Some New Synthetic Flavonoid Glycosides Related in Structure to Phlorizin

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A number of glycosides, derived from various hydroxyacetophenones, hydroxychalcones and hydroxydihydrochalcones, have been prepared by existing synthetic procedures. These compounds were synthesized in order to compare their activity with the naturally occurring hydroxydihydrochalcone glucoside, phlorizin, in blocking renal reabsorption of glucose.

The mechanism by which the hydroxydihydrochalcone, phlorizin (IIIe), inhibits the intestinal and renal absorption of glucose and other monosaccharides has been the subject of numerous investigations. Considerable evidence, recently summarized in a review,[2] now supports the thesis that phlorizin prevents the entry of hexose into the cell by blocking its transport across the cell membrane. The initial step in this transport probably involves the orientation and/or binding of the sugar to a site on the membrane[3]; presumably, phlorizin successfully competes with the sugar for this site. It is therefore of interest to know the essential structural features of an inhibitory compound necessary for activity, *i.e.*, its affinity for the membrane site. A number of polyhydroxyphenol glycosides related in structure to phlorizin have therefore been synthesized to test their effect on sugar transport and a description of the preparation and chemical properties of these analogs is the subject of this communication.

The compounds described here were prepared by modifying the procedures used by Zemplén and Bognár[4] for the synthesis of phlorizin (IIIe). The pathway for the syntheses of the new compounds is schematically represented in I → IV.
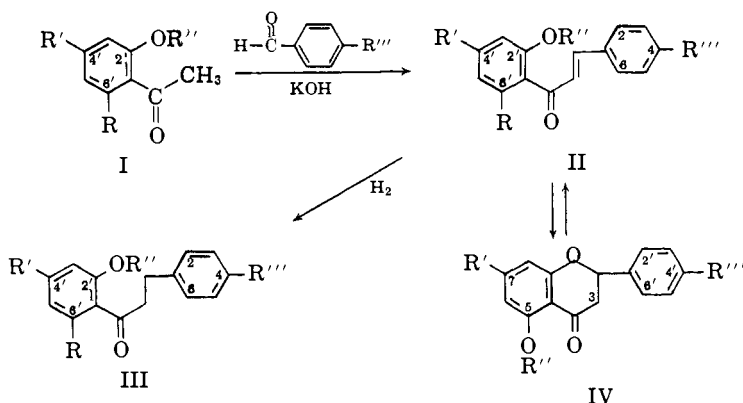
All of the hydroxyacetophenone glycosides with the exception of

(1) Department of Pharmacology, University of Kentucky, College of Medicine, Lexington, Kentucky.
(2) R. K. Crane, *Physiol. Revs.*, **40**, 789 (1960), esp. p. 808.
(3) W. Wilbrandt and T. Rosenberg, *Pharm. Revs.*, **13**, 109 (1961).
(4) G. Zemplén and R. Bognár *Ber.*, **75** 1040 (1942).

I → II → IV, III (reaction scheme with KOH, H₂)

Ia, R = OH, R′ = OCOC₆H₅, R″ = 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl

Ib, R = OH, R′ = OCOC₆H₅, R″ = 2,4,6-tri-O-acetyl-3-O-methyl-β-D-glucopyranosyl

Ic, R = OH, R′ = H, R″ = 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl

Id, R = R′ = OH, R″ = β-D-glucopyranosyl

Ie, R = H, R′ = OCOC₆H₅, R″ = 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl

IIa, R = R′ = R‴ = OH, R″ = β-D-galactopyranosyl

IIb, R = R′ = R‴ = OH, R″ = 3-O-methyl-β-D-glucopyranosyl

IIc, R = R‴ = OH, R′ = H, R″ = β-D-glucopyranosyl

IId, R = R′ = OH, R‴ = OCH₃, R″ = β-D-glucopyranosyl

IIe, R′ = R‴ = OH, R = H, R″ = β-D-glucopyranosyl

IIIa, R = R′ = R‴ = OH, R″ = β-D-galactopyranosyl

IIIb, R = R′ = R‴ = OH, R″ = 3-O-methyl-β-D-glucopyranosyl

IIIc, R = R‴ = OH, R′ = H, R″ = β-D-glucopyranosyl

IIId, R = R′ = OH, R‴ = OCH₃, R″ = β-D-glucopyranosyl

IIIe, R = R′ = R‴ = OH, R″ = β-D-glucopyranosyl

IV, R′ = OH, R‴ = OCH₃, R″ = β-D-glucopyranosyl

2′ - O - (2,3,4,6 - tetra - O - acetyl - β - D - glucopyranosyl) - 4′ - O-benzoylacetophenone (Ie) were prepared easily by treating the O-acetylglycosyl halide with the appropriate phenol in alkaline acetone. Hydrogen bonding with the carbonyl group probably explains the low activity of the *ortho* phenol function[5] and would account for the failure to synthesize this glucoside under normal conditions. However, under more strenuous conditions, *viz.* the Koenigs–Knorr synthesis in quinoline,[6] the compound was prepared without difficulty. Analogous glucosides, *o*-hydroxypropiophenone-β-D-glucoside[7] and

(5) A. G. Perkin and R. C. Storey, *J. Chem. Soc.*, 229 (1928).

(6) J. Conchie, G. A. Levy and C. A. Marsh, *Advances in Carbohydrate Chem.*, **12**, 157 (1957).

(7) G. Wagner, *Arch. Pharm.*, **290**, 625 (1957).

2'-O-(β-D-glucosyl)-4'-O-methyl-acetophenone[8] also have been formed in this manner.

One of the chalcone derivatives, 4',6'-dihydroxy-2'-O-(β-D-glucopyranosyl)-4-O-methylchalcone (IId) could not be purified by recrystallization since it isomerized readily to the corresponding flavanone (IV). On the other hand, the flavanone, by virtue of its relative insolubility in 75% ethanol, was easily isolated by repeated crystallization from alcohol. When treated with alkali, it was reconverted to the chalcone which was subsequently hydrogenated to the dihydrochalcone.

Attempts to prepare the dihydrochalcone derivative from 2'-O-(β-D-glucopyranosyl)-4,4'-dihydroxychalcone (IIe) by catalytic hydrogenation were not successful. Invariably, 2-3 times the stoichiometric amount of hydrogen was taken up. Graphical analysis clearly indicated that the reduction was not a first order reaction. This dihydrochalcone is therefore exceptional in that it is not the final product of the hydrogenation and the observations suggest that further reduction of the carbonyl group and/or the aromatic ring takes place. It seems apparent that the chelation through hydrogen bond formation between the *ortho* –OH and carbonyl groups greatly increases the stability of the dihydrochalcone and when this ring formation is not possible, the compound is susceptible to further reduction. Efforts were made to stop the reaction at the oxidation state of the dihydrochalcone by discontinuing the reduction after stoichiometric hydrogen consumption. However, a crystalline product could not be obtained from the common solvents.

The various glycosides were tested for pharmacological activity by comparing their effects with phlorizin on the maximal renal tubular reabsorptive capacity for glucose (TmG). They were administered by constant intravenous infusion to glucose-loaded dogs according to the method used by Lotspeich and Woronkow.[9] After control periods, during which TmG was determined, the glycoside was incorporated into the infusion and its effect on TmG was measured. A report on the activity of the galactose analog of phlorizin (IIIa) has been published[10]; the galactoside is much less effective in blocking glucose reabsorption in the kidney. Some of the synthetic compounds reported here have since been tested for activity, *viz.*, Id, IIIb, IIIc and IIId. When infused at a rate at which phlorizin inhibits TmG 90–100% (approximately 7×10^{-2} μmole/kg./

(8) T. Kariyone, M. Takahashi and K. Takaishi, *J. Pharm. Soc. Japan*, **76**, 917 (1956); *Chem. Abstr.*, **51**, 2770 (1957).

(9) W. D. Lotspeich and S. Woronkow, *Am. J. Physiol.*, **195**, 331 (1958).

(10) D. F. Diedrich, *Biochim. et Biophys. Acta*, **47**, 618 (1961).

min.), phloracetophenone glucoside (Id) and the 3-O-methylglucoside of phloretin (IIIb) had no effect.   The 4-O-methylphlorizin (IIId) was about 75% as active as phlorizin and the 4'-deoxy analog (IIIc) exhibited full activity in inhibiting the transport of glucose.

Experimental

Melting points were determined in a Thomas–Hoover melting point apparatus and are uncorrected.   The optical rotations were measured in a 1-dm. tube and the values have a limit of error of ±2°.   Microanalyses were performed by Clark Microanalytical Laboratory, Urbana, Illinois.

2-O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-4-O-benzoyl-6'-hydroxyacetophenone (Ia).—Purified 4-O-benzoylphloracetophenone[11] (6.1 g., 22.5 mmoles) was suspended in 30 ml. of ice cold 1.2 N potassium hydroxide and a solution of 14 g. (34 mmoles) of O-acetylgalactosyl bromide[12] in 30 ml. of acetone was added slowly at 0°.   An additional 30 ml. of acetone effected a yellow homogeneous solution which was kept at room temperature for 24 hr.   To insure completeness of reaction, the mixture was shaken mechanically for the final 3–4 hr. and then stirred into 1 l. of cold water containing 1.5 ml. of glacial acetic acid.   The mass soon solidified; it was filtered, washed with water and crystallized from methanol.   The acetylated glycoside was dissolved in a minimum amount of chloroform, separated from unreacted 4-O-benzoylphloracetophenone by filtration, and isolated by diluting the concentrated chloroform solution with hot methanol.   Final purification from methanol gave 4.1 g. (30%) of white platelets, m.p. 170–171°; $[\alpha]^{22}$D 0° (c 2.5, chloroform).

Anal. Calcd. for $C_{29}H_{30}O_{14}$: C, 57.8; H, 5.0.   Found: C, 57.3; H, 5.0.

4',6'-Dihydroxy-2'-O-(β-D-galactopyranosyl)-4-hydroxychalcone (IIa).—Compound Ia (4.2 g., 7.0 mmoles) was triturated with 3 ml. ethanol, and at 0° treated with 18 ml. of cold 10 N potassium hydroxide.   The mixture was stirred for 5–10 min. and after saponification, 1.05 g. (8.5 mmoles) p-hydroxybenzaldehyde was added.   The mixture was shaken mechanically for 24 hr. and then allowed to stand for 2 days at room temperature.   The solution was neutralized with ice cold 10% hydrochloric acid to pH 5 and the golden, feather-like chalcone crystallized rapidly.   The product was dried and repeatedly extracted with benzene to remove any benzoic acid; yield, 1.3 g. (43%).   For analysis, the compound was recrystallized from water

(11) F. W. Canter, F. H. Curd and A. Robertson. *J. Chem. Soc.*, 1245 (1931).

(12) R. G. Hanson, W. J. Rutter and P. Krichevsky, *Biochem. Preparations.* **4**, 1 (1955).

and finally dried at 80° *in vacuo;* m.p. 159–163°; $[\alpha]^{27}D$ +34° (c 1.8, 95% ethanol).

Anal. Calcd. for $C_{21}H_{22}O_{10}$: C, 58.1; H, 5.15. Found: C, 58.4; H, 5.05.

4′,6′-Dihydroxy-2′-*O*-(β-D-galactopyranosyl)-4-hydroxydihydrochalcone (IIIa).—The parent chalcone (0.4 g., 0.94 mmoles) was hydrogenated in 95% ethanol with 0.3 g. of 10% palladium-on-charcoal as the catalyst at atmospheric pressure. Hydrogen uptake was stoichiometric. After removal of the catalyst, the almost colorless solution was concentrated *in vacuo* to a small volume (2–3 ml.). Distilled water was added yielding colorless needles (0.3 g.) which were purified by repeated crystallization from water. The galactoside crystallizes with 2 moles of water which can be removed by drying at 80° *in vacuo,* m.p. 174–176° after softening at 133°; $[\alpha]^{24}D$ −40° (c 1.3, 95% ethanol).

Anal. Calcd. for $C_{21}H_{24}O_{10}$: C, 57.8; H, 5.5. Found: C, 57.7; H, 5.4.

2′-*O*-(2,4,6-Tri-*O*-acetyl-3-*O*-methyl-β-D-glucopyranosyl)-4′-*O*-benzoyl-6′-hydroxyacetophenone (Ib).—2,4,6-Tri-*O*-acetyl-3-*O*-methylglucosyl bromide,[13] obtained as a crude syrup (18 g.), was treated with 8.4 g. (31 mmoles) 4-*O*-benzoylphloracetophenone in a manner similar to that used in the preparation of Ia. The product was recrystallized from methanol yielding 7.0 g. of crude material from which 3.0 g. of unreacted 4-*O*-benzoylphloracetophenone was isolated by the chloroform extraction. The glycoside crystallized from ethanol as colorless leaflets, yield 2.5 g. (14%); m.p. 132–134°; $[\alpha]^{26}D$ −43° (c 1.1, chloroform).

Anal. Calcd. for $C_{28}H_{30}O_{13}$: C, 58.5; H, 5.3. Found: C, 58.5; H, 5.1.

4′,6′-Dihydroxy-2′-*O*-(3-*O*-methyl-β-D-glucopyranosyl)-4-hydroxydihydrochalcone (IIIb).—The chalcone was prepared from 2.3 g. (4 mmoles) of Ib as described for the preparation of IIa. Neutralization to pH 4 caused the crystallization of benzoic acid which was filtered after 0.5 hr. in the cold. Upon refrigeration of the filtrate (2–3 days), an orange-red crystalline product was harvested, dried and extracted repeatedly with benzene; yield, 1.0 g. (55%). The chalcone (IIb, m.p. 105–107°), which crystallized as a dihydrate, was not further purified and was hydrogenated as described for the preparation of IIIa. Hydrogen uptake was quantitative and from the

(13) B. Helferich and O. Lang, *J. prakt. Chem.,* **132,** 321 (1932); The 3-O-methylglucose used to prepare the halide was a generous gift from Dr. J. B. Jewell, Ayerst Laboratories, New York, New York.

almost colorless alcohol solution, 0.7 g. of pure white product was obtained. Recrystallization from water gave the analytical sample which was dried at 80° *in vacuo* over phosphorus pentoxide, m.p. 163–165°; $[\alpha]^{28}$D $-56°$ (c 1.5, 95% ethanol).

Anal. Calcd. for $C_{22}H_{26}O_{10}$: C, 58.7; H, 5.8. Found: C, 58.7; H, 5.6.

2′-O-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-6′-hydroxyaceto-phenone (Ic).—Purified 2,6-dihydroxyacetophenone[14] (5 g., 33 mmoles) was treated with 16.5 g. (40 mmoles) O-acetylglucosyl bromide[15] as previously described for the preparation of Ia and Ib. During the shaking period of 18 hr., the product crystallized from the reaction mixture and was therefore directly isolated by filtration; yield, 6.0 g. semi-crude crystalline product.[16] The compound was quite insoluble in methanol and was purified from chloroform and 5 volumes hot methanol yielding fine, colorless needles, 5.1 g. (32%), m.p. 201–203°; $[\alpha]^{28}$D $-33°$ (c 2.7, chloroform).

Anal. Calcd. for $C_{22}H_{26}O_{12}$: C, 54.8; H, 5.4. Found: C, 54.6; H, 5.3.

4,6′-Dihydroxy-2′-O-(β-D-glucopyranosyl) Chalcone (IIc).—The procedure described above for chalcone synthesis was employed except that the reaction was allowed to run for only 24 hr. Neutralization to pH 5 resulted in spontaneous crystallization of the chalcone. From 2.8 g. (5.8 mmoles) of Ic, 1.9 g. of product (78%) was obtained which was purified from 20% ethanol. The featherlike, orange-yellow needles were dried at 80° *in vacuo*, m.p. 170–171°; $[\alpha]^{26}$D $+21°$ (c 1.6, 95% ethanol).

Anal. Calcd. for $C_{21}H_{22}O_{9}$: C, 60.3; H, 5.3. Found: C, 60.2; H, 5.1.

4,6′-Dihydroxy-2′-O-(β-D-glucopyranosyl) Dihydrochalcone (IIIc).—The parent chalcone took up the theoretical amount of hydrogen and the dihydrochalcone crystallized spontaneously upon addition of water to the concentrated ethanol solution (75% yield). The compound was purified by recrystallization from water yielding soft white needles which were dried at 100° over phosphorus pentoxide *in vacuo*, m.p. 134–136° after softening at 127°; $[\alpha]^{24}$D $-33.5°$ (c 1.8, 95% ethanol).

Anal. Calcd. for $C_{21}H_{24}O_{9}$: C, 60.0; H, 5.8. Found: C, 59.7; H, 5.5.

4′,6′-Dihydroxy-2′-O-(β-D-glucopyranosyl)-acetophenone (Id).—

(14) A. Russell and J. R. Frye. *Org. Syntheses*, **21**, 22 (1941).

(15) M. E. Krahl and C. F. Cori, *Biochem. Preparations*, **1**, 33 (1949).

(16) Approximately 1 g. of product could be isolated from the aqueous alkali–acetone filtrate when it was treated in the usual manner.

The starting material, 2'-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyrano-syl)-4'-O-benzoyl-6'-hydroxyacetophenone, was prepared as described by Zemplén and Bognár.[4]   Ten grams was dissolved in 12 ml. acetone and 30 ml. of cold 10 N potassium hydroxide was added under nitrogen.   The mixture was allowed to stand tightly stoppered at room temperature for 5 hr.   It then was acidified under ice cold conditions in a stream of nitrogen with cold 15% hydrochloric acid to pH 3.5. After standing for 0.5 hr., the benzoic acid was filtered off and the filtrate was adjusted to pH 5 at 0° resulting in crystallization of the glucoside.[17]   The colorless product was collected and recrystallized from water, yield 2.8 g. (51%), m.p. 201.5–203°; $[\alpha]^{28}_{D}$ −95° (c 1.0, 95% ethanol).

Anal. Calcd. for $C_{14}H_{18}O_9$: C, 50.9; H, 5.5.   Found: C, 51.2; H, 5.3.

4',6'-Dihydroxy-2'-O-(β-D-glucopyranosyl)-4-O-methylchalcone

(IId).—Phloracetophenone glucoside (Id; 1.35 g., 4.1 mmoles) was dissolved in 6 ml. of 95% ethanol and 25 ml. of N potassium hydroxide which had been saturated with nitrogen.   Anisaldehyde (0.55 ml., 4.5 mmoles) was then added and nitrogen was bubbled through the mixture.   The stoppered flask was then shaken mechanically for 48 hr. at ambient temperature.   The solution was adjusted to pH 8, extracted a number of times with benzene and then further acidified until the first signs of turbidity appeared.   Refrigeration yielded almost immediately the golden chalcone (m.p. 190–193° after drying over calcium chloride *in vacuo*).   Further addition of acid gave second and third crops of paler yellow product; total yield, 1.5 g., 80%. Attempts to purify the compound by recrystallization from dilute alcohol failed because of its conversion to the flavanone (IV, below).

5-O-(β-D-Glucopyranosyl)-7-hydroxy-4'-O-methylflavanone   (IV).

—The crude chalcone was dissolved in 50% ethanol and acidified with acetic acid.   Pale yellow needles of the crude flavanone formed upon standing.   Repeated crystallization from 95% ethanol yielded pure flavanone in the form of colorless needles.   The product was dried *in vacuo* at 80°; m.p. 144.5–148°.

Anal. Calcd. for $C_{22}H_{24}O_{10}$: C, 58.9; H, 5.4.   Found: C, 59.1; H, 5.4.

Treatment of the flavanone with hot concentrated alkali as described by Shimokoriyama,[18] then rapid neutralization under ice cold conditions, gave a yellow gum of the chalcone which readily solidified.

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(17) Crystallization sometimes occurred only after concentration and refrigeration.

(18) M. Shimokoriyama, *J. Am. Chem. Soc.*, **79**, 4199 (1957).

4′,6′-Dihydroxy-2′-O-(β-D-glucopyranosyl)-4-O-methyldihydro-chalcone (IIId).—After quantitative hydrogenation[19] of the chalcone (1.6 g.), the dihydrochalcone was obtained by addition of a small amount of water to the alcoholic solution and concentration *in vacuo.* The product crystallized as off-white, hard leaflets (1.0 g.). Recrystallization alternately from 35% and 50% ethanol yielded pure material. After drying at 80° *in vacuo,* it melted at 199–201°; $[\alpha]^{25}$D −54° (*c* 1.6, 95% ethanol).

Anal. Calcd. for $C_{22}H_{26}O_{10}$: C, 58.7; H, 5.8. Found: C, 59.1; H, 6.0.

2′-O-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-4′-O-benzoyl-acetophenone (Ie).—A mixture of 44 g. O-acetylglucosyl bromide, 14.5 g. of freshly prepared silver carbonate[20] and 20 g. 4-O-benzoyl-resacetophenone[21] was ground in a mortar and then thoroughly mixed with 25 ml. of quinoline. The mixture was placed in a calcium chloride desiccator in the dark for 18 hr. Chloroform (100 ml.) then was added and the silver salts were removed by filtration with the aid of Celite. An additional 100 ml. of chloroform was used as a wash, and the pooled orange filtrate was extracted three times with cold 3 N sulfuric acid (300 ml.), three times with 100 ml. of cold 1 N potassium hydroxide (which extracted much of the color) and finally with water until neutral. The chloroform layer was dried with sodium sulfate and concentrated *in vacuo* to a thick syrup; 95% ethanol (50 ml.) was added and the mixture allowed to stand until crystallization ensued. The crude, yellow prisms were harvested and recrystallized from 95% ethanol giving 10.5 g. (23%) of pale yellow product, m.p. 134–136°. It was used in the chalcone synthesis without further purification. The analytical sample was obtained by triturating a portion with cold 1 N potassium hydroxide and then washing thoroughly with water. Recrystallization from 70% ethanol and finally from absolute ethanol gave colorless prisms, m.p. 137–139°; $[\alpha]^{26}$D −33° (*c* 1.0, chloroform).

Anal. Calcd. for $C_{29}H_{30}O_{13}$: C, 59.4: H, 5.15. Found: C, 59.8; H, 4.9.

(19) Usually, only 90–95% of the theoretical amount of hydrogen was taken up. When this occurred, the catalyst was removed and the alcohol evaporated. The residue was again treated with hot alkali and the newly formed chalcone was then reduced to completion.

(20) B. Helferich and W. Klein, *Ann.,* **450,** 219 (1926).

(21) The 4-O-benzoylresacetophenone was prepared in 44% yield by the procedure used to benzoylate phloracetophenone (ref. 11). The product melted at 106–108°; lit.,[22] 106–107°; 110°.[23]

(22) B. Puri and T. R. Seshardi, *J. Sci. Ind. Research* (India), **13B,** 321 (1954); *Chem. Abstr.,* **49,** 8856 (1955).

(23) R. D. Desai and V. M. Vakil, *Proc. Indian Acad. Sci.,* **12A,** 391 (1940); *Chem. Abstr.,* **35,** 3618 (1941).

4,4'-Dihydroxy-2'-O-(β-D-glucopyranosyl)-chalcone (**IIe**).—Partially purified Ie (2.9 g.) was saponified and condensed with 0.73 g. of recrystallized p-hydroxybenzaldehyde. After 24 hr. the solution was adjusted to pH 5–6 and allowed to stand in the cold for 2–3 days yielding a golden, spongy solid. It was dried over calcium chloride and extracted with benzene; yield 1.4 g. (67%). The product was recrystallized from 10% ethanol and dried at 100° *in vacuo*. It formed a red melt at 186–188°; $[\alpha]^{25}_D$ −47.5° (c 1.3, 95% ethanol).

Anal. Calcd. for $C_{21}H_{22}O_9$: C, 60.3; H, 5.3. Found: C, 60.8; H, 5.4.