127. *An Examination of the Rutaceae of Hong Kong. Part I. Flavonoid Glycosides from* Zanthoxylum *Species and the Occurrence of Optically Active Hesperetin.*

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Hesperidin and diosmin occur in barks of *Zanthoxylum* species of Hong Kong. Hydrolysis of hesperidin yields a mixture of (±)- and (−)-hesperetin from which the pure constituents have been isolated. These compounds have been characterised, and (−)-hesperetin has been racemised. Hesperetin has been converted into diosmetin by the use of *N*-bromosuccinimide.

From this work it is concluded that hesperidin is a β-7-rutinoside of (−)-hesperetin.

Hesperidin, which occurs in *Citrus* species, was first reported by Hilger.[1] Zemplén and Bognár [2] showed that it is a β-7-rutinoside of hesperetin, a flavanone synthesised by Shinoda and Kawagoye.[3] Diosmin, which occurs in hyssop plants, was isolated by Oesterle and Wander; [4] its structure was elucidated by Zemplén and Bognár [5] who converted hesperidin

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[1] Hilger, *Ber.*, 1876, **9**, 26.
[2] Zemplén and Bognár, *Ber.*, 1943, **76**, 773.
[3] Shinoda and Kawagoye, *J. Pharm. Soc. Japan*, 1928, **48**, 938.
[4] Oesterle and Wander, *Helv. Chim. Acta*, 1925, **8**, 519.
[5] Zemplén and Bognar, *Ber.*, 1943, **76**, 452.

into diosmin by a modified Kostanecki procedure; it is the glycoside of the flavanone diosmetin which was synthesised by Lovecy, Robinson, and Sugasawa.[6]

We report here that hesperidin and diosmin occur in the bark of *Zanthoxylum avicennae*, that hesperidin occurs in the bark of *Z. cuspidatum*, and that diosmin occurs in the root bark of *Z. nitidum*; the bark of the last species contains neither glycoside. The three plants are indigenous to Hong Kong.

Hydrolysis of hesperidin (from both *Z. avicennae* and *Z. cuspidatum*) with either dilute sulphuric acid or sulphuric acid in ethylene glycol yields an optically active mixture of (±)- and (—)-hesperetin from which the two have been separated by fractional recrystallisation. Both have been characterised by conversion into derivatives, and (—)hesperetin has been racemised.

Each of two authentic samples of hesperidin, kindly supplied by Professor T. R. Seshadri and Dr. J. T. Pinhey, on hydrolysis also yields a mixture of (±)- and (—)-hesperetin. Since the m. p.s for hesperetin and its derivatives stated by previous workers do not correspond throughout exactly with those of either (±)-hesperetin and its derivatives or (—)-hesperetin and its derivatives which we have obtained, we believe that much of the previous work on hesperetin has been carried out on optical mixtures, although workers who hydrolysed hesperidin under pressure in an autoclave probably obtained (±)-hesperetin. Also since recrystallisation of the optical mixture leads to a product richer in (±)-hesperetin, some previous workers may have obtained a product rich in racemate. Nevertheless no rotations have previously been recorded for hesperetin; and we describe here for the first time the preparation of both (±)- and (—)-hesperetin and their derivatives.

We conclude that hesperidin is a β-7-rutinoside of (—)-hesperetin.

Although very many naturally occurring flavanones have been reported, optically active forms are exceedingly rare. Fujise[7] isolated (—)-matteucinol and (—)-demethoxymatteucinol. Arthur[8] reported the occurrence of (—)-farrerol, Hillis[9] that of (+)-dihydrokaempferol, and Erdtman[10] that of (—)-dihydrochrysin. Whilst matteucinol, demethoxymatteucinol, and farrerol exhibit the structural regularity of methyl groups in the 6- and the 8-position, the other two and hesperetin do not. With the exception of dihydrokaempferol all naturally occurring optically active flavanones are lævorotatory.

We have converted hesperetin into diosmetin by the method of Seshadri and Bannerjee[11] and Lorette *et al.*,[12] using *N*-bromosuccinimide. This has been done previously only by a modified Kostanecki procedure.

Previous work on *Zanthoxylum* species has been surveyed by King, Housley, and King.[13]

EXPERIMENTAL

Analyses are by Dr. Zimmermann, Melbourne.

Zanthoxylum avicennae.—(a) *Isolation of hesperidin and diosmin.* Dried bark (400 g.) was extracted with boiling methanol (8 l.). The extract was reduced to 1 l. Next morning a greenish-brown semi-crystalline solid (2·4 g.) was collected and the filtrate was evaporated to 400 ml. The pale brown crystals (1·5 g.) which separated were collected.

The first crop was extracted with light petroleum (b. p. 60—80°) and then with methanol. The light brown crystalline residue (1·4 g.) was recrystallised 4 times from 50% aqueous pyridine (charcoal). Nearly colourless fine needles of diosmin hydrate, m. p. 280° (decomp.; vac.) (Found: C, 53·4; H, 5·4; OMe, 5·0. Calc. for $C_{28}H_{32}O_{15},H_2O$: C, 53·7; H, 5·4; 1OMe, 5·0%), were obtained.

The second crop treated in a similar manner yielded mainly hesperidin hydrate as almost

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[6] Lovecy, Robinson, and Sugasawa, *J.*, 1930, 817.

[7] Fujise, *Sci. Papers Inst. Phys. Chem. Res.* (*Tokyo*), 1929, **11**, 111.

[8] Arthur, *J.*, 1955, 3740.

[9] Hillis, *Austral. J. Sci. Res.*, 1952, **5**, *A*, 376.

[10] Erdtman, *Svensk Papperstidning*, 1943, **46**, 226.

[11] Bannerjee and Seshadri, *Proc. Indian Acad. Sci.*, 1952, **36**, *A*, 138.

[12] Lorette, Gage, and Wander, *J. Org. Chem.*, 1951, **16**, 930.

[13] King, Housley, and King, *J.*, 1954, 1392.

colourless fine needles, m. p. 260° (decomp.; vac.), $[\alpha]_D^{27}$ $-88{\cdot}2°$ (*c* 1·24 in pyridine) (Found : C, 53·9; H, 6·0; OMe, 4·8. Calc. for $C_{28}H_{34}O_{15},H_2O$: C, 53·5; H, 5·7; 1OMe, 4·9%), and diosmin hydrate which was less soluble in aqueous pyridine.

(b) *Diosmin.* Diosmin (5 g.) was treated with acetic anhydride (50 ml.) and pyridine (25 ml.) at room temperature for 4 days. After the product had been collected from water, it was recrystallised from ethanol, and then boiled for 2 hr. with sulphuric acid (5 ml.) in ethanol (95 ml.). The hydrolysis mixture was poured into water; the product crystallised from ethanol in fine yellow needles of diosmetin, m. p. 253—254·5° (Found : C, 63·5; H, 4·3; OMe, 10·3. Calc. for $C_{16}H_{12}O_6$: C, 64·0; H, 4·0; 1OMe, 10·3%). The triacetate formed colourless needles, m. p. 189—191° (Found : C, 61·6; H, 4·5; Ac, 30·3. Calc. for $C_{22}H_{18}O_9$: C, 62·0; H, 4·2; 3Ac, 30·3%). Both compounds gave a red colour with magnesium and hydrochloric acid in methanol. Diosmetin was also prepared from the hydrolysis of diosmin in ethylene glycol by using the method which we applied for the hydrolysis of hesperidin.

(c) *Hesperidin.* Hesperidin (10 g.) was boiled under reflux in 6% sodium hydroxide solution (200 ml.) for 2 hr. The solution was acidified, and then extracted with ether (2 l.). Crystallisation from aqueous ethanol of the residue (2·2 g.) obtained after removal of ether gave colourless needles of *iso*ferulic acid, m. p. 228—229° (Found : C, 62·1; H, 5·4; OMe, 15·9. Calc. for $C_{10}H_{10}O_4$: C, 61·8; H, 5·2; 1OMe, 16·0%) [acetate, m. p. 204—205° (Found : C, 61·5; H, 5·3. Calc. for $C_{12}H_{12}O_5$: C, 61·0; H, 5·1%)]. *iso*Ferulic acid gave a red-brown colour with ferric chloride solution. Hesperidin (5 g.) was treated at room temperature with acetic anhydride and pyridine. The product (5·2 g.), collected from water, was recrystallised from ethanol. Colourless needles of the acetate, m. p. 176—179° (Found : C, 55·7; H, 5·6; OMe, 3·4; Ac, 34·4%; *M*, 862. Calc. for $C_{44}H_{50}O_{23}$: C, 55·8; H, 5·3; 1OMe, 3·3; 8Ac, 36·4%; *M*, 946), separated.

Previous workers report difficulty in hydrolysing hesperidin but Gupta, Narasimhachari, and Seshadri[14] report an 80% yield of hesperetin from hydrolysis of hesperidin acetate. We have obtained a nearly quantitative yield of hesperetin in a smooth hydrolysis of the glycoside as follows : Hesperidin (5 g.) and ethylene glycol (100 ml.) containing sulphuric acid (5 ml.) were heated on the water-bath for 40 min. The clear yellow solution obtained was poured into water (200 ml.). The product (1·8 g.) was collected and recrystallised from ethanol. Leaf-shaped crystals of a mixture of ($\pm$)-hesperetin and ($-$)-hesperetin, m. p. 224—226° (after becoming misty at 212°), $[\alpha]_D^{26}$ $-16{\cdot}9°$ (*c* 1·42 in EtOH) (Found : C, 63·8; H, 4·8. Calc. for $C_{16}H_{14}O_6$: C, 63·6; H, 4·6%), separated.

Hesperidin (4 g.) was also hydrolysed by boiling its suspension in dilute sulphuric acid (50 ml.) for 20 hr. A mixture of ($\pm$)- and ($-$)-hesperetin was obtained from the insoluble residue, and glucose and rhamnose were isolated and separated as their osazones from the filtrate.

Authentic hesperidin obtained from other sources gave similarly on hydrolysis the optical mixture.

(d) *Separation of* ($\pm$)- *and* ($-$)-*hesperetin.* The mixture (20 g.) was fractionally recrystallised from ethanol, by a triangular scheme. The less soluble fraction (2·2 g.), obtained as hexagonal prisms, was ($\pm$)-*hesperetin*, m. p. 226—228°, $[\alpha]_D^{27}$ 0·0° (*c* 1·56 in EtOH) (Found : C, 64·1; H, 5·0; OMe, 10·3%; *M*, 347. $C_{16}H_{14}O_6$ requires C, 63·6; H, 4·6; 1OMe, 10·3%; *M*, 302). The more soluble fraction, obtained as triangular plates, was ($-$)-*hesperetin* (2·5 g.), m. p. 216—218°, $[\alpha]_D^{27}$ $-37{\cdot}6°$ (*c* 1·80 in EtOH) (Found : C, 63·8; H, 4·8; OMe, 10·3%; *M*, 346).

(e) *Derivatives of* ($\pm$)-*hesperetin.* ($\pm$)-Hesperetin (0·6 g.) was converted by means of diazomethane into the 7 : 4′-dimethyl ether (0·3 g.), m. p. 132—133° (Found : C, 65·3; H, 5·6; OMe, 27·8. Calc. for $C_{18}H_{18}O_6$: C, 65·5; H, 5·5; 3OMe, 28·2%) (Waung[15] gives m. p. 133—136° and 153—155°; Haley and Bassin[16] give m. p. 153·2—154·8°). ($\pm$)-Hesperetin (0·4 g.) was heated in aqueous ethanol with sodium acetate (0·5 g.) and hydroxylamine hydrochloride (0·5 g.) for 18 hr. The product, on recrystallisation from aqueous ethanol, yielded fine needles (0·34 g.) of the oxime, m. p. 227—228° (decomp.) (Found : C, 60·8; H, 4·7; N, 4·3. Calc. for $C_{16}H_{15}O_6N$: C, 60·6; H, 4·7; N, 4·4%). (Shinoda and Kawagoye[3] give m. p. 229—230°.) ($\pm$)-Hesperetin (1·2 g.) was acetylated by the method of Bannerjee and Seshadri,[11] using ice-cold pyridine (5 ml.) and acetyl chloride (2 ml.). The product crystallised from methanol in colourless needles (0·95 g.) of the triacetate, m. p. 139—141° (Found: C, 61·9; H, 4·8; Ac, 29·0. Calc.

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[14] Gupta, Narasimhachari, and Seshadri, *J. Sci. Ind. Res. India*, 1953, **12**, *B*, 229.

[15] Waung, *J. Pharm. Soc. Japan*, 1940, **60**, 420.

[16] Haley and Bassin, *J. Amer. Pharm. Assoc.*, 1951, **40**, 111.

for $C_{22}H_{20}O_9$: C, 61·7; H, 4·7; 3Ac, 30·1%) (Asahina, Shinoda, and Inubuse[17] give m. p. 80—82°; and Waung[15] gives m. p.s 127—129° and 165—167°).

(f) *Derivatives of* (—)-*hesperetin*. These were prepared as described for those of (±)-hesperetin and were found to be: 7 : 4′-*dimethyl ether*, m. p. 149—151°, $[\alpha]_D^{28}$ —35·6° (*c* 1·04 in $CHCl_3$) (Found : C, 65·7; H, 5·6; OMe, 27·8%); *oxime*, m. p. 219—220° (decomp.), $[\alpha]_D^{27}$ —16·1° (*c* 0·40 in EtOH) (Found : C, 60·9; H, 4·8; N, 4·3%); *triacetate*, m. p. 130—132°, $[\alpha]_D^{26}$ +21·1° (*c* 1·28 in $CHCl_3$) (Found : C, 62·0; H, 4·9; Ac, 29·8%).

(±)- and (—)-Hesperetin and their derivatives gave a bright red solution with magnesium and hydrochloric acid in methanol.

(g) *Racemisation of* (—)-*hesperetin*. A solution of (—)-hesperetin (0·4 g.) in ethylene glycol (5 ml.) was heated in a sealed tube at 250—260° for 2 hr. The reddish-brown solution was then poured into water, and the product, collected as usual, deposited (±)-hesperetin (0·1 g.), m. p. and mixed m. p. 225—227°, $[\alpha]_D^{26}$ 0·0° (*c* 0·50 in EtOH), after recrystallisation from ethanol. Solutions of (—)-hesperetin in ethanolic sodium hydroxide or ethanolic hydrochloric acid did not racemise during 3 days.

(h) *Conversion of hesperetin into diosmetin*. A solution of hesperetin triacetate (0·9 g.), *N*-bromosuccinimide (0·45 g.), and benzoyl peroxide (0·05 g.) was heated on a steam-bath for 1 hr., then more *N*-bromosuccinimide (0·3 g.) was added. The solution was boiled for another 2 hr. Next morning succinimide was removed and the filtrate was evaporated to dryness. The residue was washed with hot water, and then recrystallised 4 times from ethanol. Pale yellow crystals of diosmetin (0·08 g.), m. p. and mixed m. p. 250—253°, were obtained.

Zanthoxylum cuspidatum.—*Isolation of hesperidin*. Dried bark (400 g.) was extracted as stated for *Zanthoxylum avicennae*. After the extract had been reduced to 1 l., fine needles (1·4 g.) were formed on cooling. These were collected and then the filtrate was concentrated to 250 ml. A second crop of crystals (0·5 g.) which was deposited was combined with the first. Purification of this material yielded hesperidin hydrate, m. p. and mixed m. p. 260° (Found : C, 53·9; H, 5·9; OMe, 5·0. Calc. for $C_{28}H_{34}O_{15},H_2O$: C, 53·5; H, 5·7; 1OMe, 4·9%), which was characterised by conversion into hesperetin (also optically active).

Zanthoxylum nitidum [with C. N. MA].—(a) *Isolation of diosmin from root bark*. Milled root bark (31 kg.) was extracted with hot methanol (170 l.) for 25 hr. The extract was concentrated to 5 l. and allowed to cool. A mixture of yellow crystalline alkaloidal material (40 g.) with properties similar to those of the chelidonine group of alkaloids was collected for further investigation, and the mother-liquor was evaporated to dryness under reduced pressure. The residue was extracted with light petroleum (b. p. 60—80°), and that portion of it which was insoluble in petroleum was dried and dissolved in methanol (200 ml.). Next week fine pale brown crystals (2 g.) were collected. Crystallisation of these from aqueous pyridine gave diosmin hydrate, m. p. and mixed m. p. 283° (decomp.; vac.) (Found: C, 54·2; H, 5·9; OMe, 5·1. Calc. for $C_{28}H_{32}O_{15},H_2O$: C, 53·7; H, 5·4; OMe, 5·0%). Insufficient material was obtained for further characterisation.

(b) *Extraction of bark*. Dried bark (400 g.) was extracted as stated for *Zanthoxylum avicennae*. Neither hesperidin nor diosmin was found in the extract.

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[17] Asahina, Shinoda, and Inubuse, *J. Pharm. Soc. Japan*, 1928, **48**, 207.