CONSTITUTION OF RUVOSIDE

BY S. RANGASWAMI, F.A.SC. AND E. VENKATA RAO

(Department of Pharmacy, Andhra University, Waltair)

Received September 19, 1961

THE isolation of two new crystalline glycosides designated as peruvoside and ruvoside from the fermented seed kernels of *Thevetia neriifolia* Juss. (Fam. Apocynaceæ) was announced by the authors[1] in a preliminary communication in 1958 wherein they also mentioned the isolation of L-thevetose as a product of hydrolysis of both peruvoside and ruvoside. In a subsequent brief note[2] they adduced evidence for the presence of an aldehydic group in peruvoside and reported the formation of ruvoside on reducing peruvoside with aluminium-amalgam.

In the course of a detailed study of the chemistry of thevetin B and thevetin A obtained by the fractionation of crude thevetin from the seeds of *Thevetia neriifolia*,[3] Bloch *et al.*[4] found that thevetin B is identical with cerberoside obtained by Chen and Steldt[5] from the seed kernels of *Cerbera odollam* Gaertn. (Fam. Apocynaceæ) and is represented by the structure previously assigned by Helfenberger and Reichstein[6] to thevetin in 1948, *viz.*, digitoxigenin-L-thevetose-D-glucose-D-glucose. Thevetin A was also found to be a trioside which undergoes hydrolysis with enzymes to give D-glucose and a monoside which was identified with peruvoside of Rangaswami and Rao.[1] Reduction of the aldehyde group in peruvoside to methyl *via* the cyclic thioketal gave neriifolin (II), thus giving for peruvoside the structure α-L-thevetoside of cannogenin[7] (= 19-oxo-neriifolin) (I). It followed that thevetin A resembles thevetin B in all respects except that C_{19} exists as a methyl group in thevetin B and as an aldehyde in thevetin A.

The constitution of peruvoside having been thus settled, that of ruvoside easily follows. The reduction of peruvoside to ruvoside previously brought about by means of aluminium-amalgam has now been confirmed using sodium borohydride as the reagent. Hence the constitution of ruvoside is 19-hydroxy-neriifolin (= α-L-thevetoside of cannogenol[7]) (III).

EXPERIMENTAL

Besides the experiments leading to the conversion of peruvoside to ruvoside, other experiments pertinent to the constitution of peruvoside and

ruvoside which have only been mentioned in the form of preliminary notes[1,2] and which have not been described anywhere in the literature till now are also briefly described below.

Peruvoside (*I*).—Colourless prisms from methanol or methanol-ether m.p. 160–64°/210–16°. Legal reaction: positive; Keller-Kiliani reaction: negative; colour with conc. sulphuric acid: yellow-deep yellow-pale brown. $[\alpha]_D^{30} = -69{\cdot}6° \pm 3°$ ($c = 1{\cdot}124$ in methanol). Found: C, 65·2; H, 8·4; $-OCH_3$, 5·9%. $C_{30}H_{44}O_9$ (I) requires: C, 65·7; H, 8·1; $-OCH_3$ (1), 5·7%.

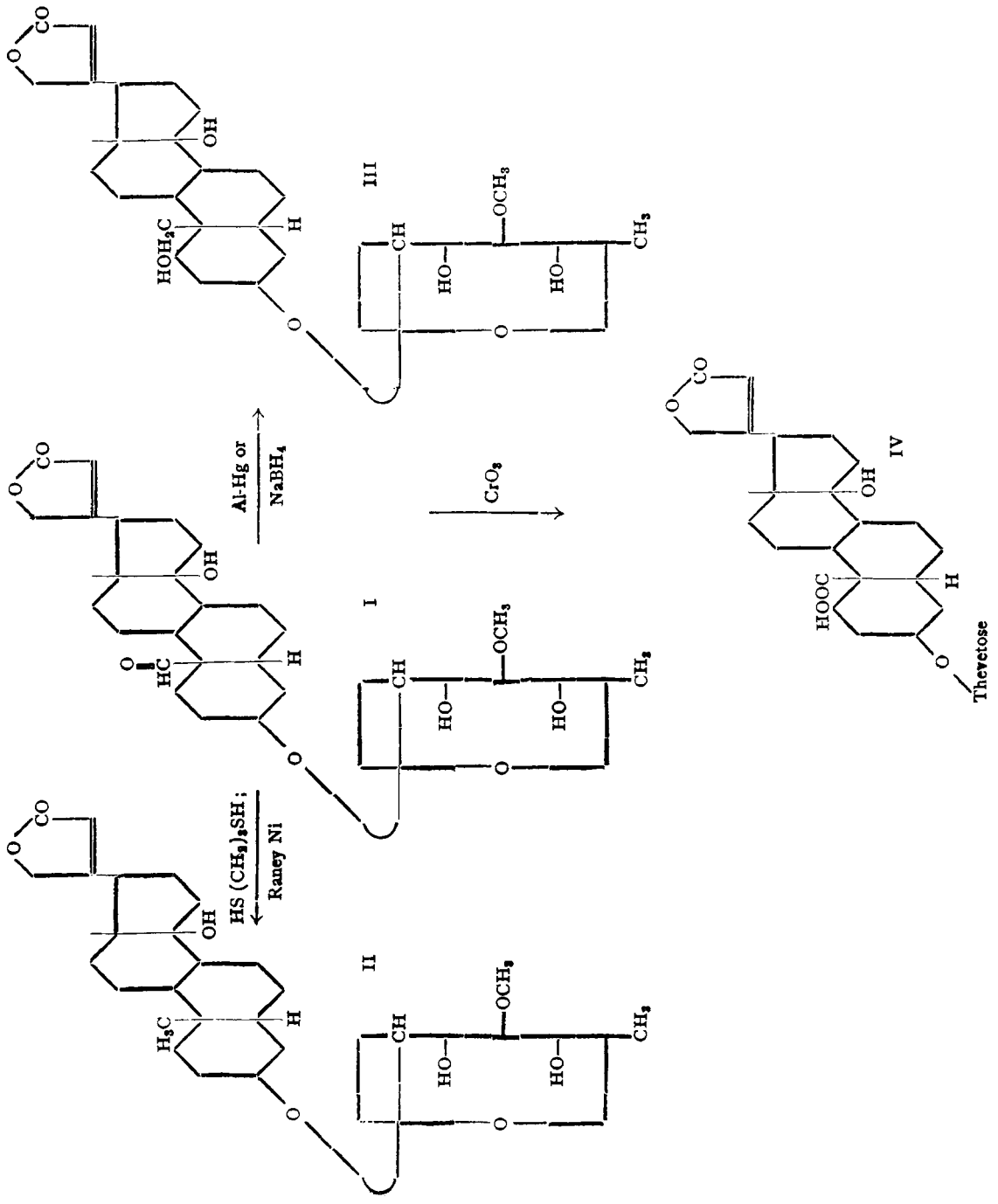
Isoperuvoside.—This was obtained by the action of potassium hydroxide in methyl alcohol employing the conditions of (*a*) Smith[8] and (*b*) Sigg *et al.*[9] It crystallized from methanol as colourless needles, m.p. 228–30°. Legal reaction: negative. $[\alpha]_D^{32} = -76{\cdot}1° \pm 2°$ ($c = 0{\cdot}812$ in methanol). Found: C, 64·7; H, 8·4; $-OCH_3$, 5·8%. $C_{30}H_{44}O_9$ requires: C, 65·7; H, 8·1; $-OCH_3$ (1) 5·7%.

Isoperuvoside acetate.—This was prepared by the action of acetic anhydride and pyridine on isoperuvoside in the cold. It crystallized from acetone-ether as colourless needles, m.p. 235–38° (decomp). $[\alpha]_D^{31} = -36{\cdot}5° \pm 3°$ ($c = 0{\cdot}602$ in methanol). Found: C, 63·5; H, 7.7; $-OCH_3$, 5·2; $-COCH_3$, 12·9%. $C_{34}H_{48}O_{11}$ requires: C, 64·5; H, 7·6; $-OCH_3$ (1), 4·9; $-COCH_3$ (2), 13·6%.

Peruvoside semicarbazone prepared by the action of semicarbazide hydrochloride and sodium acetate in methyl acohol in the cold crystallized from dilute alcohol as colourless needles, m.p. 266–68° (decomp). Found: N, 7·1%. $C_{31}H_{47}O_9N_3$ requires: N, 6·9%.

Betaine hydrazone of peruvoside using Girard reagent T.—This was prepared by treating peruvoside (100 mg.) with Girard reagent T (freshly crystallized from absolute alcohol) (62 mg.) in methanol (10 ml.) and pure acetic acid (0·5 ml.). The product was worked up as per the procedure of Schindler and Reichstein.[10] It crystallized from dilute alcohol as fine plates, m.p. 276–80° (decomp.). Found: N, 6·2%. $C_{35}H_{56}O_9N_3Cl$ requires: N, 6·0%.

Peruvosidic acid (*IV*).—This was obtained by the action of chromic acid in glacial acetic acid on peruvoside in the cold employing enough reagent to provide just over one equivalent of oxygen according to the method described by Hegedus *et al.*[11] The acid crystallized from aqueous alcohol as colourless prisms, m.p. 226–28° (decomp.). Legal reaction: positive,


Al-Hg or
NaBH4
CrO3
HS (CH2)3SH;
Raney Ni
Thevetose

$[\alpha]_D^{29} = -57° \pm 3°$ ($c = 0{\cdot}763$ in methanol). Found: C, 63·0; H, 8·2; $-OCH_3$, 5·6%. $C_{30}H_{44}O_{10}$ (IV) requires: C, 63·8; H, 7·9; $-OCH_3$ (1), 5·5%.

Peruvosidic acid methyl ester prepared by the action of ethereal diazomethane on peruvosidic acid in methanol crystallized from acetone-ether as colourless short needles, m.p. 130–34°. $[\alpha]_D^{29} = -25{\cdot}6° \pm 3°$ ($c = 0{\cdot}651$ in methanol). Found: C, 63·4; H, 8·2; $-OCH_3$, 10·8%. $C_{31}H_{46}O_{10}$ requires: C, 64·3; H, 8·0; $-OCH_3$ (2), 10·7%.

Ruvoside (III).—It crystallized from methanol-ether as colourless prisms, m.p. 228–30°. It gave positive Molisch and Legal reactions and a negative Keller-Kiliani reaction. Colour with conc. sulphuric acid: pale yellow-pink-deep pink-rose. $[\alpha]_D^{27} = -57{\cdot}8° \pm 3°$ ($c = 0{\cdot}663$ in methanol). Found: C, 64·4; H, 8·4; $-OCH_3$, 5·8%. $C_{30}H_{46}O_9$ (III) requires: C, 65·4; H, 8·4; $-OCH_3$ (1), 5·6%.

Lactone titration.—Ruvoside (20·1 mg.) was refluxed with 0·1 N alcoholic potash (10 ml.) for 2 hours and titrated with 0·1 N sulphuric acid using phenolphthalein as indicator. A blank experiment was simultaneously done. Alkali consumed: 0·401 ml. of 0·1 N alkali, corresponding to 1·1 lactone group.

Ruvoside acetate.—Ruvoside was acetylated with pyridine and acetic anhydride in the cold. The acetate could not be satisfactorily crystallized. By dissolving it in a small quantity of acetone and adding absolute ether a microcrystalline powder, m.p. 110–22° was obtained. Colour with conc. sulphuric acid: yellow-orange-pink. $[\alpha]_D^{27} = -35{\cdot}8° \pm 2°$ ($c = 0{\cdot}922$ in chloroform). Found: C, 63·5; H, 7·7; $-COCH_3$, 18·3%. $C_{36}H_{52}O_{12}$ requires: C, 63·9; H, 7·7; $-COCH_3$ (3), 19·1%.

Isoruvoside prepared by the action of cold methyl alcoholic potash on ruvoside as described earlier[8] crystallized from methyl alcohol as colourless needles, m.p. 234–37°. Legal reaction: negative. Found: C, 65·0; H, 9·0. $C_{30}H_{46}O_9$ requires: C, 65·4; H, 8·4%.

Isoruvoside acetate.—Isoruvoside was acetylated with pyridine and acetic anhydride at room temperature. The product crystallized from acetone-ether as fine short needles, m.p. 197–200°. Found: C, 63·7; H, 8·2; $-COCH_3$, 18·3%. $C_{36}H_{52}O_{12}$ requires; C, 63·9; H, 7·7; $-COCH_3$ (3), 19·1%.

Hydrolysis of ruvoside.—Ruvoside (300 mg.) was treated with Kiliani's mixture[12] (10 ml.) (acetic acid: conc. hydrochloric acid: water, 7:2:11)

and heated for 50 mts. in a boiling water-bath. The yellow solution was cooled and left in the ice-chest for 18 hours when a viscous layer settled down. The supernatant liquid was transferred to a separating funnel and the viscous layer washed with small quantities of water which were transferred to the separating funnel. The aqueous liquid was extracted with chloroform and the extracts after washing with a little water, 2 N sodium carbonate and water were mixed with the viscous layer, the chloroform solution dried over sodium sulphate and the solvent removed under vacuum. The residue (195 mg.) was chromatographed over alumina when two crystalline sugar-free fractions (negative Molisch test) melting at 228–30° and 169–72° were obtained in small yields. These fractions have not been characterized.

The aqueous liquid and the first wash-water described above were mixed and worked up for the sugar moiety as described by Rheiner *et al.*[13] under the hydrolysis of odoroside-H-monoacetate. The sugar crystallized from acetone-ether as colourless needles, m.p. 126–29°. $[\alpha]_D^{31} = -65{\cdot}8° \pm 3°$ (after 10 mts.) and $-35{\cdot}7° \pm 3°$ (after 14 hours) ($c = 0{\cdot}681$ in water). Found: C, 47·6; H, 8·1; $-OCH_3$, 17·1%. $C_7H_{14}O_5$ requires: C, 47·2; H, 7·9; $-OCH_3$ (1), 17·4%.

Mixed m.p. with authentic L-thevetose obtained from neriifolin by Kiliani hydrolysis was undepressed.

Hydrolysis of peruvoside.—Peruvoside was hydrolysed exactly as described under ruvoside. The sugar isolated in the same manner crystallized as colourless needles from acetone-ether, m.p. 125–28°. $[\alpha]_D^{27} = -67{\cdot}2° \pm 2°$ (after 10 mts.) and $-33{\cdot}2° \pm 2°$ (after 14 hours) ($c = 1{\cdot}366$ in water). Found: C, 47·2; H, 7·8; $-OCH_3$, 17·0%. $C_7H_{14}O_5$ requires: C, 47·2; H, 7·9; $-OCH_3$ (1), 17·4%.

Mixed m.p.s with L-thevetose from neriifolin and from ruvoside were undepressed. On paper chromatogram using the solvent system butanol-pyridine-water[14] authentic thevetose from neriifolin and the sugars from peruvoside and ruvoside behaved exactly alike.

Reduction of peruvoside to ruvoside.—(*a*) Using aluminium-amalgam (method of Rabald and Kraus[15]). The reduction was carried out as per details described by Doebel *et al.*[16] in their experiments on the reduction of α-antiarin to *al*-dihydro-α-antiarin. The reduction product was chromatographed over alumina. The methanol-chloroform eluates containing 1% and 2% of methanol gave unchanged peruvoside (m.p. and mixed m.p.) and the methanol-chloroform eluate containing 5% methanol, on crystalliza-

tion from methanol-ether, yielded ruvoside as colourless prisms, m.p. 232–34°. Colour reactions were same as with natural ruvoside. $[\alpha]_D^{27} = -61{\cdot}5° \pm 3°$ ($c = 0{\cdot}602$ in methanol). Found: C, 65·0; H, 8·5; $-OCH_3$, 5·9%. $C_{30}H_{46}O_9$ requires: C, 65·4; H, 8·4; $-OCH_3$ (1), 5·6%.

Mixed m.p. with natural ruvoside was undepressed. The behaviours of these two samples on paper chromatogram (solvent system: chloroform-formamide) were identical.

(*b*) *Using sodium borohydride* (*method of Hunger and Reichstein*).[17]—The reduction was carried out as per details described by Golab *et al.*[7] in their experiments on the reduction of cannogenin to cannogenol. The reduction product was chromatographed over alumina. Unchanged peruvoside and ruvoside were eluted by the same solvents as in experiment (*a*). The latter crystallized from methanol-ether as prisms, m.p. 232–34°. Colour reactions were identical with natural ruvoside. Mixed m.p. with natural ruvoside was undepressed. $[\alpha]_D^{28} = -59{\cdot}7° \pm 2°$ ($c = 0{\cdot}620$ in methanol).

Note.—Substances for analysis and rotation were dried to constant weight in high vacuum at 110° and 80° respectively.

SUMMARY

The constitution of ruvoside, a new glycoside from *Thevetia neriifolia*, has been shown to be α-L-thevetoside of cannogenol (= 19-hydroxy-neriifolin).

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