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STRUCTURE OF SANTAMARINE, A NEW SESQUITERPENE LACTONE[1,2]

A. ROMO DE VIVAR and H. JIMÉNEZ

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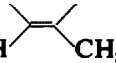
Abstract—The structure of santamarine, a new sesquiterpene lactone isolated from *Chrysantemum parthenium*, is shown to be 1β-hydroxysanta-3,11(13) dien-5,12-olide C (I).[3]

IN CONTINUATION of our studies on Compositae species growing in the valley of Mexico,[4] we investigated *Chrysantemum parthenium*,[5] cultivated in Mexico and known there as "Santamaria." *C. parthenium* was recently studied by Soucek *et al.*[6] who obtained parthenolide, (XIII) a new monocyclic lactone.

In our work we could not isolate parthenolide, but we obtained an isomer in a 0·12% yield, based on dry plant, which we called "santamarine."

That *C. parthenium* growing in Mexico is of different composition than the plant growing in Europe is not surprising. There are other examples in the literature of certain Compositae species, such as *Helenium mexicanum*[7,8] and *Iva microcephala*[9] which do not give reproducible results when collected from different regions.

Santamarine, $C_{15}H_{20}O_3$, m.p. 134–136°, [α] +96°, contains a free hydroxyl group, shown by the IR absorption at 3400 cm^{-1} and by the formation of a monoacetate (Ib). It contains two double bonds (IR bands at 1665 and 1630 cm^{-1}, uptake of two moles of hydrogen). The remaining two oxygen atoms are presumably present as part of a γ lacetone (IR band at 1760 cm^{-1}) conjugated with one of the double bonds (λ_{max} 208 mμ, ε 8090). Therefore santamarine must be a bicyclic compound.

One of the double bonds is an exocyclic methylene group (liberation of formaldehyde on ozonolysis) conjugated with the carbonyl group of lactone, as indicated by the characteristic UV absorption. The dihydrocompound (II) does not liberate formaldehyde on ozonolysis and no longer exhibits UV absorption. It contains a trisubstituted double bond (hydrogenation) as indicated by the characteristic NMR signals at 5·35 (1H) and 1·85 ppm (3H) (H  CH_3). On dehydrogenation santamarine does not produce an azulene, consequently we assumed that our substance is an eudesmanolide with the structure A or B.

[1] Contributions No. 183 from the Instituto de Química de la Universidad Nacional Autónoma de México, México 20, D. F.

[2] Taken in part from a B.Sc. Thesis to be submited by Honorio Jiménez to the Universidad nacional Autónoma de México.

[3] In this paper we employ the nomenclature suggested by Kovacks . *Czech. Chem. Comm.* **21**, 225 (1956).

[4] A. Romo de Vivar and J. Romo, *Chem. & Ind.* 882 (1959).

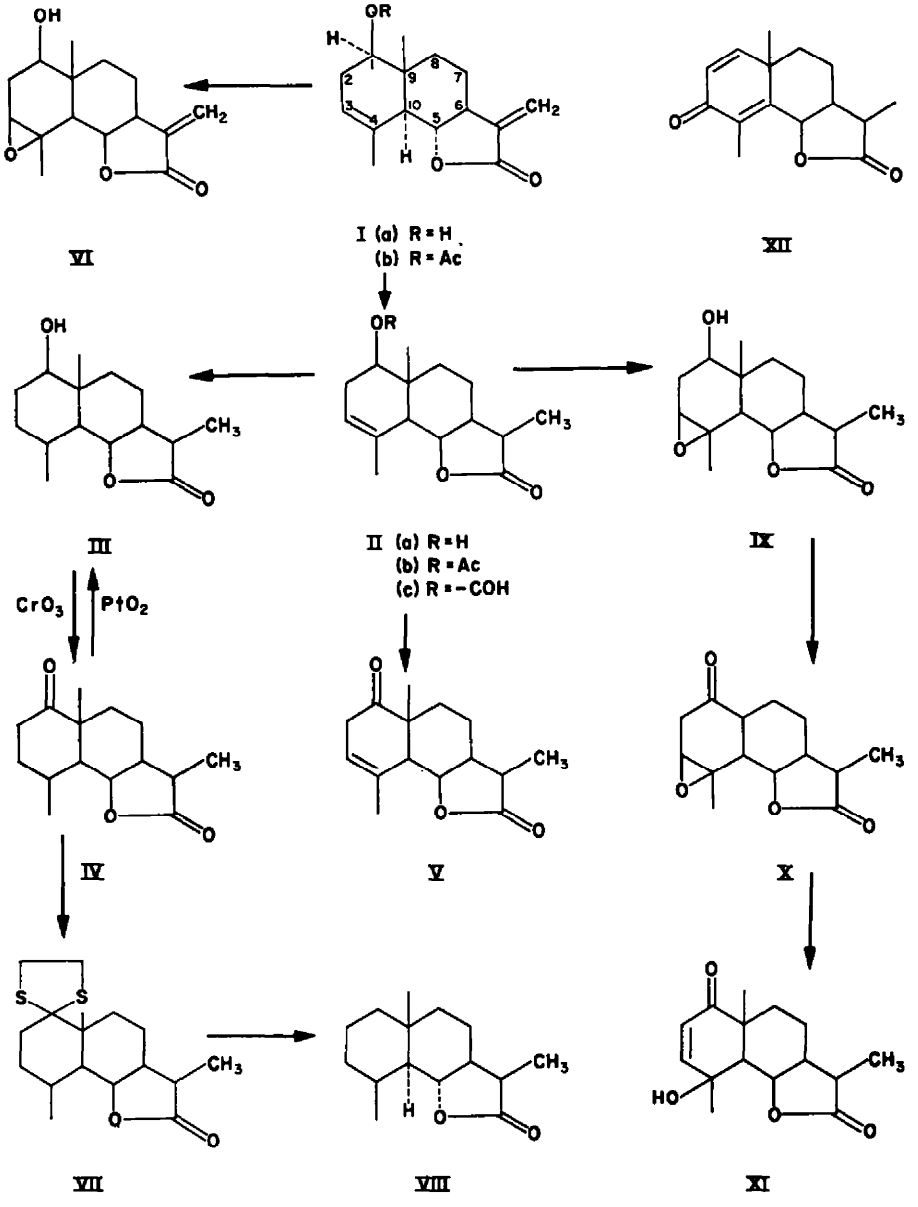
[5] Identified by Dr. F. Miranda from the Botanical Department of the Universidad Nacional Autónoma de México.

[6] M. Soucek, V. Herout and F. Sorm, *Coll. Czech. Chem. Comm.* **26**, 803 (1961).

[7] A. Romo de Vivar and J. Romo, *J. Amer. Chem. Soc.* **83**, 2326 (1961).

[8] E. Dominguez and J. Romo, *Tetrahedron* **19**, 1415 (1963).

[9] W. Herz, G. Högenauer and A. Romo de Vivar, *J. Org. Chem.* **29**, 1700 (1964).


OH
CH2
O
O
O
VI
OR
H
2
9
8
7
3
4
10
5
6
CH2
H
O
O
I (a) R = H
(b) R = Ac
XII
OR
CH3
O
O
II (a) R = H
(b) R = Ac
(c) R = -COH
OH
CH3
O
O
III
OH
CH3
O
O
IX
CrO3
PtO2
O
CH3
O
O
IV
O
CH3
O
O
V
O
CH3
O
O
X
S
S
CH3
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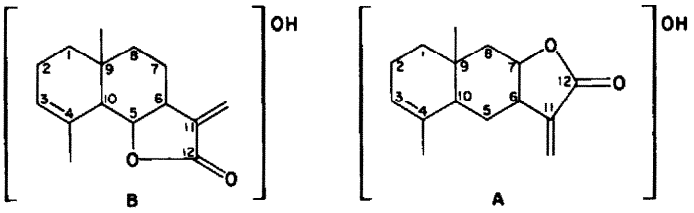
VII
CH3
H
O
O
VIII
O
CH3
HO
O
O
XI



Catalytic hydrogenation of santamarine failed to produce a pure compound. Reduction with aluminium amalgam saturated the exocyclic methylene group yielding the dihydroderivative (II) which still contains the unconjugated double bond (IR at 1660 cm^{-1}, no UV absorption). Compound II on catalytic hydrogenation with platinum oxide affords the tetrahydroderivative (III) whose IR and NMR spectra do not show the characteristic bands for the double bond. This compound on oxidation with chromic acid gives the ketoderivative (IV; IR at 1710 cm^{-1}, six membered ring ketone), confirming the secondary nature of the hydroxyl group. Desulphurization of the ethylenethioketal (VII) with Raney nickel, affords santanolide $C^{10,11}$ (VIII) identical in all respects with an authentic sample. This establishes the carbon skeleton of santamarine as in partial formula B and the stereochemistry at positions C-5, C-6, C-9 and C-10.

The NMR spectra of I, II and III completely verify the above conclusions. The spectrum of I shows the characteristic low field doublets (6·1 and 5·4 ppm) of the exocyclic methylene group conjugated with the lactone, each representing one proton. This low field absorption is absent in II and III.

Compounds I and II exhibit one singlet at 5·35 ppm (1H vinylic hydrogen at C-3) and two singlets at 0·85 (3H) and 1·85 ppm (3H) corresponding to two methyl groups at C-9 and C-4, respectively. In addition, II shows a new signal, a doublet centered at 1·21 ppm (3H; methyl group at C-11). In the NMR spectrum of III, there is a doublet centered at 1·20 ppm (3H; methyl group at C-11) and a signal at 1·03 ppm (6H; superimposed signals due to methyl groups at C-4 and C-9).

Treatment of I with *m*-chloroperbenzoic acid results in the formation of the epoxide (VI), whose UV spectrum (λ_{max} 212 mμ; ε, 8525) still shows a conjugated methylene group. The NMR spectrum exhibits the typical low field doublets of the exocyclic methylene group, with a shift of the C-4 methyl singlet to higher field due to the neighbouring epoxide group.

Tetrahydrosantamarine (III) was oxidized with chromium trioxide to the ketoderivative (IV), which gives a positive Zimmermann test indicating that the keto group is flanked by at least one methylene. In partial formula B there are four possible positions for the keto group—C-1, C-2, C-7 and C-8.

Compound IV does not produce a conjugated ketone on mild alkaline treatment[12] which would be expected if the keto group is in position C-7, and this position is therefore, eliminated.

The dihydroderivative (II) affords on oxidation the non conjugated ketone (V;

[10] W. Cocker and T. B. H. McMurray, *J. Chem. Soc.* 4549 (1956).
[11] G. H. Kulkarni, G. R. Kelkar and S. Bhattacharyya, *Tetrahedron* **20**, 2639 (1964).
[12] D. H. R. Barton, O. C. Böckman and P. de Mayo, *J. Chem. Soc.* 2263 (1960)

IR at 1710 cm^{-1}, six membered ring ketone), thus excluding position C-2 for the oxygenated function.

Of the remaining two possible positions, C-8 and C-1, the latter is favoured by the UV absorption at 205–210 mμ, characteristic of β-γ unsaturated ketones, and confirmed on the following grounds:

Chromic acid oxidation of the epoxide (IX) produces the ketoepoxide (X) which on alkaline treatment yields the α-β unsaturated γ hydroxyketone (XI; λ_{max} 215 mμ; ε, 8913 and IR at 3600 and 1680 cm^{-1}) whose NMR spectrum shows two doublets, one centered at 5·82 ppm (J = 11 c/s) corresponding to C-2 hydrogen and the other, centered at 6·1 ppm (the same J value), attributed to C-3 vinylic hydrogen. The methyl group at C-4 shows a singlet at 1·56 ppm (3H). The signal at 4·2 ppm (1H), which is present in all santamarine derivatives, is ascribed to the lactonic hydrogen at C-5. The absence of a second similar low field signal signifies that the hydroxyl group in compound XI is tertiary and can only be placed at C-4.

The above evidence confirms C-1 position for the hydroxyl group in santamarine.

Hydrogenation of the ketoderivative (IV) in acetic acid with platinum oxide gives in good yield the alcohol (III). If we assume that the hydrogen attacks the carbonyl group at C-1 from the opposite side to C-9 (β oriented methyl group), this establishes the configuration of the hydroxyl group as β equatorial. Therefore santamarine is represented by formula I.

EXPERIMENTAL[13]

Isolation of santamarine (I). The dried leaves and flowers (2·4 kg) of *C. parthenium* were extracted with 8 l. $CHCl_3$ in a soxhlet. The chloroformic solution was concentrated *in vacuo* and the residue dissolved in 800 ml EtOH, diluted with 500 ml water containing 24 g lead acetate and 4 ml acetic acid. The mixture was allowed to stand 20 hr at room temp, filtered and the clear filtrate concentrated at red. press. to one third the original volume. Subsequently it was steam distilled in order to eliminate the essential oils. Then it was taken up in $CHCl_3$ and the solvent eliminated *in vacuo*. The residue (72 g) was chromatographed on 1·2 kg alumina ALCOA F-20. From fractions 32–40 (eluted with benzene–ether 1:1), 2·9 g of a white crystalline compound was obtained with m.p. 127–128°, $[\alpha]_D$ − 96·6°; several crystallizations from ether–hexane raised the m.p. to 134–136°, λ_{max} 208 mμ (ε 8090), ν_{max} 3550, 1665 and 1630 cm^{-1}. (Found: C, 72·69; H, 8·41. Calc. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12%.)

Santamarine acetate (Ib). The acetate was prepared in the usual manner with pyridine–acetic anhydride, affording in good yields the acetate, m.p. 126–128°, ν_{max} 1770, 1735 cm^{-1}. (Found: C, 70·42; H, 7·49; O, 22·38. Calc. for $C_{17}H_{22}O_4$: C, 70·32; H, 7·64; O, 22·04.)

Ozonolysis of santamarine. Santamarine (200 mg) was ozonized at −72° in ethyl acetate solution. The ozonide was steam distilled over a methanolic solution of dimedone. The yield of formaldehyde–dimedone adduct was 35 mg with a m.p. of 193–194°. No depression was observed in a m.m.p. with an authentic sample.

1β-*hydroxysant*-3-*en*-5,12-*olide C* (II). A solution of 1 g santamarine in 160 ml EtOH was treated with 3 g freshly prepared Al—Hg and refluxed 8hr. The reaction product was filtered and crystallized from ether–hexane, yielding 400 mg of the dihydroderivative, m.p. 121–123°; the pure sample was obtained from ether–isopropyl ether and showed m.p. 124–125°, $[\alpha]_D$ +78·89°, ν_{max} 3600 and 1765 cm^{-1}. (Found: C, 71·76; H, 8·93; O, 19·44. Calc. for $C_{15}H_{22}O_3$: C, 71·97; H, 8·86; O, 19·17%.)

1β-*hydroxysantanolide C* (III). The mother liquors of 1β-hydroxysant-3-en-5,12-olide C (600 mg) were hydrogenated in EtOH with 100 mg Adams' catalyst until H_2 absorption ceased. The yield was 320 mg 1β-hydroxysantanolide C, m.p. 150° (from ether–hexane). The pure sample was prepared from ether–hexane and showed m.p. 164–166°, $[\alpha]_D$ +34°, ν_{max} 3600, 1760 cm^{-1}. (Found: C, 71·57; H, 9·76; O, 19·10. Calc. for $C_{15}H_{24}O_3$: C, 71·39; H, 9·59; O, 19·02%.)

[13] M.ps. are uncorrected rotations and IR spectra were carried out in $CHCl_3$ solution, UV spectra were run in 95% EtOH, NMR spectra were run in a Varian A-60 spectrometer apparatus in $CDCl_3$ with tetramethylsilane as internal standard. Analyses by Dr. F. Pascher, Bonn, Germany.

1-ketosantan-5,12-olide C (IV). A cold solution of III (100 mg) in 2 ml acetic acid was treated with 100 mg CrO_3 dissolved in 3 ml dil. acetic acid. During the first hr, the reaction was carried out at 10° followed by 1 hr at room temp. The reaction mixture was diluted with water and the crystalline material filtered off, affording 90 mg of IV. The pure sample (from acetone–hexane) showed m.p. 194–196°, $[\alpha]_D$ +72° and a positive Zimmermann test; ν_{max} 1760, 1700 cm^{-1}. (Found: C, 71·55; H, 8·83; O, 19·53. Calc. for $C_{15}H_{22}O_3$: C, 71·97; H, 8·86; O, 19·17.)

1-ketosant-3-en-5,12-olide C (V). A solution of 240 mg 1β-hydroxysant-3-en-5,12-olide C in 25 ml acetone and 7 ml $CHCl_3$ was treated with a solution of 90 mg CrO_3 in 5 ml water and 7 ml H_2SO_4 (50% v/v). The reaction mixture was kept 10 min at room temp, diluted with water and then extracted with $CHCl_3$. The solvent was removed and the oily residue crystallized from ether–hexane, affording 50 mg V, m.p. 134–136°, [α] +91°, ν_{max} 1770, 1710 cm^{-1}. (Found: C, 72·47; H, 8·04; O, 20·21. Calc. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12; O, 19·33%.)

Hydrogenation of 1-ketosantan-5,12-olide C. 1-ketosantanolide C (60 mg) was hydrogenated with PtO_2 (20 mg) in acetic acid. Hydrogenation ceased after an uptake of 1 mole H_2. The reaction mixture was worked up in the usual manner. Crystallization from ether–hexane afforded 40 mg of a white crystalline compound, m.p. 156–158°, identical in all respects with an authentic sample of III.

Santamarine epoxide (VI). A solution of 200 mg santamarine in 20 ml $CHCl_3$ was treated with a solution of 200 mg *m*-chloroperbenzoic acid in 20 ml $CHCl_3$ and heated under reflux for 2 hr. The solution was washed with water and $NaHCO_3$ aq, dried and evaporated. The product crystallized from acetone–ether, yield: 120 mg, m.p. 243–244°. Pure sample (from acetone–ether), m.p. 248–250°, λ_{max} 211 mμ (ε 8525), ν_{max} 1760 cm^{-1}. (Found: C, 67·90; H, 7·45; O, 24·21. Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63; O, 24·21%.)

Ethylenethioketal of 1-ketosantan-5,12-olide C (VII). 1-ketosantanolide C (100 mg) was treated with 0·5 ml ethanedithiol and 0·5 ml BF_3. The reaction mixture was allowed to stand 4 hr at room temp. Then it was poured into water and taken up in ethyl acetate, washed with $NaHCO_3$ aq and the product crystallized from ethyl acetate, yield: 80 mg, m.p. 180°. Pure sample, m.p. 186–188° (from acetone–hexane), $[\alpha]_D$ +32°, ν_{max} 1765 cm^{-1}. (Found: C, 62·38; H, 8·20; S, 19·53. Calc. for $C_{17}H_{26}O_2S_2$: C, 62·56; H, 8·03; S, 19·60%.)

Santanolide C (VIII). The ethylenethioketal prepared from 100 mg 1-ketosantanolide C, without isolation, was heated under reflux during 2 hr with 5 g Raney Ni. The catalyst was filtered off and the solution cooled. The crystalline substance was recrystallized from ether–hexane, yield: 40 mg, m.p. 152–154°, $[\alpha]_D$ +63°. It was identical with a sample of santanolide C, prepared by hydrogenation of santonine.

1-formoxysant-3-en-5,12-olide C (IIc). 1β-hydroxysant-3-en-5,12-olide C (100 mg) was treated with 2 ml formic acid and heated under reflux for 2½ hr. The violet solution was poured into water and the crystalline product filtered off, yield: 55 mg, m.p. 178–180°. After recrystallization from ether–hexane, the m.p. was raised to 180–183°, ν_{max} 1765, 1720 cm^{-1}. (Found: C, 69·09; H, 7·89; O, 23·14 Calc. for $C_{16}H_{22}O_4$: C, 69·04; H, 7·97; O, 22·99%.)

1β-hydroxysantan-3,4-oxide-5,12-olide C (IX). A solution of 500 mg 1β-hydroxysant-3-en-5,12-olide C in 20 ml $CHCl_3$ was treated with a solution of 500 mg *m*-chloroperbenzoic acid and heated under reflux for 4 hr. The chloroformic solution was washed with $NaHCO_3$ aq, dried and evaporated dryness. The residue was crystallized from chloroform–ether, yield: 350 mg, m.p. 205–208°; pure sample, m.p. 212–214°, $[\alpha]_D$ +70°, ν_{max} 3500, 1760 cm^{-1}. (Found: C, 67·46; H, 8·22; O, 24·16. Calc. for $C_{16}H_{22}O_4$: C, 67·64; H, 8·33; O, 24·03.)

1-ketosant-3-en-5,12-olide-C epoxide (X). A solution of 250 mg IX in 5 ml acetic acid was oxidized with 250 mg CrO_3 for 3 hr at room temp, washed with $NaHCO_3$ aq, dried and the product crystallized from acetone–ether, yield: 120 mg, m.p. 146–148. After recrystallization from acetone–hexane the m.p. was raised to 154–156°, $[\alpha]_D$ +65°, ν_{max} 1770, 1710 cm^{-1}. (Found: C, 67·86; H, 7·78; O, 24·58. Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63; O, 24·21%.)

Alkaline treatment of 1-ketosantan-3,4-oxide-5,12-olide C. A solution of 80 mg X in 20 ml MeOH was treated with 50 ml KOH in 2 ml water, it was acidified with dil. HCl and concentrated under red. press. The product was extracted with $CHCl_3$ and crystallized from acetone–ether, yield: 45 mg of (XI), m.p. 175–178°. Several crystallizations from the same solvents raised the m.p. to 182–183°, $[\alpha]_D$ +41·08°, λmax 215, 330 mμ (ε 8913, 58), ν_{max} 1780, 1680 cm^{-1}. (Found: C, 68·11; H, 7·68; O, 24·05. Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63; O, 24·21%.)