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New Polyether Squalene Derivatives from *Laurencia*

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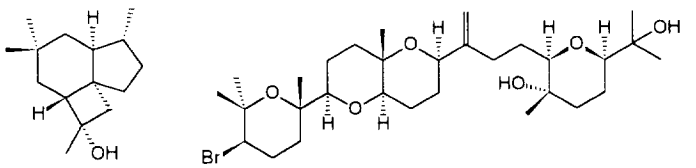
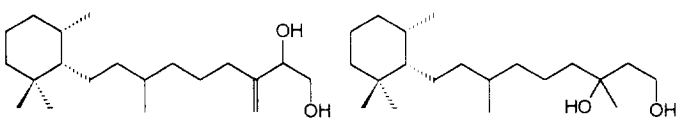
Abstract: Five new triterpenoid polyethers with a squalene carbon skeleton have been isolated from the red alga *Laurencia viridis*. Their structures were determined through the interpretation of 2D-NMR spectral data. The relative stereochemistry is proposed on the basis of ROESY and NOEDIFF data. © 1997 Elsevier Science Ltd.

Laurencia viridis sp. nov. (Ceramiales, Rhodomelaceae) is a new species described from specimens collected around the Canary Islands.[1] It is an annual plant that grows in the lower intertidal zone, intermingled with other turf algae. It occurs on exposed, overhanging rocks subject to strong wave-action, grows rapidly during winter-spring months and decays in late summer. From this alga we have isolated a new sesquiterpene, viridianol **1**,[2] the diterpenes viridiol A **2** and B **3**,[3] and a potent cytotoxic metabolite isodehydrothyrsiferol **4**.[4]



Viridianol **1** Isodehydrothyrsiferol **4**

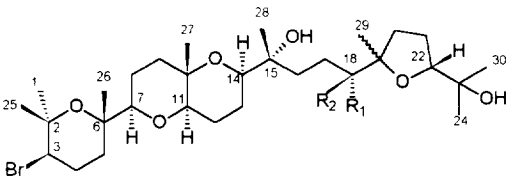


Viridiol A **2** Viridiol B **3**

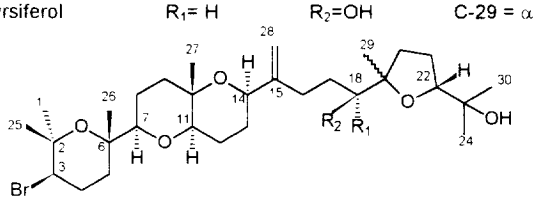
We wish to report here on the isolation and structural determination of several new polyether triterpenes with a squalene carbon skeleton related to venustatriol **5**[5] and thyrsiferol **6**.[6] The alga was collected in March 1994 at Paraíso Floral, Callao Salvaje and El Palmar, located in South Tenerife (Canary Islands). It was air-dried, ground in a Willey mill to 1mm particle size and extracted with Cl_2CH_2 in a Soxhlet apparatus and with $CHCl_3$:MeOH (1:1) at room temperature.

The crude extract was successively chomatrographed on silica gel and Sephadex LH-20 columns and the final purification was achieved, either on HPLC μ-Bondapak C-18 reverse phase, μ-Porasil and Spherisorb

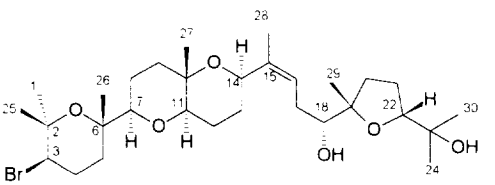
columns. This chromatographic study afforded in addition to dehydrothyrsiferol **7**,[7] five new compounds: dehydrovenustatriol **8**, 15,16-dehydrovenustatriol **9**, predehydrovenustatriol acetate **10**, 16-hydroxydehydrothyrsiferol **11** and 10-epi-15,16-dehydrothyrsiferol **12**.



5 Venustatriol R_1= OH R_2=H C-29 = β CH_3
6 Thyrsiferol R_1= H R_2=OH C-29 = α CH_3



7 Dehydrothyrsiferol R_1= H R_2=OH C-29 = α CH_3 Δ^{15-28}
8 Dehydrovenustatriol R_1= OH R_2=H C-29 = β CH_3 Δ^{15-28}

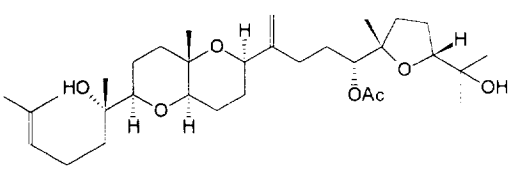


9 15,16-Dehydrovenustatriol

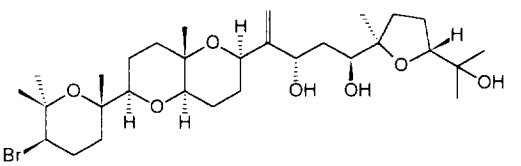
Dehydrovenustatriol **8** was isolated as an amorphous white solid, $[\alpha]^{25}_{D}$= + 4.6 (*c* 0.26, $CHCl_3$) and the molecular formula $C_{30}H_{51}O_6Br$ was established on the basis of its molecular ion. Its spectroscopic data were closely related to those of dehydrothyrsiferol **7** and suggesting a similar planar structure. The relative stereochemistry for **8** was determined by means of a ROESY spectrum which established the same ABC-ring system as that of thyrsiferol. However, the chemical shifts of H-18, Me-29 and H-22 together with the coupling constant values of methines H-18 and H-22 were very similar to those observed for the venustatriol series. Moreover, the cis relationship between Me-29 and H-22 was confirmed by the ROE correlation observed between them.

Compound **9**, 15,16-dehydrovenustatriol, proved to be an isomer of compounds **7** and **8**, as established by HRMS. A detailed comparison of the spectral properties of **8** and **9** showed that the difference between them was the presence of a trisubstituted double bond in compound **9**, evident on the basis of ^{1}H-NMR [δ 1.73 (s, 3H); 5.42 (dd, J= 7.7 and 7.6 Hz) and ^{13}C-NMR δ 126.3 (d); 137.8 (s) and 18.73 (q)]. The Z-configuration of the double bond was assigned by the intense ROE correlation observed between Me-28 and H-16 as well as by the ^{13}C-NMR chemical shift of the vinyl methyl group. Also, the following ROE correlations: H-16 with H-18; H-18 with Me-29; H-14 with H-17β; H-16 with H-17α could only be accommodated by this Z-configuration.

Undoubtedly, the most interesting metabolite, from the biogenetic point of view, was predehydrovenustatriol acetate **10**. It was isolated as a colourless oil $[\alpha]^{25}_{D}$ = + 20.6 (*c* 0.33, $CHCl_3$) and the molecular formula $C_{32}H_{54}O_7$ was established by HRMS. The absorption at 3691, 3455 and 1725 cm^{-1} in the I.R. spectrum indicated the presence of two hydroxy and one ester group, respectively. The ^{1}H-NMR spectrum revealed the presence of six methyls in α position to the oxygen at δ 1.25, 1.24, 1.19, 1.15 and 1.09, two olefinic methyls at δ 1.61 and 1.67, and an acetate methyl group at δ 2.07; five oxygenated methines at δ 4.95 (dd, J= 2.3 and 10 Hz), 4.15 (dd, J= 5.5 and 9.1 Hz), 3.97 (dd, J= 6 and 9 Hz), 3.81 (dd, J= 7 and 7.1 Hz) and 3.45 (dd, J= 2 and 11.6 Hz) and three olefinic protons at δ 5.10 (1H, t, J= 7 Hz) and 4.73 (2H, bs). The ^{13}C-NMR spectrum confirmed the presence of these groups and a detailed analysis of 2D-NMR spectral data showed that the structure of the BC-rings as well as that of the side chain was identical with that of dehydrovenustatriol acetate. The important feature to emphasize was the absence of the bromine atom in the position C-3 together with the presence of a trisubstituted double bond between the carbons C-2/C-3. In order to confirm the presence of this system as well as the relative configuration at carbon C-6, compound **8** gave the 18-monoacetate by treatment with Ac_2O/Py and then by reaction with zinc powder and acetic acid in ethanol afforded a tricyclic compound identical in all respects with compound **10**, thus confirming its structure as a venustatriol derivative.



10 Predehydrovenustatriol acetate



11 16-Hydroxydehydrothyrsiferol

The next compound 16-hydroxydehydrothyrsiferol **11** was isolated, as the previous one, as an amorphous solid whose molecular formula $C_{30}H_{51}O_7Br$ was established by HRMS. Comparison of its NMR spectra with those of dehydrothyrsiferol, revealed the disappearance of the allylic methylene group which was substituted by a new methine group in α position to a hydroxy group. The allylic nature of the secondary hydroxyl group was established on the basis of its ^{1}H-NMR chemical shift at δ 4.86 and its localization at C-16 was deduced unequivocally from its 2D-NMR data. Thus, in the COSY experiment connectivities could be established between proton H-16 (δ 4.86, dd, J= 4.2 and 7.6 Hz) and the protons of the methylene H-17 (δ 1.88 and 1.69) which in turn were connected with the proton of the methine H-18 (δ 3.82, dd, J= 1.5 and 10.1 Hz), forming an isolated system that ends in two quaternary carbons. These carbons were identified as C-15 (δ 149.29) and C-19 (δ 85.54) by their HMBC correlations with H-16 and H-18, respectively. The relative stereochemistry at chiral centres with the exception of C-16, was established as being identical with that observed for dehydrothyrsiferol in accordance with the correlations established in the ROESY experiment. Thus, the proton H-16 (δ 4.86) was correlated with the protons H-13 (δ 1.85), H-14 (δ 4.50), H-17 (δ 1.69) and H-18 (δ 3.82). Furthermore, the proton H-17' (δ 1.88) showed a ROE connectivity with H-18. This sequence of ROE correlations can only be explained if the relative configuration at C-16 is S*, as shown in **Figure 1**.

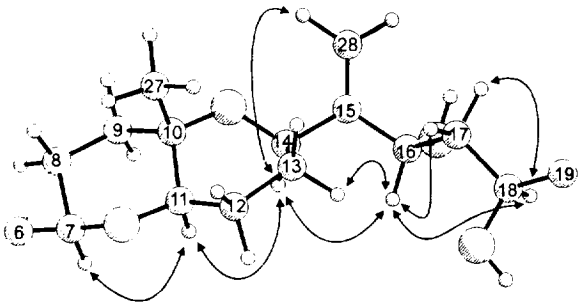
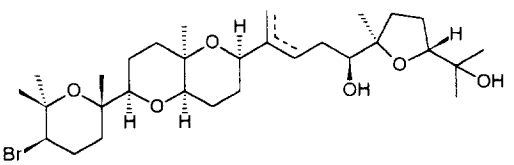


Figure 1

The last compound isolated, 10-epi-15,16-dehydrothyrsiferol **12**, is an isomer of 10-epidehydrothyrsiferol **13**,[4] published recently. Interpretation of COSY, HMQC and HMBC data allowed assignments of ^{1}H and ^{13}C-NMR signals, which readily demonstrated that the sole difference between them was the position of the double bond. The NMR data of compound **12** clearly established the presence of an E-trisubstituted double bond between carbons C-15/C-16, instead of the present in compound **11** between carbons C-15/C-28. The relative stereochemistry of all their chiral centres was identical. The isolation of these compounds reinforces the hypothesis that the biosynthesis of these squalene-polyether derivatives through the cyclization of the squalene tetraepoxide precursor may not be concerted.



12 10-Epi-15,16-dehydrothyrsiferol Δ^{15-16}
13 10-Epidehydrothyrsiferol Δ^{15-28}

EXPERIMENTAL PART

General methods. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. IR spectra were measured on a Bruker IFS55 spectrometer. The NMR spectra were obtained with a Bruker AMX-400 instrument. Chemical shifts are reported relative to TMS and coupling constants are given in Hz. HRMS were performed on a Kratos MS-80RFA spectrometer. HPLC was carried out with a LKB 2248 system equipped with a differential diffractometer detector. Silica gel CC and TLC were performed on Silica gel Merck 60 G. TLC plates were visualised by spraying with H_2SO_4/H_2O/AcOH (1:4:20) and heating. All solvents were purified by standard techniques.

Plant material. Seeds of *Laurencia viridis* were collected in April 1994 in the intertidal zone at Callao Salvaje, Paraíso Floral, El Palmar (Tenerife, Canary Islands). Dried material of sterile plants, sporophytes and gametophytes is deposited at TFC Phyc (Herbario de la Universidad de La Laguna, Departamento de Biología Vegetal, Botánica, Tenerife).

Extraction. The dried alga (4 kg) was extracted with CH_2Cl_2 in a Soxhlet apparatus for 24 h each and after with $CHCl_3$:MeOH (1:1) at room temperature. The combined extracts were evaporated *in vacuo* to leave a dark-green viscous oil (52.0 g, 1.3 % dry weight).

Chromatographic separation. The crude extract was chromatographed on a silica gel column using n-hexane-EtOAc mixtures of increasing polarity. The *n*-hexane:EtOAc (3:2) eluate, after solvent evaporation, was successively chromatographed with a Sephadex LH-20 column (600 x 70 mm∅), with *n*-hexane:$CHCl_3$:MeOH (2:1:1) as eluent; and medium pressure silica gel chromatography, collecting 25 ml fractions: *n*-hexane:EtOAc (7:3), fractions 1-70; *n*-

hexane:EtOAc (1:1), fractions 71-100; EtOAc, fractions 101-150. Fractions exhibiting similar tlc profiles were combined and each one was rechromatographed on a medium pressure reverse-phase Lobar LiChropred RP-8 (310 x 25 mm∅), with MeOH:H_2O (9:1) as eluent and later on a μ-Bondapak C-18 (150 x 19 mm∅) column HPLC reverse phase chromatography with acetonitrile:H_2O (9:1) as eluent. Fractions 40-51 (816 mg) yielded pure **10** (3.3 mg) and impure **8** and **9**; final purification was carried out by HPLC employing μ-Porasil (150 x 19 mm∅) prepacked column and using *n*-hexane:EtOAc (7:3), affording pure **8** (6.5 mg) and **9** (26.2 mg). Fractions 52-82 (750.7 mg) yielded pure **7** (219.8 mg). Fractions 83-107 (344 mg) yielded pure **11** (2.3 mg) and impure **4** and **13**, which were rechromatographed by HPLC on μ-Porasil, *n*-hexane:EtOAc (3:2), affording pure **4** (2.3 mg) and **13** (23.2 mg). Fractions 108-130 (1.235 g) gave, after final purification by HPLC employing a Spherisorb Silica 5 μ (300 x 6.5 mm∅) prepacked column in *n*-hexane:EtOAc (1:1), pure **12** (2.2 mg).

Compound 8. Dehydrovenustatriol: amorphous white solid, $[\alpha]_D^{25}$ = + 4.6 (*c* 0.26, $CHCl_3$); IR υ_{max} ($CHCl_3$): 3689, 3649, 3020, 2928, 2854, 2359, 2341, 1734, 1601 and 1466 cm^{-1}; HRMS: 588.28693 (calc. $C_{30}H_{51}O_6{}^{81}Br$ 588.28485 [M^+]), 586.28496 (calc. $C_{30}H_{51}O_6{}^{79}Br$ 586.28690 [M^+]), 506.36071 (calc. $C_{30}H_{50}O_6$ 506.36074 [M^+-HBr]); FAB (NBA+NaI) MS at m/z: 611, 609, 529, 329, 279, 207, 176, 143; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.13 (s, 3H, H-24), 1.16 (s, 3H, H-29), 1.20 (s, 3H, H-26), 1.23 (s, 3H, H-27), 1.26 (s, 3H, H-30), 1.27 (s, 3H, H-1), 1.40 (s, 3H, H-25), 1.45 (m, H-17), 1.48 (m, H-8), 1.51 (m, H-9), 1.60 (m, H-20), 1.63 (m, H-12), 1.66 (m, H-17'), 1.73 (m, 2H, H-5), 1.75 (m, H-8'), 1.80 (m, H-12'), 1.82 (m, H-9'), 1.83 (m, H-13), 1.92 (m, 2H, H-21), 2.06 (m, H-13'), 2.11 (m, H-4), 2.17 (m, H-20'), 2.20 (m, H-16), 2.25 (m, H-4'), 2.43 (m, H-16'), 3.08 (dd, J= 11.4, 2.6 Hz, H-7), 3.45 (dd, J= 11.3, 5.6 Hz, H-11), 3.59 (dd, J= 10.5, 1.9 Hz, H-18), 3.83 (dd, J= 7.3, 7.3 Hz, H-22), 3.90 (dd, J= 12.3, 4.2 Hz, H-3), 4.30 (dd, J= 8.0, 4.0 Hz, H-14), 4.89 (s, H-28), 5.04 (s, H-28'); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 20.02 (q, C-26), 20.49 (q, C-27), 22.14 (t, C-12), 23.35 (t, C-8), 24.06 (q, C-29), 24.06 (q, C-25), 25.73 (q, C-24), 26.51 (t, C-13), 27.26 (t, C-21), 27.97 (q, C-30), 28.65 (t, C-4), 29.71 (t, C-16), 31.20 (t, C-17), 31.41 (q, C-1), 31.87 (t, C-20), 37.47 (t, C-5), 39.04 (t, C-9), 59.45 (d, C-3), 72.37 (s, C-23), 72.91 (d, C-14), 73.36 (s, C-10), 74.79 (s, C-6), 75.36 (s, C-2), 76.90 (d, C-18), 79.03 (d, C-11), 84.93 (d, C-22), 86.69 (s, C-19), 87.07 (d, C-7), 110.64 (t, C-28), 151.47 (s, C-15).

Compound 9, 15-16-Dehydrovenustatriol: amorphous white solid, $[\alpha]_D^{25}$ = - 5.4 (*c* 0.26, $CHCl_3$); IR υ_{max} ($CHCl_3$): 3571, 2981, 2866, 1732, 1602, 1460, 1378 and 1321 cm^{-1}; HRMS: M^+ not observed, 570.27450 (calc. $C_{30}H_{49}O_5{}^{81}Br$ 570.27429 [M^+-H_2O]), 506.35715 (calc. $C_{30}H_{50}O_6$ 506.36074 [M^+-HBr]), 445.17512 (calc. $C_{22}H_{36}O_4{}^{81}Br$ 445.17765 [M^+-$C_8H_{15}O_2$]), 443.17607 (calc. $C_{22}H_{36}O_4{}^{79}Br$ 443.17970 [M^+-$C_8H_{15}O_2$]), 399.15331 (calc. $C_{20}H_{32}O_3{}^{79}Br$ 399.15348 [M^+-$C_{10}H_{19}O_3$]); MS at m/z: 570, 506, 473, 445, 443, 403, 399, 363, 334, 291, 207, 143; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.14 (s, 3H, H-24), 1.18 (s, 3H, H-29), 1.21 (s, 3H, H-26), 1.22 (s, 3H, H-30), 1.23 (s, 3H, H-27), 1.27 (s, 3H, H-1), 1.41 (s, 3H, H-25), 1.44 (m, H-8), 1.54 (m, H-5), 1.56 (m, H-12), 1.56 (m, H-9), 1.60 (m, H-13), 1.73 (s, 3H, H-28), 1.76 (m, H-8'), 1.79 (m, H-9'), 1.81 (m, H-5'), 1.85 (m, 2H, H-21), 1.95 (m, H-12'), 2.05 (m, H-20), 2.08 (m, H-13'), 2.11 (m, H-4), 2.12 (m, H-20'), 2.18 (m, H-17), 2.24 (m, H-4'), 2.27 (m, H-17'), 3.09 (dd, J= 11.4, 2.2 Hz, H-7), 3.40 (dd, J= 9.9, 2.2 Hz, H-18), 3.66 (dd, J= 11.0, 7.4 Hz, H-11), 3.76 (dd, J= 8.7, 6.9 Hz, H-22), 3.89 (dd, J= 12.2, 4.1 Hz, H-3), 4.77 (dd, J= 12.3, 2.7 Hz, H-14), 5.42 (dd, J= 7.7, 7.6 Hz, H-16), ^{13}C-NMR (100 MHz, $CDCl_3$) δ 18.73 (q, C-28), 20.06 (q, C-26), 21.26 (q, C-27), 21.53 (t, C-12), 22.28 (q, C-29), 22.68 (t, C-8), 22.98 (q, C-25), 23.64 (q, C-24), 24.17 (t, C-13), 26.49 (t, C-21), 27.63 (q, C-30), 28.24 (t, C-4), 30.13 (t, C-17), 31.02 (q, C-1), 34.32 (t, C-20), 37.05 (t, C-5), 38.64 (t, C-9), 59.01 (d, C-3), 67.35 (d, C-14), 70.56 (s, C-23), 72.22 (s, C-10), 74.38 (s, C-6), 74.96 (s, C-2), 76.35 (d, C-18), 76.44 (d, C-11), 85.31 (s, C-19), 86.52 (d, C-7), 86.89 (d, C-22), 126.23 (d, C-16), 137.83 (s, C-15).

Compound 10, Predehydrovenustatriol acetate: colourless oil; $[\alpha]_D^{25}$ = + 20.6 (*c* 0.33, $CHCl_3$); IR υ_{max} ($CHCl_3$): 3691, 3580, 3456, 2978, 2931, 2872, 2359, 1727, 1456 and 1249 cm^{-1}; HRMS: M^+ not observed, 548.37054 (calc. $C_{32}H_{52}O_7$ 548.37130 [M^+-2H]), 532.37182 (calc. $C_{32}H_{52}O_6$ 532.37639 [M^+-H_2O]), 473.32361 (calc. $C_{29}H_{45}O_5$ 473.32670 [M^+-C_3H_7O-H_2O]); MS at m/z: 548, 532, 522, 473, 405, 345, 321, 282, 261, 227, 183; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.09 (s, 3H, H-24), 1.15 (s, 3H, H-26), 1.19 (s, 3H, H-29), 1.24 (s, 3H, H-30), 1.25 (s, 3H, H-27), 1.35 (m, H-5), 1.47 (m, H-13), 1.51 (m, H-17), 1.53 (m, H-5'), 1.54 (m, H-8), 1.58 (m, H-20), 1.61 (s, 3H, H-25), 1.63 (m, H-21), 1.67 (m, H-12), 1.67 (s, 3H, H-1), 1.71 (m, H-4), 1.80 (m, H-8'), 1.87 (m, H-20'), 1.89 (m, H-12'), 1.91 (m, H-17'), 1.93 (m, H-13'), 1.98 (m, H-21'), 2.07 (s, 3H, H-32), 2.11 (m, H-4'), 2.16 (m, H-16), 2.32 (m, H-16'), 3.45 (dd, J= 11.6, 2.0 Hz, H-7), 3.81 (dd, J= 7.0, 6.9 Hz, H-22), 3.97 (dd, J= 9.0, 5.9 Hz, H-11), 4.15 (dd, J= 9.1, 5.5 Hz, H-14), 4.73 (bs, 2H, H-28), 4.95 (dd, J= 10.0, 2.3 Hz, H-18), 5.10 (dd, J= 7.1, 7.0 Hz, H-3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 17.65 (q, C-25), 21.19 (q, C-32), 22.07 (t), 22.17 (q, C-29), 23.22 (q, C-26), 24.31 (q, C-27), 24.92 (q, C-24), 25.68 (q, C-1), 26.33 (t), 26.47 (t), 27.08 (t), 27.49 (t, C-21), 27.89 (q, C-30), 27.96 (t, C-4), 28.32 (t, C-16), 32.63 (t), 34.81 (t,

C-20), 36.75 (t, C-5), 71.34 (s, C-23), 73.33 (s, C-6), 75.13 (d, C-7), 77.75 (d, C-18), 77.77 (s, C-10), 79.16 (d, C-14), 84.01 (s, C-19), 84.60 (d, C-11), 85.39 (d, C-22), 109.42 (t, C-28), 124.79 (d, C-3), 131.33 (s, C-2), 146.03 (s, C-15), 170.84 (s, C-31).

To a solution of product **8** (3 mg, 5.7 μmol) in dry pyridine (250 μl) was added acetic anhydride (250 μl) and the mixture was stirred for 5 h at rt under nitrogen. Usual work-up gave an oily residue.To a solution of this residue, in 1 ml of EtOH at 0° C, were added 5 mg of Zn powder and 50 μl of AcOH. The mixture was stirred for 3 hr, filtered off and the solvent evaporated to give an oily residue which was chromatographed, affording 1.9 mg of pure compound **10**.

Compound 11, 16-Hydroxydehydrothyrsiferol: amorphous white solid; $[\alpha]_D^{25}$ = - 2.2 (*c* 0.23, $CHCl_3$); IR υ_{max} ($CHCl_3$): 3789, 3694, 3514, 3020, 2929, 2856, 1726, 1602, 1461, 1381 and 1221 cm^{-1}; HRMS: 604.27987 (calc. $C_{30}H_{51}O_7{}^{81}Br$ 604.27976 [M^+]), 602.28189 (calc. $C_{30}H_{51}O_7{}^{79}Br$ 602.28181 [M^+]), 522.35563 (calc. $C_{30}H_{50}O_7$ 522.35565 [M^+-HBr]); FAB (NBA+NaI) MS: m/z: 627, 625; 609; 593; 577; 561; 545; 357; 301; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.13 (s, 3H, H-24), 1.14 (s, 3H, H-29), 1.21 (s, 3H, H-26), 1.22 (s, 3H, H-30), 1.28 (s, 3H, H-1), 1.30 (s, 3H, H-27), 1.41 (s, 3H, H-25), 1.47 (m, H-8), 1.55 (m, H-9), 1.57 (m, H-5), 1.61 (m, H-12), 1.69 (m, H-17), 1.75 (m, H-8′), 1.77 (m, H-9′), 1.81 (m, H-5′), 1.84 (m, 2H, H-21), 1.85 (m, H-13), 1.87 (m, H-12′), 1.88 (m, H-17′), 2.10 (m, H-4), 2.12 (m, 2H, H-20), 2.22 (m, H-13′), 2.25 (m, H-4′), 3.09 (dd, J= 11.6, 2.7 Hz, H-7), 3.58 (dd, J= 11.1, 6.4 Hz, H-11), 3.77 (dd, J= 10.1, 6.2 Hz, H-22), 3.82 (dd, J= 10.1, 1.5 Hz, H-18), 3.90 (dd, J= 12.3, 4.2 Hz, H-3), 4.50 (dd, J= 10.6, 3.4 Hz, H-14), 4.86 (dd, J= 7.6, 4.2 Hz, H-16), 5.25 (s, H-28), 5.30 (s, H-28′); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 20.05 (q, C-26), 20.43 (q, C-27), 21.61 (t, C-12), 22.93 (q, C-29), 23.25 (t, C-8), 23.65 (q, C-25), 24.00 (q, C-24), 25.98 (t, C-13), 26.48 (t, C-21), 27.67 (q, C-30), 28.21 (t, C-4), 31.00 (q, C-1), 32.12 (t, C-20), 34.96 (t, C-17), 37.08 (t, C-5), 36.62 (t, C-9), 58.94 (d, C-3), 70.46 (s, C-23), 72.09 (d, C-14), 72.83 (d, C-18), 73.39 (s, C-10), 74.35 (s, C-6), 75.00 (s, C-2), 77.21 (d, C-11), 82.96 (d, C-16), 85.54 (s, C-19), 86.66 (d, C-7), 87.52 (d, C-22), 114.72 (t, C-28), 149.29 (s, C-15).

Compound 12, 10-Epi-15,16-dehydrothyrsiferol: amorphous white solid; $[\alpha]_D^{25}$ = + 8.0 (*c* 0.20, $CHCl_3$); IR υ_{max} ($CHCl_3$): 3789, 3694, 3572, 3022, 2928, 2857, 1726, 1666 and 1602 cm^{-1}; HRMS: M^+ not observed, 506.36410 (calc. $C_{30}H_{50}O_6$ 506.36074 [M^+-HBr]); FAB (NBA+NaI) MS: m/z: 759; 685; 641; 633; 625; 611; 609; 593; 591; 545; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.13 (s, 3H, H-24), 1.16 (s, 3H, H-27), 1.17 (s, 3H, H-29), 1.22 (s, 3H, H-26), 1.22 (s, 3H, H-30), 1.28 (s, 3H, H-1), 1.41 (s, 3H, H-25), 1.56 (m, H-21), 1.60 (m, H-9), 1.61 (m, H-5), 1.63 (s, 3H, H-28), 1.64 (m, H-8), 1.66 (m, H-13), 1.71 (m, H-12), 1.85 (m, H-21′), 1.86 (m, H-20), 1.87 (m, H-5′), 1.89 (m, H-12′), 1.91 (m, H-13′), 1.92 (m, H-8′), 1.95 (m, H-9′), 2.11 (m, H-20′), 2.12 (m, H-4), 2.14 (m, H-17), 2.23 (m, H-4′), 2.24 (m, H-17′), 3.58 (dd, J= 9.7, 2.8 Hz, H-18), 3.68 (dd, J= 7.1, 7.1 Hz, H-7), 3.76 (dd, J= 10.0, 6.0 Hz, H-22), 3.82 (dd, J= 6.7, 6.7 Hz, H-11), 3.89 (dd, J= 12.2, 4.0 Hz, H-3), 4.23 (dd, J= 7.6, 7.6 Hz, H-14), 5.60 (dd, J= 6.7, 6.7 Hz, H-16); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 11.88 (q, C-28), 20.77 (q, C-29), 23.45 (q, C-27), 23.71 (q, C-25), 23.99 (q, C-24), 26.43 (t, C-21), 26.54 (t, C-8), 27.39 (t, C-12), 27.71 (q, C-26), 27.71 (q, C-30), 28.14 (t, C-4), 30.18 (t, C-13), 30.39 (t, C-17), 30.95 (q, C-1), 32.02 (t, C-20), 34.00 (t, C-9), 35.77 (t, C-5), 59.06 (d, C-3), 70.45 (s, C-23), 74.80 (s, C-2), 74.82 (s, C-6), 76.68 (d, C-18), 84.51 (d, C-14), 84.69 (d, C-11), 84.70 (s, C-10), 85.79 (s, C-19), 87.55 (d, C-22), 88.23 (d, C-7), 122.94 (d, C-16), 137.31 (s, C-15).

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