276. *Natural Glycosides. Part VI. The Hexose Residue of Phloridzin.*

By Alexander Müller and Alexander Robertson.

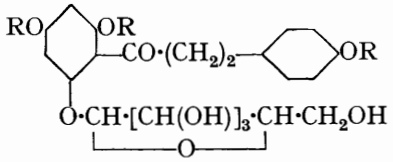
In Part I of this series (J., 1930, 21; compare Wessely and Sturm, *Monatsh.*, 1929, **53**, 554) the position of the hexose residue in phloridzin was established, and since on decomposition with baryta the glucoside yields phlorin, which is identical with synthetical phloroglucinol β-glucoside (Cremer and Seuffert, *Ber.*, 1912, **45**, 2565; Fischer and Strauss, *ibid.*, 1912, **45**, 2467), it may be reasonably assumed in agreement with the classification of Euler ("Chemie der Enzyme," 1922, Vol. 2, 220) that phloridzin is a normal β-glucopyranoside (I). This conclusion, however, appears to be inconsistent with the behaviour of the compound towards mineral acids and enzymes. As the result of a quantitative study of their acid hydrolysis, Moelwyn-Hughes (*J. Gen. Physiol.*, 1930, **13**, 807; cf. *Trans. Faraday Soc.*, 1929, **25**, 81) has shown that, of a number of glycosides (including biosides) examined, phloridzin is the most labile and more closely resembles the γ-fructosides than the normal β-glucopyranosides.

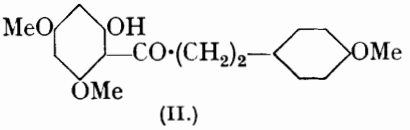
In connexion with the enzymatic hydrolysis of phloridzin, the observations of Bourquelot and Hérissey (*Compt. rend. Soc. Biol.*, 1895, **47**, 578) on the action of the "emulsin" of *Aspergillus niger*, and those of Giaza and his co-workers (*ibid.*, 1906, **60**, 1038; 1907, **62**, 1197) on the action of certain extracts of animal origin, may be neglected from a diagnostic point of view, since these agents appear to be mixtures of enzymes. Regarding the action of emulsin, it has been maintained by Hérissey, Maquenne, and Bourquelot respectively (for refs., see Bridel, *Bull. soc. Chim. Biol.*, 1930, **12**, 921) that phloridzin is not decomposed by this enzyme, while Bridel (*loc. cit.*) asserts that the glucoside is hydrolysed, indicating that it is a β-glucoside, but that owing to the low solubility of the compound the hydrolysis can only be detected polarimetrically after a long period. On the other hand, Moelwyn-Hughes (*loc. cit.*) states that, although phloridzin is unaffected by emulsin, it is hydrolysed by maltase containing saccharase and by maltase-free saccharase at p_H 4·45, at which the α-glucase of the saccharase is supposed to be inactive, thus indicating that the compound is a derivative of a γ-hexose. This hexose, he finds, differs from glucose in that it has a considerably lower specific rotation, $[\alpha]_D$ + 44·61° instead of + 52·58°, and is not immediately fermented by *Bacillus pestis*. Hesse (*Annalen*, 1875, **176**, 89; 1878, **192**, 173) has recorded a similarly low value $[\alpha]_D$ + 45·86° (cf., however, *Annalen*, 1893, **277**, 302), while Rennie (J., 1887, **51**, 634) gives $[\alpha]_D$ + 57·9°. In view of the unsatisfactory evidence regarding the nature of the hexose residue of phloridzin, the present investigation was undertaken (cf. Armstrong and Armstrong, "The Glycosides," 1931, p. 16).

We have examined the sugar obtained by the acid hydrolysis of phloridzin, and found that it is identical with glucose and yields on acetylation according to Fischer's directions (*Ber.*, 1916, **49**, 584) the well-known β-penta-acetate.

The methylation of phloridzin was effected by the application of the Purdie reaction to the trimethyl ether (I, R = Me); this indirect route was employed to prevent the *C*-methylation of the phloroglucinol residue. On hydrolysis, the resulting syrupy *hepta-methyl* ether gave rise to *O*-trimethyl phloretin (II) and 2 : 3 : 4 : 6-tetramethyl glucose, thus clearly showing that in phloridzin the glucose residue has a normal pyranose structure.

(I.)





(II.)

Confirmation of this structure was obtained in the synthesis of trimethyl phloretin β-glucoside (I, R = Me) which was accomplished by the interaction of (II) and *O*-tetra-acetyl α-glucosidyl bromide in the presence of silver oxide and quinoline, and subsequent hydrolysis of the resulting *tetra-acetate*. The synthetical glucoside (I, R = OMe) is identical in every way with the natural trimethyl ether of phloridzin. Although the synthetical tetra-acetate of (I, R = Me) isolated from the reaction mixture melts 6—7° higher than

the natural derivative, yet the product obtained by reacetylation of the synthetical glucoside was in every way identical with the natural compound. Both the natural and the synthetical phloridzin trimethyl ether are hydrolysed by emulsin in aqueous alcohol at 37°, yielding trimethyl phloretin (II) and glucose.

As a result of our experiments, it is clearly established that trimethyl phloridzin (I, R = Me) is a normal β-glucopyranoside, and therefore, since there is no reason to believe that the structure of the hexose residue is modified in the course of the phenolic methylation by the methyl iodide–potassium carbonate method, it is equally certain that phloridzin is also a β-glucopyranoside (I, R = H). Although we are unable to account for the observations of Moelwyn-Hughes (*loc. cit.*), yet we are of the opinion in regard to the behaviour of phloridzin towards emulsin that, taking into account the variability in the rate of hydrolysis of different β-glucosides by this enzyme, the absence of reaction, or alternatively the extremely low rate observed by Bridel (*loc. cit.*), is due to the nature of the aglucone residue. In this connexion, it is noteworthy that Dann and Quastel (*Biochem. J.*, 1928, **22**, 245) have found that, of several glucosides examined, phloridzin exerted by far the highest retarding action on the rate of glucose fermentation by zymin.

Experimental.

Isolation of Glucose from Phloridzin.—A solution of the glucoside (30 g.) in 0·2*N*-sulphuric acid (440 c.c.) was refluxed for 90 minutes; after cooling, the crystalline phloretin was filtered off, the aqueous liquor neutralised with barium carbonate, filtered, and evaporated in a vacuum at 40—45°, and the residue extracted with boiling alcohol. On cooling, the extract deposited anhydrous glucose, which, on recrystallisation from acetic acid, had m. p. 144—145°, $[\alpha]_{5461}^{19°}$ + 112·3° after 10 minutes, + 68·48° (20 hours), $[\alpha]_{D}^{19°}$ + 52·46° (20 hours) (c = 1·395 in water). Acetylation of a specimen with acetic anhydride and sodium acetate on the steam-bath for ¼ hour gave rise to β-penta-acetyl glucose, identical with an authentic specimen, m. p. and mixed m. p. 131°, $[\alpha]_{D}^{19°}$ + 3·79° (c = 1·554 in chloroform) (Found : C, 49·2; H, 5·9. Calc. for $C_{16}H_{22}O_{11}$: C, 49·0; H, 5·7%).

Methylation of Phloridzin and Hydrolysis of O-*Heptamethyl Phloridzin.*—Methylation of the hydrate (20 g.) with methyl iodide (30 c.c.) and potassium carbonate (22 g.) in boiling acetone (100 c.c.) for 10—14 hours gave rise to the trimethyl ether, which separated from aqueous acetone as the monohydrate in slender needles (21 g., air-dried), m. p. 75—76° after sintering at 73°, $[\alpha]_{5461}^{23°}$ − 39·27° in alcohol (c, 0·991) (Johnson and Robertson, *loc. cit.*, give m. p. 63—65°).

A solution of the trimethyl ether (21 g.) in acetone (40 c.c.) containing methyl iodide (8 c.c.) and silver oxide (10 g.) was refluxed for 12 hours; two further portions of oxide (10 g.) and one of iodide (10 c.c.) were added during the first 2 hours. A solution of the product in acetone (200 c.c.) was filtered from silver salts and, after the addition of a little silver oxide, evaporated under diminished pressure, and the residue was again methylated in the same manner. The product was then methylated 8—10 times without an extraneous solvent until a constant methoxyl value was obtained; each time 50—70 c.c. of methyl iodide were used and 30 g. of silver oxide added in 3 portions during 24 hours. The *heptamethyl* ether was obtained as a pale yellow syrup (12 g.) [Found : OMe, 40·2. $C_{21}H_{17}O_3(OMe)_7$ requires OMe, 40·6%].

In view of the extreme ease with which trimethyl phloridzin and the partially methylated mixture derived therefrom are hydrolysed by traces of hydrogen iodide formed by the slight decomposition of the methyl iodide in the course of the evaporation of the acetone solutions obtained in the early stages of the methylation process, it is essential that these evaporations should be carried out in the presence of silver oxide.

A solution of the syrup in acetone (100 c.c.) containing 10% sulphuric acid (25 c.c.) was refluxed for 6 hours, and after several hours at room temperature the dark red oil which had separated crystallised. On isolation, the solid was found to be *O*-trimethyl phloretin, m. p. and mixed m. p. 109—110°, after recrystallisation from acetone (Johnson and Robertson, *loc. cit.*). The acid filtrate from the crude trimethyl phloretin was diluted with 2% sulphuric acid (80 c.c.) and refluxed for 4 hours, cooled, filtered, and neutralised with a slight excess of lead carbonate. After removal of the lead salts, the solution was evaporated in a vacuum at 30° in the presence of a little lead carbonate, the residue extracted with boiling alcohol, the extract evaporated, the residual oil dissolved in light petroleum (b. p. 60—80°) (200 c.c.), and the solution decanted from a little insoluble material. Distillation of the solvent left a pale yellow oil, which, on being kept over-night at 0°, partly crystallised. The crystalline material was found to consist of 2 : 3 : 4 : 6-tetramethyl glucose, which, twice crystallised from light

petroleum containing a little ether, had m. p. 92—94°, $[\alpha]_D^{21°}$ + 113·6° in alcohol (*c*, 3·559), after 4 days + 83·65° [Found: OMe, 52·3. Calc. for $C_6H_8O_2(OMe)_4$: OMe, 52·6%].

2-O-*Tetra-acetyl-β-glucosidoxy*-4 : 6 : 4′-*trimethoxy-β-phenylpropiophenone* (O-*Tetra-acetyl Trimethyl Phloridzin*).—Silver oxide (10 g.) was added with stirring to an intimate mixture of *O*-trimethyl phloretin (7 g.), *O*-tetra-acetyl α-glucosidyl bromide (20 g.), and freshly distilled quinoline (40 c.c.), the reaction initiated by warming to 30—35°, the mixture stirred for 15 minutes, kept in a desiccator for 3 hours, and dissolved in warm acetic acid (70 c.c. at 40°), and the filtered solution poured into water (350 c.c.). The resulting dark oil, which soon solidified, was extracted with warm alcohol, and the filtered extract cooled. The unchanged trimethyl phloretin which first separated was removed, and the *acetate* of the glucoside then crystallised in the course of 10—12 hours, forming colourless needles (0·9 g.). Recrystallised from 50% alcohol, it had m. p. 94—95°, $[\alpha]_{5461}^{21°}$ − 44·64° in chloroform (*c*, 1·074) [Found: C, 59·7; H, 6·0; OMe, 15·1. $C_{29}H_{29}O_{11}(OMe)_3$ requires C, 59·4; H, 5·9; OMe, 14·4%]. The compound does not reduce Fehling's solution until it has been boiled with hydrochloric acid, and does not give a ferric chloride reaction.

2-β-*Glucosidoxy*-4 : 6 : 4′-*trimethoxy-β-phenylpropiophenone* (*Trimethyl Phloridzin*).—A solution of the foregoing acetate (0·5 g.) in chloroform (2 c.c.) was cooled in an ice–salt mixture and treated with a cooled 0·5*N*-solution of sodium methoxide in methyl alcohol (3 c.c.). 20 Minutes later, the reaction mixture was diluted with ice-water and acidified with dilute acetic acid, and, on the removal of the chloroform and methyl alcohol at room temperature by means of a current of air, a theoretical yield of the glucoside quickly separated in colourless needles. Twice recrystallised from dilute acetone, it had m. p. 75—76° after sintering at 73°, alone or mixed with an authentic specimen, $[\alpha]_{5461}^{21°}$ − 38·85° in alcohol (*c*, 3·306) [Found, in air-dried specimen: OMe, 19·4; H_2O, 3·6. Calc. for $C_{21}H_{21}O_7(OMe)_3,H_2O$: OMe, 18·8; H_2O, 3·6%. Found, in material dried in high vacuum at 56°: C, 60·6; H, 6·3; OMe, 19·6. Calc. for $C_{21}H_{21}O_7(OMe)_3$: C, 60·2; H, 6·3; OMe, 19·5%]. The compound does not reduce Fehling's solution until hydrolysed with mineral acid. Acetylation of the synthetical glucoside with acetic anhydride and pyridine at room temperature gave the tetra-acetate, which, after repeated recrystallisation from dilute alcohol, had m. p. 87—88°, $[\alpha]_{5461}^{20°}$ − 44·52° in chloroform (*c*, 1·003). Mixed with the aforementioned specimen, m. p. 94—95°, it melted at 87—89°.

Acetylation of Natural Trimethyl Phloridzin.—The glucoside (9 g.) was acetylated with acetic anhydride (45 c.c.) and pyridine (50 c.c.) during 2 days at room temperature, and the crude product isolated and dissolved in warm alcohol. The small amount of oily material which separated first on cooling was removed, and the acetate then crystallised in needles (6·3 g.). Recrystallised, the compound had m. p. 87—88°, $[\alpha]_{5461}^{21°}$ − 44·77° in chloroform (*c*, 0·994), unchanged by exhaustive purification (Found: C, 59·3; H, 5·9; OMe, 14·8%). Mixed with the synthetical tetra-acetate of m. p. 94—95° and 87—88°, it melted at 87—95° and 87° respectively. Acetylation at 100° with use of sodium acetate gave the same product.

Deacetylation of the natural tetra-acetate by the method used for the synthetical compound gave rise to the monohydrate of trimethyl phloridzin, m. p. and mixed m. p. 75—76° after slight sintering at 73°, $[\alpha]_{5461}^{21°}$ − 39·11° in alcohol (*c*, 3·401) (Found, in material dried over calcium chloride: C, 58·1; H, 6·6; H_2O, 3·6. Calc. for $C_{24}H_{30}O_{10},H_2O$: C, 58·2; H, 6·5; H_2O, 3·6%. Found, in specimen dried in high vacuum at 56°: C, 60·6; H, 6·4; OMe, 19·5%). Treatment of this material with acetic anhydride and pyridine or sodium acetate regenerates the acetate, m. p. 87—88°.

Hydrolysis of Synthetical and Natural Trimethyl Phloridzin with Emulsin.—Water (7 c.c.) containing emulsin (0·2 g.) was added to a solution of the synthetical glucoside (0·2 g.) in alcohol (3 c.c.) and toluene (0·2 c.c.), the mixture kept at 37° for 24 hours and diluted with water (50 c.c.), and the solid collected and extracted with boiling alcohol (2 c.c.). The filtered extract was mixed with water, and on cooling, deposited colourless needles of *O*-trimethyl phloretin (II), m. p. 109°, identical in every way with an authentic specimen. The aqueous filtrate from the crude solid reduced Fehling's solution. Control experiments gave negative results.

Natural trimethyl phloridzin was hydrolysed in the same manner with emulsin, yielding trimethyl phloretin and glucose.

The authors are grateful to the Chemical Society for research grants, and to the Rockefeller International Education Board for a Research Fellowship to one of them (A. M.).

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[*Received, June 17th*, 1933.]