# Structure and the Absolute Configuration of a New Diterpene, (-)-2(*S*),8(*R*)-Dihydroxyverrucosane, from the Liverwort *Gyrothyra underwoodiana* (Gyrothyraceae)[1]

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The structure of a new diterpene, (-)-2(*S*),8(*R*)-dihydroxyverrucosane, isolated from the liverwort *Gyrothyra underwoodiana* (Gyrothyraceae) has been established by $^1H$-$^1H$ 2D NMR spectroscopy and was confirmed by X-ray diffraction. The absolute configuration was determined by using the dibenzoate chirality method[7] for the bis(*p*-bromobenzoate) derivative.

*Gyrothyra underwoodiana* Howe[2] is the only representative of a liverwort family (Gyrothyraceae) endemic to the western United States. It grows primarily on vertical soil banks of sandstone derivation in coastal and near-coastal regions. The affinities of this family and therefore the genus and species are uncertain. Morphological and developmental studies[3] suggest possible relationships with families as diverse as the Southbyaceae, Geocalycaceae, and Balantiopsidaceae, but resolution awaits other approaches, perhaps chemical or biochemical. In the present report we describe the isolation, structure determination, and absolute configuration of a new diterpene (-)-2(*S*),8-(*R*)-dihydroxyverrucosane (**1**) from this liverwort. A few compounds with the same carbon skeleton have been identified from *Mylia verrucosa*, a liverworth of the Jungermanniaceae family.[4] This family is not thought to be closely related to Gyrothyraceae.

The major compound (**1**, mp 158.5-160.0 °C; $[\alpha]_D$ -108.4°) was isolated from a diethyl ether extract of fresh *G. underwoodiana* by using both silica gel column chromatography and $C_{18}$ reversed-phase low-pressure liquid chromatography. The presence of two secondary hydroxy groups ($\nu$ 3620 $cm^{-1}$; $\delta$ 77.0, 73.7), three tertiary methyl groups ($\delta$ 0.88, 0.96, 1.20), an isopropyl group ($\nu$ 1390 $cm^{-1}$; $\delta$ 0.90, 0.83), and a cyclopropane ring ($\nu$ 1025, 865 $cm^{-1}$; $\delta$ 0.20, 0.48) were indicated by the usual IR, $^1H$ NMR, and $^{13}C$ NMR criteria. On the basis of the EI-MS spectrum which showed a molecular ion peak at $m/z$ 306, **1** was assumed to be a saturated tetracyclic diterpenoid.

The $^1H$ NMR spectral assignments of **1** were obtained by using $^1H$-$^1H$ shift correlated spectroscopy (COSY)[5] (Figure 1). Two signals at $\delta$ 0.20 and 0.48, assigned to the methylene protons at the 4 position in the cyclopropane ring, showed coupling with the $\delta$ 1.00 signal which was assigned to the 5-H on the cyclopropane ring (Figure 2). Also the $\delta$ 0.20 signal showed long range coupling with the methyl signal at $\delta$ 1.20. The methine proton geminal to the isopropyl methyls (15-H, $\delta$ 2.24) was coupled with the $\delta$ 2.06 signal (13-H), the coupling multiplicity of which (dddd, $J$ = 2.4 Hz, 4.8 Hz, 10.8 Hz, 10.8 Hz) indicated the presence of three other vicinal protons (14-H, 12$\alpha$-H, 12$\beta$-H). The 14 proton signal at $\delta$ 1.32 (dd, $J$ = 10.8 Hz, 10.8 Hz) showed coupling with the signal at $\delta$ 1.12 (dd, $J$ = 10.8 Hz, 9.6 Hz) which was also coupled with the hydroxy methine proton (2-H) at $\delta$ 3.67. These above are strong evidence that **1** has a verrucosane skeleton[4] possessing a hydroxy group at the 2 position (Figure 4).

The other hydroxy methine proton's signal of **1** was observed at $\delta$ 3.42. To investigate the position of this hydroxy group, **1** was oxidized to a hydroxy ketone (**2**) (Figure 3). In the $^1H$-$^1H$ 2D NMR spectrum of **2**, the signal of the hydroxy methine proton at the 2-position was unchanged, similar to the oxidation of (-)-2$\beta$,9$\alpha$-dihydroxyverrucosane reported by Matsuo et al.[4a,c] Two new sets of signals characteristic of AB type coupling were observed at $\delta$ 2.2m and 2.42 and were assigned to the methylene protons adjacent to the carbonyl group in **2**. This suggested that the hydroxy group of **1** was attached to the 8- or 9-position. Since the physical and spectral data of **2** were not in agreement with those of (-)-2$\beta$-hydroxy-9-oxoverrucosane,[4a,c] the hydroxy group must be attached to the 8-position. The small coupling constants of the hydroxy methine proton's signal of **1** ($\delta$ 3.42, ddd, $J$ = 3.6 Hz, 3.6 Hz, 3.6 Hz) indicated that the hydroxy group at the 8-position has the $\beta$ axial orientation. Thus, the structure of **1** was determined to be (-)-2$\beta$,8$\beta$-dihydroxyverrucosane.

The $\delta$ 0.96 and 0.88 methyl signals of **1** were assigned to the 20- and 19-methyl groups respectively, because the 20-methyl protons could be affected by a through space effect of the 8$\beta$-hydroxy group. This $\delta$ 0.96 methyl signal of **1** shifted to $\delta$ 0.66 in **2** is caused by the disappearance

(1) Work at the University of California was supported in part by a U.S. Publich Health Service Biomedical Grant to I.K., and work at Humboldt State University was supported by a grant from the Darrel Nielson Institute.

(2) A voucher specimen of this species is deposited in the Humboldt State University, Norris No. 68750B.

(3) (a) Crandall-Stotler, B. *J. Hattori Bot. Lab.* **1976**, *40*, 355. (b) Schuster, R. M. *The Biologist* **1955**, *58*, 137.

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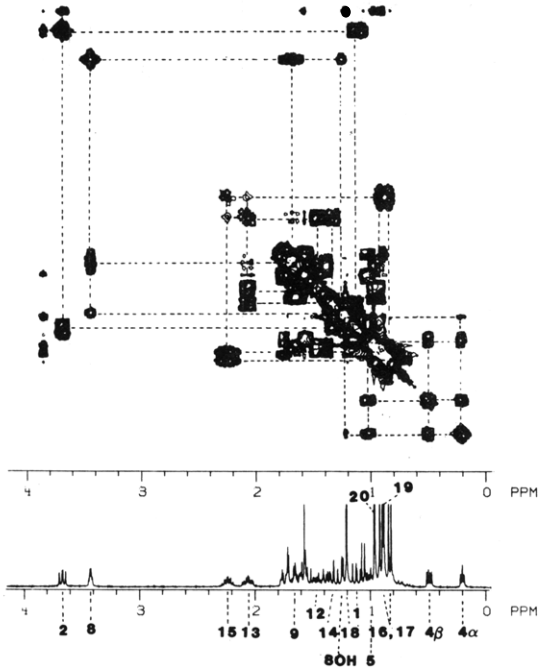


**Figure 1.** $^1H$-$^1H$ shift correlated spectroscopy (COSY) of 1 in $CDCl_3$.

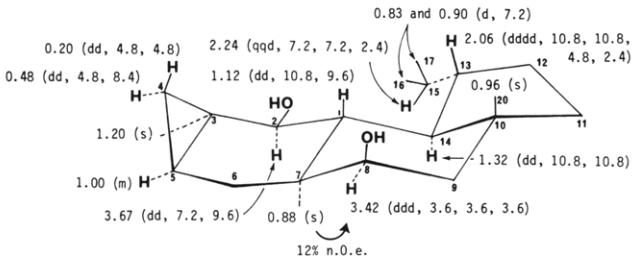


**Figure 2.** $^1H$ NMR assignments of 1 in $CDCl_3$. Chemical shifts are shown in $\delta$ and coupling constants are in Hz.

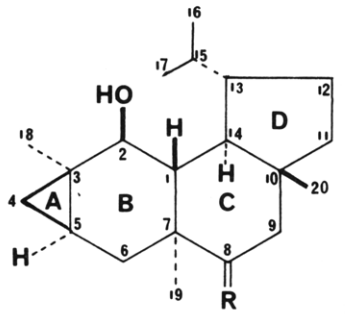


**Figure 3.** Verrucosane skeleton. 1, R = α-H, β-OH. 2, R = O.

of the 8β-hydroxy group's effect. The NOE (12%) observed between the 19-methyl protons and the 8α-proton supports these assignments (Figure 2).

This structure was confirmed by X-ray analysis of 1. Figure 4 is a perspective drawing of 1 with atomic numbering and shows 1 has a molecular constitution similar to that of (−)-2β,9α-dihydroxyverrucosane.[4d,6] The ring B is considerably flattened from the ideal chair form as might be expected from the fusion of the cyclopropane ring (ring A). Torsion angles indicate that ring C is in a slightly distorted chair form, but ring B adopts the 1,2-diplanar form where C(1), C(2), C(3), C(5), and C(6) are coplanar

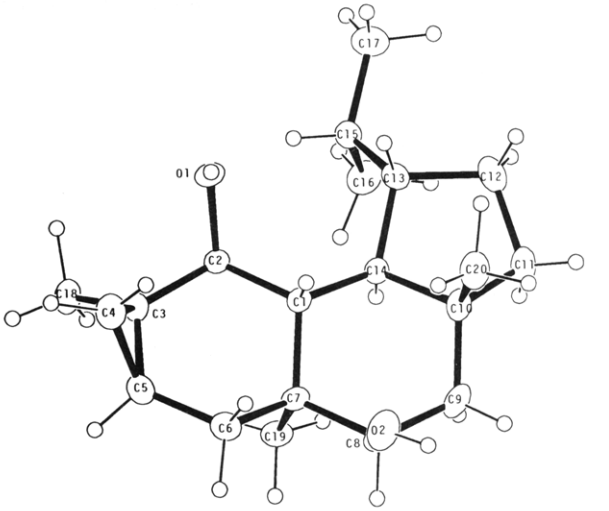

**Figure 4.** Perspective drawing of 1.

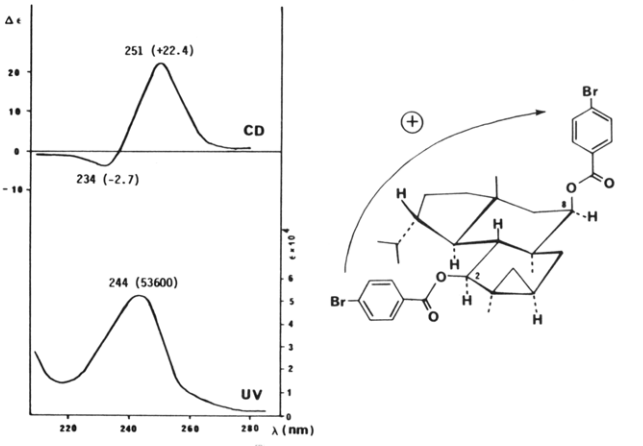


**Figure 5.** CD and UV spectra of the 2,8-bis(*p*-bromobenzoyl)-verrucosane (3) in EtOH.

within 0.036 (4) Å with C(7) deviating by 0.725 (45) Å from this plane. A closing of torsion angle C(1)–C(2)–C(3)–C(5) compared with that of C(3)–C(53)–C(6)–C(7) is mainly due to the transannular interaction between O(1) and C(15)H (O(1)–H-C(15) = 2.48 (2) Å). The cyclopentane ring (ring D) has an envelope conformation with C(10) as the "flap" atom.

The shortening of the bonds, C(2)–C(3) = 1.518 (3) Å, C(3)–C(18) = 1.525 (4) Å, and C(5)–C(6) = 1.505 (4) Å, is consistent with the partial $sp^2$ hybridization expected in a three-membered ring. The mean value of the endocyclic bond angles at C(2), C(3), and C(5) is 118.7 (2) Å, which corresponds to the flattening of the ring B at this region. All other bond distances and angles agree well with accepted values for given bond types. There is one intramolecular hydrogen bond, O(2)–H—O(1) of 3.009 (3) Å. All other intermolecular distances correspond to van der Waals contacts.

The absolute configuration of 1 was examined by using the dibenzoate chirality method[7] on the *p*-bromobenzoate derivative (3) of 1. The molecular ion peak at $m/z$ 672 in the EI–MS of 3, the IR spectrum ($\nu$ no OH), and the molar extinction coefficient ($\epsilon$ 53 600) at 244 nm in the UV spectrum showed 3 is a dibenzoate derivative. Figure 5 shows the CD spectrum of 3. It has a positive first Cotton effect at 251 nm and negative second Cotton effect at 234

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nm. The positive sign of the first Cotton effect leads to the conclusion that the exciton chirality between the transition moment of two *p*-bromobenzoate chromophores was postive. Therefore, 1 was determined to be (−)-2-(*S*),8(*R*)-dihydroxyverrucosane.

## Experimental Section

Melting points were determined by using a Sybron Thermolyne Mp-12615 and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 and 241 MC polarimeter. The UV spectrum was recorded on a Hitachi 100-80 spectrophotometer and IR spectra were recorded on a Nicolet 7199 FT-IR. Mass spectra were obtained on a Hitachi RMU 6-MG apparatus. CD was measured with a Jasco J-40 spectropolarimeter. $^1$H NMR spectra were determined on a Nicolet NT-300 spectrometer and homonuclear $^1$H connectivities were determined by a COSY experiment as described by Bax.[5] The $^{13}$C NMR spectrum was recorded on a Joel FX-100. Tetramethylsilane was used as internal reference for NMR measurements. The X-ray measurement was determined by a Philips PW 1100 four-circle diffractometer.

**Isolation of 1 from *G. underwoodiana*.** Fresh *G. underwoodiana* (1.7 kg) was collected in September 1983, in Arcata, CA. After a storage in diethyl ether for one wook, the mixture was filtered. Subsequent removal of the ether gave a residue of 2.25 g. Silica gel chromatography (silica gel 60, E. Merck) of the residue using *n*-hexane–ethyl acetate eluent gave a fraction (300 mg dry weight) which contained the major compound 1. This fraction was purified by $C_{18}$ reversed-phase low-pressure liquid chromatography (pump, Fluid Metering, Inc.; column, SR 10/50, Pharmacia Fine Chemicals packed with Lichroprep RP-18, particle size 25–40 μm, E. Merck) with a $H_2O$–MeOH mixture solvent to give 96 mg of pure 1: mp 158.5–160.0 °C (from *n*-hexane); $[\alpha]^{20}_D$ −108.4° (*c* 0.83, $CHCl_3$); IR ($CHCl_3$) 3620, 1390, 1025 and 865 cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 0.20 (dd, 1 H, *J* = 4.8 Hz, 4.8 Hz, 4α), 0.48 (dd, 1 H, *J* = 4.8 Hz, 8.4 Hz, 4β), 0.83 (d, 3 H, *J* = 7.2 Hz, 16 or 17), 0.88 (s, 3 H, 19), 0.90 (d, 3 H, *J* = 7.2 Hz, 16 or 17), 0.96 (s, 3 H, 20), 1.00 (m, 1 H, 5), 1.12 (dd, 1 H, *J* = 9.6 Hz, 10.8 Hz, 1), 1.20 (s, 3 H, 18), 1.24 (d, 1 H, *J* = 3.6 Hz, 8-OH), 1.32 (dd, 1 H, *J* = 10.8 Hz, 10.8 Hz, 14), 1.47 (m, 1 H, 12e, 2.06 (dddd, 1 H, *J* = 2.4 Hz, 4.8 Hz, 10.8 Hz, 1.8 Hz, 13), 2.24 (qqd, 1 H, *J* = 7.2 Hz, 7.2 Hz, 2.4 Hz, 15), 3.42 (ddd, 1 H, *J* = 3.6 Hz, 3.6 Hz, 3.6 Hz, 8), 3.67 (dd, 1 H, *J* = 7.2 Hz, 9.6 Hz, 2); $^{13}$C NMR ($CDCl_3$) δ 77.0 (d), 73.7 (d), 48.0 (d), 44.0 (:), 43.5 (s), 43.2 (s), 42.9 (d), 41.8 (t), 40.6 (t), 34.7 (t), 28.1 (d), 25.7 (q), 23.3 (q), 22.8 (t), 22.1 (s), 20.8 (d), 20.4 (q), 19.7 (q), 18.7 (t), 15.1 (q); these coupling patterns were supported by the partially relaxed Fourier Transformation (PRFT) decoupled $^{13}$C NMR spectrum; EI-MS, *m/z* 306 ($M^+$), 288, 270, 255, 191, 123, 81, 43.

**(−)-(2*S*)-2-Hydroxy-8-oxoverrucosane (2).** The diol 1 (20 mg) was mixed with an excess of Jones' reagent (0.1 mL) in acetone (1 mL), and the mixture was stirred at 0 °C for 5 min. Isopropyl alcohol (1 mL) was then dropped into the mixture to reduce the remaining Jones' reagent. After filtration of the mixture, the solvent was removed from the filtrate. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc) and was recrystallized from *n*-hexane–EtOAc to give 13 mg of colorless needles (2): sublimed 165–180 °C; $[\alpha]^{24}_D$ −133.3° (*c* 0.03, $CHCl_3$); IR ($CHCL_3$) 3480, 2960, 1690, 1050, and 875 cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 0.18 (dd, 1 H, *J* = 4.8 Hz, 4.8 Hz, 4α), 0.49 (dd, 1 H, *J* = 4.8 Hz, 8.4 Hz, 4β), 0.66 (s, 3 H, 20), 0.89 (d, 3 H, *J* = 7.2 Hz, 16 or 17), 0.93 (d, 3 H, *J* = 7.2 Hz, 16 or 17), 1.00 (m, 1 H, 1), 1.06 (s, 3 H, 19), 1.23 (s, 3 H, 18), 1.30 (m, 1 H, 14), 2.10–2.20 (m, 2 H, 13 and 15), 2.27 (d, 1 H, *J* = 21 Hz, 9β), 2.42 (d, 1 H, *J* = 21 Hz, 9α), 3.79 (dd, 1 H, *J* = 7.2 Hz, 9.6 Hz, 2); EI-MS, *m/z* 304 ($M^+$), 289, 271, 243, 177, 123, 81.

**X-ray Analysis of 1.** Crystals of 1 were grown from *n*-hexane solution. These crystals are orhorhombic and belong to the chiral space group $P2_12_12_1$. Accurate cell constants determined from a least-squares fit of 16 high angle reflections were: *a* = 18.515 (1e Å, *b* = 15.490 (1) Å, and *c* = 6.327 (1) Å. All unique diffraction maxima with $2\theta \leq 50°$ were recorded in the $\omega/2\theta$ scan mode using a four circle diffractometer and graphite monochromated Mo Kα X-rays. Three reference reflections monitored every 240 min displayed neither systematic nor significant deviations from their initial intensities. Of the 1839 reflections surveyed, 1451 (79%) were judged observed ($I > 3\sigma(I)$) after correction for Lorentz, polarization, and background effects.

The structure was solved by MULTAN.[8] All hydrogen atoms were located on a difference electron density synthesis and included in subsequent calculations. Full-matrix least-squares refinement with anisotropic temperature factors for the non-hydrogen atoms and isotropic temperature factors for hydrogen converged to a conventional crystallographic discrepancy index of 0.038.[9] Additional crystallographic details such as the positional and thermal parameters, bond distances and angles, and observed and calculated structure factors are available as supplementary material.

**2,8-Bis(*p*-bromobenzoyl)verrucosane (3).** *p*-Bromobenzoyl chloride (29 mg) and 1 (10 mg) were dissolved in dry pyridine (1 mL), and the mixture was stirred at 50 °C for 1 day. The solvent was evaporated in vacuo and the major product (3, 10 mg) was separated by preparative TLC (silica gel G uniplate, 250 micron layer, 20 cm × 20 cm, Analtech, In.) with *n*-hexane–EtOAc (1:1, v/v): IR ($CHCl_3$) no OH, 1705, 1580, 1475, 1390, 1265–1280 (br), 1098, 1010, and 840 cm$^{-1}$; EI-MS, *m/z* 672 ($M^+$), 472, 470, 289, 288, 271; UV (EtOH) $\lambda_{max}$ 244 nm ($\epsilon$ 5.31 × 10$^4$); CD curve (EtOH) $[\theta]_{251}$ +74000 (positive maximum), $[\theta]_{234}$ −8900 (negative maximum).

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**Registry No.** 1, 92545-04-5; 2, 92545-05-6; 3, 92545-06-7.

**Supplementary Material Available:** Fractional coordinates, temperature factors, bond distances, bond angles (4 pages). Ordering information is given on any current masthead page.

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