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STRUCTURAL ELUCIDATION AND CONFORMATIONAL ANALYSIS OF GERMACRANE-TYPE DITERPENOIDS FROM THE BROWN ALGA PACHYDICTYON CORIACEUM

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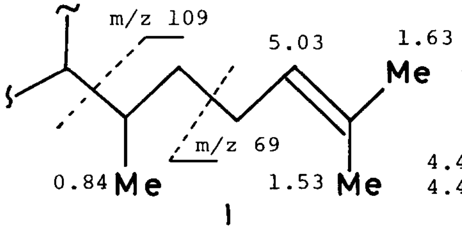
Summary: From the brown alga, Pachydictyon coriaceum, three new 'germacrane-type' diterpenes, acetoxypachydiol (4), 3-hydroxyacetyldilophol (9), and dilophol acetate (11), have been isolated, and the stereochemical features of them as well as obscuronatin (13) have been elucidated.

Natural products possessing a cyclodecane framework are frequently found as germacrane sesquiterpenoids in terrestrial plants.[1] On the contrary, diterpenoids having a ten-membered ring are much rarer, and it is only ten years ago that the first germacrane-type diterpene, dilophol,[2] was isolated from an alga. Since then, several other diterpenoids having a ten-membered ring have been obtained from marine resources.[3-6] In the course of our study on the constituents of the brown alga, Pachydictyon coriaceum, we have isolated three new germacrane-type diterpenes together with a known diterpene, obscuronatin,[3] and this paper describes the structural elucidaton of the new compounds, as well as the conformational analysis of these compounds.

Acetoxypachydiol (4), $C_{22}H_{36}O_4$ (m/z for M^+, 364.248), $[\alpha]_D$ -76.4° (c 0.55, $CHCl_3$), was obtained as a colorless oil from the methanol extract of P. coriaceum. The IR spectrum (CCl_4) implies the presence of acetoxy (1750 and 1240 cm^{-1}) and hydroxy (3300-3600 cm^{-1}) groups. On acetylation (Ac_2O/Py), two acetyl groups were incorporated (^1H-NMR), indicating that two hydroxy groups were present. Fragments at m/z 109 and 69, and the ^1H-NMR signals depicted in the partial structure suggest the existence of the side chain 1.[7] Decoupling works (400 MHz; -50 °C) infered additional partial structures 2 and 3. The downfield (δ 6.10) chemical shift of the olefin proton in moiety 2 indicates the cis relationship between this olefin proton and the acetoxymethyl group. Other three carbons and five protons which are not discussed above must be two CH_2's and one CH, because all of five methyl groups that exist in acetoxypachydiol (^1H and ^{13}C-NMR) are found in 1 to 3. Consideration of these facts and biogenesis of diterpenes



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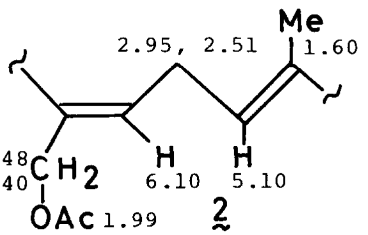

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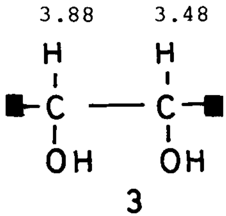

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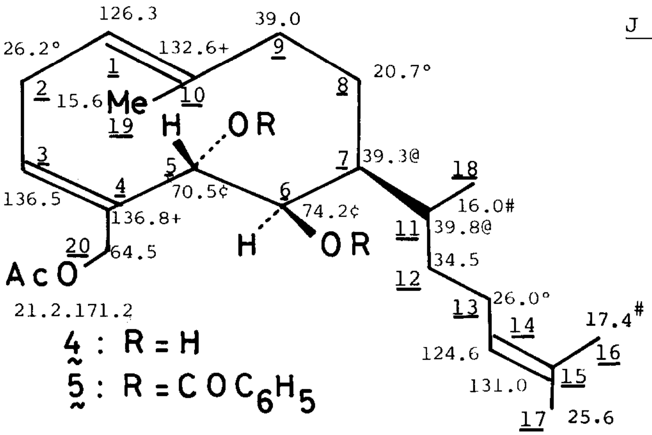


4 : R = H

5 : $R = COC_6H_5$

J values (-50°C) of protons

$J_{2\alpha-2\beta}$ = 13.0 Hz

$J_{2\alpha-1}$ = 0 Hz

$J_{2\beta-1}$ = 11.4 Hz

$J_{2\alpha-3}$ = 9.2 Hz

$J_{2\beta-3}$ = 4.8 Hz

$J_{20\alpha-20\beta}$ = 12.1 Hz

J_{5-6} = 9.9 Hz

J_{6-7} = 0 Hz

led to the gross structure 4 (without stereochemistry) or its isomer in which the glycol moiety is located on C-8 and C-9. In the 400 MHz ^{1}H-NMR spectrum measured at 27°C, several protons appear as broad signals, indicating that acetoxypachydiol takes two or more conformations, which invert in a moderate rate on an NMR time scale.[8] Interestingly, when the spectrum was taken at -50°C, only one set of signals were observed. Similar phenomena were observed in the ^{13}C-NMR spectra; while the spectrum measured at 27°C (100 MHz) showed several broad signals (Fig. 1-A) and the number of carbon signals was less than the one expected from the molecular formula, the spectrum recorded at -50°C (Fig. 1-B) exhibited 22 sharp signals. These facts suggest that acetoxypachydiol takes a major (possibly >98%) conformation at -50°C, and the minor conformer(s), whose population increases as the temperature rises, is not observable in the NMR spectra at that low temperature. Double resonance experiments (400 MHz) performed at -50°C provided the spin coupling constants of the protons (see structure 4). The coupling constants are interpretable only when the conformation and relative configurations of the substituents are supposed as seen in 6 or 7. In order to differenciate the two, NOE experi-

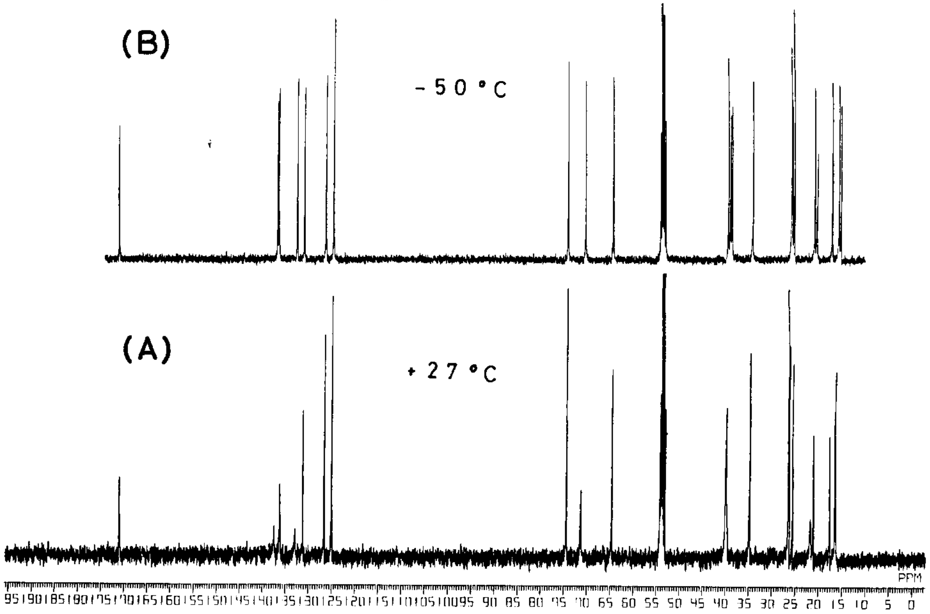
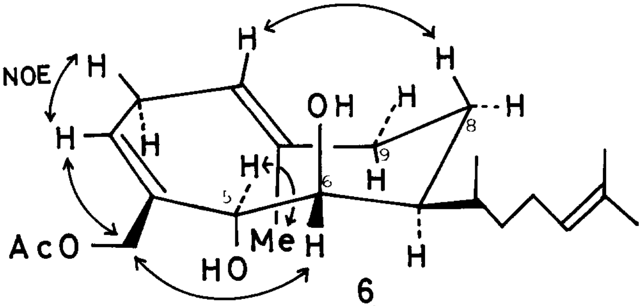


Fig. 1 ^{13}C-NMR spectra (100 MHz; CD_2Cl_2) of acetoxypachydiol (4).



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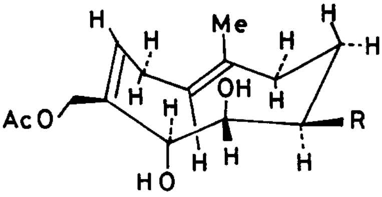
(7; 8β,9α-diol instead of 5,6-diol)



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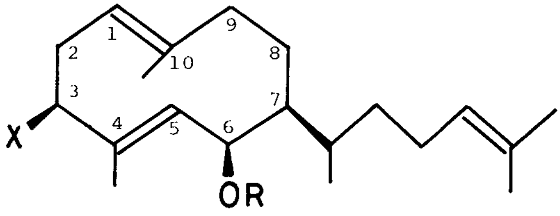
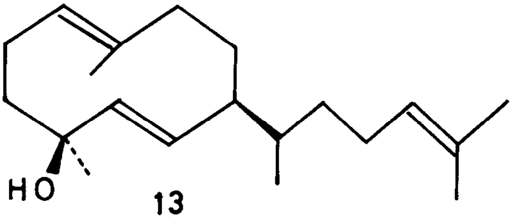
ments were carried out at -50°C using a 400 MHz spectrometer, but, unexpectedly, no NOE was observed between any pairs of protons by this high field spectrometer. On the other hand, when a 250 MHz instrument was used, small but distinct NOEs between several pairs of protons were observed.[9] These findings (depicted in 6) confirmed the structure and stereochemistry of acetoxypachydiol to be 6. MM2 calculations showed that the most stable conformation was exactly the same as 6, and free energy difference between 6 and the next favorable conformation 8 was ca. 1 kcal. The dibenzoate 5 exhibited negative splitted Cotton effects (λ_{ext} 237 nm, $\Delta\varepsilon$ -9.8; λ_{ext} 222 nm, $\Delta\varepsilon$ +9.8), and, hence, the absolute configuration of acetoxypachydiol was determined as depicted in 6.[10]

Acetoxypachydiol (4) is the first example of diterpene that possesses a 1,4-cyclodecadiene skeleton.

3-Hydroxyacetyldilophol (9), $C_{22}H_{36}O_3$, $[\alpha]_D$ -7.2° (c 0.64, $CHCl_3$), IR ($CHCl_3$) 3600, 1730, 1240 cm^{-1}, MS m/z 348 (M^+), 306, 288, 270, 177, 159, 109, 69 (base), exhibited the ^1H-NMR spectrum very similar with that of 3-acetoxyacetyldilophol (10).[6] Noticiable differences were that (1) only one acetyl signal was found in 9 and (2) the chemical shift of 3-H in 10 (δ ∿5.0) moved up to δ 4.36 (t, J = 3 Hz) in 9. On the basis of these properties, the structure 9 was assigned for 3-hydroxyacetyldilophol, and the structure was confirmed by conversion of 9 into 10 by acetylation.



9 ; X = OH , R = Ac
10 ; X = OAc , R = Ac
11 ; X = H, R = Ac
12 ; X = H, R = H



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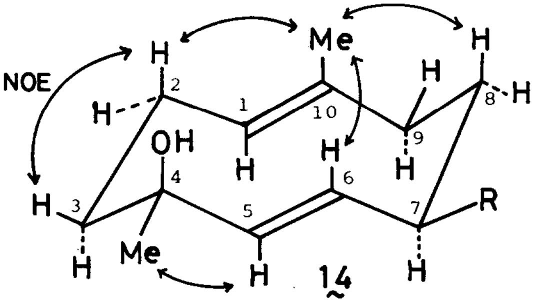

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From a less polar fraction, dilophol acetate (11), $[\alpha]_D$ -30.9° (c 0.57, $CHCl_3$), IR ($CHCl_3$) 1730, 1240 cm^{-1}, was isolated. Hydrolysis (KOH/MeOH) of dilophol acetate produced dilophol (12),[2] $[\alpha]_D$ -26.1° (c 0.44, $CHCl_3$). It is noteworthy that dilophol acetate changed into a compound on standing with silica gel (Merck, Kieselgel 60, Art. 7734, CH_2Cl_2) at room temperature for 24 hr. The product was characterized as obscuronatin (13),[3] $[\alpha]_D$ -112° (c 0.49, $CHCl_3$), by comparison of the spectral properties. The relative stereochemistry of obscuronatin has been recently confirmed by synthesis,[11] and since the absolute configuration of dilophol (12) has been established,[6] the absolute configuration of obscuronatin in the present alga was determined as in 13 by the above transformation.[12] The 400 MHz ^1H-NMR spectrum (27°C) of 13 reveals the well-defined signals. Also the ^{13}C-NMR spectrum (22.5 MHz at 27°C) exhibited sharp signals. These indicate that obscuronatin takes one stable conformation in contrast with other germacrane-type diterpenes. The conformation of obscuronatin was determined as depicted in 14, by analysis of the spin coupling patterns of the key protons (1-H, 2β-H, 5-H, 6-H) and observation of NOEs between the protons as shown in 14. Experiments using a paramagnetic shift reagent [Eu(fod)$_3$] also supported the conformation 14.[13]

Formation of obscuronatin (13) from dilophol acetate (11) is reasonably interpreted by (i) intramolecular [1,5] shift of the acetoxy group to C-4 followed by hydrolysis, or (ii) attack of water to C-4 with concomitant removal of the acetoxy group.

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1. N. H. Fischer, E. J. Oliver, and H. D. Fischer, "Progress in the Chemistry of Natural Products", Vol. 38, pp 47, Springer-Verlag, Wien New York, 1979.
2. V. Amico, G. Oriente, M. Piatteli, C. Tringali, E. Fattorusso, S. Magno, L. Mayol, J. Chem. Soc. Chem. Comm., 1976, 1024.
3. Y. Kashman, A. Groweiss, J. Org. Chem., 1979, 45, 3814.
4. H. H. Sun, W. Fenical, J. Org. Chem., 1979, 44, 1354.
5. R. Kazlauskas, P. T. Murphy, R. J. Wells, J. F. Blount, Tetrahedron Lett., 1978, 4155.
6. N. Enoki, H. Shirahama, A. Furusaki, K. Suehiro, E. Osawa, R. Ishida, T. Matsumoto, Chemistry Lett., 1984, 459.
7. M. Ishitsuka, T. Kusumi, H. Kakisawa, Y. Kawakami, Y. Nagai, T. Sato, J. Org. Chem., 1983, 48, 1937.
8. K. Tori, I. Horibe, H. Minato, K. Takeda, Tetrahedron Lett., 1971, 4355.
9. At low temperature, molecular motion becomes slow, which will result in increase in correlation time (τ_c). Under these conditions, NOE will become very small if ω_0 (frequency of observation system) is large enough to make $(\omega_0\tau_c)^2 \simeq 1$. Use of a low frequency spectroscopy [small ω_0; $(\omega_0\tau_c)^2 < 1$, +NOE] or lowering more the temperature [larger τ_c; $(\omega_0\tau_c)^2 > 1$, -NOE] would solve such problem.
10. T. Yoshida, J. Nobuhara, M. Uchida, T. Okuda, Tetrahedron Lett., 1976, 3717.
11. M. Kodama, K. Okumura, Y. Kobayashi, T. Tsunoda, S. Itô, Tetrahedron Lett., 1984, 25, 5781.
12. Chiloptical properties of obscuronatin from a soft coral Xenia obscuronata are not known (private communication from Prof. Y. Kashman).
13. Δδ values (ppm, $CDCl_3$) on addition of 1.4 molar equivalent of Eu(fod)$_3$: 2.82 (4-Me), 2.55 (3β-H), 2.46 (6-H), 1.85 (2β-H), 1.55 (5-H), 1.11 (1-H). Other signals did not show significant shifts.

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