form), X-ray powder diffraction data[14]: 7.97[15] −20[16], 7.10–5, 6.55–5, 5.63–10, 5.50–10, 4.65–10, 4.29–100, 3.98–40, 3.75–20, 3.66–10, 3.41–10, 3.20–10, 3.01–10, 2.84–5, 2.70–10, 2.37–5, 2.16–10, 2.01–5. The substance is therefore identified as maltitol nonaacetate.[5]

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(14) Reported in ref. 5 but herein further developed with more lines.
(15) Interplanar spacing, Å., CuK_α radiation.
(16) Relative intensity as percentage strongest line; estimated visually.

[CONTRIBUTION FROM THE BOTANICAL INSTITUTE, FACULTY OF SCIENCE, UNIVERSITY OF TOKYO]

Studies on Flavanone Glycosides. IV. The Glycosides of Ripe Fruit Peel and Flower Petals of *Citrus Aurantium* L.

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From the ripe fruit peel of Japanese bitter orange (*Citrus aurantium* f. *Kabusu* and *C. aurantium* var. *cyathifera*), two flavonoid glycosides have been isolated, namely naringin and rhoifolin. Details of the isolation and identification of these two glycosides are reported. The finding of these two flavonoid glycosides in the ripe fruit peel of the Japanese bitter orange is contradictory to previous reports of other workers that hesperidin is the only flavanone glycoside present in the ripe fruit of the *Citrus aurantium* cultivated in Europe. In the flower petals, however, of *C. aurantium* f. *Kabusu*, hesperidin has been found to be the only flavonoid glycoside present.

Introduction

Kolle and Gloppe[1] have reported that the ripe fruit peel of *Citrus aurantium* L., cultivated in Europe, contains one flavanone glycoside, hesperidin. The unripe fruit peel, however, contains not only the hesperidin, but also its isomer neohesperidin. Zemplén and Tettamanti[2] tentatively gave neohesperidose, the disaccharide of neohesperidin, a structure of 1-L-rhamnoside-4-D-glucose.

In order to see if the peel of Japanese bitter oranges contain either hesperidin or neohesperidin, or both, the ripe fruits of *C. aurantium* f. *Kabusu* and var. *cyathifera* have been investigated. Contrary to expectation, neither a trace of hesperidin nor of neohesperidin could be found. Rather, two different flavonoid glycosides, naringin and rhoifolin have been isolated and identified. Rhoifolin is the 7-rhamnoglucoside of apigenin and has been previously isolated by Hattori and Matsuda[3] from the leaves of *Rhus succedenea*.

Naringin has also been previously isolated from the fruit peels of an edible citrus, *Citrus grandis* Osbeck.[4] Asahina and Inubuse[5] also found naringin in the flower petals of this citrus. From the flower petals of *C. Aurantium* f. *Kabusu*, however, not naringin, but rather hesperidin has been the only flavanone glycoside obtained.

Experimental

Isolation of the Glycosides from the Ripe Fruit Peel.—Two kg. of finely divided fresh peels of *C. aurantium* f. *Kabusu*, obtained from 25 ripe fruit in February, 1951, was extracted five times with hot ethanol (a total of 11 l.) for one hour. After concentration *in vacuo* of the combined extracts to about 400 ml., 2 l. of ethanol was added and the precipitate then filtered off. The ethanolic filtrate was again evaporated, this time to 250 ml., water was added, and the mixture was further evaporated to about 200 ml. The resulting aqueous solution was treated with ether, and then saturated with chloroform by shaking. After 2–3 days, the colorless needles which had precipitated were filtered, and washed with a small quantity of water and then with ether; yield 10 g. Approximately the same yield was obtained from 2 kg. of the peels of *C. aurantium* var. *cyathifera*.

When 8 g. of the crude crystals was extracted with 20 parts of boiling ethyl acetate, approximately 4 g. dissolved. The residue was extracted again with 50 ml. of the solvent, and the mixture filtered. The filtrate was evaporated, and this new residue was redissolved in ethyl acetate and filtered. On evaporation to 40 ml., crystallization occurred. After repeated recrystallizations from water, colorless crystals of this glycoside I (naringin) were obtained; yield about 3 g.

The residue (of the original 8 g. of crude crystals) after the ethyl acetate extractions, was recrystallized from 20 ml. of 50% ethanol (or methanol). Almost colorless, minute, crystalline needles of the glycoside II (rhoifolin) were obtained; yield about 2.5 g.

Identification of the Glycosides of the Ripe Fruit Peel.—The crystals of glycoside I melted first at 80–83°, then solidified, and melted finally at 193–194°. A mixture of this glycoside with an authentic sample of naringin showed no depression of melting point.

Anal. Calcd. for $C_{27}H_{32}O_{24}\cdot 6H_2O$: C, 47.08; H, 6.44; water of crystallization, 15.7. Found: C, 47.08; H, 6.12; water of crystallization, 15.9.

Hydrolysis of the glycoside I yielded naringenin and the sugars glucose and rhamnose. The latter were identified by their osazones, and part of the aglycon was converted to its diacetate, m.p. 140–143°.[6] Mixed melting point determination of authentic naringenin with the aglycon, m.p. 245–247°; and of authentic naringenin diacetate with the diacetate of the aglycon showed no change. Glycoside I is, therefore, naringin.

Rhoifolin.—The crystals of glycoside II melted at 250–265° after sintering at 200–205°. When mixed with rhoifolin isolated from the leaves of *Rhus succedanea*, this substance showed no depression of melting point.

Anal. Calcd. for $C_{27}H_{30}O_{14}\cdot 6H_2O$: C, 47.08; H, 6.44; water of crystallization, 16.3. Found: C, 47.16; H, 6.12; water of crystallization, 15.7.

Hydrolysis of glycoside II produced rhamnose and glucose, which were identified as the osazones, and an aglycon with a melting point above 340°. The aglycon gave a diacetate, m.p. 192–193°,[7] and a triacetate, m.p. 180–182°. The melting points of the diacetate and the triacetate were not altered when mixed with authentic apigenin di- and triacetate (m.p. 192–193° and 180–182°). Glycoside II, therefore, was apigenin-7-rhamnoglucoside, which is rhoifolin.

For the determination of the position of the sugar residue in rhoifolin (glycoside II), 0.4 g. of the glycoside was put into a flask with 4 ml. of dimethyl sulfate. To this mixture was then added, drop by drop, a solution of 4 g. of sodium

(1) F. Kolle and K. E. Gloppe, *Pharm. Zentralhalle*, **77**, 421 (1936).
(2) G. Zemplén and A. K. Tettamanti, *Ber.*, **71**, 2511 (1939).
(3) S. Hattori and H. Matsuda, *Arch. Biochem. Biophys.*, in press.
(4) S. Hattori, M. Hasegawa and M. Kanao, *Acta Phytochimica*, **15**, 199 (1949).
(5) Y. Asahina and M. Inubuse, *J. Pharm. Soc. Japan*, **48**, 868 (1938).
(6) M. Shimokoriyama, *J. Chem. Soc. Japan*, **70**, 234 (1949).
(7) M. Shimokoriyama, *Bull. Chem. Soc. Japan*, **16**, 284 (1941).

hydroxide in 6 ml. of water. The methylated derivative was extracted with ethyl acetate and these extracts were distilled off *in vacuo*. The residue was immediately dissolved in 10 ml. of 50% ethanol, mixed with 10 ml. of 20% hydrochloric acid, and heated on a water-bath for hydrolysis. Pale yellow crystals gradually separated. After several recrystallizations, the yellow needles melted at 266–270°, and showed the properties of apigenin 5,4′-dimethyl ether. The sugar residue, therefore, was attached to position 7 of apigenin.

Isolation of Hesperidin from the Flower Petals.—Flower petals of Japanese bitter oranges, which had fallen upon the ground after pollination, were collected (June 3, 1950) at Ninomiya, Kanagawa Prefecture of the Faculty of Agriculture of the University of Tokyo. Two hundred and fifty grams of petals was twice extracted with 500-ml. portions of boiling ethanol. After the removal of the ethanol by vacuum distillation, water was added to the light-brown colored solution, and, on standing, a crystalline precipitate resulted. After 2–3 days, the precipitate was filtered, washed with acetone to remove resinous brown material, and repeatedly recrystallized from pyridine–water. The resulting colorless needles had a melting point of 250–251°. No change in melting point resulted on mixture with an authentic sample of hesperidin.

From the original mother liquor of hesperidin, no other flavonoid aglycone or glycoside was obtained.

Anal. Calcd. for $C_{28}H_{34}O_{15}$: C, 55.08; H, 5.57. Found: C, 54.75; H, 5.87.

Discussion

The results obtained with the fruit peel are contradictory to the generally accepted idea that hesperidin is contained in the fruit peels of the bitter orange. They are in sharp contrast to the report of Kolle and Gloppe who observed the presence of both neohesperidin and hesperidin in immature orange fruit peels. Whether or not neohesperidin plus hesperidin are really present in immature fruits and naringin and rhoifolin appear in their place in mature fruits, still needs experimental investigation.

In regard to the biogenetic relation between naringin and rhoifolin, there appears to be but one difference. Naringenin, the aglycon of naringin is 5,7,4′-trihydroxyflavanone; apigenin, the aglycon of rhoifolin is 5,7,4′-trihydroxyflavone. The sugar residue and its position are the same in both. There may be some possibility that in plant cells, rhoifolin may be derived from naringin by dehydrogenation; or naringin from rhoifolin by hydrogenation. Experiments are in progress to determine what glycosides are present in the fruit peel in the younger and immature stages.

Bitter orange, botanically designated as *Citrus aurantium* is now believed to be native to North India. It was transplanted from there, in ancient times, into Europe and the Far East. At present, taxonomists ordinarily consider that the European bitter orange (bitter Seville) and the Asian one represent, respectively, a variety or a horticultural form. This conclusion apparently was drawn from the morphological viewpoint, and we can find sufficient reason for it in comparison with our results. In view of the fact that the two bitter orange varieties common to Japan contain the same flavonoid glycosides in the fruit peel, they probably belong to the same phylogeny.

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Preparation of Hydantoins Containing a Cycloalkyl Substituent[1]

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The initial synthesis is reported of hydantoins having a 5-cyclopropyl, 5-cyclobutyl or 5-cyclopentyl substituent.

Despite the rather large number of 5,5-disubstituted hydantoins recorded in the literature, no examples are known of hydantoin derivatives containing a 5-cycloalkyl substituent other than cyclohexyl. The present paper reports the synthesis of six members of the series having a cyclic grouping possessing three, four or five carbon atoms.

From cyclopropanecarbonitrile, by means of the Grignard reaction, have been prepared cyclopropyl phenyl ketone and cyclopropyl isoamyl ketone. In turn, these ketones were converted into the corresponding 5-cyclopropyl-5-phenyl- and 5-isoamylhydantoins. Similarly, cyclobutyl phenyl ketone yielded 5-cyclobutyl-5-phenylhydantoin which, through catalytic hydrogenation, was converted into 5-cyclobutyl-5-cyclohexylhydantoin. Finally, methyl 2-methylcyclopentyl ketone and methyl 2,3-dimethylcyclopentyl ketone, respectively, were converted into 5,5-disubstituted hydantoins. In the first instance, higher and lower melting (in all probability the *cis* and *trans*) forms of 5-methyl-5-(2-methylcyclopentyl)-hydantoin were obtained. In the other, purification or separation of the isomers was much more difficult; although, again, a higher melting and a lower melting fraction were obtained, these do not necessarily represent pure diastereoisomers.

Experimental

Preparation of Cyclopropyl Phenyl Ketone.—Trimethylene bromide was converted into γ-bromobutyronitrile[3,4] in 56% yield, b.p. 75° (5 mm.); 208° (754 mm.); $n^{25}D$

(1) From a portion of the Ph.D. dissertation of Cecil Winston Gayler, University of Texas, June, 1943.

(2) Parke, Davis and Company Research Fellow, 1941–1943.

(3) C. Derrick and R. Hess [THIS JOURNAL, **40**, 537 (1918)] reported b.p. 100–102° (20 mm.).

(4) S. Gabriel [*Ber.*, **22**, 3336 (1889)] reported b.p. 205° (atm. press.).