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Two New Antitumoral Polyether Squalene Derivatives

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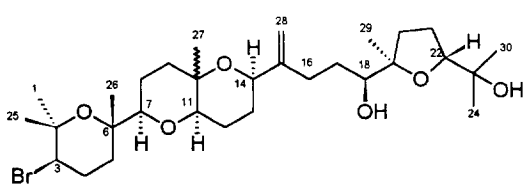
Abstract: Two new polyether squalene derivatives isodehydrothyrsiferol **2** and 10-epidehydrothyrsiferol **3** have been isolated from the red alga *Laurencia viridis*. Their structures were determined through the interpretation of 2D NMR spectra and their antitumoral activity was established. Copyright © 1996 Elsevier Science Ltd

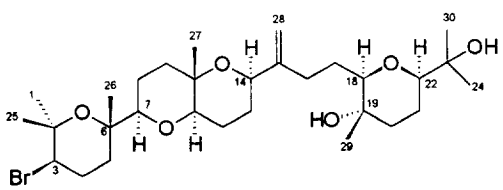
Red seaweeds of the genus *Laurencia* are known to produce the interesting and active squalene-derived metabolites thyrsiferol and venustatriol[1]. These bromine-containing polyethers show strong cytotoxic and antiviral properties, respectively.

We wish to report on the isolation and structure determination by spectral methods of two new polyether triterpenes with a squalene carbon skeleton which exhibited a significant pharmacology activity. These compounds were isolated from acetone extracts of *Laurencia viridis* sp. *nov* (Ceramiales, Rhodomelaceae), a new species described from specimens collected around the Canary Islands, in Macaronesia[2].

Isodehydrothyrsiferol **2** did not show a molecular ion in its mass spectrum. Its molecular formula was established as $C_{30}H_{51}O_6Br$ on the basis of the fragmentations observed in its HRMS at m/z 506.36127 (M^+-HBr) and the two isotopic peaks at m/z 570.25401/572.25524 (M^+-CH_3)[3]. These results establish that this compound is an isomer of dehydrothyrsiferol **1** [4] and the comparison of the NMR spectral data of these two compounds showed that the differences between them were localized around the isolated tetrahydrofuran ring system present in compound **1**. Thus, in the ^{1}H-NMR spectrum of compound **2**, the proton signals H-7, H-18 and H-22 were overlapped and centred at δ 3.08, 3.13 and 3.15, while in compound **1**, these signals were centred at δ 3.08, 3.51, and 3.75, respectively. Moreover, in the ^{13}C-NMR spectrum was observed



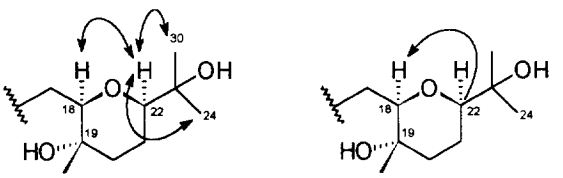
1 C_{27} = β- CH_3
3 C_{27} = α- CH_3



2

a significative change in the chemical shifts of carbons bearing an oxygen atom C-18 and C-19. These carbon chemical signals in compound **1** were centred at δ 76.31 and 86.71 for the tertiary (C-18) and quaternary (C-19) carbons, while in compound **2,** they were centred at δ 84.10 and 70.15, respectively. These data suggested that the heterocyclic ring has changed in this compound to a tetrahydropyran ring system instead of the tetrahydrofuran ring system present in compound **1**. It was confirmed by the combination of the HMQC and HMBC experiments. In the HMQC experiment, the proton signals assigned to protons H-18 (δ 3.13) and H-22 (δ 3.15) were correlated with the carbon chemical shifts at δ 84.10 (C-18) and 84.42 (C-22), while in the HMBC experiment was observed the correlation between C-22 and H-18. This result established the ether link between carbons C-18 and C-22.

The conformation of this tetrahydropyran ring was established as a chair with the side chains in the equatorial orientation as deduced not only from the observed coupling constant values but also by the analysis of nuclear Overhauser enhancements. Since the ^{1}H-NMR signals for H-18 and H-22 in deuterated chloroform overlap, the experiments were carried out in deuterated pyridine. In this spectrum H-18 was centred at δ 3.51 (dd, J= 1.17 and 10.37 Hz) and H-22 at δ 3.38 (dd, J= 1.24 and 11.2 Hz), thus establishing the axial orientation of these methines in accordance with the coupling constant values. In the nOe experiment, the irradiation of the proton signal H-22 causes enhancement of the proton signals for the methyl groups Me-24/Me-30 and for H-18, while the irradiation of H-18 only causes enhancement of H-22. (**Figure 1**). These results allowed us to establish that the relative orientation of the methyl group Me-29 must be axial and on the opposite face relative to methines H-18 and H-22. Moreover, from the biogenetic point of view, there are two possibilities for the formation of the dihydropyran ring and only one of them yielded the two protons of the ring closure on the same face, whose that the methyl group is on the other face.



nOe HMBC

Figure 1

10-Epidehydrothyrsiferol **3**, proved to be an isomer of compounds **1** and **2** on the basis of its molecular ion at m/z 587/589 (MH^+) in the FAB mass spectrum, NBA being the most suitable matrix. Its spectroscopic data[5] showed that the chemical structure was closely related to the value that observed for dehydrothyrsiferol **1**. Thus, the fragmentations observed in the mass spectrum and the absorptions observed in the infrared spectrum were identical. In the ^{1}H-NMR spectrum, the chemical shifts and the coupling constant values for methines H-3, H-18 and H-22 together with those for the vinylic methylene protons H-28 were also identical. However, the proton signals for methines at δ 3.69 (H-7), δ 3.84 (H-11) and δ 4.27 (H-14) showed differences either for the chemical shifts or for the coupling constant values. These proton chemical shifts were assigned on the basis of the observed HMBC correlations. Thus, carbon C-3 (δ 59.04), which was identified by its HMQC correlation with H-3 (δ 3.90), showed correlation with the methylene H-5's (δ 1.61) which, in turn, was correlated with the methyl group Me-26 (δ 20.74). The HMBC correlation between this carbon methyl group and the proton signal centred at δ 3.69 permit us to identify H-7. Taking into account the characteristic chemical shift for the allylic methine H-14 (δ 4.27), the remaining signal at δ 3.84 must be H-11. The HMBC

correlation between this proton signal and the carbon methyl signal centred at δ 23.56 allowed us to identifiy the proton signal for Me-27 at δ 1.17.

The comparison between these proton signals and those established for dehydrothyrsiferol, showed differences in the coupling constant values. Thus, while in compound **1** the proton signals for H-7 and H-11 were solved as a double of doublets with two different coupling constant values (one large and the other small), in compound **3**, the proton signals H-7 and H-11 showed a double of doublets with identical coupling constant values. This fact must be due to a change in the conformation of the fused tetrahydropyran rings. It was confirmed in the ROESY experiment by the correlations observed of H-11 with H-7, H-14 and Me-27. This last correlation established that the two heterocycles must be cis-fused with a flexible conformation, instead of the trans-fused system present in compound **1**.

The biogenesis of these squalene-polyether derivatives has been proposed as a result of an attack of bromonium ion on the terminal C-2/C-3 double bond of squalene tetraepoxide, which induces concerted cyclizations to occur, thus forming the frame-work of these polyethers[6]. Thus, all compounds isolated from *Laurencia* species showed identical relative stereochemistry at the C-2/C-15 moiety, the differences being established at carbons C-18 and C-19 in the thyrsiferol and venustatriol series or in the absence of the tetrahydrofuran ring system in the magireol series[7].

The isolation of compounds **2** and **3** established a new type of these squalene-polyether derivatives and the stereochemistry at carbon C-10 in compound **3** indicated that the proposed biogenesis through the cyclization of the squalene tetraepoxide precursor may not be concerted.

A simple screening procedure has been carried out to determine and compare the tumoral activity of these compounds, using an adapted form of the method described by Bergeron et al[8]. The antitumor cells employed were P-388 (suspension culture of a lymphoid neoplasm from DBA/2 mouse), A-549 (monolayer culture of human lung carcinoma), HT- 29 (monolayer culture of a human colon carcinoma) and MEL-28 (monolayer culture of human melanoma). Compound **2** demonstrated an important and selective activity in P-388 cells line (**Table 1**).

	IC 50 μg/ml			
Compounds	P-388	A-549	HT-29	MEL-28
Isodehydrothyrsiferol (**2**)	0.01	2.5	2.5	2.5
10-Epidehydrothyrsiferol (**3**)	1	5	5	5

Table 1

Acknowledgements

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3. **Compound 2:** Oil; $[\alpha]^{25}_D$ = + 6.5 (*c* 0.23, $CHCl_3$); IR υ_{max} ($CHCl_3$): 3490, 2956, 2857, 1725, 1462, and 1381 cm^{-1}; HRMS: M^+ no observed, 572.25524 (calc. $C_{29}H_{47}O_6{}^{81}Br$ 572.25355 [M^+-CH_3]), 570.25401 (calc. $C_{29}H_{47}O_6{}^{79}Br$ 570.25560 [M^+-CH_3]), 506.36127 (calc. $C_{30}H_{50}O_6$ 506.36074 [M^+-HBr]), 473.32621 (calc. $C_{29}H_{45}O_5$ 473.32670 [M^+-HBr-CH_3-H_2O]), 445.17358 (calc. $C_{22}H_{36}O_4{}^{81}Br$ 445.17765 [M^+-$C_8H_{15}O_2$]), 443.17453 (calc. $C_{22}H_{36}O_4{}^{79}Br$ 443.17970 [M^+-$C_8H_{15}O_2$]), 363.25452 (calc. $C_{22}H_{35}O_4$ 363.25353); MS at m/z: 572, 570, 506, 473, 445, 443, 403, 363, 332, 265, 143; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.04 (s, H-28), 4.88 (s, H-28'), 4.30 (dd, J= 4.3, 7.0 Hz, H-14), 3.90 (dd, J= 4.2, 12.3 Hz, H-3), 3.43 (dd, J= 5.9, 11.5 Hz, H-11), 3.15 (dd, J= 1.7, 10.7 Hz, H-22), 3.13 (dd, J= 1.7, 10.6 Hz, H-18), 3.08 (dd, J= 2.6, 11.2 Hz, H-7), 2.42 (m, H-16), 2.25 (m, H-4), 2.13 (m, H-16'), 2.12 (m, H-4'), 2.09 (m, 2H, H-21), 2.06 (m, H-13), 1.88 (m, H-20), 1.88 (m, H-17), 1.85 (m, H-13'), 1.82 (m, H-5), 1.81 (m, H-9), 1.80 (m, H-12), 1.78 (m, H-8), 1.60 (m, H-12'), 1.55 (m, H-5'), 1.55 (m, H-20'), 1.51 (m, H-9'), 1.50 (m, H-17'), 1.49 (m, H-8'), 1.40 (s, 3H, H-25), 1.27 (s, 3H, H-1), 1.22 (s, 3H, H-27), 1.20 (s, 3H, H-30), 1.20 (s, 3H, H-26), 1.17 (s, 3H, H-29), 1.15 (s, 3H, H-24); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 19.83 (q, C-27), 20.48 (q, C-26), 20.64 (q, C-29), 22.23 (t, C-12), 23.34 (t, C-8), 24.01 (q, C-25), 24.41 (q, C-24), 24.99 (t, C-21), 26.72 (t, C-13), 26.77 (q, C-30), 27.28 (t, C-17), 28.66 (t, C-4), 29.96 (t, C-16), 31.42 (q, C-1), 37.50 (t, C-5), 39.10 (t, C-9), 40.22 (t, C-20), 59.47 (d, C-3), 70.15 (s, C-19), 72.13 (s, C-23), 72.66 (d, C-14), 73.31 (s, C-10), 74.79 (s, C-6), 75.35 (s, C-2), 79.37 (d, C-11), 84.10 (d, C-18), 84.42 (d, C-22), 87.08 (d, C-7), 110.40 (t, C-28), 151.68 (s, C-15).

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5. **Compound 3:** Oil; $[\alpha]^{25}_D$ = + 20.7 (*c* 0.76, $CHCl_3$); IR υ_{max} ($CHCl_3$): 3438, 2978, 1644, 1455, and 1374 cm^{-1}; HRMS: M^+ no observed, 529.23585 (calc. $C_{27}H_{44}O_5{}^{81}Br$ 529.23516 [M^+-C_3H_7O]), 527.23762 (calc. $C_{27}H_{44}O_5{}^{79}Br$ 527.23721 [M^+-C_3H_7O]), 506.36318 (calc. $C_{30}H_{50}O_6$ 506.36074 [M^+-HBr]), 446.18054 (calc. $C_{22}H_{37}O_4{}^{81}Br$ 446.18547 [M^+-$C_8H_{14}O_2$]), 444.18748 (calc. $C_{22}H_{37}O_4{}^{79}Br$ 444.18752 [M^+-$C_8H_{14}O_2$]), 363.25223 (calc. $C_{22}H_{35}O_4$ 363.25353); MS at m/z: 529, 527, 506, 445, 363, 291, 209, 143; FAB MS m/z: 587/589 [MH^+]; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.13 (s, H-28), 4.83 (s, H-28'), 4.27 (dd, J= 7.4, 7.4 Hz, H-14), 3.90 (dd, J= 3.6, 12.3 Hz, H-3), 3.84 (dd, J= 7.0, 7.0 Hz, H-11), 3.76 (dd, J= 6.0, 9.7 Hz, H-22), 3.69 (dd, J= 6.8, 6.8 Hz, H-7), 3.53 (d, J= 10.3 Hz, H-18), 2.39 (m, H-16), 2.25 (m, H-4), 2.11 (m, H-4'), 2.06 (m, H-16'), 2.08 (m, H-20), 2.05 (m, H-13), 1.94 (m, H-9), 1.92 (m, H-8), 1.90 (m, H-12), 1.88 (m, H-21), 1.85 (m, H-5), 1.84 (m, H-20'), 1.70 (m, H-12'), 1.67 (m, H-17), 1.66 (m, H-13'), 1.64 (m, H-8'), 1.61 (m, H-5'), 1.57 (m, H-21'), 1.50 (m, H-17'), 1.41 (s, 3H, H-25), 1.29 (s, 3H, H-1), 1.22 (s, 3H, H-26), 1.22 (s, 3H, H-30), 1.17 (s, 3H, H-27), 1.15 (s, 3H, H-29), 1.13 (s, 3H, H-24); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 20.74 (q, C-26), 23.56 (q, C-27), 23.70 (q, C-25), 23.76 (q, C-24), 26.43 (t, C-8), 26.56 (t, C-21), 27.07 (t, C-12), 27.69 (q, C-30), 28.13 (t, C-4), 28.94 (t, C-16), 30.34 (t, C-17), 30.93 (q, C-1), 31.22 (q, C-29), 31.63 (t, C-13), 31.91 (t, C-20), 33.80 (t, C-9), 35.83 (t, C-5), 59.04 (d, C-3), 70.46 (s, C-23), 74.78 (s, C-2), 74.89 (s, C-6), 76.27 (d, C-18), 82.21 (d, C-14), 84.64 (s, C-10), 84.78 (d, C-11), 86.08 (s, C-19), 87.60 (d, C-22), 88.25 (d, C-7), 109.17 (t, C-28), 149.63 (s, C-15).

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