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THREE VERRUCOSANE DITERPENOIDS, VERRUCOSANE TRIOL AND RELATED COMPOUNDS FROM THE LIVERWORT *MYLIA VERRUCOSA*

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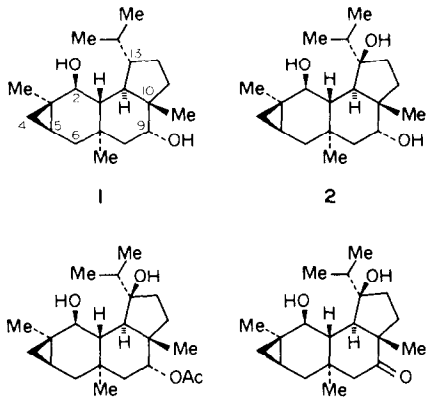
Key Word Index—*Mylia verrucosa*; Bryophyta; Hepaticae; diterpenoids; 2β,9α,13β-trihydroxyverrucosane; 9α-acetoxy-2β,13β-dihydroxyverrucosane; 2β,13β-dihydroxy-9-oxoverrucosane.

Abstract—Three new diterpenoids of the verrucosane class were isolated as minor constituents of the methanol extract of the liverwort *Mylia verrucosa* and their structures shown to be 2β,9α,13β-trihydroxyverrucosane, 9α-acetoxy-2β,13β-dihydroxyverrucosane and 2β,13β-dihydroxy-9-oxoverrucosane.

INTRODUCTION

Liverworts contain several oil bodies in the cells of the gametophytes which are characteristic of the species. They usually elaborate mono-, sesqui- and diterpenoids as well as esters of fatty acids and aromatic acids. New sesquiterpenoids of various types were isolated from several liverworts and most of the liverwort sesquiterpenoids are the enantiomeric forms corresponding to antipodes of those from higher plants [1–4]. Few investigations have been conducted on the diterpenoids of these plants Previously, we isolated the diterpene diol (−)-2β, 9α-dihydroxyverrucosane (**1**), $C_{20}H_{34}O_2$, and the related compounds consisting of a novel verrucosane carbon skeleton from the liverwort *Mylia verrucosa* belonging to the Jungermanniaceae [5–8]. They had one or two oxygenated functional groups at the C-2 and C-9 or C-11 positions in the verrucosane skeleton having a 3,6,6,5-tetracyclic framework substituted with three tertiary methyls and one isopropyl group. The present paper deals with the isolation and structure determination of three diterpenoids containing three oxygenated functional groups on C-2, C-9 and C-13 of the verrucosane structure. On the basis of the following chemical and spectral evidence their structures were elucidated as 2β,9α,13β-trihydroxyverrucosane (**2**), 9α-acetoxy-2β,13β-dihydroxyverrucosane (**3**) and 2β,13β-dihydroxy-9-oxoverrucosane (**4**), respectively.



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RESULTS AND DISCUSSION

The three diterpenoids, (−)-2β,9α,13β-trihydroxyverrucosane (**2**), (−)-9α-acetoxy-2β,13β-dihydroxyverrucosane (**3**) and (−)-2β,13β-dihydroxy-9-oxoverrucosane (**4**), were isolated as minor constituents from a polar fraction of the liverwort extract by a combination of CC and prep. TLC.

The most polar component of the three was characterized by the spectral evidence as a diterpene triol having two secondary hydroxy and one tertiary hydroxy groups. The spectral properties were, furthermore, very close to those of the major diol (−)-2β,9α-dihydroxyverrucosane (**1**) except for the presence of an extra tertiary hydroxy group. The structure was, therefore, deduced to be a triol (**2**) containing one more tertiary hydroxy group than in the diol molecule (**1**). The spectra of another two compounds (**3** and **4**) suggested both contained the same tetracyclic verrucosane skeleton and **3** was an acetoxy derivative of the triol (**2**) while **4** was a carbonyl compound. In fact, acetylation of the triol (**2**) gave the acetoxy-diol (**3**), and the keto-diol (**4**) was produced by Jones' oxidation of the triol (**2**).

The dihydroxyketone (**4**) was refluxed with sulphuric acid in acetone to afford a tricyclic homoallyl alcohol (**5**), through a homoallylic ring opening reaction of the cyclopropyl carbinol [9]. The other homoallyl alcohol (**6**), having additional tertiary and secondary hydroxy groups was obtained from the acetoxy-diol (**3**) by the same reaction. Formation of such homoallyl alcohols (**5** and **6**) by homoallylic ring expansion demonstrated not only the position but also the stereochemistry of the cyclopropane ring and the C-2 secondary hydroxy group to hold a *cis*-relationship in the original molecule [10]. By base treatment of the hemiacetal (**7**), which was produced by

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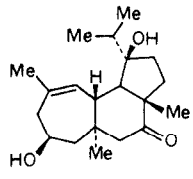
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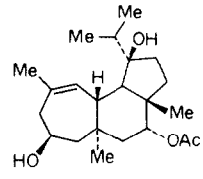
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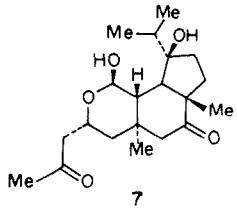
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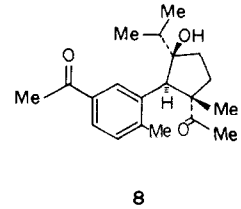
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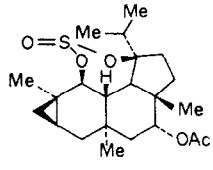
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oxidation of the homoallyl alcohol (**5**) with osmium tetraoxide–sodium periodate, a trisubstituted benzenoid (**8**) was produced in a high yield, the mechanism of formation of which should be the same as that in (−)-5β-hydroxy-9-oxohomoverrucos-2-ene reported previously [5, 7]. From this reaction the carbonyl group was established to be on C-9 of the verrucosane structure. The configuration of the C-9 secondary hydroxy, and the C-9 acetoxy group in the other two compounds, was assigned to be α-(axial) based on the ^{1}H NMR coupling constants of the methine protons.

Finally, the position and stereochemistry of the remaining tertiary hydroxy group was determined by the following spectral and chemical methods. The benzyl proton of the acetophenone (**8**) appeared at δ 3.84 as a singlet in the ^{1}H NMR spectrum suggesting the C-13 position of the tertiary hydroxy group. The configuration of the C-13 hydroxy group was deduced from the results of solvent shifts. The ^{1}H NMR spectra of the natural products **2**–**4** and the tricyclic products **5** and **6** were determined in both deuterochloroform and deuteropyridine solvents. The C-10 methyl signals of the three tertiary methyls in the molecules revealed a much larger deshielding effect by the pyridine as shown in Table 1. This result suggested a 1,3-diaxial relationship of the C-13 tertiary hydroxy group and the C-10 methyl. Furthermore, the sulphite (**9**) was formed in a higher yield, when the acetoxy-diol (**3**) was treated with thionyl chloride–pyridine. The C-13 tertiary hydroxy group, therefore, possessed the same β-configuration as that of the C-2 secondary hydroxy group.

Table 1. Pyridine-induced solvent shifts (Δ-values) of the three tertiary methyls for **2**–**6**

	2	**3**	**4**	**5**	**6**
C-3 methyl	−0.07	−0.02	−0.03	—	—
C-7 methyl	−0.29	−0.04	−0.09	−0.08	−0.04
C-10 methyl	−0.37	−0.34	−0.14	−0.22	−0.30

$\Delta = \delta(CDCl_3) - \delta(C_5D_5N)$.

It is interesting in consideration of the systematic relationship between the liverworts and the algae that the 3,6,6,5-tetracyclic verrucosane diterpenoids of the liverworts are chemically in a more modified stage than the 11,5-bicyclic dolabellanes isolated from the algae.

EXPERIMENTAL

Mps are uncorr. Merk Kieselgel 60 was used for CC and Merk Kieselgel 60 PF_{254} was used for TLC and prep. TLC. Analytical plates were visualized under UV light or were sprayed with 10% H_2SO_4 in EtOH and heated at 100° for 10 min. IR spectra were recorded on a grating spectrometer and optical rotations were taken on an automatic polarimeter in $CHCl_3$. ^{1}H NMR spectra were measured at 60 or 90 MHz in $CDCl_3$, unless otherwise stated, with TMS as the int. standard. MS were determined at 70 eV.

Extraction and isolation. The liverwort *Mylia verrucosa* (4.8 kg), which was collected at Akaishi-san in Ehime prefecture and dried in the shade, was digested × 3 with EtOH (18 l.) at room temp. for 3 days. The neutral dark green viscous oil (75 g) was washed with 3% aq. NaOH to remove acidic material and then chromatographed over a column of Si gel using a mixture of hexane and EtOAc to yield 15 fractions. By a further combination of CC and prep. TLC fractions 14 and 15 gave the three new diterpenoids, (−)-2β,9α,13β-trihydroxyverrucosane (**2**, 90 mg), (−)-9α-acetoxy-2β,13β-dihydroxyverrucosane (**3**, 80 mg) and (−)-2β,13β-dihydroxy-9-oxoverrucosane (**4**, 50 mg).

(−)-2β,9α,13β-Trihydroxyverrucosane (**2**), $C_{20}H_{34}O_3$, mp 243.5–244.5°; $[\alpha]_D$ −72.6° (*c* 1.9); IR ν_{max}^{KBr} cm^{-1}: 3450, 3250, 3070, 1035 and 1020; ^{1}H NMR: δ 0.1–0.7 (3H, complex), 0.95 and 0.99 (each 3H, *d*, *J* = 7.0 Hz), 1.05, 1.05 and 1.24 (each 3H, *s*), 3.60 (1H, *t*, *J* = 3.0 Hz) and 3.76 (1H, *d*, *J* = 10.0 Hz); MS *m/z* (rel. int.): 304 $[M-18]^+$ (3), 286 (3), 279 (12), 261 (100), 243 (85), 225 (8), 201 (13), 187 (10), 163 (20), 149 (68), 133 (19), 119 (19), 107 (24), 93 (18), 81 (29), 71 (27), 55 (30) and 43 (48). (−)-9α-Acetoxy-2β,13β-dihydroxyverrucosane (**3**), $C_{22}H_{36}O_4$, mp 234.5–235.5°; $[\alpha]_D$ −46.2° (*c* 1.5); IR ν_{max}^{KBr} cm^{-1}: 3220, 3050, 1735, 1243 and 1030; ^{1}H NMR: δ 0.2–0.6 (3H, complex), 0.93 and 0.98 (each 3H, *d*, *J* = 7.0 Hz), 0.98, 1.08 and 1.23 (each 3H, *s*), 3.82 (1H, *d*, *J* = 10.0 Hz) and 4.72 (1H, *t*, *J* = 3.5 Hz); MS *m/z* (rel. int.): 348 $[M]^+$ (3), 324 (6), 305 (56), 288 (6), 273 (4), 270 (4), 263 (5), 245 (100), 237 (15), 203 (14), 189 (17), 177 (49), 167 (31), 149 (54), 133 (31), 119 (36), 107 (37), 91 (23), 81 (42), 71 (25), 55 (32) and 43 (83). (−)-2β,13β-Dihydroxy-9-oxoverrucosane (**4**), $C_{20}H_{32}O_3$, mp 222.5–223.0°; $[\alpha]_D$ −107.2° (*c* 1.3); IR ν_{max}^{KBr} cm^{-1}: 3275, 3070, 1710 and 1019; ^{1}H NMR: δ 0.2–0.7 (3H, complex), 0.88 and 0.99 (each 3H, *d*, *J* = 7.0 Hz), 0.88, 1.27 and 1.33 (each 3H, *s*) and 3.83 (1H, *d*, *J* = 10.5 Hz); MS *m/z* (rel. int.): 302 $[M]^+$ (2), 284 (2), 259 (100), 241 (13), 217 (16), 199 (13), 175 (9), 159 (8), 145 (7), 133 (15), 119 (22), 107 (17), 93 (17), 81 (29), 69 (14), 55 (19) and 43 (42).

Acetylation of (−)-2β,9α,13β-*trihydroxyverrucosane* (**2**). The triol, **2** (20 mg), was dissolved in pyridine (2 ml) and Ac_2O (0.5 ml) was added to the soln. After being set aside for two nights at room temp., the mixture was worked-up in the usual way to give a crude product. The acetate, **3** (10 mg), was purified by means of prep. TLC. It was identical (IR and ^{1}H NMR spectra) with (−)-9α-acetoxy-2β,13β-dihydroxyverrucosane (**3**) isolated from the same liverwort.

Jones' oxidation of the triol (**2**). The triol, **2** (35 mg), was mixed with an excess of Jones' reagent (0.5 ml) in Me_2CO (10 ml) and the mixture was stored at 0° for 5 min. The reaction mixture was

then poured into H_2O and then extracted with Et_2O. The Et_2O soln was worked-up in the usual way to afford a crude carbonyl substance. The oxidation product (30 mg), which was purified by prep. TLC, was identical with the naturally occurring (−)-2β,13β-dihydroxy-9-oxoverrucosane (**4**) (IR and ^{1}H NMR spectra).

Acid treatment of the dihydroxyketone (**4**). The keto-diol, **4** (80 mg), was dissolved in 0.5 N H_2SO_4–Me_2CO (1:4) (10 ml) and heated under reflux for 24 hr. The reaction mixture was poured into a large vol. of H_2O and taken-up into Et_2O. The Et_2O soln was washed with 5% $NaHCO_3$ soln and satd aq. NaCl, and dried over dry Na_2SO_4. The usual work-up afforded a crude crystalline substance which was purified by prep. TLC and recrystallized from hexane and EtOAc to give the homoallyl alcohol (**5**, 75 mg), $C_{20}H_{32}O_2$, as colourless prisms: mp 223.0–225.0°; $[\alpha]_D$ −33.3° (*c* 1.3); IR ν_{max}^{KBr} cm^{-1}: 3450, 1695, 1025 and 900; ^{1}H NMR: δ 0.89 and 0.99 (each 3H, *d*, *J* = 7.0 Hz), 0.96 and 1.36 (each 3H, *s*), 1.84 (3H, *br s*), 3.61 (1H, *br t*, *J* = 10.0 Hz) and 5.38 (1H, *br d*, *J* = 6.0 Hz); MS *m/z* (rel. int.): 320 $[M]^+$ (2), 302 (6), 259 (5), 194 (6), 175 (6), 133 (5), 119 (6), 107 (5), 71 (12), 69 (11), 57 (100) and 43 (63).

Acid treatment of the acetoxy-diol (**3**). The acetoxy-diol (**3**, 30 mg) was treated with an Me_2CO soln of H_2SO_4 in the same manner described above to produce the homoallyl alcohol (**6**, 25 mg), $C_{22}H_{36}O_4$, as a colourless viscous substance: IR ν_{max}^{film} cm^{-1}: 3300, 1735, 1245 and 1027; ^{1}H NMR: δ 0.94 and 0.99 (each 3H, *d*, *J* = 7.0 Hz), 0.96 and 1.19 (each 3H, *s*), 1.82 (3H, *br s*), 2.02 (3H, *s*), 3.12 (1H, *br t*, *J* = 10.0 Hz), 4.80 (1H, *t*, *J* = 3.0 Hz) and 5.45 (1H, *br d*, *J* = 6.0 Hz).

Oxidation of the homoallyl alcohol (**5**). To a soln of **5** (70 mg) in a mixed solvent of dry C_6H_6 (10 ml) and dry pyridine (1 ml), a soln of CrO_3 (300 mg) in C_5H_5N (4 ml) was added under cooling in an ice bath and the mixture was allowed to react at room temp. for 4 days. The solvent was distilled out and the residual substance was dissolved in EtOH (20 ml). The EtOH soln was mixed with a soln of Na_2SO_3 (2.5 g) in H_2O (10 ml). The mixed soln was heated under reflux for 2 hr and the filtrate, after removal of the ppt, was extracted with $CHCl_3$. The soln was washed with satd aq. NaCl and the solvent was evaporated off. The residual substance thus obtained was dissolved in MeOH (15 ml) and the soln was mixed with a soln of $NaIO_4$ (160 mg) in H_2O (4 ml). The mixture was allowed to stand at room temp. for 2 days and was extracted with EtOAc after dilution with H_2O. The usual work-up afforded the hemiacetal (**7**, 85 mg), $C_{20}H_{32}O_5$, as colourless needles which were recrystallized from hexane and EtOAc: mp 161.5–162.5°; $[\alpha]_D$ −47.8° (*c* 2.8); IR ν_{max}^{KBr} cm^{-1}: 3400, 3280, 1708, 1585, 1565, 1085, 1040, 995 and 970; ^{1}H NMR: δ 0.82 and 0.97 (each 3H, *d*, *J* = 7.0 Hz), 1.04 and 1.38 (each 3H, *s*), 2.19 (3H, *s*), 4.62 (1H, *m*, $W_{\frac{1}{2}}$ = 12.0 Hz) and 5.07 (1H, *d*, *J* = 7.5 Hz); MS *m/z* (rel. int.): 344 $[M-18]^+$ (9), 291 (82), 249 (15), 231 (15), 149 (17), 139 (15), 123 (17), 119 (11), 109 (17), 97 (17), 86 (24), 81 (19), 71 (32), 67 (14), 57 (27) and 43 (100).

Formation of the 3,4-*disubstituted acetophenone* (**8**). To a soln of the hemiacetal (**7**, 80 mg) in MeOH (5 ml), a soln of 5% KOH in MeOH (5 ml) was added and the mixture was allowed to react at room temp. for 6 hr. The reaction mixture was diluted with H_2O, extracted with $CHCl_3$ and the $CHCl_3$ soln, after being dried, was evaporated. The reaction product was purified by prep. TLC to give the 3,4-disubstituted acetophenone (**8**, 63 mg), $C_{20}H_{28}O_3$, as a viscous substance: $[\alpha]_D$ +52.9° (*c* 3.0); IR ν_{max}^{film} cm^{-1}: 3520, 1675, 1600, 1565, 1360, 1265, 1045, 820 and 755; ^{1}H NMR: δ 0.78 and 0.89 (each 3H, *d*, *J* = 7.0 Hz), 1.24 (3H, *s*), 2.31 (3H, *s*), 2.36 (3H, *s*), 2.52 (3H, *s*), 3.84 (1H, *s*), 7.14 (1H, *d*, *J* = 8.0 Hz), 7.64 (1H, *dd*, *J* = 8.0 and 2.0 Hz) and 8.32 (1H, *d*, *J* = 2.0 Hz); MS *m/z* (rel. int.): 316 $[M]^+$ (11), 278 (17), 235 (7), 231 (16), 213 (9), 200 (6), 189 (82), 175 (7), 169 (4), 147 (16), 129 (6), 115 (6), 105 (4) and 43 (100).

Formation of the sulphite (**9**). To a soln of acetoxy-diol, **3** (40 mg), in pyridine (4 ml), $SOCl_2$ (0.4 ml) was added dropwise at 0° and the mixture left at room temp. for 20 min. The mixture was poured into ice–water and was taken-up into Et_2O. The Et_2O soln was worked-up in the usual way to give a viscous substance showing two spots on TLC. By prep. TLC the less polar component was separated to yield crystalline compound **9** (10 mg), $C_{32}H_{34}O_5S$, mp 186.0–188.0°; IR ν_{max}^{KBr} cm^{-1}: 3070, 1720, 1240, 1175 and 1160; ^{1}H NMR: δ 0.6–0.9 (3H, complex), 1.01 and 1.15 (each 3H, *d*, *J* = 7.0 Hz), 1.01, 1.18 and 1.33 (each 3H, *s*), 2.05 (3H, *s*), 3.42 (1H, *d*, *J* = 13.5 Hz) and 4.89 (1H, *t*, *J* = 3.0 Hz).

REFERENCES

1. Hayashi, S. and Matsuo, A. (1975) *Kagaku No Ryoiki* **29**, 46
2. Matsuo, A., Sato, S., Nakayama, M. and Hayashi, S. (1979) *J. Chem. Soc. Perkin Trans.* 1, 2652.
3. Matsuo, A., Atsumi, K., Nakayama, M. and Hayashi, S (1981) *J. Chem. Soc. Perkin Trans.* 1, 2816.
4. Matsuo, A. (1982) *J. Hattori Bot. Lab.* **53**, 295.
5. Matsuo, A., Nozaki, H., Nakayama, M., Hayashi, S. and Takaoka, D. (1978) *J. Chem. Soc. Chem. Commun.* 198.
6. Hayashi, S., Matsuo, A., Nozaki, H., Nakayama, M. Takaoka, D. and Hiroi, M. (1978) *Chem. Letters* 953.
7. Takaoka, D. (1979) *J. Chem. Soc. Perkin Trans.* 1, 2711.
8. Nozaki, H., Matsuo, A., Kushi, Y., Nakayama, M., Hayashi S., Takaoka, D. and Kamiya, N. (1983) *J. Chem. Soc. Perkin Trans.* 2, 763.
9. Gašić, M., Whalen, D., Johnson, B. and Winstein, S. (1967) *J. Am. Chem. Soc.* **89**, 6382.
10. Demarco, P. V., Farkas, E., Doddrell, D., Mylari, B. L. and Wenkert, E. (1968) *J. Am. Chem. Soc.* **90**, 5480.