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Cytotoxic Squalene-Derived Polyethers from the Marine Red Alga *Laurencia obtusa* (Hudson) Lamouroux[1)]

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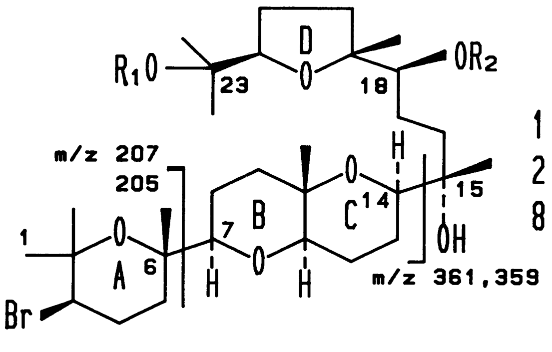
Five new cytotoxic triterpenoids with squalene carbon skeleton have been isolated from the title alga. The structures of 15(28)-anhydrothyrsiferyl diacetate, 15-anhydrothyrsiferyl diacetate, and magireol-A were confirmed by the spectral and chemical methods. The structures of magireol-B and -C were deduced from the spectral data.

In our continuing work on the neutral extract of *Laurencia obtusa* (Magiresozo in Japanese) which exhibited the remarkably cytotoxic properties, we previously reported[2)] the isolation of strongly active squalene derivatives, thyrsiferyl 23-acetate (1) and thyrsiferol (2),[3)] together with a novel *meso* compound, teurilene. Further investigation of this extract using the assay of cytotoxicities against P388 in vitro cell line has led to the isolation of five new compounds which are structurally related to thyrsiferol (2). In this paper, we wish to describe the structures of these active metabolites,[4)] 15(28)-anhydrothyrsiferyl diacetate (3), 15-anhydrothyrsiferyl diacetate (4), magireol-A (5), magireol-B (6), and magireol-C (7).

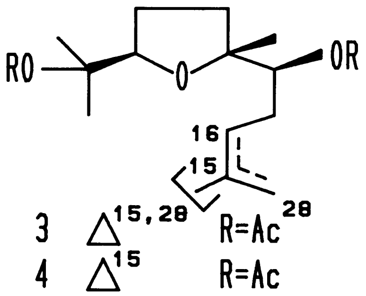
15(28)-Anhydrothyrsiferyl diacetate (3) (0.5% of the extract), $C_{34}H_{55}O_8Br$, mp 94-95 °C, $[\alpha]_D$ +8.8° (c 1.00; $CHCl_3$), displayed in its spectra[5)] the presence of two acetyl groups [ν_{max} 1735, 1720, and 1240 cm^{-1}; δ 1.97 and 2.07 (each 3H, s); δ 170.3 (s) and 170.7 (s)] and an *exo*-methylene group [ν_{max} 1645 and 895 cm^{-1}; δ 4.86 and 5.03 (each 1H, br s); δ 110.1 (t) and 150.9 (s)]. The ^1H and ^{13}C NMR spectra of 3 were similar to those of thyrsiferyl 23-acetate (1).

15-Anhydrothyrsiferyl diacetate (4) (0.4%), $C_{34}H_{55}O_8Br$, mp 163-164 °C, $[\alpha]_D$ +12.9° (c 1.00), had the same molecular formula as that of 3. The presence of two acetyl groups and a trisubstituted double bond was evident on the basis of ^1H NMR [δ 1.97, 2.01 (each 3H, s), 1.63 (3H, br s), and 5.35 (1H, br dd, J=7.0, 7.0 Hz)] and ^{13}C NMR [δ 170.4 (s) x 2, 139.0 (s), 121.8 (d), and 12.3 (q)] spectra. On detailed comparison of the spectral properties[5)] of 3 and 4 with those of 1, formula 3 and 4 could readily be assigned for these diacetates, respectively. The *E*-configuration of the double bond at C-15 in 4 was indicated by the ^{13}C NMR chemical shift (δ 12.3) of the vinyl methyl group which was observed in a considerably high magnetic field region.[6)] The structures of 3 and 4 were confirmed by the following reaction. Treatment of thyrsiferyl 18,23-diacetate (8)$^{2)}$ with thionyl chloride in pyridine at 0-5 °C for 10 h afforded the mixture of two dehydrated products (4:1). The major product was found to be identical with 4 in all respects, and the minor one was identified as 3 by comparison of the spectral data.$^{7)}$

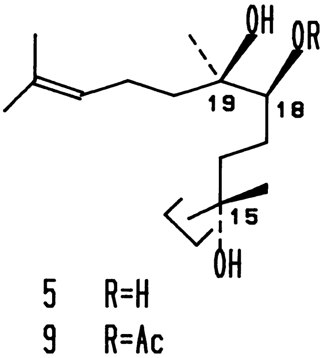
Magireol-A (5) (0.2%), $C_{30}H_{53}O_6Br$, HR-MS, m/z 552.2834, calcd for $C_{30}H_{49}O_4$ ^{79}Br, M^+-2H_2O, 552.2814, mp 98.5-100 °C, $[\alpha]_D$ 0° and $[\alpha]_{577}$ -7.0° (c 1.00), showed the following spectral characteristics; ν_{max} 3350, 1195, 1130, 1065, and 1030 cm^{-1}; δ 1.12, 1.17, 1.18, 1.20, 1.27, 1.40 (each 3H, s), 1.63, 1.69 (each 3H, br s), 3.05 (1H, dd, J=11.0, 2.2 Hz), 3.33 (1H, dd, J=9.2, 1.8), 3.56 (1H, dd, J=11.2, 7.1), 3.70 (1H, dd, J=12.3, 3.1), 3.89 (1H, dd, J=12.1, 4.4), and 5.14 (1H, br dd, J=7.1, 7.1); δ CH_3: 17.7, 20.1, 21.5, 22.9, 23.3, 23.7, 25.8, and 31.1, CH_2: 20.7, 21.1, 22.2, 23.1, 25.2, 28.3, 33.9, 36.3, 37.1, and 38.6, CH: 59.0, 76.0, 76.3, 79.2, 86.6, and 124.9, C: 72.0, 73.5, 74.4, 74.6, 75.0, and 131.6; m/z 445, 443 (0.7:0.7), 361, 359 (0.7:0.6), 207, 205 (5:5), 125 (79), and 69 (40). Since the IR spectrum of 5 showed the absence of carbonyl group, six oxygen atoms were assumed to be involved in 5 as hydroxyl and ether groups. Acetylation of 5 with acetic anhydride and pyridine gave the monoacetate 9, $C_{32}H_{55}O_7Br$, whose IR spectrum revealed absorptions due to acetyl and hydroxyl groups at 1730 and 3450 cm^{-1}, indicating the presence of one or more tertiary hydroxyl groups in 9. The presence of a prenyl moiety, $(CH_3)_2C=CH-CH_2-$, was shown by the ^1H NMR [δ 1.63 and 1.69 (each 3H, br s), and 5.14 (1H, br dd, J=7.1, 7.1 Hz)], ^{13}C NMR [δ 124.9 (d) and 131.6 (s)], and MS [m/z 69 ($C_5H_9^+$)] spectra. Furthermore, the ^{13}C NMR spectrum revealed that there were no double bonds other than the above-mentioned trisubstituted double bond and hence magireol-A (5) must be a tricyclic compound because of four degrees of unsaturation. The MS spectrum of 5 showed the fragment ions at m/z 361, 359 ($M^+-C_{13}H_{25}O_3$) and 207, 205 ($M^+-C_{22}H_{39}O_5$), which are also observed in the MS spectra of thyrsiferol (2) and its derivatives 1 and 8 as the characteristic fragments



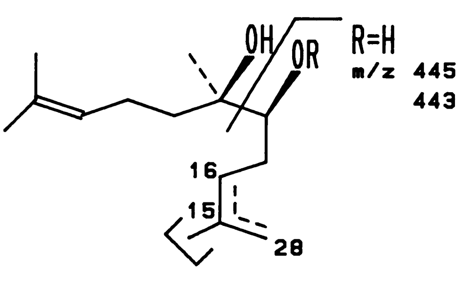
1 R_1=Ac R_2=H
2 R_1=R_2=H
8 R_1=R_2=Ac



3 $\Delta^{15,28}$ R=Ac
4 Δ^{15} R=Ac



5 R=H
9 R=Ac



6 $\Delta^{15,28}$ R=H
7 Δ^{15} R=H
10 $\Delta^{15,28}$ R=Ac
11 Δ^{15} R=Ac

resulting from cleavage at the C_{14}-C_{15} and C_6-C_7 bonds. Above results strongly suggested that the same A, B, and C ring system as that of thyrsiferol (2) is present in the molecule of 5, and consequently formula 5 could be proposed for magireol-A. Confirmation of the structure of 5 was carried out as follows. Upon epoxidation with m-chloroperbenzoic acid in dichloromethane, magireol-A (5) yielded epoxides which, without separation, were successively treated with p-toluenesulfonic acid to afford a mixture of two products (1:1). The more polar product was found to be identical with thyrsiferol (2) in all respects. Therefore, the structure of magireol-A is represented by formula 5.

Magireol-B (6) (0.2%), mp 64.5-66 °C, $[\alpha]_D$ +7.8° (c 1.00), and magireol-C (7) (0.2%), mp 69-70 °C, $[\alpha]_D$ + 6.4° (c 1.00), had the same molecular formula $C_{30}H_{51}O_5Br$. Both compounds 6 and 7 gave the corresponding monoacetates 10 and 11, $C_{32}H_{53}O_6Br$, respectively, on acetylation with acetic anhydride and pyridine. The ^1H and ^{13}C NMR spectra[8] of 6 and 7 were very similar to those of magireol-A (5), except for the signals due to an exo-methylene group [δ 4.89 and 5.04 (each 1H, br s); δ 110.4 (t) and 151.1 (s)] in 6 and an additional trisubstituted double bond [δ 1.66 (3H, br s) and 5.52 (1H, br dd, J=7.3, 6.6 Hz); δ 12.8 (q), 122.6 (d), and 139.6 (s)] in 7, respectively. Moreover, the MS spectra of 6 and 7 showed the same fragments at m/z 445, 443, 207, 205, 125, and 69 as those of 5. In view of the above-mentioned spectral properties together with co-occurrence of 3 and 4 in the same alga, formulae 6 and 7 would be most probable for the structures of magireol-B and magireol-C, repectively.

References

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3) J. W. Blunt, M. P. Hartshorn, T. J. McLennan, M. H. G. Munro, W. T. Robinson, and S. C. Yorke, Tetrahedron Lett., 1978, 69. The relative configuration at C-14, C-15, C-19, and C-22 of thyrsiferol have been mistranscribed from the X-ray view of thyrsiferyl 18-acetate (Dr. M. H. G. Munro, priv. commun., Feb. 11, 1985), and therefore the structures of thyrsiferyl 23-acetate and thyrsiferol should be revised as formula 1 and 2 , respectively, as shown in this paper. More recently, venustatriol[9] with the same carbon framework as that of thyrsiferol has been isolated from L. venusta along with thyrsiferol (2) and thyrsiferyl 23-acetate (1).

4) Cytotoxicity value, ED_{50}: 3, 50 ng/ml; 4, 100 ng/ml; 5, 30 ng/ml; 6, 30 ng/ml; 7, 30 ng/ml.

5) 3: IR (Nujol), ν_{max} 1735, 1720, 1645, 1275, 1240, 1170, 1140, 1110, 1050, 1030, 910, 895, and 840 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$), δ 1.18, 1.19 x 2, 1.26, 1.39, 1.42, 1.44, 1.97, 2.07 (each 3H, s), 3.07 (1H, dd, J=11.0, 2.6 Hz) 3.41 (1H, dd, J=11.5, 5.7), 3.89 (1H, dd, J=12.1, 4.0), 4.01 (1H, dd, J=8.8, 5.9), 4.26 (1H, br dd, J=7.5, 4.0), 4.86 (1H,br s), 4.93 (1H, dd,

J=10.3, 2.9), and 5.03 (1H, br s); ^{13}C NMR (67.8 MHz, $CDCl_3$), δ CH_3: 19.6, 20.1, 21.2, 21.7, 22.4, 22.5, 22.9, 23.7, and 31.1, CH_2: 21.8, 23.0, 26.3, 26.5, 28.3, 28.5, 28.8, 34.4, 37.1, 38.8, and 110.1, CH: 59.0, 72.5, 77.5, 78.8, 85.0, and 86.7, C: 72.7, 74.4, 75.0, 82.6, 84.3, 150.9, 170.3, and 170.7; MS (70 eV), m/z (rel. intensity) 207, 205 (15:14), 185 (19), and 125 (100); HR-MS, m/z 670.3064, calcd for $C_{34}H_{55}O_8{}^{79}Br$, M^+, 670.3079.

4: ν_{max} 1739, 1712, 1280, 1240, 1170, 1140, 1110, and 1030 cm^{-1}; δ 1.19 x 2 1.20, 1.26, 1.39, 1.42, 1.45, 1.97, 2.01 (each 3H, s), 1.63 (3H, br s), 3.06 (1H, dd, J=11.4, 2.9 Hz), 3.47 (1H, dd, J=11.0, 6.2,), 3.89 (1H, dd, J=12.1, 4.0), 4.02 (1H, dd, J=8.6, 5.7), 4.20 (1H, br dd, J=9.4, 3.7), 4.95 (1H, dd, J=9.5, 3.7), and 5.35 (1H, br dd, J=7.0, 7.0); δ CH_3: 12.3, 20.1 x 2, 21.1, 21.7, 22.4 x 2, 22.8, 23.7, and 31.0, CH_2: 21.7, 23.0, 25.6, 26.5, 28.3, 28.7 34.5, 37.1, and 38.8, CH: 59.0, 75.4, 77.4, 78.0, 85.0, 86.6, and 121.8, C: 72.2, 74.4, 75.0, 82.6, 84.1, 139.0, and 170.4 x 2; m/z 207, 205 (7:6), 185 (5), and 125 (100); HR-MS, m/z 670.3064.

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7) The deacetyl derivative of 3 has been previously isolated from L. pinnatifida. A. G. Gonzalez, J. M. Arteaga, J. J. Fernandez, J. D. Martin, M. Norte, and J. Z. Ruano, Tetrahedron, 40, 2751, (1984).

8) 6: ν_{max}3440, 3320, 1640, 1190, 1095, and 900 cm^{-1}; δ 1.16, 1.20, 1.23, 1.27, 1.40 (each 3H, s), 1.62, 1.69 (each 3H, br s), 3.08 (1H, dd, J=11.0, 2.6 Hz), 3.39 (1H, d, J=9.9), 3.44 (1H, dd, J=11.4, 5.5), 3.89 (1H, dd, J=12.1, 4.0), 4.31 (1H, br dd, J=8.9, 3.9), 4.89, 5.04 (each 1H, br s), and 5.13 (1H, br dd J=7.0, 7.0); δ CH_3: 17.7, 19.5, 20.1, 23.3, 23.7, 25.7, and 31.0, CH_2: 21.7, 22.1, 22.9, 26.1, 28.3, 29.6, 30.0, 36.1, 37.1, 38.6, and 110.4, CH: 59.0, 72.5, 78.2, 78.8, 86.7, and 124.7, C: 73.1, 74.4, 74.6, 75.0, 131.6, and 151.1; m/z 445, 443 (2.2:2.4), 207, 205 (34:36), 125 (65), and 69 (43); HR-MS, m/z 570.2915, calcd for $C_{30}H_{51}O_5{}^{79}Br$, M^+, 570.2917.

7: ν_{max}3550, 3500, 1130, 1110, and 1060 cm^{-1}; δ 1.19, 1.20, 1.22, 1.27, 1.40, (each 3H, s), 1.63, 1.66, 1.69 (each 3H, br s), 3.07 (1H, dd, J=11.0, 2.2 Hz), 3.45 (1H, dd, J=9.7, 3.2), 3.49 (1H, dd, J=11.2, 6.8), 3.89 (1H, dd, J=12.1, 4.4), 4.26 (1H, br dd, J=9.7, 3.5), 5.13 (1H, br dd, J=7.1, 7.1), and 5.52 (1H, br dd, J=7.3, 6.6); δ CH_3: 12.8, 17.7, 20.1, 20.3, 23.4, 23.7, 25.7, and 31.1, CH_2: 21.8, 22.2, 23.0, 25.8, 28.3, 30.0, 36.4, 37.1, and 38.8, CH: 59.0, 75.4, 77.7, 77.9, 86.6, 122.6, and 124.6, C: 72.3, 74.4 x 2, 74.9, 131.7, and 139.6; MS, m/z 445, 443 (2.2:2.4), 207, 205 (27:28), 125 (79), and 69 (50); HR-MS, 570.2914.

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