
9	36.7	37.2[c]	42.0	45.9	42.6	36.6
10	42.2[c]	43.2	42.7	42.7	42.1	42.4
11	40.8	38.0[c]	37.7	37.9	37.9	41.0
12	25.6	34.8[b]	35.1	35.2	38.0	25.8
13	30.8[b]	85.5	84.6	84.9	83.5	29.8
14	50.7	60.2	59.4	59.7	59.6	50.7
15	46.6[a]	35.3	35.3	35.3	40.9	45.1
16	21.4	18.5	18.4	18.5	13.9	21.3
17	23.8	19.9	19.8	19.9	67.1	23.9
18	20.3[d]	20.5	20.3	20.5	20.3	25.4
19	17.7	17.7	12.5	11.5	12.6	17.1
20	20.6[d]	19.3	20.2	20.7	20.2	20.4
			170.4		170.4	
			21.0		21.1	
					170.9	
					20.9	

[a–d] Values may be interchanged within the same column.

an alcohol and an acetoxy group, can be placed at C-13 and C-16. Steric conditions as observed in a Dreiding model, render it difficult to interpret the NOE spectra in order to establish the correct substitution pattern.

Chemical oxidation should distinguish between alcoholic or acetylic substitution on each of the relevant carbon atoms. In the case of alcoholic substitution at C-16, oxidation with PCC aluminium hydroxide should give an aldehyde; an acetylic substitution in this position, however, would show no reaction. The reaction was carried out, using the method of Tietze and Eicher [7]. Structure elucidation of the semisynthetic product led to structure **8**, thus confirming structure **5** for this compound.

5β-Hydroxy-*epi*-neoverrucosane (**6**) was identified as the previously known 5β-hydroxy-*epi*-neoverrucosane by comparison of physical and spectral data [8].

5,18-Dihydroxy-*epi*-homoverrucosane (**7**) on Cl^+-MS gave a fragment at *m/z* 289 $[M+H]^+$ and an intense one at *m/z* 271 $[M+H-18]^+$, thus indicating the loss of an H_2O molecule. The ^1H NMR spectrum contained two resonances of methyl groups forming doublets at δ 0.77 and 0.78, two singlet methyl groups at δ 0.84 and 0.85, and a signal of a one-proton triplet at δ 3.65, indication of an hydroxyl substituent. A broad doublet δ 5.53 provided evidence of unsaturation and the sharp two-proton singlet at δ 4.00 pointed to a $-CH_2OH$ moiety.

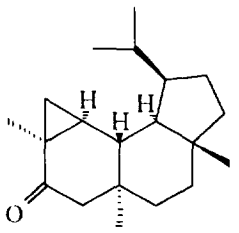
Unfortunately, the small amount of substance available did not allow us to record a conventional ^{13}C spectrum. In view of the finely resolved ^1H NMR spectrum, we carried out 2D NMR experiments (H,H COSY, (heteronuclear multiple bond connectivity (HMBC), and heteronuclear single quantum coherence (HSQC)) in order to elucidate the structure from the ^1H, ^1H and inverse ^1H, ^{13}C coupling patterns. Analysing and combining the facts from different spectra, some connectivities could be established, indicating structure **7** (Fig. 4).

The NOE spectra were used to elucidate the stereochemistry at C13. A NOE at H-2 strongly affected the resonance of H-13. Referring to Dreiding model, this experiment assigned H-13 as H-13α. Thus, the molecule could be assigned to be 5,18-dihydroxy-*epi*-homoverrucosane (**7**), which in addition would be the most suitable conformation of biogenetic grounds.

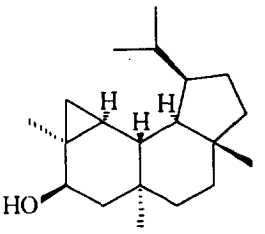
In view of the possible structural isomerization, we had to clarify the biological origin of compound **7**. In the isolation process, acid treatment of a cyclopropane moiety could result in a homoallylic ring expansion, resulting in a tricyclic skeleton as observed in compound **7**. In analogy to this reaction, we dissolved 300 mg of compound **3** (80% purity) in 0.5 N H_2SO_4–acetone (1:4) and heated the mixture under reflux for 5 hours [9]. Isolation and structure elucidation of products **9** and **10** gave no indication of a ring expansion, not even after a second reflux for 48 hours. These results, combined with the fact that the unusual substitution pattern of **7** did not match that of any other isolated compound, made us assign compound **7** as a natural product from *F. alaskana*.

Verrucosane-type diterpenes and related structures form a rare class of terpenoid skeletons. Recently, a verrucosane was reported from a phototrophic bacterium [10] and neoverrucosanes were reported from an Okinawan sponge [11]. Within the plant kingdom, the entire group consisting of verrucosanes, neoverrucosanes and homoverrucosanes seems to be restricted to the class of liverworts (Table 1). On chemotaxonomic grounds, it is interesting to note that this is the first report of verrucosane-type diterpenoids within the order of Metzgeriales, whereas all other publications deal with representatives of the Jungermanniales.

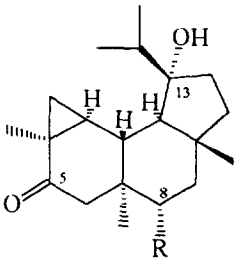
The species under investigation belongs to the genus *Fossombronia* Raddi (*Fossombroniaceae* Schuster, formerly *Codoniaceae*), a genus classified by Schuster to be 'one of the most sharply isolated hepatic genera' [12]. Comparative terpenoid chemistry of this taxon is rare, due to the limited number of species investigated [3, 13, 14]. The occurrence of rare chemical constituents comprising hopanes, sacculatanes or *epi*-neoverrucosanes, emphasizes the isolated position of *Fossombronia* within the Metzgeriales, thus supporting Schuster's phylogenetic classification. Considering the limited research on *Fossombronia*, the occurrence of substances like the *epi*-neoverrucosanes, emphasizes the somewhat exceptional position of *F.*



1 5-Oxo-*epi*-neoverrucosane



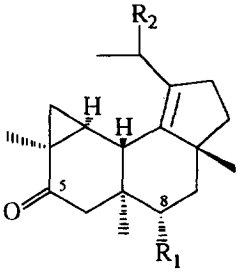
6 5ß-Hydroxy-*epi*-neoverrucosane



2 R = H 13-Hydroxy-5-oxo-*epi*-neo-verrucosane

3 R = OAc 8-Acetoxy-13-hydroxy-5-oxo-*epi*-neoverrucosane

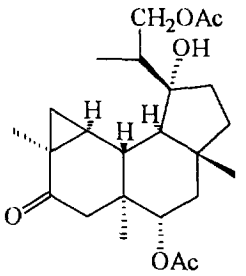
4 R = OH 8,13-Dihydroxy-5-oxo-*epi*-neoverrucosane



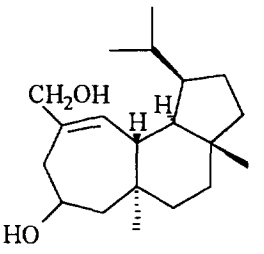
8 R_1 = OAc, R_2 = CH_2OAc 8,16-Diacetoxy-5-oxo-*epi*-neo-verrucos-(13)-ene

9 R_1 = OAc, R_2 = CH_3 8-Acetoxy-5-oxo-*epi*-neoverrucos-(13)-ene

10 R_1 = OH, R_2 = CH_3 8-Hydroxy-5-oxo-*epi*-neo-verrucos-(13)-ene



5 8,16-Diacetoxy-13-hydroxy-5-oxo-*epi*-neoverrrucosane



7 5,18-Dihydroxy-*epi*-homoverrucosane

Fig. 4. *epi*-Neoverrucosanes and *epi*-homoverrucosanes from *F. alaskana*.

alaskana within the genus *Fossombronia*, first discussed by Steere and Inoue [1], based on some morphological features.

Until now, little effort has been made to elucidate the biogenetic pathway of *epi*-neoverrucosane-type structures in plants. The system of axenically cultured *Fossombronia* provides exceptional promise for this biosynthetic research. Unlike classical cell culture experiments, this system provides the possibility to handle fully differentiated organisms, thus using a system equipped with complete enzyme and constituent patterns. Biosynthetic studies are in progress.

EXPERIMENTAL

General. Mps: uncorr. Optical rotation: $CHCl_3$. EI-MS: direct inlet, 70 eV, or in case of compound **7** positive chemical ionization Cl. ^{1}H NMR: 400 MHz or 500 MHz. ^{13}C NMR spectra at 100 MHz or 125 MHz, using $CDCl_3$ as solvent unless stated otherwise. In other solvents or mixtures of solvents used, spectra were recorded with addition of TMS. TLC and VLC [15]: silica gel H 60, average particle size 15 μm. HPLC: 250 × 4 mm steel columns packed with silica gel (5 μm) or modified (diol) silica gel (7 μm).

Fossombronia alaskana (190 *g*). Gametophytes were cultivated on agar (0.9%) under aseptic conditions and continuous illumination at 18°. Cultures were propagated on Gamborg B5 medium [16] supplemented with 1% sucrose. The axenic culture was initiated and kindly provided by Prof. D. Basile, New York Botanical Garden. A voucher specimen of the culture was deposited at Pharmakognosie und Analytische Phytochemie, Universität des Saarlandes, Saarbrücken, Germany.

Extraction. Gametophytes of axenically cultured *Fossombronia alaskana* were air-dried and pulverized. Percolation with Et_2O afforded 12 g of crude extract. Successive CC on Sephadex LH 20 (CH_2Cl_2–MeOH 1:1) and gradient VLC (100% *n*-hexane to 100% EtOAc) yielded several fractions, which were subjected to HPLC purification. Fraction 3, upon HLPC (silica gel, hexane–tert-butylmethylether 97:3) gave 5 mg of **1**. Fr. 6 was repeatedly chromatographed on HPLC (silica gel hexane–EtOAc 94.5:5.5; diol hexane–tert-butylmethylether 94:6, silica gel hexane–EtOAc 92:8) and gave 3 mg of **6**. Fr. 8 was submitted to VLC on RP-18 material, eluating with MeOH–H_2O (4:1) and after HPLC on silica gel gave 297 mg of **2**. Fr. 9 was crystallized from *n*-hexane EtOAc and yielded 400 mg of **3**. Successive HPLC of fr. 10 on silica gel (hexane–EtOAc 7:3; 3:2) afforded 3 mg of **5**. Finally, fr. 11 (silica gel, hexane–EtOAc 3:7) gave 33 mg of **4** and 0.3 mg of **7**.

5-*Oxo*-epi-*neoverrucosane* (**1**). Orthorhombic crystals (−15°, *n*-hexane), mp 92°, $[\alpha]_D^{20}$ +177.2 (*c* 0.21). EI-MS *m/z* (rel. int.): 288 (4.6), 273 (6.5), 245 (9.9), 205 (27.8), 161 (12.9), 149 (14.0), 121 (29.9), 40 (100). IR ν_{max} (FT) cm^{-1}: 1683, 1464, 1390, 1392, 1371, 1352, 1339, 1286. ^{1}H NMR and ^{13}C NMR: Tables 2 and 3. ^{1}H NMR in C_6H_6: 0.58 (1H, *t*, *J* = 4.8 Hz, H-3), 0.72 (3H, *s*), 0.74 (3H, *s*), 0.81 (1H, *m*, H-3), 0.86 (3H, *d*, *J* = 6.6 Hz, H-16), 0.94 (3H, *d*, *J* = 6.7 Hz, H-17), 1.14 (3H, *s*, H-18). X-ray crystallographic analysis: $C_{20}H_{32}O$, orthorhombic, $P2_12_12_1$, a = 10.018(5), b = 11.642(7), c = 15.226(8) Å, D_x = 1.079 mg m^{-3}, Z = 4; four circle diffractometer Siemens AED2, MoK_α radiation, $\bar{\omega}/\theta$ scan, 2θ range 3.0–48.0°. A total of 1611 reflections were collected, among which 1249 reflections ($F > 2.0\sigma$ (I)) were stored as observed. The R values of the mixed isotropic/anisotropic refinement of the non-hydrogen atoms gave R = 0.082, R_w = 0.081. The atomic coordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Center, University Chemical Laboratory.

13-*Hydroxy*-5-*oxo*-epi-*neoverrucosane* (**2**). Prism, mp 104°, $[\alpha]_D^{20}$ +123.4 (*c* 1.7). EI-MS *m/z* (rel. int.): 304 (2.2), 286 (2.8), 271 (5.5), 261 (3.8), 219 (3.1), 205 (6.1), 191 (1.8), 189 (1.8), 161 (7.2), 149 (9.5), 43 (100). IR ν_{max} (FT) cm^{-1}: 1678, 1456, 1421, 1386, 1341, 1300, 1282, 1242, 1217. ^{1}H NMR and ^{13}C NMR: Tables 2 and 3. ^{1}H NMR (C_6H_6): 0.61 (1H, *t*, *J* = 5.1 Hz, H-3), 0.64 (3H, *s*, H-19 or H-20), 0.80 (3H, *d*, *J* = 7.1 Hz, H-16), 0.82 (3H, *s*, H-19 or H-20), 0.82 (1H, *m*, H-3), 0.97 (3H, *d*, *J* = 7.0 Hz), 1.23 (3H, *s*, H-18), 1.57 (1H, *d* = 15.4 Hz, H-6), 1.83 (1H, *d*, *J* = 12.8 Hz, H-14), 2.05 (1H, *d*, *J* = 15.3 Hz, H-6), 2.28 (1H, *sept*, H-15).

8α-*Acetoxy*-13-*hydroxy*-5-*oxo*-epi-*neoverrucosane* (**3**). Prism, mp 165°, $[\alpha]_D^{20}$ +71.3 (*c* 1.3). EI-MS *m/z* (rel. int.): 362 (0.1), 344 (2.1), 319 (0.3), 287 (0.8), 302 (2.3), 284 (2.2), 269 (18.1), 203 (6.5), 191 (3.3), 189 (2.7), 149 (3.8), 43 (100). IR ν_{max} (FT) cm^{-1}. 1731, 1683, 1468, 1443, 1388, 1376, 1244. ^{1}H NMR and ^{13}C NMR: Tables 2 and 3. ^{1}H NMR in C_6H_6: 0.64 (1H, *t*, *J* = 5.1 Hz, H-3), 0.78 (3H, *s*, H-19 or H-20), 0.81 (3H, *d*, *J* = 6.7 Hz, H-16), 0.83 (1H, *m*, H-3), 0.96 (3H, *s*, H-19 or H-20), 0.99 (3H, *d*, *J* = 6.7 Hz, H-17), 1.19 (3H, *s*14-, H-18), 1.27 (1H, *dd*, *J* = 12.4/7.63 Hz), 1.82 (1H, *m*), 1.97 (1H, *d*, *J* = 12.9 Hz, H-14), 2.25 (1H, *sept*, H-15), 2.29 (1H, *d*, *J* = 15.3 Hz, H-6), 4.78 (1H, *dd*, *J* = 8.2 and 3.6 Hz, H-8).

8α,13-*Dihydroxy*-5-*oxo*-epi-*neoverrucosane* (**4**). Prism, mp 115°, $[\alpha]_D^{20}$ +108.1 (*c* 1.2). EI-MS *m/z* (rel. int.): 320 (0.3), 305 (0.1), 302 (82.0), 287 (4.1), 277 (80.9), 284 (0.7), 269 (2.3), 205 (1.8), 203 (6.1), 191 (1.9), 189 (5.1), 161 (8.8), 149 (9.5), 43 (100). IR ν_{max} (FT) cm^{-1}: 1675, 1566, 1548, 1536, 1513, 1503, 1466, 1443, 1423, 1386, 1344, 1279, 1247. ^{1}H NMR and ^{13}C NMR: Tables 2 and 3.

8α,16-*Diacetoxy*-13-*hydroxy*-5-*oxo*-epi-*neoverrucosane* (**5**). Prism, mp 125°, $[\alpha]_D^{20}$ +118.1 (*c* 0.11). EI-MS *m/z* (rel. int.): 420 (0.2), 377 (0.1), 360 (1.3), 300 (1.2), 203 (12.4), 191 (1.9), 189 (3.4), 161 (8.7), 149 (8.7), 43 (100). ^{1}H NMR and ^{13}C NMR: Tables 2 and 3.

5β-*Hydroxy*-epi-*neoverrucosane* (**6**). Prism, mp 147°, $[\alpha]_D^{20}$ +42.1 (*c* 0.10). EI-MS *m/z* (rel. int.): 290

Table 3. ^{1}H NMR data for *epi*-neoverrucosanes*

H	1	2	3	4	5	6
1	–†	1.60 *dd* (12.7, 4.8)	1.72 *dd* (12.9, 4.3)	1.66 *m* ‡	–†	–†
2	1.65 ‡	1.45 ‡	1.53 ‡	1.55 ‡	–†	0.79 ‡
3	0.96 *t* (4.8, 4.8)	0.94 ‡	0.93 ‡	0.97 ‡	0.94 ‡	0.29 *t* (4.7, 4.7)
	1.18 ‡	1.12 ‡	1.14 ‡	1.17 *dd* (12.6, 4.7)	1.21 ‡	0.58 *dd* (8.2, 4.5)
5	–	–	–	–	–	4.01 *dd* (10.6, 7.4)
6	1.94 *d* (15.3)	1.91 *d* (15.4)	1.77 *d* (15.4)	1.80 *d* (15.4)	–	–†
	2.02 *d* (15.3)	2.00 *d* (15.4)	2.10 *d* (15.4)	2.51 *d* (15.4)	2.20 *d* (15.3)	
8	–†	1.07 *d* (13.4, 3.3/3.2)	4.71 *dd* (11.8, 4.3)	3.48 *dd* (11.7, 4.3)	4.74 *dd* (11.8, 4.3)	–†
9	–†	1.35 *m*	1.61 *dd* (12.1, 4.4)	1.66 *m*	–†	–†
		1.45 *m*	1.40 *m*	1.55 *m*		
11	–†	1.40 *m*	1.35 *m*	1.45 *m*	–†	–†
	–†	1.45 *m*	1.45 *m*	1.55 *m*		
12		1.35 *m*	1.30 *m*	1.45 *m*	–†	2.08 ‡
	–†	1.95 *m*	1.90 *m*	2.00 *m*		
13	2.12 *m*	–	–	–	–	–†
14	–†	1.84 *d* (12.5)	1.92 *d* (12.8)	1.89 *d* (12.8)	–†	–†
15	2.07 *sept*	2.27 *sept*	2.20 *sept*	2.29 *sept*	2.15 *sext*	2.08 ‡
16	0.87 *d* (6.7)	0.90 *d* (7.0)	0.90 *d* (7.0)	0.92 *d* (7.0)	3.82 *dd* (10.8, 8.4)	0.84 *d* (6.4)
					4.31 *dd* (10.8, 3.2)	
17	0.94 *d* (6.7)	0.95 *d* (7.1)	0.94 *d* (6.9)	0.97 *d* (6.9)	1.16 *d* (6.7)	0.91 *d* (6.4)
18	1.22 *s*	1.17 *s*	1.17 *s*	1.21 *s*	1.23 *s*	1.19 *s*
19	0.82 *s*	0.84 *s*	0.89 *s*	0.85 *s*	0.94 *s*	0.79 *s*
20	0.87 *s*	0.79 *s*	0.90 *s*	0.87 *s*	0.98 *s*	0.81 *s*
			1.93 *s* OAc		1.99 *s* OAc	
					2.06 *s* OAc	

* Coupling constants (Hz) in parentheses.
† Could not be assigned.
‡ Spin systems not entirely resolved.

(2.3), 275 (5.9), 272 (1.9), 257 (5.9), 247 (4.7), 43 (95.5), 41 (100). NMR data were in good accordance with the literature.

5,18-*Dihydroxy*-epi-*homoverrucosane* (**7**). Needles. *m/z* 289 $[M+H]^+$. ^{1}H NMR: 2.48 (1H, *dd*, J = 10.6 and 4.2 Hz, H-1), 5.53 (1H, *d*, J = 4.1 Hz, H-2), 2.25 (1H, *bd*, J = 10.7 Hz, H-4), 2.42 (1H, *t*, J = 8.9 Hz, H-4), 3.65 (1H, *t*, J = 8.6 Hz, H-5), 1.50 (1H, *m*, H-6), 1.90 (1H, *m*, H-6), 1.25 (1H, *m*, H-8), 1.50 (1H, *m*, H-8), 1.30 (1H, *m*, H-9), 1.40 (1H, *dt*, J = 2.6/7.3, H_3, H-9), 1.15 (1H, *m*, H-11), 1.52 (1H, *m*, H-11), 1.51 (1H, *m*, H-12), 1.71 (1H, *m*, H-12), 2.00 (1H, *m*, H-13), 1.60 (1H, *dd*, J = 7.7/10.8 Hz, H-14), 1.75 (1H, *sept*, H-15), 0.78 (3H, *d*, J = 5.3 Hz, H-16), 0.77 (3H, *d*, J = 5.2 Hz, H-17), 4.00 (2H, *bd*, H-18), 0.84 (3H, *s*, H-19), 0.85 (3H, *s*, H-20). ^{13}C NMR: 41.3 (*d*, C-1), 134.4 (*d*, C-2), 133.8 (*s*, C-3), 38.7 (*t*, C-4), 66.0 (*d*, C5), 58.4 (*t*, C-6), 37.4 (*t*, C-8), 36.9 (*t*, C-9), 40.7 (*t*, C-11), 23.8 (*t*, C-12), 44.6 (*d*, C-13), 51.6 (*d*, C-14), 29.8 (*d*, C-15), 21.4 (*q*, C-16), 19.8 (*q*, C-17), 68.2 (*t*, C-18), 18.5 (*q*, C-19), 19.3 (*q*, C-20). C-7 and C-10 could not be detected by inverse correlations.

8α,16-*Diacetoxy*-5-*oxo*-epi-*neoverrucosane*-13-*en* (**8**). 0.9 mg. ^{1}H NMR: 0.77 (3H, *s*), 1.03 (3H, *d*, J = 6.8 Hz, H-17), 1.03 (1H, *m*), 1.13 (3H, *s*), 1.23 (1H, *m*), 1.26 (3H, *s*), 1.99 (3H, *s*, –OAc), 2.02 (3H, *s*, –OAc), 2.13 (1H, *d*, J = 14.6 Hz, H-1), 2.33 (2H, *m*), 2.54 (1H, *bs*), 3.28 (1H, *sext*, H-15), 3.83 (1H, *dd*, J = 10.7/7.6 Hz, H-16), 4.14 (*dd*, J = 10.8 and 7.7 Hz, H-16), 4.82 (1H, *dd*, J = 11.9 and 4.8 Hz, H-8). ^{1}H NMR (C_6H_6): 0.52 (1H, *t*, J = 5.25 Hz, H-3), 0.83 (3H, *d*, J = 6.8 Hz, H-17), 0.84 (3H, *s*), 0.86 (1H, *m*, H-3), 1.04 (3H, *s*), 1.37 (3H, *s*), 1.50 (2H, *m*), 1.61 (3H, *s*), 1.63 (3H, *s*), 1.67 (1H, *dd*, J = 14.2 and 7.6 Hz), 1.77 (1H, *d*, J = 14.3 Hz), 2.04 (4H, *m*), 2.33 (1H, *bs*), 2.34 (1H, *b*, J = 14.2 Hz, H-1), 3.31 (1H, *sext*, H-15), 3.67 (1H, *dd*, J = 10.7 and 7.6 Hz, H-16), 4.14 (1H, *dd*, J = 7.7 and 10.8 Hz, H-16), 4.88 (1H, *dd*, J = 11.9 and 4.8 Hz, H-8).

8α-*Acetoxy*-5-*oxo*-epi-*neoverrucosane*-13-*en* (**9**). 25 mg. ^{1}H NMR: 0.76 (3H, *s*), 0.99 (3H, *d*, J = 6.9 Hz, H-16), 0.99 (3H, *d*, J = 6.9 Hz, H-17), 1.01 (1H, *m*), 1.11 (3H, *s*), 1.20 (1H, *m*), 1.25 (3H, *s*), 1.41–1.68 (4H, *m*), 1.95 (2H, *m*), 1.97 (3H, *s*, –OAc), 2.12 (1H, *d*, J = 14.6 Hz, H-1), 2.31 (2H, *m*), 2.48 (1H, *bs*), 3.02 (1H, *sept*, H-15), 4.80 (1H, *dd*, J = 12.0 and 4.8 Hz, H-8). ^{13}C NMR: 12.9, 19.6, 21.1, 21.7, 21.7, 25.1, 27.3, 27.7, 28.4, 29.4, 30.1, 38.2, 40.7, 44.8, 47.0, 47.1, 49.4, 75.8 (C-8), 134.6 and 141.0 (C-13 and C-14), 170.4 (–OAc), 209.2 (C-5).

8α-*Hydroxy*-5-*oxo*-epi-*neoverrucosane*-13-*en* (**10**). 25 mg. ^{1}H NMR: 0.69 (3H, *s*), 1.03 (3H, *d*, J = 6.4 Hz, H-16), 1.03 (3H, *d*, J = 6.4 Hz, H-17), 1.07 (3H, *s*), 1.25 (3H, *s*), 2.46 (1H, *d*, J = 14.7 Hz, H-1), 3.03 (1H, *sept*, H-15), 3.51 (1H, *dd*, J = 4.7 and 12.1 Hz, H-8). ^{13}C NMR: 11.8, 19.7, 21.7, 21.7, 25.4, 27.2, 27.9,

28.4, 29.3, 30.1, 38.3, 44.7, 47.2, 48.6, 49.4, 74.3 (C-8), 135.0 and 140.5 (C-13 and C-14), 209.8 (C-5).

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