CCXXVIII.—*Natural Glucosides. Part III. The Position of the Biose Residue in Hesperidin.*

By Frederick E. King and Alexander Robertson.

The discovery by Will (*Ber.*, 1887, **20**, 1186) that hesperidin on hydrolysis gives rise to rhamnose in addition to glucose and hesperitin led Tanret (*Bull. Soc. chim.*, 1888, **49**, 20) to propose the

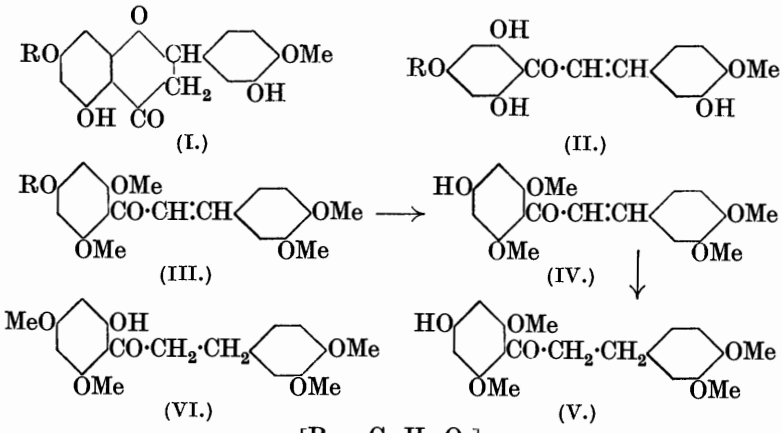
empirical formula $C_{50}H_{60}O_{27}$ for the glucoside. This complex molecule he considered to break down on hydrolysis thus :

$$C_{50}H_{60}O_{27} + 2H_2O = 2C_{16}H_{14}O_6 + 2C_6H_{12}O_6 + C_6H_{12}O_5.$$

Asahina and Inubuse (*J. Pharm. Soc. Japan*, 1929, **49**, 11) have shown, however, that hesperidin invariably forms a very stable hydrate which has the formula $C_{28}H_{34}O_{15},H_2O$ and that it is a simple rhamnoglucoside of hesperitin, analogous to naringin.

Our preliminary experiments on the constitution of the glucoside were similar to those described by Asahina and Inubuse and entirely confirmed their results. It is reasonable to assume that the compound is a bioside and not a diglucoside and therefore a study of the constitution must, to be exhaustive, now take into consideration the two points, (a) the position of the biose residue and the nature of the linking involved, and (b) the structure of the biose. In this communication proof is given of the position of the biose residue.

On methylation by means of methyl sulphate and sodium hydroxide hesperidin gave a product which did not contain a free phenolic group and in which the biose residue was partially methylated. Hydrolysis of the crude material with dilute sulphuric acid gave rise to *4-hydroxy-2 : 6-dimethoxyphenyl 3 : 4-dimethoxystyryl ketone* (IV).



[R = $C_{12}H_{21}O_9$]

The methylation of glucosides of polyhydric phenols by the methyl iodide–potassium carbonate method has been shown to attack only the phenolic groups (J., 1930, 21, 2434), and when hesperidin was treated in this way a thick syrup was obtained which on hydrolysis afforded the styryl ketone (IV). It is likely that the intermediate product of methylation consisted mainly of (III) and hence hesperidin

may be represented by either formula (I) or (II). Asahina, Shinoda, and Inubuse (*J. Pharm. Soc. Japan*, 1928, **48**, 207) suggest that hesperitin is normally a flavanone which readily isomerises to a chalkone when submitted to methylation or acetylation. By analogy we consider it probable that the bioside has the flavanone structure (I).

The orientation of the styryl ketone (IV) is conclusively established by its synthesis from 4-hydroxy-2 : 6-dimethoxyacetophenone (Canter, Curd, and Robertson, this vol., p. 1249) and veratraldehyde. Catalytic reduction of (IV) with hydrogen in the presence of palladium gave rise to the *ketone* (V). The latter compound was prepared by an independent method : condensation of phloroglucinol dimethyl ether and β-3 : 4-dimethoxyphenylpropionitrile by the method of Hoesch produced a mixture of ketimine hydrochlorides, which on hydrolysis and separation gave (V) and the isomeride (VI). To effect a separation of the ketones (V) and (VI) advantage was taken of the different rates of hydrolysis of the ketimine hydrochlorides (compare Johnson and Robertson, *loc. cit.*).

Experimental.

Hesperidin.—The commercial glucoside supplied by Merck of Darmstadt gave a purple coloration with alcoholic ferric chloride. The impurity responsible for this was readily removed by washing with hot water and the pure glucoside was obtained by extracting the washed material in a Soxhlet apparatus with 95% methyl alcohol. Crystallised from this solvent or from acetic acid, it formed colourless needles, m. p. 251—252°, decomposing at 254° (Found : C, 53·4; H, 5·7. Calc. for $C_{28}H_{34}O_{15},H_2O$: C, 53·5; H, 5·7%. Found in a specimen dried at 135° : C, 55·1; H, 5·7. Calc. for $C_{28}H_{34}O_{15}$: C, 55·1; H, 5·6%. Found : loss on drying, 3·3. Monohydrate requires loss, 2·9%).

Acetylation by means of acetic anhydride and sodium acetate on the steam-bath during 4 hours gave an *acetyl* derivative. At first this compound separated from warm methyl alcohol as a gel which gradually crystallised, but ultimately it readily formed colourless slender needles, m. p. 175—176° (Found : C, 55·8; H, 5·3. The octa-acetate, $C_{44}H_{50}O_{23}$, requires C, 55·8; H, 5·3. The nona-acetate, $C_{46}H_{52}O_{24}$, requires C, 55·9; H, 5·3%). It is sparingly soluble in alcohol and easily soluble in ethyl acetate.

Methylation of Hesperidin.—(A) Methyl sulphate (35 c.c.) was gradually added to a solution of the glucoside (10 g.) in 5% aqueous sodium hydroxide (40 c.c.); the temperature was maintained at 40—45° and the solution was kept alkaline by the addition of 20% aqueous sodium hydroxide. When the greater part of the methyl

sulphate had been added, the product separated as a yellow viscous mass; this was dissolved in alcohol, and the solution treated with charcoal, filtered, and evaporated to dryness. The amorphous residual solid was more soluble in cold than in warm water and insoluble in sodium hydroxide. $[\alpha]_D^{18°}$ — 84·5° in 50% acetone (c = 0·5914 g. in 50 c.c.) (Found: OMe, 32·4%). Acetylation of this substance gave rise to an amorphous product (Found: OMe, 23·0%).

The methylated glucoside (6·5 g.) was hydrolysed by boiling with 50% aqueous methyl alcohol (75 c.c.) containing 5% of sulphuric acid for 2 hours. The cooled mixture was diluted with water and next day the reddish gum (3 g.) which separated was collected and dissolved in 3% aqueous sodium hydroxide. After filtration the solution was acidified with acetic acid and the semi-solid precipitate was collected and repeatedly crystallised from methyl alcohol. *4-Hydroxy-2 : 6-dimethoxyphenyl 3 : 4-dimethoxystyryl ketone* (IV) was finally obtained in lemon-yellow, prismatic needles, m. p. 194° [Found: C, 66·2; H, 5·6; OMe, 35·4. $C_{15}H_8O_2(OMe)_4$ requires C, 66·3; H, 5·8; OMe, 36·0%]. This substance dissolves in concentrated hydrochloric or sulphuric acid, forming a bright red solution which becomes colourless on extreme dilution with water. In dilute aqueous sodium hydroxide, it forms an orange to orange-yellow solution, and it does not give a ferric chloride reaction. Acetylation with acetic anhydride and sodium acetate on the steam-bath during 3 hours afforded the *acetate*, which crystallised from alcohol in almost colourless needles, m. p. 175° (Found: C, 65·0; H, 5·7. $C_{21}H_{22}O_7$ requires C, 65·3; H, 5·7%).

(B) A suspension of hesperidin (5 g.) and well-ground potassium carbonate (5 g.) in a mixture of acetone (100 c.c.) and methyl iodide (10 c.c.) was heated under reflux on the steam-bath for 65 hours; after 25 hours, methyl alcohol (50 c.c.) and further quantities of iodide (10 c.c.) and carbonate (5 g.) were added. The solution was filtered from inorganic salts and evaporated in a vacuum. The resulting pale yellow syrup was readily soluble in warm water but insoluble in cold sodium hydroxide solution. It was hydrolysed by refluxing with 50% aqueous ethyl alcohol (50 c.c.) containing 5% of hydrogen chloride on the water-bath for 1·5 hours and the styryl ketone (IV) was isolated and purified as described in method (A), forming lemon-yellow prismatic needles, m. p. and mixed m. p. 194° (Found: C, 66·4; H, 5·7%).

Synthesis of 4-Hydroxy-2 : 6-dimethoxyphenyl 3 : 4-Dimethoxystyryl Ketone (IV).—4-Hydroxy-2 : 6-dimethoxyacetophenone (Canter, Curd, and Robertson, *loc. cit.*) (4 g.) and veratraldehyde (3·5 g.) were added to a solution of sodium ethoxide (3 g.) in alcohol (40 c.c.), and the mixture refluxed on the steam-bath for 1 hour.

After cooling, the orange-red solution was acidified with acetic acid, and the ketone precipitated by the addition of water. Crystallised from methyl alcohol, it formed pale yellow, prismatic needles, m. p. 194° alone or mixed with a specimen prepared from hesperidin (Found: C, 66·4; H, 5·8%). The acetate had m. p. and mixed m. p. 175° (Found: C, 65·2; H, 5·7%).

4-*Hydroxy*-2 : 6 : 3′ : 4′-*tetramethoxy*-β-*phenylpropiophenone* (V).—The foregoing styryl ketone (1 g.) was dissolved in alcohol (150 c.c.) containing palladium (0·1 g.) in suspension and shaken in an atmosphere of hydrogen: absorption was almost theoretical and complete in 15—20 minutes. After the greater part of the solvent had been evaporated, the residual solution was filtered and the *ketone* precipitated with water, forming a mass of colourless needles, m. p. 104°. After repeated crystallisation from dilute alcohol it had m. p. 109° (Found: C, 65·9; H, 6·8. $C_{19}H_{22}O_6$ requires C, 65·9; H, 6·4%). This *compound* is readily soluble in ether and alcohol and does not give a ferric chloride reaction.

2-*Hydroxy*-4 : 6 : 3′ : 4′-*tetramethoxy*-β-*phenylpropiophenone* (VI).—2-Hydroxy-4 : 6-dimethoxyphenyl 3 : 4-dimethoxystyryl ketone was obtained by condensing 2-hydroxy-4 : 6-dimethoxyacetophenone (Canter, Curd, and Robertson, *loc. cit.*) (4 g.) and veratraldehyde (3·5 g.) by means of sodium ethoxide (3 g.) in boiling alcohol. It crystallised from alcohol in orange-yellow plates (6·2 g.), m. p. 157° (Found: C, 65·4; H, 5·7%) (compare Kostanecki and Tambor, *Ber.*, 1904, **37**, 793). The acetate had m. p. 118°.

The styryl ketone (1 g.) was quantitatively reduced in ethyl acetate (100 c.c.) by means of palladium (0·1 g.) and hydrogen; absorption was complete in 10—15 minutes. The product (VI) crystallised from alcohol in colourless hexagonal plates, m. p. 127° (Found: C, 66·1; H, 6·7%) (compare Freudenberg and Cohn, *Ber.*, 1923, **56**, 2127).

Condensation of β-3 : 4-*Dimethoxyphenylpropionitrile* and *Phloroglucinol Dimethyl Ether*.—β-3 : 4-Dimethoxyphenylpropionamide was prepared by the method of Haworth and Perkin (J., 1926, 1775), but the yield (88% of the theoretical) was improved by extracting the compound from the aqueous solution with chloroform. Dehydration of the amide (22 g.) in boiling chloroform (150 c.c.) by means of phosphorus pentachloride (22 g.) during 15 minutes afforded the nitrile (10 g.) as a colourless thick oil, b. p. 220°/20 mm. Attempts to dehydrate the amide with thionyl chloride were entirely unsuccessful.

A solution of the nitrile (10 g.) and phloroglucinol dimethyl ether (7 g.) in dry ether (75 c.c.) was saturated with hydrogen chloride in the presence of zinc chloride (3 g.). The semi-solid mixture of

ketimine hydrochlorides gradually separated and after 2 days a further quantity was precipitated by the addition of ether (150 c.c.). A suspension of the product in water (100 c.c.) was heated on the steam-bath for 20 minutes, and after cooling, the aqueous layer was decanted. A solution of the residual solid in warm alcohol on cooling deposited 2-hydroxy-4 : 6 : 3′ : 4′-tetramethoxy-β-phenylpropiophenone (5·4 g.), m. p. and mixed m. p. 127° after purification (Found : C, 65·8; H, 6·5%). With alcoholic ferric chloride this ketone gives a brownish-red coloration which changes to brown on dilution with water.

The alcoholic mother-liquor from the foregoing crude ketone was evaporated to small bulk and on addition of ethyl acetate (200 c.c.) the isomeric ketimine (1·5 g.) separated. It was hydrolysed by boiling for 20 minutes with water (50 c.c.) and on cooling 4-hydroxy-2 : 6 : 3′ : 4′-tetramethoxy-β-phenylpropiophenone solidified, m. p. and mixed m. p. 109° after crystallisation from dilute alcohol (Found : C, 66·3; H, 6·5%).

We are indebted to the Chemical Society for grants which have in part defrayed the cost of this investigation.

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE,
UNIVERSITY OF LONDON. EAST LONDON COLLEGE.

[*Received, May* 21*st*, 1931.]