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First *seco*-C Oleananes from Nature

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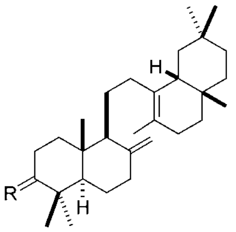
ABSTRACT



1 : R = H, β-OH
2 : R = H, β-OAc
3 : R = O

The triterpenes 8,14-*seco*-oleana-8(26),13-dien-3*β*-ol (1) and its acetyl derivative 2 were isolated from *Stevia viscida* and *Stevia eupatoria*, respectively. Their structures were elucidated by 2D NMR, including carbon–carbon connectivity experiments, and confirmed by X-ray diffraction analysis of ketone 3. The absolute configuration was determined by NMR analysis of the Mosher esters of 1. The biogenetic implications of the new substances are discussed.

The discovery of *seco*-triterpenoids as natural products has been useful for understanding the biogenetic pathways of live organisms. The isolation of these apparently unfinished new skeletons may, in some cases, confirm hypotheses or provide evidence to reinforce the biosynthetic approaches for specific groups of natural substances.[1] To date, several *seco*-A,[2] *seco*-A,B,C,[3] *seco*-B,C,[4] and *seco*-E[5] oleananes have been found in diverse species. However, no *seco*-C oleananes have been isolated yet. In other triterpene families there are relevant groups of *seco*-C derivatives, such as the onoceranes,[6] which belong to the gammacerane family. This paper describes the isolation, structure elucidation, and NMR spectral assignment of 8,14-*seco*-oleana-8(26),13-dien-3*β*-ol (**1**) and 8,14-*seco*-oleana-8(26),13-dien-3*β*-ol acetate (**2**) from species of the genus *Stevia*[7] (Chart 1).

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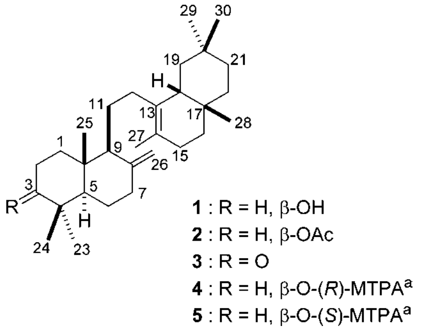
Chart 1



1 : R = H, β-OH
2 : R = H, β-OAc
3 : R = O
4 : R = H, β-O-(*R*)-MTPA[a]
5 : R = H, β-O-(*S*)-MTPA[a]

[a] MTPA = α-methoxy-α-(trifluoromethyl)phenylacetyl

Table 1. ^{13}C NMR Assignments of *seco*-C Oleananes **1**−**3** in $CDCl_3$ at 75.4 MHz

carbon	1	2[a]	3	carbon	1	2[a]	3
1	36.8	36.5	37.6	16	26.4	26.5	26.5
2	28.0	24.3	34.9	17	31.5	31.5	31.6
3	78.9	80.9	216.7	18	42.9	43.0	43.1
4	39.1	38.0	47.8	19	42.9	42.9	43.0
5	54.7	54.8	55.3	20	31.0	31.0	31.1
6	24.1	23.9	25.2	21	34.6	34.6	34.6
7	38.2	38.0	38.0	22	36.5	36.5	36.6
8	148.3	148.1	147.6	23	28.3	28.2	26.0
9	56.9	56.7	56.1	24	15.4	16.5	21.8
10	39.5	39.4	39.5	25	14.5	14.5	14.1
11	22.9	22.9	23.4	26	106.6	106.7	107.4
12	30.8	30.7	30.8	27	18.8	18.7	18.9
13	134.3	134.2	133.9	28	27.2	27.3	27.3
14	123.5	123.6	123.7	29	33.1	33.1	33.2
15	29.5	29.5	29.5	30	24.1	24.1	24.2

[a] OAc: δ 171.0 and 21.3

8,14-*seco*-Oleana-8(26),13-dien-3β-ol (**1**) was obtained as a white solid, mp 134−135 °C and $[\alpha]_D$ +30, after chromatography of the hexane extracts of the roots of *Stevia viscida*.[8] The molecular formula was determined as $C_{30}H_{50}O$ by HREIMS (found 426.3868, calcd 426.3862). The 300 MHz ^{1}H NMR spectrum displayed signals at δ 4.87 and δ 4.62 for an exocyclic methylene, a signal at δ 3.24 for a hydrogen geminal to a hydroxyl group (dd, J = 11.7, 4.4 Hz), a signal at δ 1.55 for a vinylic methyl group, and six singlets between δ 0.98 and 0.66 for six tertiary methyl groups. The ^{13}C NMR spectrum (Table 1) in combination with a DEPT spectrum indicated the presence of an exocyclic double bond (δ 148.3 and 106.6) and a tetrasubstituted double bond (δ 134.3 and 123.5). The ^{13}C NMR sp^3 region revealed the presence of four quaternary carbons, four methines, 11 methylenes, and seven methyl groups in agreement with a tetracyclic triterpene structure.

8,14-*seco*-Oleana-8(26),13-dien-3β-ol acetate (**2**) was obtained as a white solid, mp 96−98 °C and $[\alpha]_D$ +45, after chromatography of the hexane extracts of the roots of two collections of *Stevia eupatoria*[9] from different locations. The molecular formula was determined as $C_{32}H_{52}O_2$ by HREIMS (found 468.3966, calcd 468.3967). The ^{1}H NMR spectrum displayed signals at δ 4.86 and 4.63 for the exocyclic methylene. The signal for the hydrogen geminal to the acetate group at C-3 appeared shifted downfield (δ 4.50) with respect to the spectrum of **1**. A signal at δ 1.54 for a vinylic methyl group and six singlets between δ 0.86 and 0.69 for six tertiary methyl groups were consistent with structure **2**. The ^{13}C NMR spectrum showed the presence of the acetate group signals at δ 171.0 and 21.3, the exocyclic double bond at δ 148.1 and 106.7, and the endocyclic olefin at δ 134.2 and 123.6. As expected, treatment of **1** with acetic anhydride and pyridine afforded acetate **2**, while alkaline hydrolysis of **2** yielded alcohol **1**.

Given that both triterpenes contain a new hydrocarbon skeleton, their structure and ^{13}C NMR spectral assignment (Table 1) were secured by means of HSQC, HMBC, DEPT, and particularly by a ^{13}C−^{13}C connectivity experiment of compound **2**, obtained using the INADEQUATE pulse sequence. The signal-to-noise ratio of the INADEQUATE spectrum was improved by the use of the FRED program,[10] which allowed us to detect the C4−C24 correlation and to clearly evidence all the sp^2−sp^3 and sp^3−sp^3 ^{13}C-$^{-3}$C connectivities, except that for C18−C19 due to chemical shift proximity. These two carbon atoms were distinguished by means of the DEPT spectrum. The sp^2−sp^2 connectivities were not observed because the ^{13}C−^{13}C coupling constant value was set at 35 Hz for the INADEQUATE acquisition. Nevertheless, the olefinic carbons C8, C13, C14, and C26 were clearly assigned from their respective connectivities with the sp^3 carbons and from the HMBC data. The ^{13}C NMR assignments of rings D and E of the new *seco*-C oleananes **1** and **2** were compared with those of *seco*-A,B,C-oleanane **9** isolated from the fern *Polypodiodes formosana*.[3] This comparison revealed that the ^{13}C NMR assignment for the C12−C13−C14−C15 fragment of **9** may be revised to δ 31.7, 133.6, 123.9, and 29.5, respectively. In turn, the ^{13}C NMR assignments of rings A and B of **1**−**3** were compared to those of 3β-hydroxylabda-8(20),13-dien-15-oic acid,[11] 3β-acetyloxy-labda-8(20),13-dien-15-oic acid, and labda-8(17),12*Z*,14-trien-3-one,[12] being in full agreement with our results.

The structure and stereochemistry of **1** and **2** were confirmed by X-ray diffraction analysis[13] of ketone **3**,

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obtained by treatment of alcohol **1** with the CrO_3−pyridine complex which yielded colorless needles, mp 129−131 °C. The X-ray structure of **3** (Figure 1) evidenced the preferred conformation in the solid state of this peculiar substance, in which two carbocyclic units are linked by an ethylene chain. The C9−C11−C12−C13 dihedral angle was found to be +166°, while rings A, B, D, and E showed chair, chair, half-chair, and chair conformations, respectively. Density functional theory calculations[14] of ketone **3** indicated that the global minimum energy structure was very similar to that found in the solid state. The root-mean-square error between the X-ray structure of **3** and the minimum energy DFT molecular model, considering the non-hydrogen atoms, was 0.238 Å. The conformational search was carried by using molecular mechanics (MMFF) and a Monte Carlo procedure which afforded nine different conformations within a range of 5 kcal/mol. The geometry of these structures was optimized by DFT calculations using the B3LYP/6-31G* level of theory. The DFT global minimum (E_T = −1247.32417 hartrees) showed a C9−C11−C12−C13 dihedral angle of +174° and rings A, B, D, and E in chair, chair, half-chair, and chair conformations, respectively. Two other relevant minima were found within a 2 kcal/mol range. A geometrical analysis of these structures indicated that the first local minimum (ΔE_T = 1.15 kcal/mol) showed a variation in the C11−C12−C13−C18 dihedral angle with respect to conformer A (−93° vs −160°), while the second local minimum (ΔE_T = 1.37 kcal/mol) presented a variation in the C9−C11−C12−C13 dihedral angle (+72° vs +174°).

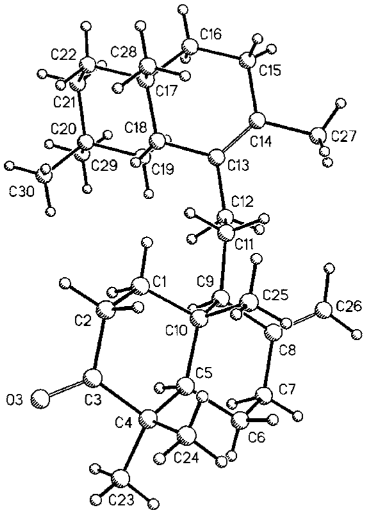

Figure 1. X-ray structure of 8,14-*seco*-oleana-8(26),13-dien-3-one (**3**).

The absolute configuration of the new substances was established as 5*R*,9*S*,10*R*,17*R*,18*R* by 2D NMR analysis of the Mosher esters[15] **4** and **5**, which were prepared by treatment of alcohol **1** with either (*R*)- or (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid in the presence of dicyclohexylcarbodiimide and 4-(dimethylamino)pyridine. Table 2 shows the ^{1}H NMR chemical shift differences for selected signals of both esters.

Table 2. Selected ^{1}H NMR Chemical Shifts for **4** and **5**

proton	**4** (*R*)	**5** (*S*)	$\Delta_{(R-S)}$
H-1α(ax)	1.22	1.22	+0.00
H-1β(eq)	1.81	1.79	+0.20
H-2α(eq)	1.76	1.60	+0.16
H-2β(ax)	1.88	1.81	+0.07
Me-23(eq)	0.81	0.90	−0.09
Me-24(ax)	0.77	0.78	−0.01

The isolation of this kind of molecules may be indicative of the presence of alternative pathways for the triterpene biosyntheses in the genus *Stevia*. In the present case, the squalene cyclization may proceed as represented in Scheme 1 through a pathway similar to that proposed for the biosynthesis of the preoleanatetraene **9**.[3] In analogy to the biosynthesis of **9** and the well-established route for oleananes,[1] we can surmise that monocyclization of squalene (**4**) will produce intermediate **5** which can be designated as a predammarenyl cation. Similarly, intermediate **6** can be considered as a prebaccharenyl cation which may cyclize to the prelupenyl cation **7**. The E-ring expansion will lead to a preoleanyl cation **8** forming the preoleananetetraene **9** via two hydrogen shifts. Finally, epoxidation of the terminal

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(13) Crystal data: $C_{30}H_{48}O$, monoclinic, space group $P2_1$, a = 13.401(1) Å, b = 7.508(3) Å, c = 13.903(2) Å, V = 1352.0(3) Å^3, ρ_{calcd} = 1.04 g/cm^3, Z = 2, M = 424.68, $F(000)e^-$ = 472. Crystal size: 0.50 × 0.42 × 0.30 mm^3. Collected reflections: 1950 within a θ range of 3.29−54.93° on a Bruker-Nonius CAD4 diffractometer with Cu Kα radiation (λ = 1.541 84 Å). Unique reflections: 1851 with R_{int} = 3.9%. Observed reflections: 1845 with $[I > 4\sigma(I)]$. The structure was solved by direct methods using SHELXS97 (Sheldrick, G. M. *Programs for Crystal Structure Analysis*; Institut für Anorganische Chemie der Universität: Göttingen, Germany, 1998). Final discrepancy indices were R_F = 3.9% and R_W = 10.6% refining 373 parameters. Crystallographic data are deposited with the Cambridge Crystallographic Data Centre. The CCDC deposition no. is 226154. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK. Fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk.

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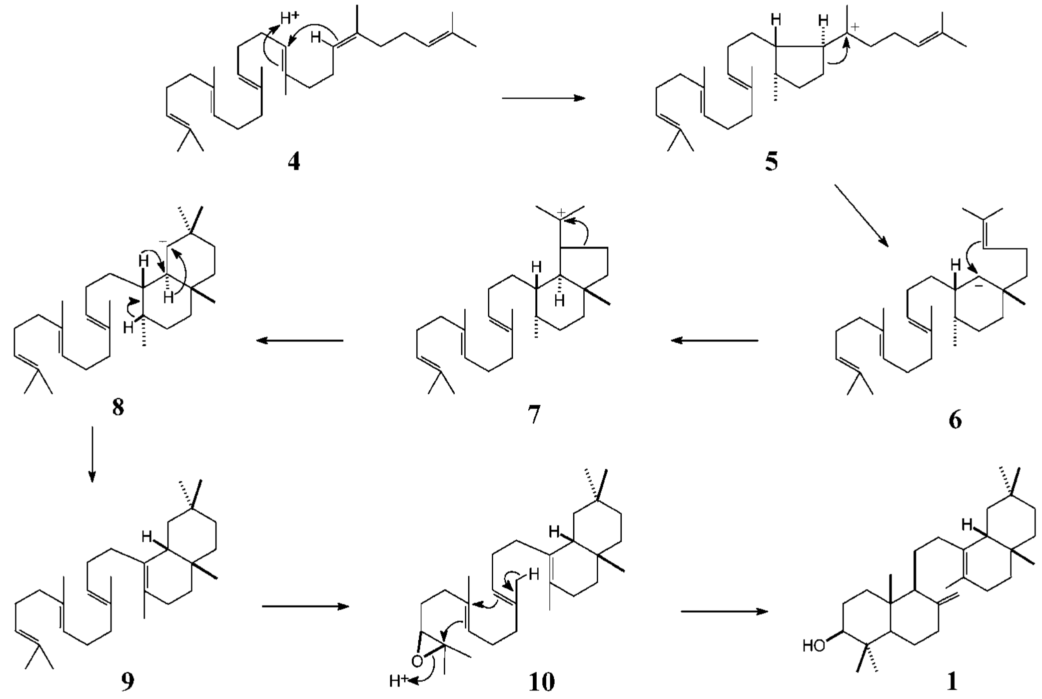
Scheme 1



double bond as in **10**, followed by protonation and opening of the epoxide moiety, will induce formation of rings A and B, as has been proposed for the biosynthesis of onoceranes.[16] In agreement with this hypothesis, several species of the genus *Stevia* produce triterpenes belonging to the dammarane,[17] lupane,[18] and oleanane[19] families.

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Supporting Information Available: Experimental details on the isolation of **1** and **2**, physical and spectral data of **1**−**3**, and preparation of Mosher esters **4** and **5**. HSQC, HMBC, ^{13}C-$^{-3}$C connectivity spectrum, and FRED analysis of **2**. X-ray and DFT atomic coordinates of **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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