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ISOLATION AND STRUCTURE OF PERUVIN

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Abstract—Peruvin, a constituent of *Ambrosia peruviana* has been established to be a sesquiterpene lactone, represented by formula I.

THE occurrence of sesquiterpene lactones such as ambrosin[2-4] and coronopilin[5] in the genus Ambrosiae has been reported. For this reason, the examination of several Ambrosiae species widely distributed in the Valley of Mexico was undertaken. The results which led to the structure of peruvin, a constituent of *Ambrosia peruviana*, obtained by chromatography of the chloroform extract of the plant are reported in this paper.

Peruvin (I) is a crystalline product ($C_{15}H_{20}O_4$), m.p. 191–193°, $[\alpha]_D$ +155°. Its spectral features are very similar to coronopilin.[5] The IR spectrum of peruvin (I) has a band at 1750 cm^{-1} (double strength) and a weak band at 1655 cm^{-1}, attributed to a cyclopentanone and an α,β-unsaturated-γ-lactone. The UV maximum of peruvin (I) at 214 mμ (ε, 9200), coupled with the formation of a pyrazoline (II), demonstrate the presence of an exocyclic methylene group conjugated with the lactone. Further confirmation was obtained by examination of the NMR spectrum[6] of peruvin (I). It shows two low field doublets at 6·26 and 5·67, corresponding to the vinyl protons of the exocyclic methylene group. Two lateral doublets and a central triplet, centered at 5·02 (intensity one proton), indicates a lactone closure at C-8[7,8] if a coronopilin carbon skeleton is assumed. A tertiary methyl group is responsible for a singlet at 1·1 and a secondary methyl group for a doublet (J = 7 c/s) centered at 1·11.

From the Pd–C catalyzed hydrogenation of peruvin (I), only isoperuvin (III; λ_{max} 219 mμ; ε, 15800) could be isolated. The vinyl methyl group of III is responsible in the NMR spectrum for a singlet at 1·86. The fourth oxygen atom of peruvin (I) is involved in a tertiary hydroxy function (IR band at 3470 cm^{-1}) attached at C-1, as shown by the following evidence. Peruvin (I) did not afford an acetate under basic acetylation conditions. Brief treatment of isoperuvin (III) with thionyl chloride gave

[1] Taken in part from a thesis to be submitted by P. Joseph-Nathan to the Universidad Nacional Autónoma de México.

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[4] Abu-Shady and T. O. Soine, *J. Amer. Pharm. Assoc.* **42**, 387 (1953).

[5] W. Herz and G. Högenauer, *J. Org. Chem.* **26**, 5011 (1961).

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

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

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

a mixture of two anhydro derivatives (IV and V). The position of the double bond in both anhydro derivatives is deduced from the examination of their NMR spectra. Lactone IV shows a broad signal at 6·3 ascribed to the vinyl proton at C-2. The vinyl methyl group is responsible for a singlet at 1·92, the secondary methyl group exhibits a doublet centered at 1·3. A singlet at 1·2 corresponds to the tertiary methyl grouping. Lactone V does not show vinyl proton signals. A singlet at 1·86 (intensity six protons), is attributed to the C_{10} and C_{11} vinyl methyl groups. The tertiary methyl group exhibits a singlet at 1·08.

The relative position of the five membered keto group was ascertained by selenium dioxide oxidation of V or a mixture of IV and V yielding the dienone (VI; λ_{max} 215, 295 mμ; ε, 20500, 13800). The IR spectrum is in accord with structure VI. It shows bands at 1760 cm^{-1} (γ-lactone), at 1700 cm^{-1} (cyclopentenone) and at 1640 cm^{-1} (C=C double bonds). In the NMR spectrum, the dienone (VI) exhibits two low field doublets, centered at 8·17 and at 6·28, ascribed to two vinyl protons. In the methyl region, two singlets with different chemical shifts, at 2·19 and at 1·93, corresponding to the C-10 and C-11 vinyl methyl groups, respectively are present. A singlet at 1·11 is ascribed to the tertiary methyl group. The dienone (VI) was correlated with mexicanin A (X)[8] in the following manner. Dihydromexicanin A (XI)[8] was treated with mesyl chloride in pyridine solution. Elimination of the elements of methanesulfonic acid with collidine in the crude mesylate (XIb), followed by selenium dioxide oxidation, furnished a product, identified as the dienone (VI). Therefore peruvin is a pseudoguaianolide with a constitution represented by formula I.

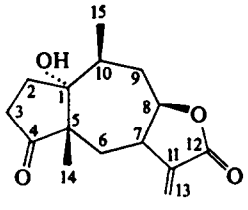
When the crude product obtained from the hydrogenation of peruvin (I) is dehydrated with thionyl chloride followed by selenium dioxide oxidation, a mixture of the dienones (VI and VII) was obtained. The dienone (VII; λ_{max}; 296 mμ; ε, 12000) shows in the NMR spectrum two low field doublets at 8·08 and 6·2, assigned to the vinylic protons. In the methyl region, a singlet at 2·11 ascribed to the vinyl methyl group is present. A secondary and a tertiary methyl group are responsible for a doublet centered at 1·2 and a singlet at 1·27, respectively.

Hydrogenation of the dienone (VI) in acetic acid solution with Adams catalyst saturated the dienone chromophore, yielding the ketone (VIII; λ_{max} 218 mμ; ε, 16600). Its NMR spectrum exhibits a singlet at 1·86 corresponding to a vinyl methyl group. A secondary methyl group is responsible for a doublet centered at 1·07, partially superimposed on a singlet at 1·0, the latter being ascribed to the tertiary methyl group. The configuration of the C-10 methyl group in the ketone (VIII) is identical to that of the same substituent in peruvin (I), since hydrogenation of the anhydro derivative (IV) also afforded the ketone (VIII).

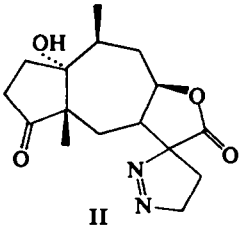
Treatment of peruvin (I) with formic acid or with hydrochloric acid in acetone solution yielded a carboxylic acid ($C_{15}H_{20}O_4$; λ_{max}; 205 mμ). Its structure (IXa) could be inferred from its spectral features. In the IR spectrum it shows bands at 1700 cm^{-1} (α,β-unsaturated carboxylic acid), at 1740 cm^{-1} (five membered ketone) and at 1625 cm^{-1} (C=C double bond). The exocyclic methylene protons are responsible in the NMR spectrum for two low field singlets at 6·47 and 5·7. Two broad signals centered at 4·41 and 2·98 (intensity one proton each) are ascribed to the hydrogens at C_8 and C_7, respectively. A doublet centered at 1·24 corresponds to the secondary methyl group. The tertiary methyl group is responsible for a singlet at 1·1. Brief treatment of the acid (XIa) with ethereal diazomethane furnished the methyl ester

(IXb). It was recovered unchanged after treatment with acetic anhydride–pyridine. The NMR singlet at 3·8 showed by IXb corresponds to the methyl ester.[9]

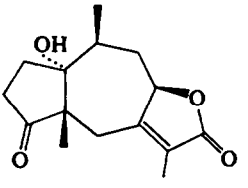
We can assign to peruvin and its derivatives a *trans* ring junction as shown in formula (I), since the ORD curve of peruvin (I) and of the lactone (VIII) show positive Cotton effects[10] of an amplitude similar to those exhibited by related lactones such as tetrahydroparthenin,[11] coronopilin[11] and tetrahydrohelenalin[8,12] of known stereochemistry at the ring junction. As the lactone (VIII) derives from peruvin (I) as well as from mexicanin A (X), and as in the series of reactions described above which lead to VIII, the C-5 asymmetric center has not been affected, we can assume that peruvin (I) and mexicanin A (X) have the same beta configuration at C-5. Apparently tetrahydromexicanin A, whose ORD curve exhibits a weak negative Cotton effect, possesses a system of *cis* fused rings as shown in formula (XII). The lactone closure of peruvin (I) has the same beta configuration as in mexicanin A (X) and helenalin.[8] Also it can be inferred that the C-10 methyl group of peruvin (I) has a beta configuration, since its derived lactone (VIII) differs from dihydroisoaromatin (correlated with helenalin),[13] which possesses the same gross structure as VIII and whose C-10 methyl group has an alpha configuration. Biogenetical considerations render it very probable that peruvin has the same stereochemistry at C-7 as that found in other pseudoguaianolides.[14]



I



II



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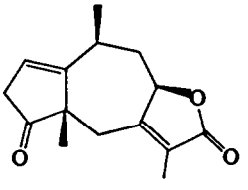

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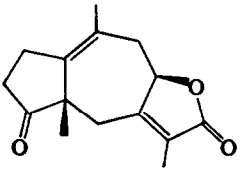
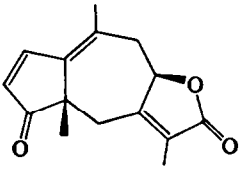


V



VI

[9] Coronopilin[5], which has the lactone closure at C–6, under these conditions afforded coronopilic acid, a naphthalenoid derivative [see T. A. Geissman and J. Turley, *J. Org. Chem.* **29**, 2553 (1964)].

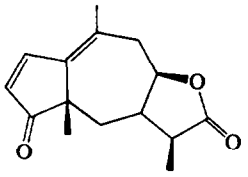
[10] The ORD curve of the 7,11-saturated derivative of lactone (VIII) had also a positive Cotton effect. [See J. Romo, P. Joseph-Nathan and G. Siade, *Tetrahedron* **22**, 1499 (1966).]

[11] W. Herz, H. Watanabe, M. Miyazaki and Y. Kishida, *J. Amer. Chem. Soc.* **84**, 2601 (1962).

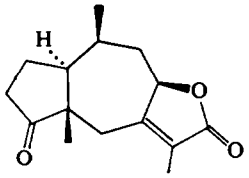
[12] C. Djerassi, J. Osiecki and W. Herz, *J. Org. Chem.* **22**, 1361 (1957).

[13] J. Romo, P. Joseph-Nathan and F. Díaz, *Tetrahedron* **20**, 79 (1964).

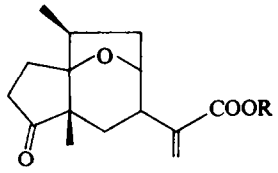
[14] W. Herz, A. Romo de Vivar, J. Romo and N. Viswanathan, *Tetrahedron* **19**, 1359 (1963).



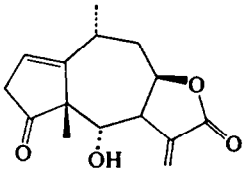
VII



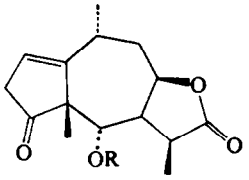
VIII



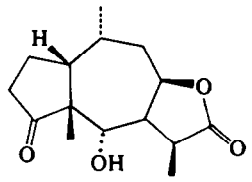
IX a, R = H
b, R = Me



X



XI



XII

EXPERIMENTAL[15]

Isolation of peruvin (I). *Ambrosia peruviana*[16] was collected in August (1963) in the vicinity of Pachuca (Estado de Hidalgo). The dried plant (10 Kg) was extracted with $CHCl_3$ (30 l) for 20 hr under reflux. The extract was filtered and evaporated to dryness. The residue dissolved in EtOH (5 l) was treated with a solution of lead acetate (120 g) in water (4 l.), left at room temp overnight, filtered, diluted with water (6 l.) and extracted with $CHCl_3$. The extract was evaporated to dryness and the residue dissolved in benzene was chromatographed on alumina. The crystalline fractions, eluted with benzene and increasing proportions of ether and with $CHCl_3$, were combined and crystallized from acetone–ether, yielding I (4·3 g), m.p. 168–170°,[17] $[\alpha]_D$ +155°; λ_{max} 214 mμ; ϵ, 9200; IR bands at 3470 cm^{-1} (hydroxyl group), at 1750 cm^{-1} (cyclopentanone and α,β-unsaturated-γ-lactone) and at 1655 cm^{-1} (C=C double bond); rotatory dispersion (in dioxan); $[\alpha]_{400}$ +590°; $[\alpha]_{320}$ +1108°; $[\alpha]_{315}$ +1230°; $[\alpha]_{310}$ +1364°; $[\alpha]_{307\cdot5}$ +1364°. (Found: C, 68·04; H, 7·55; O, 24·28. Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63; O, 24·21%.)

Pyrazoline of peruvin (II). Peruvin (I; 100 mg) in MeOH (5 ml) was treated with an ethereal solution of diazomethane, left at 4° overnight, evaporated to dryness *in vacuo* and the residue crystallized from acetone-hexane, yielding needles (45 mg), m.p. 150° (dec): $[\alpha]_D$ +347°; λ_{max}; 322 mμ; ϵ, 190. (Found: C, 62·87; H, 7·13; O, 21·15; N, 9·07. Calc. for $C_{16}H_{22}O_4N_2$: C, 62·72; H, 7·24; O, 20·89; N, 9·14%.)

Isoperuvin (III). A solution of I (1 g) in AcOEt (40 ml) was hydrogenated with 10% Pd–C (100 mg) overnight. The solution was filtered, evaporated and the residue crystallized from acetone–ether, yielding III (410 mg), m.p. 208–210°. Further crystallizations from acetone–ether raised the m.p. to 221–222°; $[\alpha]_D$ +79°; λ_{max} 219 mμ; ϵ, 15800: IR bands at 3400 cm^{-1} (hydroxyl group), at 1730 cm^{-1} (cyclopentanone) with a shoulder at 1750 cm^{-1} (γ-lactone) and at 1670 cm^{-1} (C=C double bond). (Found: C, 68·04; H, 7·48; O, 24·06. Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63; O, 24·21%.)

[15] M.ps are uncorrected. Analyses by Dr. Franz Pascher, Bonn, Germany. IR spectra and rotations were run in $CHCl_3$, UV spectra in 95% EtOH. The alumina used in the chromatograms was Alcoa, F-20 (washed with AcOEt). We are grateful to Syntex, S.A., for the determination of the rotations and ORD curves.

[16] We are grateful to the late Dr. Faustino Miranda for the identification of the plant.

[17] This material appeared to be pure, as judged by TLC. Careful chromatography on alumina did not raise the m.p. However, from some fractions of the chromatogram of the extract, a small amount of a product m.p. 191–193° was obtained. It was identified as peruvin (I) by the standard methods. Its pyrazoline and the acid (IXa), prepared with this sample were identical with those obtained from the lower melting peruvin (I).

Dehydration of isoperuvin (III) *with* $SOCl_2$. A solution of III (1·7 g) in pyridine (15 ml) was treated at 0° with $SOCl_2$ (2 ml), left for 10 min, poured into ice-water and extracted with AcOEt. The organic layer was washed with dil HCl, water, dried and evaporated to dryness *in vacuo*. Repeated crystallizations of the residue from acetone–ether yielded the *anhydro derivative* (V; 370 mg), m.p. 156–158°; $[\alpha]_D$ −84°; λ_{max} 216 mμ; ϵ, 15500: IR bands at 1750 cm^{-1} (cyclopentanone and γ-lactone) and at 1680 cm^{-1} (C=C double bonds). (Found: C, 73·16; H, 7·43; O, 19·44. Calc. for $C_{15}H_{18}O_2$: C, 73·15; H, 7·37; O, 19·48%.)

Repeated crystallizations from acetone–hexane of the mother liquors of V afforded the isomeric IV (190 mg), m.p. 105°: $[\alpha]_D$ −30·5°: λ_{max} 217 mμ; ϵ, 15400: IR bands at 1750 cm^{-1} (cyclopentanone and γ-lactone) and at 1680 cm^{-1} (C=C double bond). (Found: C, 73·21; H, 7·26; O, 19·67. Calc. for $C_{15}H_{18}O_2$: C, 73·15; H, 7·37; O, 19·48%.)

Treatment of the anhydro derivative V *with* SeO_2. A solution of V (150 mg) in AcOH (8 ml), was treated with SeO_2 (150 mg), heated on the steam bath for 20 min, diluted with water and extracted with AcOEt. The organic layer was washed with cold 5% NaOH, water, dried, evaporated to dryness and the residue dissolved in benzene was passed over alumina. Crystallization from acetone–hexane yielded the *dienone* (VI) as yellow prisms (60 mg), m.p. 197–200°; $[\alpha]_D$ −178°; λ_{max} 215, 295 mμ; ϵ, 20500, 13800; IR bands at 1760 cm^{-1} (γ-lactone), at 1700 cm^{-1} (cyclopentenone) and at 1640 cm^{-1} (C=C double bonds). (Found: C, 73·69; H, 6·77; O, 19·58. Calc. for $C_{15}H_{16}O_2$: C, 73·75; H, 6·60; O, 19·65%.)

Treatment with SeO_2 (300 mg) of the mixture of the anhydro derivatives (300 mg) obtained by dehydration of isoperuvin (III) afforded 100 mg of the dienone (VI), m.p. 195–197°.

Dienone VII. The crude product obtained from the hydrogenation of I (1 g) was dissolved in pyridine (10 ml) and treated with $SOCl_2$, following the procedure described above. The gummy product (690 mg) dissolved in AcOH (10 ml) was oxidized with SeO_2 (700 mg). Crystallization from acetone–hexane afforded 190 mg of VI, m.p. 193–195°. From the mother liquors there was obtained 50 mg of VII, m.p. 154–158° (yellow plates from acetone–hexane), $[\alpha]_D$ +77°; λ_{max} 296 mμ; ϵ, 12000; IR bands at 1770 cm^{-1} (γ-lactone), at 1700 cm^{-1} (cyclopentenone) and a shoulder at 1660 cm^{-1} (C=C double bonds). (Found: C, 73·43; H, 7·30; O, 19·36. Calc. for $C_{15}H_{18}O_2$: C, 73·15; H, 7·37; O, 19·48%.)

Dienone VI *from dihydromexicanin A* (XIa). A cold solution of XIa (350 mg) in pyridine (6 ml) was treated with $MeSO_2Cl$ (1 ml), left at room temp for 3 hr, poured in ice-water and extracted with AcOEt. The organic layer was washed with dil HCl, water, dried and evaporated to dryness *in vacuo*. The mesylate (XIb) did not crystallize. It was dissolved in γ-collidine, heated under reflux for 8 hr, diluted with AcOEt, washed with dil HCl, water, dried and evaporated to dryness. The residue (180 mg) was dissolved in AcOH, treated with SeO_2 (180 mg), heated for 20 min on the steam bath, diluted with water and extracted with $CHCl_3$. The organic layer was washed with 5% NaOH, water, dried and evaporated to dryness. Crystallization of the residue from acetone–ether afforded yellow prisms, m.p. 193–195°; $[\alpha]_D$ −174°; λ_{max}; 214, 295 mμ; ϵ, 19000, 13200. Mixed m.p. with VI was undepressed and the IR spectra were superimposable.

Hydrogenation of the dienone (VI). A solution of VI (300 mg) in AcOH (20 ml) was hydrogenated with PtO_2 (50 mg) until the uptake of H_2 ceased. The solution was filtered and evaporated to dryness *in vacuo*. Crystallization of the residue from acetone–hexane yielded the *lactone* (VIII) as prisms, m.p. 166–169°; $[\alpha]_D$ +81°; λ_{max} 218 mμ; ϵ, 16600; IR bands at 1750 cm^{-1} (cyclopentanone and γ-lactone) and at 1680 cm^{-1} (C=C double bond); rotatory dispersion (in dioxan); $[\alpha]_{400}$ +344°; $[\alpha]_{330}$ +1811°; $[\alpha]_{328\cdot5}$ +2925°; $[\alpha]_{317\cdot5}$ +2475°. (Found: C, 72·29; H, 7·82; O, 19·56. Calc. for $C_{15}H_{20}O_2$: C, 72·55; H, 8·12; O, 19·33%.)

Hydrogenation of IV (150 mg) in AcOH with PtO_2 as described above furnished 70 mg of VIII, m.p. 167–169°, undepressed on admixture with a sample of the lactone obtained by hydrogenation of VI. The IR spectra were superimposable.

Acid IXa. A solution of I (70 mg) in acetone (6 ml) was treated with 5 drops of conc. HCl, left at room temp overnight, concentrated *in vacuo* and diluted with water. The precipitate was collected, washed with water and crystallized from MeOH–ether. This yielded prisms, m.p. 220°; soluble in $NaHCO_3$aq; $[\alpha]_D$ +60°; λ_{max} 205 mμ; ϵ, 5540; IR bands at 3140 and 1700 cm^{-1} (α,β-unsaturated carboxylic acid), at 1740 cm^{-1} (cyclopentanone) and at 1625 cm^{-1} (C=C double bond). (Found: C, 68·03; H, 7·87; O, 24·46. Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63; O, 24·21%.)

A solution of I (150 mg) in HCOOH (8 ml) was heated under reflux for 3 hr, diluted with water

and extracted with AcOH. The organic layer was washed with water and evaporated to dryness. Crystallization of the residue from acetone–ether yielded IXa (90 mg), m.p. 217–220°, identified by the standard methods with that obtained above.

Methyl ester (IXb). This was obtained in nearly quantitative yield by brief treatment of IXa with ethereal diazomethane and crystallized as needles from ether–hexane, m.p. 89°, $[\alpha]_D$ +58°; λ_{max} 202 mμ; ϵ, 7470. The IR spectrum showed a band with peaks at 1750 cm^{-1} (cyclopentanone), 1725 cm^{-1} (α,β-unsaturated ester group) and 1635 cm^{-1} (C=C double bond). (Found: C, 69·09; H, 8·03; O, 22·89. Calc. for $C_{16}H_{22}O_4$: C, 69·04; H, 7·97; O, 22·99%.)