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STRUCTURE OF VEPRISONE, A CONSTITUENT OF *VEPRIS BILOCULARIS*

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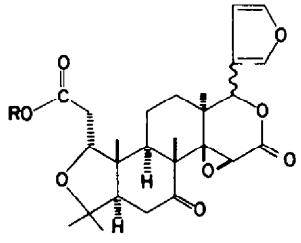
Abstract—Veprisone a neutral crystalline constituent of *Vepris bilocularis* has been shown to be methyl epi-isoobacunoate having structure I.

VEPRIS BILOCULARIS Engler (Rutaceae) is a large tree found commonly on the West Coast of India. Extraction of the powdered, defatted stem bark of this tree with ether gave a mixture of alkaloids from which the furanoquinoline bases kokusaginine, flindersiamine and skimmianine were isolated in a pure form.[1] The ether extract after removal of basic matter also afforded a neutral crystalline compound m.p. 180–181°, $[\alpha]_D^{30}$ −18°, named veprisone in a yield of 0·06%. On the basis of the studies reported in this paper veprisone is shown to be the hitherto-unknown methyl epi-isoobacunoate (I).

Analyses of veprisone and its derivatives like the oxime and semicarbazone are in agreement with the molecular formula $C_{27}H_{34}O_8$ (mol. wt. by mass spectrum, 486). In saponification experiments two equivalents of alkali were consumed. Based on Zeisel determinations the IR data, the presence of one methoxyl group, a carbomethoxy group and a δ-lactone system was deduced. (see below).



I R = CH_3

II R = H

The UV absorption spectrum of veprisone shows maxima at 207 and 282 mμ (ε, 7013 and 84). The IR absorption spectrum of veprisone (CH_2Cl_2) has bands at 3130, 1596, 1505 and 880 cm^{-1} suggesting the presence of a furan ring. Other prominent bands are 1740 cm^{-1} (δ-lactone and/or ester) and 1710 cm^{-1} (cyclohexanone).

The NMR spectrum of veprisone (Fig. 1) shows signals in the region 1·3 δ (15H) corresponding to five quarternary methyl groups. A signal at 3·71 δ (3H) should be ascribed to the ester methyl group. Signals at 7·57 δ (2H) and 6·48 δ (1H) corresponded to the α- and β-protons of a β-monosubstituted furan ring. A close relationship to

[1] T. R. Govindachari and V. N. Sundararajan, *J. Sci. Ind. Res., India* **20B**, 298 (1961).

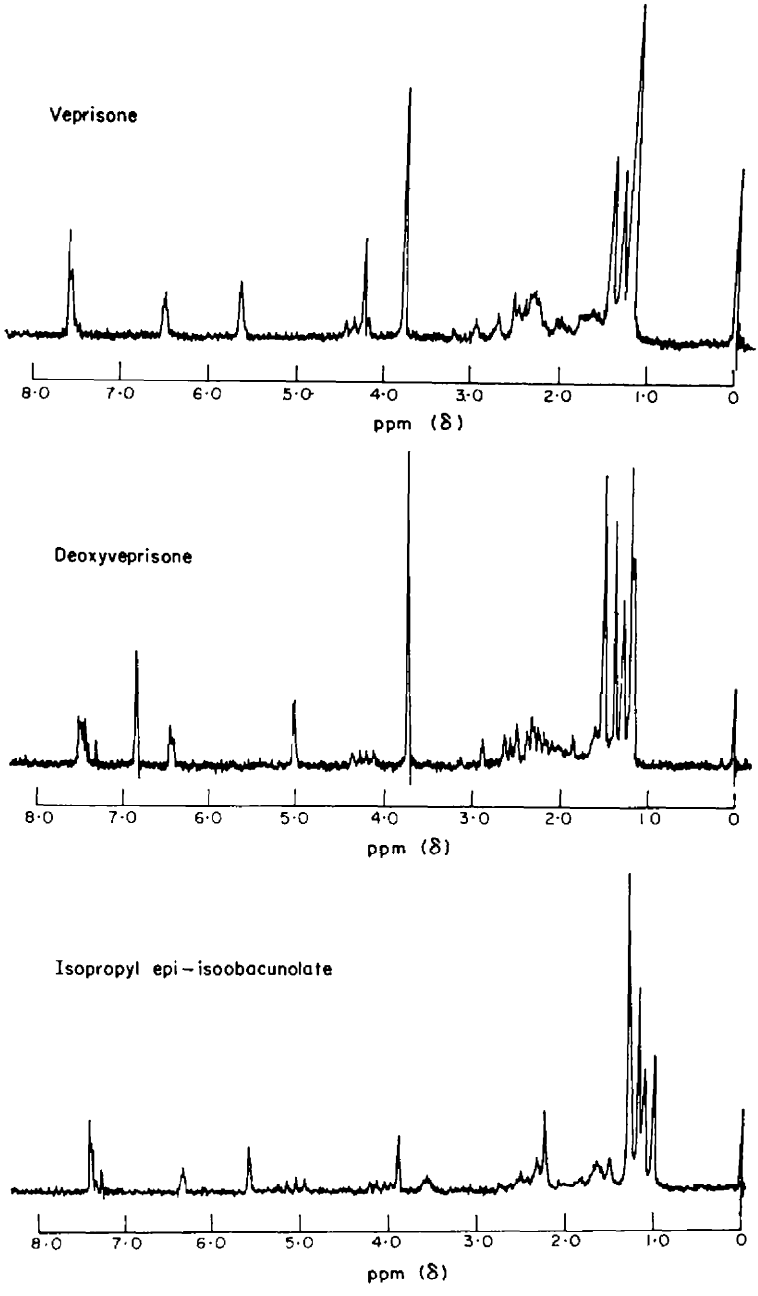
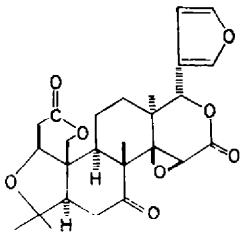
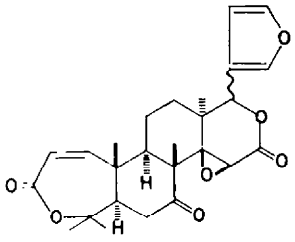


FIG. 1. The NMR spectra taken in $CDCl_3$ on a Varian A 60 spectrometer using tetramethylsilane (TMS) as an internal standard.

the bitter principles limonin[2] (III) and obacunone (IV)[3] is apparent and further borne out by the presence of signals at 5·6 δ (singlet, corresponding to the proton at C_{17} in limonin) and 4·25 δ (singlet, corresponding to the proton at C_{15} in limonin). A well resolved quartet is present at 4·3 δ (1H, J = 5 c/s)



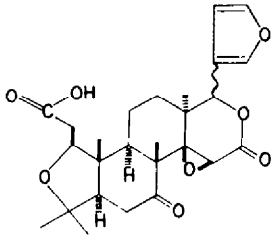
III



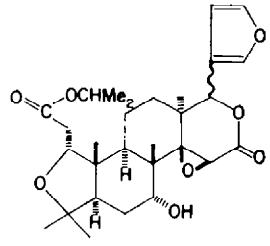
IV

Catalytic reduction of veprisone (10% Pd/C) or reduction with lithium aluminium hydride or sodium borohydride yields only amorphous products. Reduction of veprisone with hydriodic acid in acetic acid gives an acid $C_{26}H_{32}O_7$, m.p. 182–183°, formed by removal of an oxygen atom and hydrolysis of the ester function. The acid may be converted to deoxyveprisone, $C_{27}H_{34}O_7$ by treatment with diazomethane. The NMR spectrum of deoxyveprisone (Fig. 1) shows signals in the region of 1·3 δ (15H: five quarternary methyl groups), 7·48 δ and 6·8 δ (3 protons of the furan ring), a signal at 5·02 δ (1H, corresponding to the C_{17} proton in limonin) and a well split quartet at 4·21 δ (1H, J = 5 c/s.). There are no signals in the 4·0 δ region indicating the disappearance of a proton attached to an epoxy system. Instead, a signal appeared downfield at 6·82 δ. These results indicate the presence of an epoxy ring in veprisone in an environment perhaps identical with that in limonin.

Hydrolysis of veprisone with methanolic potassium hydroxide yields a crystalline carboxylic acid $C_{26}H_{32}O_8$, m.p. 157–158° whose identity with isoobacunoic acid (V) was established by comparison with an authentic specimen.* Methylation of this compound with diazomethane gives, however, an amorphous product, which is not identical with veprisone. The NMR spectrum of isoobacunoic acid is similar to that of veprisone, except that the signal at 4·0 δ is an ill-defined multiplet, instead of the well-defined quartet seen in the latter compound.



V



VI

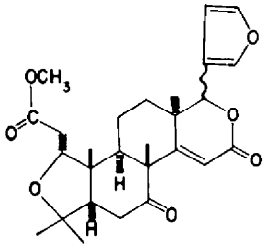
* Kindly furnished by Professor D. H. R. Barton to whom our thanks are due.

[2] D. H. R. Barton, S. K. Pradhan, S. Sternhell and J. F. Templeton, *J. Chem. Soc.* 255 (1961).

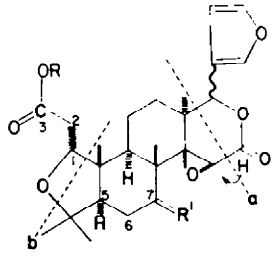
[3] T. Kamikawa and T. Kubota, *Tetrahedron* **12**, 262 (1961); T. Matsuura, T. Kamikawa and T. Kubota, *Ibid.* **12**, 269 (1961); T. Kubota, T. Matsuura, T. Tokoroyama and T. Matsumoto, *Tetrahedron Letters* No. 10, 325 (1961).

It has been reported that mild treatment of obacunoic acid with alkali gives epi-isoobacunoic acid (II) which is transformed under more vigorous conditions to isoobacunoic acid (V). Veprisone, likewise on brief treatment with barium hydroxide gives a crystalline acid, m.p. 276–277°, which should be epi-isoobacunoic acid.* On treatment with diazomethane, the acid gives veprisone in quantitative yield.

Reduction of veprisone by the Meerwein-Ponndorf method gives a compound, m.p. 187–188°, identical with an authentic speciment† of isopropyl epi-isoobacunolate (VI). The NMR spectrum of VI (Fig. 1) shows in addition to the signals present in veprisone a doublet at 1·05 δ (6H, J = 7 c/s) due to the isopropyl methyl groups, and a triplet at 5·05 δ which may be ascribed to the proton on the carbon bearing the hydroxyl group. These results prove conclusively that veprisone should be constituted as methyl epi-isoobacunoate (I). Epi-isoobacunoic acid (II) on treatment with hydriodic acid in acetic acid yields a crystalline acid identical with that obtained by similar treatment of veprisone (see above). In deoxyveprisone the configuration at C_1 should, therefore, be the same as in veprisone and the former could be assigned structure VII. The quartets seen in the NMR spectra of veprisone, deoxyveprisone and isopropyl epi-isoobacunolate in the region of 4·3 δ should be ascribed to the proton at C_1 in these compounds.



VII



I R = CH_3, R' = O

V R = H, R' = O $C_1 - C_2$ β

VI R = $CHMe_2$, R' = H, OH

The mass spectra of veprisone (I), isoobacunoic acid (V) and isopropyl epi-isoobacunolate (VI) show peaks corresponding to the loss of the side chains at C_1, in addition to weaker peaks at M-15, M-18 and M-28.

A dominant peak in the mass spectrum of veprisone is that due to loss of 123 mass units. Apparently, this could occur by fission of the molecule along line *a*, with concommitant transfer of a hydrogen. The loss of 123 mass units is dominant in the spectra of isoobacunoic acid and isopropyl epi-isoobacunolate. Another interesting feature in the mass spectra of both veprisone and isoobacunoic acid is an intense peak at 261 mass units, which could result by fission along *a* (and hydrogen transfer) and also along *b* of these molecules. The corresponding peak in the case of isopropyl epi-isoobacunolate occurs at 263.

* The product melts at 189–191° when heated rapidly. Kubota *et al*, report the m.p. of epi-isoobacunoic acid as 270–271°.

† Kindly furnished by Professor Kubota to whom our thanks are due.

EXPERIMENTAL

Isolation of veprisone. Powdered bark of *Vepris bilocularis* (35 kg) was extracted thrice with pet. ether (40–60°) to remove fatty matter. The dried material was then soaked in ether and kept at room temp for 4–5 days. The ether extract after concentration was extracted with 2N HCl till all the alkaloidal material was removed. This basic fraction gave kokusaginine as the major alkaloid. The ether layer was then washed with water, concentrated to a small volume and left in the refrigerator overnight. The neutral compound that separated was collected, washed with cold ether and recrystallized from ethanol as colourless plates of veprisone (20·7 g), m.p. 180–181°. When a benzene solution of veprisone was chromatographed on a column of silica gel or alumina, polymorphic crystals m.p. 162–163°, changing to m.p. 180°, on crystallization from ethanol were obtained; $[\alpha]_D^{30}$ −18° (*c*. 0·82 in $CHCl_3$). (Found: C, 66·5; 66·6; H, 7·1; 7·3; O, 26·2. $C_{27}H_{34}O_6$ (M.W. 486) requires: C, 66·7; H; 7·0; O, 26·3%).

Mol. wt. by saponification method 484·4; by X-ray, 486·8 ± 4; mass spectrum, 486.

Veprisone was characterized as the oxime, m.p. 197° with dec. (Found: C, 64·7; H, 7·2. $C_{27}H_{35}O_6N$ requires: C, 64·7; H, 7·0%) and the semicarbazone, m.p. 222–224° softening at 204°. (Found: C, 61·8; H, 7·1. $C_{28}H_{37}O_6N_3$ requires: C, 61·9; H, 6·8%).

Epi-isoobacunoic acid (II, R = H). A solution of $Ba(OH)_2$ (1 g in 10 ml water) was added to a solution of veprisone (1 g in 35 ml methanol) and refluxed for 15 min. After cooling, the solution was filtered and diluted with water (20 ml). The methanol was removed *in vacuo* and the solution acidified with HCl aq. The precipitate was collected, washed with water and dissolved in methylene chloride. This was extracted with a sat. $NaHCO_3$ aq, the bicarbonate solution acidified with dil. HCl aq and extracted with methylene chloride. The methylene chloride after drying (Na_2SO_4) and evaporation gave a residue (600 mg) which on crystallization from methylenechloride–hexane gave colourless needles m.p. 276–277° (slow heating) m.p. 189–191° (when the temp was raised quickly) $[\alpha]_D^{30}$ −61·0° (*c*. 1·04 in CH_2Cl_2) ν_{max} 1730 (δ-lactone), 1704 (ketone, carboxylic acid) 3080, 1590 and 870 (furan) cm^{-1}. (Found: C, 65·5; H, 6·9; $C_{26}H_{32}O_6$ requires: C, 66·1; H, 6·8%).

Methyl epi-isoobacunoate (veprisone) I; R = CH_3). The carboxylic acid (II; R = H; 100 mg) was dissolved in ether and treated with a solution of diazomethane in ether. This was kept 1 min and after decompositon (2 drops of acetic acid) of the excess diazomethane the solvent was removed. This gave a residue which crystallized from ethanol–ether giving colourless needles (90 mg) m.p. 180°. The mixed m.p. with veprisone was undepressed. Thin layer chromatography on silica gel (benzene: 5% methanol) showed that the methylation product and veprisone had identical R_f values (0·44). The IR spectra were also identical.

Isoobacunoic acid (V). Veprisone (2 g) was refluxed with methanolic KOH (600 mg in 35 ml) for 20 min giving a pale yellow solution. The methanol was removed *in vacuo* and water (50 ml) was added. This was treated with a solution of I_2 (750 mg) in KI (800 mg in 40 ml water). After 3 min a small amount of Na_2SO_3 was added and the solution acidified with HCl aq. The colourless precipitate was collected, washed with water and dried (1·8 g). This was dissolved in methylene chloride (50 ml) shaken with $NaHSO_3$ aq, then water and extracted with sat. $NaHCO_3$ aq (25 ml × 3). The methylene chloride was washed with water and dried. This gave a neutral substance (0·408 g). The bicarbonate solution was acidified with HCl aq and the precipitate extracted with methylene chloride, washed with water and dried. This gave a colourless solid (0·724 g) which crystallized from methylene chloride–hexane (40–60°) as colourless needles m.p. 155–157°; $[\alpha]_D^{30}$ −49° (*c*, 1·02 in $CHCl_3$); λ_{max} 215 and 278 mμ (ε, 3571 and 408) ν_{max} 1740 (δ-lactone), 1708 (ketone, carboxylic acid), 3120, 1590, 1500 and 878 cm^{-1} (furan). (Found: C, 65·7; H, 6·9. $C_{26}H_{32}O_6$ requires: C, 66·1; H, 6·8%).

The hydrolysed compound from veprisone had a superimposable IR spectrum with isoobacunoic acid and the mixed m.p. with an authentic sample was not depressed. The R_f values were also identical when thin layer chromatograms were run on silica gel.

Isopropyl epi-isoobacunolate (VI). To a suspension of veprisone (1·0 g) in isopropanol (6 ml) freshly distilled aluminium isopropoxide (6 ml) was added. The flask was heated very gradually in an oil bath at 80–90° in such a way that about 1 drop of the distillate was collected in about 10 min. The distillate was tested for the removal of acetone. After heating for about 5 hr no more acetone separated. The reaction mixture was diluted with water and extracted with methylene chloride. This gave a colourless solid (500 mg). On chromatographic separation on a short column of deactivated alumina (10 g) and elution with benzene, a crystalline solid was obtained which after recrystallization from benzene melted at 186–187°. ν_{max} 3620 (hydroxyl), 1720 (δ-lactone, ester), 876 cm^{-1}

(furan). (Found: C, 67·6; H, 7·7. $C_{29}H_{40}O_8$ requires: C, 67·4; H, 7·8%). The mass spectrum indicated an m/e peak at 516. The mixed m.p. with an authentic sample of isopropyl epi-isoobacunolate, m.p. 186°, was undepressed. The IR spectra of the two samples were identical.

Deoxyepi-isoobacunoic acid. Veprisone (2·4 g) in glacial acetic acid (60 ml) was heated with HI (40 ml) at 60° for 4 hr. The reaction mixture was poured into water, extracted with chloroform and the chloroform layer washed with 5% $NaHSO_3$ aq and water. It was then extracted thrice with sat. $NaHCO_3$ aq. The bicarbonate solution was acidified with HCl aq, the precipitate collected and dried (1·4 g). Crystallization from methylene chloride–hexane (40–60°) gave a product m. p. 182–183° (dec) $[\alpha]_D^{30}$ + 1·4° (*c*. 0·88 in $CHCl_3$) λ_{max} 215 mμ (ϵ, 12,860); ν_{max} 1720 (δ-lactone), 1700 (ketone carboxylic acid) 3100, 1610, 1500 and 877 cm^{-1} (furan). (Found: C, 68·3; H, 7·2; $C_{26}H_{32}O_7$, requires: C, 68·4; H, 7·1%).

Deoxyveprisone (VII). The above carboxylic acid (500 mg) was dissolved in dry ether (20 ml) and methanol (10 ml) and an excess of diazomethane in ether added. Removal of the solvent and crystallization from methanol gave colourless needles (450 mg) m.p. 242–243°. $[\alpha]_D^{30}$ −51·89° (*c*. 1·04 in $CHCl_3$) λ_{max} 214 mμ (ϵ, 12,700), ν_{max} 1715 (δ-lactone), 1702 (ketone), 3120, 1600, 1500 and 876 cm^{-1} (furan). (Found: C, 69·1; H, 7·3; $C_{27}H_{34}O_7$ requires: C, 68·9; H, 7·3%).

Deoxyepi-isoobacunoic acid. Epi-isoobacunoic acid (300 mg) was heated with glacial acetic acid (7 ml) and HI (*d*, 1·7; 5 ml) at 60° for 4 hr. The solution was poured into water and extracted with methylene chloride. This was washed with water, $NaHSO_3$ aq and finally with water. The methylene chloride was then extracted with sat. $NaHCO_3$ aq (25 ml × 3) and the bicarbonate layer acidified with HCl aq and extracted with methylene chloride (25 ml × 4). This was washed with water, dried (Na_2SO_4) and the solvent evaporated *in vacuo*. The residue (125 mg) crystallized from methylene-chloride–hexane in colourless needles m.p. 181–182° (dec). The mixed m.p. with deoxyepi-isoobacunoic acid obtained from veprisone was undepressed. The two compounds had also the same R_f value when a thin layer chromatogram was run on silica gel.

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