The same two products were formed when the hydrolysis was carried out in aqueous sodium hydroxide.

2. **Reaction of XVI with Base.**—A solution of 0.05 g. of XVI in 3 ml. of sodium hydroxide (0.22 *N*) was refluxed until completion of the reaction. Sodium ions were removed as described above, and the product subjected to ionophoretic separation in borate buffer. After four hours at 600 volts and 17–20 milliamp. only one spot was observed under ultraviolet light, anodic migration, −1.5 cm.

When XVI was refluxed in 3 ml. of ethanol and 2 ml. of sodium hydroxide (1.1 *N*) for 30 minutes, and the product evaluated as above (700 volts, 13 milliamp. for four hours) a single spot with anodic migration, −1.5 cm., was observed.

3. **Reaction of VIII with Base.**—A sample of 0.01 g. of VIII was refluxed with ethanolic aqueous sodium hydroxide as described under experiment 1 above, and an aqueous solution of the product, free of sodium ions, was subjected to separation by paper electrophoresis, 700 volts, 14–16 milliamp. for 180 minutes, as described above. Only one spot, anodic migration −0.7 cm. (1-β-D-arabinosyluracil, −0.7 cm.), was observed.

4. **Reaction of VI with Acid (HCl–DMF–Water), Followed by Reaction with Base.**—A sample of 0.025 g. of VI in 5 ml. of DMF and 1.0 ml. of hydrochloric acid (2 *N*) was heated 15 minutes at 80–90°. The solution was taken to dryness *in vacuo* and triturated with water. The ultraviolet absorption spectrum (230/260, 2.83) of the residue indicated complete cleavage of the anhydro bond. The residue was treated with ethanol–water–sodium hydroxide as described above. Ionophoretic separation of the product after removal of sodium ions (700 volts, 17–19 milliamp. for 240 minutes) gave the following anodic migrations: −2.8 cm. (1-β-D-arabinosyluracil, −2.6 cm.), + 5.9 cm. (1-β-D-xylosyluracil, + 6.0 cm.), + 9.8 cm. (1-β-D-ribosyluracil, + 10.0 cm.). No spot was found at the migration spot of 1-β-D-lyxosyluracil, + 14.3 cm.

A similar experiment carried out upon the residue after refluxing VI with sodium benzoate in DMF for 4.5 hours demonstrated the presence of the same three isomers; 1-β-D-lyxofuranosyluracil was again absent.

5. **Reaction of XII with Base.**—A sample of the amorphous residue obtained after the removal of VI and VII from the reaction mixture of V and sodium benzoate in refluxing DMF (representing about 35% by weight of the starting material V) was subjected to alkaline hydrolysis in ethanol–water–NaOH as described under (1) above. The solution was freed of sodium ions and subjected to ionophoretic separation in borate buffer, *p*H 6–6.05, as described previously. Spots having the following anodic migrations after 192 minutes at 700 volts and 18–21 milliamp. were observed: −2.3 cm. (1-β-D-arabinosyluracil, −2.2 cm.), + 4.7 cm. (1-β-D-xylosyluracil, + 4.6 cm.), + 8.2 cm. (1-β-D-ribosyluracil, + 8.2 cm)., + 12.1 cm. (1-β-D-lyxosyluracil, + 12.2 cm.).

The spots were cut from the paper and eluted with water. The relative intensities of the resulting solutions, measured at 262 mμ were: arabinosyl 1.0, xylosyl 1.2, ribosyl 0.9 and lyxosyl 1.1.

The results of a similar experiment carried out under only slightly different conditions (900 volts, 10–13 milliamp., 285 minutes) are shown in Fig. 6.

6. **Hydrolysis of the "5′-Deoxy" Residue.**—A sample of 0.20 g. of the residue remaining after isolation of XVI from the reaction of XV with sodium benzoate in refluxing DMF was refluxed with two drops of hydrochloric acid (2 *N*) in 10 ml. of water until cleavage of the anhydro bond was complete, as determined spectrophotometrically. To the mixture was added 1 ml. of sodium hydroxide (1.13 *N*) until removal of the benzoyl groups was complete.

Paper electrophoresis of the product (600 volts, 17–20 milliamp., 4 hours) gave the following spots: −1.4 cm., + 9.1 cm. (1-(5′-deoxy-β-D-ribofuranosyl)-uracil, + 9.5 cm.), + 12.1 cm. (1-(5′-deoxy-β-D-lyxofuranosyl)-uracil, + 12.1 cm.).

Polarimetric Determinations.—Techniques and equipment previously described[2] were used for all optical rotations. Calculations of the rotation of the dialdehydes of 1-β-D-xylofuranosyluracil and 1-β-D-arabinofuranosyluracil was based upon the concentrations of the nucleosides. Readings were taken until constancy was reached.

Ultraviolet Spectrophotometric Data.—The curves shown in Fig. 3 were made using the Cary recording spectrophotometer, model 11. All molar extinction coefficients were calculated from optical density values obtained using the Beckman model DU spectrophotometer.

[CONTRIBUTION FROM THE FRUIT AND VEGETABLE CHEMISTRY LABORATORY, WESTERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION, AGRICUTURAL RESEARCH SERVICE, U. S. DEPARTMENT OF AGRICULTURE]

Flavonoid Compounds of Citrus. III. Isolation and Structure of Eriodictyol Glycoside

BY ROBERT M. HOROWITZ AND BRUNO GENTILI

RECEIVED OCTOBER 8, 1959

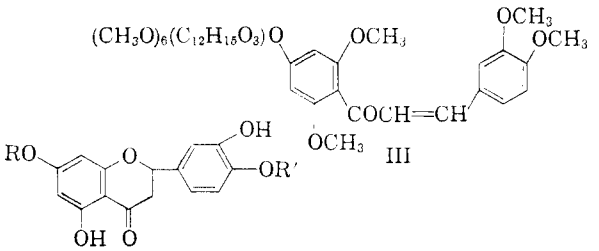
Lemon peel contains an appreciable quantity of a glycoside of eriodictyol which can be isolated by chromatography on silicic acid. The structure of this glycoside ("eriocitrin") is shown to be eriodictyol 7-β-rutinoside. A convenient procedure is described for preparing eriodictyol by hydrolyzing the crude mixed glycosides with hemicellulase.

Some years ago Bruckner and Szent-Györgyi[1,2] discussed the composition of "citrin," a mixture of flavonoid glycosides which had been isolated earlier from lemon peel.[3] Citrin was said to contain the flavanones hesperidin and "eriodictin" (considered to be a glycoside of eriodictyol), although no supporting experimental evidence was given. Hesperidin had long been recognized as a major constituent of lemons and oranges, but neither eriodictyol nor its glycosides had been previously reported to occur in citrus. Since citrin, or, at least, certain of its components, was thought to have an effect on capillary permeability, and was even regarded for a time as a vitamin concerned with the proper functioning of the capillaries,[3] it was of importance to establish whether eriodictyol was actually present. Several years after the appearance of the papers cited above, Mager[4] described the isolation from citrin of crystalline eriodictyol rhamnoside and its hydrolysis to eriodictyol and rhamnose, while later Higby[5] also mentioned the isolation of eriodictyol from a non-crystalline glycoside obtained from lemon peel. In both instances, however, the experimental data were fragmentary and the occurrence of this flavonoid has never been regarded as firmly established. Recently, there has been renewed interest in the question since the discovery by Masri and DeEds[6] that eriodictyol and related compounds having *o*-dihydroxyl groups

(1) V. Bruckner and A. Szent-Györgyi, *Nature*, **138**, 1057 (1936).
(2) A. Szent-Györgyi, *Z. physiol. Chem.*, **255**, 126 (1938).
(3) S. Rusznyák and A. Szent-Györgyi, *Nature*, **138**, 27 (1936).
(4) A. Mager, *Z. physiol. Chem.*, **274**, 109 (1942).
(5) R. H. Higby, *J. Am. Pharm. Assoc., Sci. Ed.*, **32**, 74 (1943).
(6) M. S. Masri and F. DeEds, *Proc. Soc. Exp. Biol. Med.*, **99**, 707 (1958).

cause involution of the thymus gland in young rats. On re-examining the problem we find that lemon peel does contain a substantial quantity of eriodictyol glycoside; that the structure of this glycoside is eriodictyol 7-β-rutinoside; and that eriodictyol itself, although it occurs only in the glycosidic form, may be prepared in good yield from the enzymatic hydrolysate of the crude mixed glycosides of lemon peel.

Of the ten to fifteen flavonoid glycosides occurring in lemons, only the very insoluble hesperidin and diosmin[7] have been isolated without applying elaborate purification procedures. Identification and metabolic studies of the remaining flavonoids may be simplified considerably by hydrolyzing various crude extracts of the glycosides and isolating the aglycones. In our experience, this hydrolysis must be carried out enzymatically, for the use of acid on these extracts leads invariably to tarry materials from which no crystalline aglycones can be obtained. Fungal hemicellulase at *p*H 4.5 is an effective enzyme for this purpose and is capable of acting on all the glycosides in lemons, while β-glucosidase appears to be almost totally inactive. After completion of the hydrolysis the aglycones may be separated into an ether-soluble and an ether-insoluble fraction. The latter fraction consists largely of racemic eriodictyol (5,7,3′,-4′-tetrahydroxyflavanone) (I), which crystallizes readily from dilute alcohol.[8] Its identity was proved unequivocally by comparison with a sample



III

I, R = R′ = H (eriodictyol)
II, R = H; R′ = CH_3 (hesperetin)
IV, R = 6-*O*-β-L-rhamnosyl-D-glucosyl; R′ = CH_3 (hesperidin)
V, R = 6-*O*-β-L-rhamnosyl-D-glucosyl; R′ = H (eriocitrin)

of authentic eriodictyol made by demethylating hesperetin (5,7,3′ - trihydroxy - 4′ - methoxyflavanone) (II). On treatment with N-bromosuccinimide, eriodictyol was dehydrogenated in good yield to the expected luteolin (5,7,3′,4′ - tetrahydroxyflavone). A number of other derivatives were prepared, including 7,3′,4′-triacetyl- and 5,7,-3′,4′-tetraacetyleriodictyol, the oxime, the 7-methyl ether and triacetyleriodictyol 7-methyl ether.

The glycoside of eriodictyol was isolated by chromatographing unhydrolyzed extracts of lemon peel on silicic acid using chloroform containing increasing amounts of methanol as eluent. Silicic acid has seldom been used for the chromatography of flavonoid glycosides and aglycones although, with the proper choice of solvent, it is capable of giving very satisfactory separations, and it has the advantage of being available in a pure, standardized form. The glycoside was obtained from the column as a gum, which changed to a pale yellow, water-soluble solid after several precipitations from acetone–ethyl acetate. Though it failed to crystallize from solvents, on paper chromatograms it appeared to be essentially homogeneous, with mere traces of impurities visible. The acetyl derivative crystallized at once from ethanol, m.p. 135–136.5°. We propose to call this glycoside *eriocitrin*, to avoid confusing it with eriodictyol rhamnoside,[4] which, in various compilations, has been referred to as "eriodictin."

Hydrolysis of eriocitrin with hemicellulase yielded eriodictyol, rhamnose and glucose. That the glycoside was composed of one mole each of these constituents was shown by analytical data for the free glycoside and its acetyl derivative. Eriocitrin obviously contained free 3′,4′-*o*-dihydroxyl groups, since it reduced Tollens reagent immediately and gave deeply colored oxidation products in the presence of air and alkali. Its absorption maximum at 285 mμ shifted to 306 mμ when aluminum chloride was added, while no shift occurred when sodium acetate was added. Based on unpublished spectral studies of a large number of flavanones, these results mean that the compound has a free 5- and a covered 7-hydroxyl group, respectively. Thus, eriocitrin must have its sugars attached to the 7-hydroxyl group in the form of a disaccharide. By analogy with the other recognized flavanone glycosides of citrus, we should expect this to be either of the two known rhamnoglucoses, rutinose (6-*O*-β-L-rhamnosyl-D-glucose) or neohesperidose, the structure of which will be discussed in a later article.

In order to identify the disaccharide, eriocitrin was methylated in two stages, first with methyl iodide and potassium carbonate in acetone to cover the sensitive *o*-hydroxyl groups, then with methyl iodide and silver oxide in dimethylformamide to methylate the sugar hydroxyl groups. During the methylation the flavanone was converted to the corresponding chalcone. The resulting eriocitrin chalcone decamethyl ether crystallized readily from methanol (m.p. 176–178°) and proved to be identical with hesperidin chalcone nonamethyl ether (III), the crystalline derivative obtained by exhaustive methylation of hesperidin.[10] Since hesperidin is the 7-β-rutinoside of 5,7,3′-trihydroxy-4′-methoxyflavanone[11] (IV), it follows that eriocitrin also contains rutinose and that its structure must be eriodictyol 7-β-rutinoside (V).

Next to hesperidin, eriocitrin is probably the most abundant flavonoid in lemons. We have not been able to confirm the occurrence of the eriodictyol rhamnoside described by Mager,[4] nor have we obtained evidence for the presence of other possible glycosides of eriodictyol, such as the neohesperidose derivative. Certainly our experiments do not preclude the possibility of their occurrence at certain stages of growth or in different varieties

(7) R. M. Horowitz, *J. Org. Chem.*, **21**, 1184 (1956).

(8) The ether-soluble fraction contained a number of aglycones and, on concentration and standing, yielded a crystalline flavonol, limocitrin, m.p. 274–275°. This was shown earlier to be 3′,8-dimethoxy-3,5,7,4′-tetrahydroxyflavone.[9]

(9) R. M. Horowitz, THIS JOURNAL, **79**, 6561 (1957).

(10) G. Zemplén and A. K. Tettamanti, *Ber.*, **71B**, 2511 (1938).

(11) G. Zemplén and R. Bognar, *ibid.*, **76B**, 773 (1943).

of lemons. It is of interest that an extract of Valencia orange peel appeared to contain no eriodictyol or eriodictyol glycoside, as indicated by paper chromatography.

Experimental

Isolation of Eriodictyol. A.—"Calcium Flavonate Glycoside, Lemon"[12] (100 g.), in 0.1 *M* acetate buffer (1800 ml., *p*H 4.6) was warmed to about 50° to effect partial solution. The warm mixture was filtered through Celite to yield a dark reddish-brown filtrate, the *p*H of which was readjusted to 4.6 with acetic acid. Hemicellulase (3 g., Nutritional Biochemicals Corp.), suspended in a small volume of acetate buffer, was added with shaking and the mixture kept for 2 days at room temperature. The hydrolysis then appeared essentially complete, as indicated by Bryant's test.[13] A further addition (0.5 g.) of hemicellulase was made and the mixture allowed to stand one more day. It was then extracted with 4 × 250 ml. of ethyl acetate, the ethyl acetate filtered, dried ($MgSO_4$) and evaporated to dryness. The residue (6 g.) was a yellow-brown semi-crystalline solid which, on a paper chromatogram prepared with 50% acetic acid, was resolved into five major spots. Three of these reduced Tollens reagent, one of the reducing substances being a flavanone made visible by spraying with sodium borohydride.[14] The solid was broken up and boiled under reflux for 1 hour with ether (50 ml.). Filtration gave a yellow ethereal solution (containing limocitrin[9] and other flavonoids), and an undissolved crystalline solid (3.4 g.). This was recrystallized from methanol–water as lens-shaped, buff-colored crystals (2.5 g.), m.p. 267–268°, raised to 270–271° (colorless) by sublimation or further recrystallization; reported[15] m.p. 267°; λ_{max}^{EtOH} 289 mμ. The mixed m.p. with authentic eriodictyol prepared by demethylating hesperetin (see below) was not depressed and both specimens had the same infrared spectrum.

Anal. Calcd. for $C_{15}H_{12}O_6$: C, 62.5; H, 4.20. Found: C, 62.3; H, 4.38.

B.—A methanolic extract of dried lemon peel[16] was allowed to stand in the refrigerator for several weeks until most of the hesperidin had crystallized. The concentrated extract (100 g.) was suspended in 0.1 *M* acetate buffer (1800 ml.), filtered and treated with hemicellulase (5 g.). After 3 days at room temperature the mixture was worked up as in part A to give the characteristic lens-shaped crystals of eriodictyol, m.p. 269–270°. This sample of eriodictyol, which had not been exposed to strong acid or alkali at any stage in its preparation, was optically inactive, as was the sample of part A.

5,7,3′,4′-Tetra-O-acetyleriodictyol.—Eriodictyol (0.5 g.) dissolved in a mixture of acetic anhydride (5 ml.) and pyridine (5 ml.) was kept at room temperature for 2 days, then was warmed on the steam-bath for 1 hour and diluted with water. The product crystallized from ethyl acetate–ether as colorless needles, m.p. 141–142°, reported[15] m.p. 137°; λ_{max}^{EtOH} 259, 314 mμ.

Anal. Calcd. for $C_{23}H_{20}O_{10}$: C, 60.6; H, 4.42; CH_3CO, 37.7. Found: C, 60.5; H, 4.42; CH_3CO, 37.7.

7,3′,4′-Tri-O-acetyleriodictyol.—Following the general procedure of Shimokoriyama,[17] a mixture of eriodictyol (0.5 g.), acetic anhydride (3 ml.) and pyridine (4 drops) was shaken in a cold water-bath for 15 minutes until the solution became clear. Dilution with water gave a solid, m.p. 116–117° from methanol. Further recrystallization from methanol, benzene–ligroin and ethyl acetate–ether produced a different form, m.p. 146–147°, λ_{max}^{EtOH} 274, 338 mμ. The compound gave a positive ferric test, but was unaffected by ethereal diazomethane.

Anal. Calcd. for $C_{21}H_{18}O_9$: C, 60.9; H, 4.38; CH_3CO, 31.2. Found: C, 61.3; H, 4.34; CH_3CO, 32.3.

Eriodictyol Oxime.—Eriodictyol (150 mg.) dissolved in a mixture of ethanol (6 ml.), water (2 ml.), hydroxylamine hydrochloride (0.3 g.) and sodium acetate (0.3 g.), was heated 5 hours on the steam-bath, when it no longer gave a positive test for flavanones with sodium borohydride. The solution was partially evaporated, diluted with water and set aside to crystallize. The product was recrystallized from ether–hexane as pale yellow rods (95 mg.), m.p. 199–200°, λ_{max}^{EtOH} 281 mμ, $\lambda_{max}^{EtOH\text{-}HCl}$ 314 mμ. It has been reported[18] that eriodictyol does not form an oxime.

Anal. Calcd. for $C_{15}H_{13}NO_6$: C, 59.5; H, 4.33; N, 4.63. Found: C, 59.9; H, 4.98; N, 4.21.

7-O-Methyl-5,3′,4′-tri-O-acetyleriodictyol.—Eriodictyol (0.4675 g.) in dry acetone (250 ml.) was treated with methyl sulfate (0.16 ml., 1 mole) and potassium carbonate (2 g.) and the mixture boiled under reflux for 1 hour. The gummy residue obtained after filtration and evaporation was acetylated in hot acetic anhydride–pyridine to give colorless needles, m.p. 152–153° from ethyl acetate–ether; λ_{max}^{EtOH} 273, ~ 308 mμ.

Anal. Calcd. for $C_{22}H_{20}O_9$: C, 61.7; H, 4.71; CH_3O, 7.23. Found: C, 61.9; H, 4.72; CH_3O, 7.69.

7-O-Methyleriodictyol.—The previous compound was warmed in a small volume of ethanol containing concentrated hydrochloric acid. On cooling, a solid was obtained which crystallized from dilute ethanol as colorless needles, m.p. 213–215°, reported[19] m.p. 215°, λ_{max}^{EtOH} 287 mμ.

Anal. Calcd. for $C_{16}H_{14}O_6$: C, 63.6; H, 4.67; CH_3O, 10.2. Found: C, 63.6; H, 4.90; CH_3O, 11.9.

Dehydrogenation of Eriodictyol.[20]—5,7,3′,4′-Tetra-*O*-acetyleriodictyol (4.3 g.) in carbon tetrachloride (200 ml.) was treated with N-bromosuccinimide (4.3 g.) and benzoyl peroxide (100 mg.). The solution was boiled under reflux for 90 minutes, then placed in a refrigerator to crystallize. The product, recrystallized from ethanol (500 ml.), weighed 2.9 g. and had m.p. 231–233°, raised to 232–233° by recrystallization from acetone–ethanol; reported[15] m.p. of **tetra-O-acetylluteolin** 226–227°, λ_{max}^{EtOH} 259, 298 mμ.

Anal. Calcd. for $C_{23}H_{18}O_{10}$: C, 60.9; H, 3.99. Found: C, 60.7; H, 3.92.

The above tetraacetyl derivative (1 g.) was warmed in a mixture of glacial acetic acid (12 ml.) and concentrated hydrochloric acid (5 ml.) until crystallization occurred. Several recrystallizations of the product from methanol afforded **luteolin** as pale yellow plates, m.p. 333–335°, reported[15] m.p. 328–330°; λ_{max}^{EtOH} 256, ~ 268, 349 mμ. The compound was indistinguishable from authentic luteolin on paper chromatograms: R_f 0.55 in acetic acid–water (1:1), R_f 0.66 in ethanol–acetic acid–water (5:1:6).

Anal. Calcd. for $C_{15}H_{10}O_6$: C, 62.9; H, 3.52. Found: C, 62.6; H, 3.60.

Benzoylation of the luteolin with benzoyl chloride in warm pyridine followed by evaporation and crystallization of the residue from ethanol gave **tetra-O-benzoylluteolin**, m.p. 204–206°, reported[15] m.p. 200–201°; λ_{max}^{EtOH} 234, 300 mμ.

Anal. Calcd. for $C_{43}H_{26}O_{10}$: C, 73.5; H, 3.73. Found: C, 73.1; H, 3.79.

Demethylation of Hesperetin.—A solution of hesperetin (1.25 g.) in acetic anhydride (20 ml.) containing phenol (0.1 g.) was brought to a boil and treated gradually with 47% hydriodic acid (12.5 ml.). After 5 mintues the solution was poured into a large volume of aqueous sodium bisulfite and the mixture was warmed. The tar which separated on cooling was filtered out and the filtrate extracted with 3 × 100 ml. of ethyl acetate. Evaporation of the ethyl acetate followed by acetylation of the residue with acetic anhydride–sodium acetate gave colorless needles (140 mg.) of tetra-*O*-acetyleriodictyol, m.p. 144–144.5° from ethyl acetate–ether. Deacetylation of this compound in methanol–concentrated hydrochloric acid (10 minutes warming) afforded eriodictyol, m.p. 266–267° from dilute methanol. Paper chromatograms of the eriodictyol showed only traces of impurities.

(12) A mixture of the calcium salts of the flavonoid glycosides of lemon peel manufactured by Exchange Lemon Products Co., Corona, Calif. This material contains relatively little hesperidin. Mention of specific products or brands does not constitute endorsement by the Department of Agriculture.

(13) E. F. Bryant, *J. Am. Pharm. Assoc., Sci. Ed.*, **39**, 480 (1950).

(14) R. M. Horowitz, *J. Org. Chem.*, **22**, 1733 (1957).

(15) I. Heilbron, Editor, "Dictionary of Organic Compounds," Oxford University Press, New York, N. Y., 1953.

(16) Extract D of ref. 7.

(17) M. Shimokoriyama, *Bull. Chem. Soc. Japan*, **16**, 284 (1941).

(18) J. Shinoda and S. Sato, *J. Pharm. Soc. Japan*, **49**, 64 (1929).

(19) N. Narasimhachari and T. R. Seshadri, *Proc. Indian Acad. Sci.*, **32A**, 17 (1950).

(20) The dehydrogenation procedure is that of N. B. Lorette, T. B. Gage and S. H. Wender, *J. Org. Chem.*, **16**, 930 (1951).

Isolation of Eriocitrin.—"Calcium Flavonate Glycoside, Lemon" (100 g.) mixed with water (1000 ml.) was heated on the steam-bath for one hour, then was cooled in an ice-bath while the *p*H was adjusted to 3 with hydrochloric acid. The mixture was extracted with 5 × 200 ml. of *n*-butyl alcohol and the combined extract was washed with 3 × 25 ml. of 5% aqueous sodium bicarbonate and 3 × 25 ml. of water. Evaporation of the butanol in a vacuum gave a mixture of glycosides (7.4 g.) as a light yellow gum. An additional quantity (2 g.) was obtained by prolonged extraction of the aqueous layer with ethyl acetate using a continuous liquid–liquid extractor.

To the gummy mixture of glycosides (6 g.) dissolved in methanol (60 ml.) was slowly added 100-mesh silicic acid (30 g.) (Mallinckrodt analytical reagent) with continuous shaking. The methanol was then removed as completely as possible at room temperature under oil-pump vacuum. The dried product was ground in a mortar under chloroform (150 ml.) and the slurry was added slowly to the chloroform layer on top of a silicic acid column which had been prepared as follows: a slurry of 100-mesh silicic acid (1090 g.) in U.S.P. chloroform (4000 ml.) was poured into an 8-cm. (diameter) chromatographic tube forming a column 43 cm. high. (In certain runs the mixture of glycosides dissolved in a minimum of methanol was added directly to the column, but, in general, better results were obtained by preadsorbing the glycosides on silicic acid. It should be noted that the glycosides are almost completely insoluble in chloroform.)

The column was eluted with 36 liters of chloroform–methanol, which varied in concentration from 2 to 16% methanol (v./v.). A total of 2200 fractions was taken by means of an automatic fraction collector. Every tenth fraction was examined on duplicate paper chromatograms which were developed with 10% acetic acid and were sprayed with Tollens reagent and methanolic sodium borohydride, respectively.[14] Eriocitrin was eluted with 13% methanol (fractions 1636 to 1790), while hesperidin was eluted with 12% methanol (fractions 1250 to 1620). Several other glycosides were obtained which will be described in another paper.

The fractions containing eriocitrin were combined and taken to dryness. The residual gum (1.1 g.) was precipitated first from *n*-butyl alcohol, then several times from ethyl acetate–acetone, from which it was obtained as a light yellow, rather granular solid, melting range 154–164°; λ_{max}^{EtOH} 285, 330 (low) mμ; $\lambda_{max}^{EtOH-AlCl_3}$ 306, 382 mμ; $\lambda_{max}^{EtOH-NaOAc}$ 285, 330 (low) mμ; R_f 0.70 in 10% acetic acid. It was very soluble in water and methanol.

Anal. Calcd. for $C_{27}H_{32}O_{15}$: C, 54.4; H, 5.41; CH_3O, nil. Found: C, 54.1; H, 5.47; CH_3O, 0.90.

Hydrolysis of Eriocitrin.—A solution of eriocitrin (50 mg.) in 0.1 *M* acetate buffer (10 ml.) was treated with hemicellulase (40 mg.) and the mixture kept overnight at room temperature. It was then extracted with 3 × 20 ml. of ethyl acetate and the combined extract taken to dryness. On crystallization of the residue from dilute methanol there was obtained 19 mg. (79%) of eriodictyol as lens-shaped crystals, m.p. 270–271°, unchanged on mixing with authentic material. The infrared spectrum was identical with that of an authentic sample.

In another run, eriocitrin (0.1 g.) was hydrolyzed in hot *N* hydrochloric acid for 15 minutes. The mixture was extracted with ethyl acetate and the aqueous layer partially evaporated and chromatographed on paper. The presence of rhamnose and glucose was shown as previously described.[7]

Nona-acetyleriocitrin.—Eriocitrin (0.1 g.) was mixed with acetic anhydride (2 ml.) and sodium acetate and the mixture warmed on a steam-bath for 2 hours, then was heated to boiling for 1 minute. The solution was diluted with ice-water and allowed to stand until the product had solidified. The crude product (0.146 g., m.p. 127–130°) crystallized from ethanol as fine, colorless needles, m.p. 133–134°, raised to 135–136.5° by two further recrystallizations; λ_{max}^{EtOH} 268, 310 mμ.

Anal. Calcd. for $C_{45}H_{50}O_{24}$: C, 55.4; H, 5.17; 10 CH_3CO, 39.7; CH_3O, nil. Found: C, 55.3; H, 5.13; CH_3CO, 39.4; CH_3O, 0.40.

Eriocitrin Chalcone Decamethyl Ether.—A mixture of eriocitrin (75 mg.), methyl iodide (2 ml.), potassium carbonate (1 g.) and acetone (30 ml.) was boiled under reflux overnight in an atmosphere of nitrogen. The mixture was filtered and the potassium salts washed with hot acetone. Evaporation of the acetone filtrate gave a gum which was redissolved in N,N-dimethylformamide (1 ml.) and treated with methyl iodide (0.5 ml.) and silver oxide (0.5 g.).[21] After shaking the mixture overnight at room temperature, it was filtered and the silver salts were washed with dimethylformamide and chloroform. The combined filtrate was washed with dilute aqueous potassium cyanide, then with water and was finally taken to dryness under vacuum. The residual gum (33 mg.) was crystallized from a small volume of methanol; m.p. 176–178°, undepressed on mixing with hesperidin chalcone nonamethyl ether. The infrared spectrum was identical with that of hesperidin chalcone nonamethyl ether.

Anal. Calcd. for $C_{37}H_{52}O_{15}$: C, 60.3; H, 7.11. Found: C, 60.6; H, 7.25.

Hesperidin Chalcone Nonamethyl Ether.—A mixture of hesperidin (10 g.), N,N-dimethylformamide (120 ml.), methyl iodide (45 ml.) and silver oxide (45 g.) (added in portions during 30 minutes) was stirred overnight at room temperature, then was filtered and the product worked up as in the previous experiment. It crystallized readily from methanol as small prisms, m.p. 175–177°, reported[10] m.p. 180–181°, λ_{max}^{EtOH} 340 mμ.

Anal. Calcd. for $C_{37}H_{52}O_{15}$: C, 60.3; H, 7.11; CH_3O, 42.1. Found: C, 60.2; H, 7.17; CH_3O, 41.1.

We wish to thank L. M. White for the analytical data.

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(21) The methylation procedure using dimethylformamide is that of R. Kuhn, I. Löw and H. Trischmann, *Chem. Ber.*, **88**, 1492 (1955).