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STEVIN, A NEW PSEUDOGUAIANOLIDE ISOLATED FROM *STEVIA RHOMBIFOLIA* H.B.K.[1]

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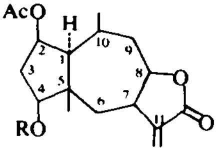
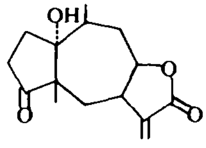
Abstract—Stevin a constituent of *Stevia rhombifolia* H.B.K. has been shown to be a new pseudoguaianolide with structure Ia.

IN PREVIOUS papers[2–4] we have reported the structures of the pseudoguaianolides peruvin (II), peruvinin (III) and cumanin (IV) isolated from *Ambrosia peruviana* Willd and *A. cumanensis* H.B.K. (tribe Heliantheae). They are characterized by a lactone grouping oriented at C-8 in contrast to the pseudoguaianolides found in other *Ambrosia* species which possess a lactone group closed at C-6.[5]

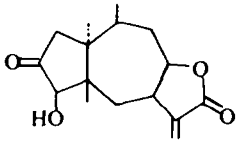
We now describe the structure determination of a new pseudoguaianolide with its lactone grouping oriented at C-8 which we have named *stevin*. It was isolated from a collection of *Stevia rhombifolia* H.B.K. (Eupatorieae).



I a, R = H
b, R = Ac



II



III

Stevin (Ia) m.p. 184–186°, $[\alpha]_D$ +161°, analyzed for $C_{17}H_{24}O_5$. It contains a secondary OH group (IR band at 3600 cm^{-1}) which affords an acetate and was oxidized to a ketone.

Stevin (Ia) exhibits UV (λ max 212 mμ; ε, 10,080) and IR spectra (bands at 1750 and 1652 cm^{-1}) typical of an α,β-unsaturated γ-lactone. The other two oxygen atoms of the empirical formula (Ia) are involved in an acetoxy group as shown by an IR band at 1730 cm^{-1} and corroborated by its NMR spectrum.[6] It shows in the Me region a singlet at 2·03 corresponding to an acetyl group. A singlet at 0·96 and a doublet (J = 6 c/s) at 1·07 indicates the presence of a tertiary and a secondary

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[1] Contribution No. 243 from the Instituto de Química de la Universidad Nacional Autónoma de México.

[2] P. Joseph-Nathan and J. Romo, *Tetrahedron* **22**, 301 (1966).

[3] J. Romo, P. Joseph-Nathan and G. Siade, *Tetrahedron* **22**, 1499 (1966).

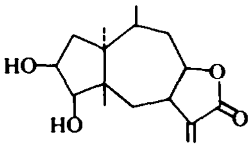
[4] J. Romo, A. Romo de Vivar, P. Joseph-Nathan and C. Alvarez, *Tetrahedron* **22**, (1966).

[5] W. Herz, H. Watanabe, M. Miyasaki and Y. Kishida, *J. Am. Chem. Soc.* **84**, 2601 (1962); W. Herz and G. Högenauer, *J. Org. Chem.* **26**, 5011 (1961); M. Suchý, V. Herout and F. Šorm, *Coll. Czech. Chem. Comm.* **28**, 2257 (1963); T. J. Mabry, W. Renold, H. E. Miller and H. B. Kagan, *J. Org. Chem.* **31**, 681 (1966) and Refs cited therein.

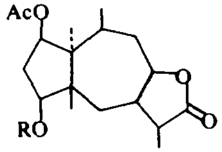
[6] The NMR spectra were determined by Mr. Eduardo Díaz on a Varian A-60 spectrometer in $CDCl_3$ soln. using TMS as an internal reference. All chemical shifts are reported in ppm as δ values (c/s 60).

Me group. The exocyclic methylene protons showed a pair of low field doublets ($J = 2$ c/s) at 6·26 and 5·62. A complex signal at 5·17 was assigned to the hydrogen on the carbon bearing the acetoxy group, therefore this function is attached to a secondary carbon atom. A triplet ($J = 9$ c/s) centered at 3·95 attributed to the proton on the carbon carrying the OH group is shifted downfield on acetylation. An allylic proton is responsible for a broad signal at 3·17. A signal at 2·50 ascribed to the OH group is not observed after equilibration with deuterium oxide. The chemical shift and the multiplicity of a signal centered at 4·70 which in the NMR spectrum of dehydrostevin (VI) (obtained by chromium trioxide oxidation of an acetone solution of Ia is clearly observed at 4·67 as a pair of lateral doublets and a central triplet with smaller doubling[7] indicates a lactone oriented to C-8. The NMR spectrum of VI also exhibits the following signals: Two methylene low field doublets at 6·28 and 5·67, a triplet with smaller doubling at 5·29 (hydrogen on the carbon bearing the acetoxy grouping), a singlet at 2·07 (acetyl group) and a doublet at 1·12 partially superimposed with a singlet at 1·06 (secondary and tertiary Me groups).

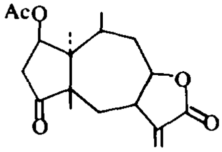
Catalytic hydrogenation of stevin (Ia) yielded a dihydroderivative (Va) which does not show high absorption at 212 mμ in the UV spectrum. The methylene low field doublets observed in the NMR spectrum of Ia are not present in the spectrum of Va. A new doublet at 1·14 assigned to the 11-Me group is superimposed on the other Me signals.



IV

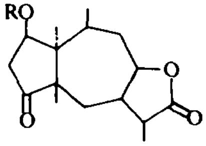


V a, R=H
b, R=Ac

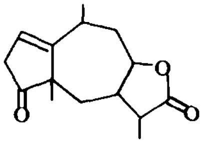


VI

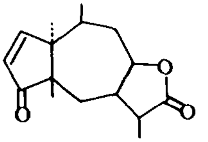
The relative position of the acetoxy and OH groups in the 5-membered ring of stevin as shown in formula Ia was elucidated in the following way: The IR spectrum of dehydrostevin (VI) exhibits a strong carbonyl band at 1750 cm^{-1} corresponding to a cyclopentanone, a γ-lactone and an acetyl group, which in dehydrodihydrostevin (VIIb) appears at 1745 cm^{-1} with a shoulder at 1767 cm^{-1}. Mild treatment of an ethanolic solution of dehydrodihydrostevin (VIIb) with hydrochloric acid gave the



VII a, R=H
b, R=Ac



VIII



IX

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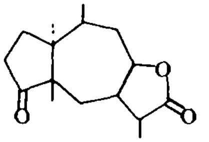
[7] W. Herz, W. A. Rohde, K. Rabindran, P. Jayaraman and N. Viswanathan, *J. Am. Chem. Soc.* **84**, 3857 (1962).

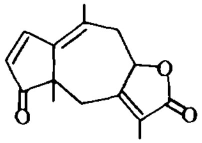
desacetyl derivative (VIIa). It has IR bands at 3640 cm^{-1} (OH group) and a strong carbonyl band at 1750 cm^{-1} (γ-lactone and cyclopentanone). More drastic acid treatment of an acetone solution of VIIb with *p*-toluenesulfonic acid eliminated the elements of acetic acid yielding the β,γ-unsaturated cyclopentanone VIII. The facile elimination of the ester group indicated that the latter is located in a β-position relative to the ketone. The above treatment produced the α,β-unsaturated cyclopentanone IX, however, the acidic conditions caused isomerization of the double bond to C-1, since the derivative VIII showed end absorption in the UV spectrum[8] (λ max 205 mμ; ε, 6763) and exhibits an NMR singlet with smaller doubling at 5·90 corresponding to only one vinyl proton. The isomerization of the double bond under acid conditions of α,β-unsaturated cyclopentanones of the pseudoguaianolide series have been previously described.[9–11]

Treatment of dehydrodihydrostevin (VIIb) with γ-collidine afforded a cyclopentenone. It shows a UV max at 228 mμ (ε, 8060) and IR bands at 1770 cm^{-1}, at 1710 and 1600 cm^{-1}. The position of the double bond in the α,β-unsaturated 5-membered ketone as shown in formula IX was deduced from the NMR spectrum. It exhibits two vinylic protons that were involved in an ABX system, observed as two quadruplets centered at 7·57 and 6·08. The complex signals at 4·70 and 3·17 were ascribed to the C-8 and C-1 hydrogen atoms. In the Me region a singlet at 1·30 was assigned to the angular Me group. The secondary Me groups were responsible for two superimposed doublets (J = 7 c/s) centered at 1·19 and 1·16.

All the above data supported structure Ia for stevin. Final proof of structure was provided by catalytic hydrogenation of the cyclopentenone (IX). The resulting ketone proved to be identical with the lactone X derived from cumanin (IV)[3] of known constitution and stereochemistry. Therefore stevin (Ia) possesses structure Ia with the asymmetric centers at C-1, C-5, C-7, C-8 and C-10 oriented as in cumanin (IV).[3]



X



XI

The mother liquors remaining after crystallization of dihydrostevin (Va) contained an isoderivative which was not isolated. However, chromium trioxide oxidation of these mother liquors followed by successive treatment with *p*-toluenesulfonic acid and selenium dioxide without isolation of the intermediary products afforded in small yield the isodienone (XI) identified with a product obtained previously from peruvin (II).[2] In this manner stevin (Ia) was also correlated with the peruvin series.

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[8] H. Labhart and G. Waginère, *Helv. Chim. Acta* **42**, 2219 (1959).

[9] W. Herz, A. Romo de Vivar, J. Romo and N. Viswanathan, *J. Am. Chem. Soc.* **85**, 19 (1963).

[10] W. Herz, M. V. Lakshmikantham and R. N. Mirrington, *Tetrahedron* **22**, 1709 (1966).

[11] A. Romo de Vivar, L. Rodriguez-Hahn, J. Romo, M. V. Lakshmikantham, R. N. Mirrington, J. Kagan and W. Herz, *Tetrahedron*, **22**, 3279 (1966).

EXPERIMENTAL[12]

Isolation of stevin (Ia). *Stevia rhombifolia* H.B.K. was collected in November (1965), in the vicinity of Cuautla (Estado de Morelos).[13] The aereal part of the plant (4 kg) was extracted twice with EtOH (8 l.) for 12 hr. The extract was filtered, concentrated to a small volume (2 l.) and treated with a soln of lead acetate (40 g) in water (2 l.). The mixture was left overnight, filtered, diluted with water (3 l.) and extracted twice with $CHCl_3$. The extract was evaporated to dryness, the residue was dissolved in benzene and chromatographed on alumina (800 g). The more polar fractions eluted with benzene crystallized. They were combined and recrystallized from acetone–ether yielding 3·750 g of Ia, m.p. 184–186, $[\alpha]_D$ +161, λ max: 212 mμ: (ε, 10080); IR bands at 3600 cm^{-1} (OH group), at 1750 cm^{-1} (γ-lactone), at 1730 cm^{-1} (acetyl group) and at 1652 cm^{-1} (weak, C=C double bond). (Found: 66·22; H, 7·73; O, 26·09 Calc. for $C_{17}H_{24}O_5$: C, 66·21; H, 7·84; O, 25·95%.)

Stevin acetate (Ib). Acetylation of Ia with Ac_2O-pyridine afforded Ib, m.p. 116; $[\alpha]_D$ +106·8; λ max. 212 mμ: (ε, 8500): IR bands at 1770 cm^{-1} (γ-lactone), at 1740 cm^{-1} (acetyl groups), at 1660 cm^{-1} (weak, C=C double bond). (Found: C, 65·35; H, 7·45; O, 27·50. Calc. for $C_{19}H_{26}O_6$: C, 65·12; H, 7·48; O, 27·40%.)

Dehydrostevin (VI). A soln of Ia (200 mg) in acetone (15 ml) was oxidized with 8N CrO_3 (0·5 ml) at 5 until the persistence of an orange colour. After 5 min the soln was diluted with water and extracted with AcOEt. The organic layer was washed with water, $NaHCO_3$aq and evaporated to dryness. Crystallization of the residue from ether–hexane yielded small needles (160 mg), m.p. 103–107. Further crystallizations from ether–hexane raised the m.p. to 112; λ max 212 mμ: (ε, 10403); IR bands at 1750 cm^{-1} (strong, γ-lactone, cyclopentanone and acetyl group), at 1660 cm^{-1} (C=C double bond). (Found: C, 66·76; H, 7·29; O, 26·29. Calc. for $C_{17}H_{22}O_5$: C, 66·65; H, 7·24; O, 26·11%.)

Dihydrostevin (Va). A soln of Ia (400 mg) in AcOEt (60 ml) was hydrogenated with 5% Pd-C (70 mg) until the uptake of H_2 ceased. The soln was filtered, evaporated to dryness and the solid residue was crystallized from acetone–hexane. This yielded 310 mg of Va, m.p. 148–150. Further crystallizations from acetone–ether raised the m.p. to 161–162, $[\alpha]_D$ +100·6; IR bands at 3620 cm^{-1} (OH group), a broad band with two peaks at 1765 and at 1740 cm^{-1} (γ-lactone and acetyl group). (Found: C, 65·99; H, 8·30; O, 25·73 Calc. for $C_{17}H_{26}O_5$: C, 65·78; H, 8·44; O, 25.78%.)

Dihydrostevin acetate (Vb). Acetylation of Va with Ac_2O pyridine for 1 hr on the steam bath yielded Vb, m.p. 157–158, $[\alpha]_D$ +61·7; IR bands at 1775 cm^{-1} (γ-lactone), at 1740 cm^{-1} (acetyl groups). (Found: C, 64·52; H, 8·08; O, 27·04 Calc. for $C_{19}H_{28}O_6$: C, 64·75; H, 8·01; O, 27·24%).

Dehydrodihydrostevin (VIIb). Oxidation of Va (310 mg) as described above for VI, furnished VIIb (170 mg) (brilliant plates from acetone–hexane), m.p. 162–163; $[\alpha]_D$ +146·2; IR band at 1745 cm^{-1} with a shoulder at 1767 cm^{-1} (γ-lactone carbonyl, acetyl group and cyclopentanone). (Found: C, 66·31; H, 7·70; O, 25·74. Calc. for $C_{17}H_{24}O_5$: C, 66·21; H, 7·84; O, 25·95%.)

Desacetyldehydrodihydrostevin (VIIa). A mixture of VIIb (200 mg), EtOH (25 ml) and HCl (1 ml) was heated under reflux for 30 min, diluted then with water and extracted with AcOEt. The organic layer was washed with $NaHCO_3$aq, dried and evaporated to dryness. The residue crystallized from acetone–ether yielding plates (60 mg), m.p. 196; $[\alpha]_D$ +153·6; IR bands at 3640 cm^{-1} (OH group) and at 1750 cm^{-1} (strong γ-lactone and cyclopentanone). (Found: C, 67·79; H, 8·35; O, 24·17. Calc. for $C_{15}H_{22}O_4$: C, 67·64; H, 8·33; O, 24·03%.)

Treatment of VIIb *with γ-collidine.* A soln of VIIb (150 mg) in γ-collidine (6 ml) was heated under reflux for 4 hr. The cold soln was diluted with water and extracted with AcOEt. The organic layer was washed with dil HCl, water and evaporated to dryness. The residue crystallized from acetone–pentane. This yielded IX (70 mg) as brilliant plates, m.p. 167–168; $[\alpha]_D$ −29·7; λ max: 228 mμ; (ε, 8060); IR bands at 1770 cm^{-1} (γ-lactone), at 1710 and 1600 cm^{-1} (cyclopentenone). (Found: C, 72·34; H, 8·03; O, 19·52 Calc. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12; O, 19·33%.)

Hydrogenation of the lactone IX. A soln of IX (50 mg) in AcOEt (15 ml) and containing 5% Pd-C (30 mg) was hydrogenated. Crystallization from acetone–ether afforded 35 mg of X, m.p. 147–148. This product

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[12] The mps are uncorrected, the rotations were determined at 20 in $CHCl_3$ solution. The IR spectra were recorded in $CHCl_3$. The UV spectra were determined in 95% EtOH. The microanalyses were performed by Dr. Franz Pascher, Bonn, Germany. The chromatograms were carried out with alumina Alcoa F-20 (washed with AcOEt). We are grateful to Syntex, S.A., for the determination of the rotations.

[13] We are grateful to Dr. Arturo Gómez Pompa of the Instituto de Biologia (U.N.A.M.) for the classification of the plant. Voucher Number TR No. 1, Herbario Nacional de la U.N.A.M. (MEXU).

showed no depression in mixed m.p. determination with XIII of the cumanin series and the IR spectra were identical (see ref. 3)

Isodienone XI. The mother liquors remaining after the crystallization of Va (130 mg) were oxidized in acetone soln with 8N CrO_3. The gummy residue (90 mg) dissolved in acetone (10 ml) was treated with *p*-toluenesulfonic acid (90 mg) and heated under reflux for 20 min. The soln was diluted with water and extracted with AcOEt. The organic layer was washed with $NaHCO_3$aq and evaporated to dryness. The residue dissolved in AcOH (7 ml) was treated with SeO_2 (100 mg) and heated on the steam bath for 20 min. The soln was diluted with water and extracted with AcOEt. The extract was washed with water, $NaHCO_3$aq, dried and evaporated. Chromatography on alumina (6 g) afforded 10 mg of XI (prisms from acetone-hexane), m.p. 194–196. Identified by the standard methods with VI of the peruvin series.[2]

Treatment of dehydrodihydrostevin VIIb *with* p-*toluenesulfonic acid*. A soln of VIIb (100 mg) in acetone (10 ml) was treated with *p*-toluenesulfonic acid (100 mg) and heated under reflux for 30 min. The soln was diluted with water and extracted with AcOEt. The organic layer was washed with $NaHCO_3$aq, dried and evaporated. The residue dissolved in hexane was chromatographed on alumina (8 g). The crystalline fractions obtained in the less polar fractions were combined and recrystallized from ether–pentane. This yielded VIII (60 mg), m.p. 113–114, $[\alpha]_D$ +45·33; λ max 205 mμ; (ε, 6763); IR band at 1765 cm^{-1} (strong, γ-lactone and cyclopentanone). (Found: C, 72·70; H, 8·22; O, 19·49. Calc. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12; O, 19·33%.)

The yield of this reaction was erratic. The best yield of VIII is reported. In another experiment a small amount of IX was obtained.