807. *Dihydrochalcones of* Malus *Species.*

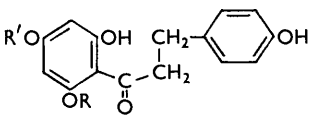
By A. H. Williams.

Two new dihydrochalcone glucosides have been isolated from the leaf of certain species of *Malus*. One, from *M. trilobata*, is a glucoside of phloretin and is isomeric with phloridzin; the other, from *M. sieboldii*, is a glucoside of 3-hydroxyphloretin, an aglycone not previously known to occur naturally. Various chromone derivatives have been prepared from these compounds.

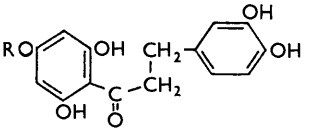
The dihydrochalcone glucoside phloridzin (I) was reported by de Koninck [1a] to occur in the bark of apple, pear, cherry, and plum trees. He later [1b] described its isolation from root bark of the apple tree, which is still the richest source known. No confirmation of its presence in pear, cherry, and plum was published and it is now recognised [2] that the apple is the only one of the fruit trees in which phloridzin occurs, although the original erroneous report still persists in some modern text-books.

Phloridzin is the main phenolic compound in the leaf and bark of all varieties of cultivated apple (*M. pumila*), but in a survey of the leaf of other apple species by paper chromatography it has been found to be wholly or partly replaced in certain species by two other compounds. One [3] of these (II) occurs only in *M. trilobata*, from the leaf of which normal phloridzin is entirely absent. The other [4] compound (III) occurs in four of the twenty-five *Malus* species listed by Rehder,[5] comprising series 3, Sieboldianæ, (Rehd.), and in all hybrids of these four species that have been examined. The species concerned are from East Asia and Japan.

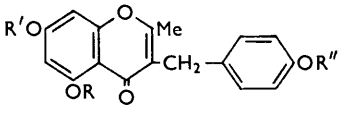
Both these compounds are easily isolated from alcoholic extracts of the appropriate leaf. Since they occur in place of phloridzin, it seemed likely that they might be dihydrochalcones. Hydrolysis with dilute acid or emulsin gave in each case glucose and another compound.



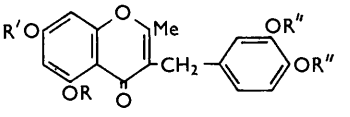
(I) R = β-D-glucosyl, R′ = H
(II) R = H, R′ = β-D-glucosyl
(IV) R = R′ = H



(III) R = β-D-glucosyl
(VIII) R = H



(V) R = R″ = Ac,
R′ = tetra-*O*-acetyl-β-D-glucosyl
(VI) R = R′ = R″ = H
(VII) R = tetra-*O*-acetyl-β-D-glucosyl,
R′ = R″ = Ac



(IX) R = R′ = R″ = Ac
(X) R = R″ = Ac,
R′ = tetra-*O*-acetyl-β-D-glucosyl
(XI) R = R′ = R″ = H

The aglycone from the *M. trilobata* compound (II) was recognised as phloretin (IV) by its chromatographic behaviour, and its identity confirmed by comparison with authentic phloretin and comparison of acetylation products. Quantitative hydrolysis showed one molecule of phloretin to be combined with one molecule of glucose; the glucoside can therefore differ from phloridzin only in the point of linkage of the glucose to phloretin.

[1] de Koninck, *Annalen*, 1835, **15**, (*a*) 75, (*b*) 258.
[2] Williams, in " Phenolics in Plants in Health and Disease," Pergamon Press, Oxford, 1960, p. 3.
[3] Williams, *Chem. and Ind.*, 1960, 934.
[4] Williams, *Chem. and Ind.*, 1956, 1306.
[5] Rehder, " Manual of Cultivated Trees and Shrubs," The Macmillan Co., New York, 2nd ed., 1954, p. 389.

Examination of the products of alkali breakdown of the glucoside (II) showed the presence of a compound chromatographically indistinguishable from phlorin; the glucose must therefore be attached to the phloroglucinol ring, and since phloridzin is the 2′-glucoside, the new compound must be the 4′-glucoside. This has been confirmed by methylation of the glucoside, followed by acid hydrolysis, which yielded the 4,2′,6′-trimethyl ether of phloretin, identical with a synthetic sample.

A compound of apparently the same structure as the *M. trilobata* glucoside (II) was synthesised by Zemplén and Bognár,[6] but its melting point and rotation are higher than those found for the compound (II), and unlike the latter it failed to give a crystalline acetylation product. Unfortunately a direct comparison has not been possible. Jorio [7] has prepared a glucoside, by partial hydrolysis of dihydronaringin, which is chromatographically indistinguishable from our product (II); but on repeating the synthesis of Bognár and Zemplén she obtained a substance differing widely in chromatographic behaviour.

Attempts to prepare an acetyl derivative of the glucoside (II) with acetic anhydride and sodium acetate at 100° gave only gums; the hepta-acetate was, however, obtained crystalline by the use of acetic anhydride and pyridine at room temperature. Heating the glucoside with acetic anhydride and sodium acetate under reflux gave a crystalline compound, different from the hepta-acetate, and identified as 5-acetoxy-3-4′-acetoxybenzyl-2-methyl-7-(tetra-*O*-acetyl-β-D-glucosyloxy)chromone (V), since on deacetylation and hydrolysis with acid it gave the already known 5,7-dihydroxy-3-4′-hydroxybenzyl-2-methylchromone (VI) which can be prepared from phloretin. Phloridzin gave at 160° with acetic anhydride and sodium acetate a crystalline derivative which must be 7-acetoxy-3-4′-acetoxybenzyl-2-methyl-5-(tetra-*O*-acetyl-β-D-glucosyloxy)chromone (VII), since it too gave the hydroxychromone (VI) on deacetylation and hydrolysis. The formation of the hydroxychromone (VI) by these three routes is a further confirmation of the interrelationships of phloretin and its glucosides.

The glucoside (III) from *M. sieboldii*, and its aglycone (VIII) were judged by their chromatographic behaviour to have probably one more phenolic hydroxyl group than phloridzin and phloretin. From the alkaline decomposition of the aglycone (VIII) β-(3,4-dihydroxyphenyl)propionic acid was isolated, and phloroglucinol identified chromatographically. Methylation of the aglycone gave 2′-hydroxy-3,4,4′,6′-tetramethoxydihydrochalcone, identified by comparison with an authentic specimen; the aglycone (VIII) is therefore 3-hydroxyphloretin (3,4,2′,4′,6′-pentahydroxydihydrochalcone), and was found to be combined with one molecule of glucose in the natural product (III). Methylation of the latter, followed by acid hydrolysis, gave 4′-hydroxy-3,4,2′,6′-tetramethoxydihydrochalcone, identified by comparison with a synthetic sample. The glucose molecule must therefore be attached at the 4′-position as in (II), and the compound is 3-hydroxyphloretin 4′-glucoside. Zemplén, Bognár, and Szegö [8] synthesised a compound which should be the same as (III), but it appears to differ in melting point and some other properties. No direct comparison has been possible.

It is a curious fact that the aglycones phloretin (IV) and 3-hydroxyphloretin (VIII) both retain a free 2′-hydroxyl on methylation, whereas the free phenolic hydroxyl groups of the corresponding glucosides (II and III) are methylated completely under similar conditions. Phloridzin too is known to undergo complete methylation of its free phenolic hydroxyls, and hence on subsequent acid hydrolysis it gives the same phloretin trimethyl ether as is obtained by the methylation of that aglycone.[9,10]

Acetylation of 3-hydroxyphloretin (VIII) with acetic anhydride and pyridine gave the

[6] Zemplén and Bognár, *Ber.*, 1942, **75**, 645.
[7] Jorio, *Ann. Chim.* (*Italy*), 1959, **49**, 1929.
[8] Zemplén, Bognár, and Szegö, *Ber.*, 1943, **76**, 1112.
[9] Wessely and Sturm, *Monatsh.*, 1929, **53/54**, 554.
[10] Johnson and Robertson, *J.*, 1930, 21.

penta-acetate, but heating with acetic anhydride and sodium acetate under reflux gave another compound, which by analogy with phloretin must be 5,7-diacetoxy-3-(3,4-diacetoxybenzyl)-2-methylchromone (IX). The glucoside (III) gave by the pyridine method an octa-acetate, and by the sodium acetate method 5-acetoxy-3-(3,4-diacetoxybenzyl)-2-methyl-7-(tetra-*O*-acetyl-β-D-glucosyloxy)chromone (X). Acid hydrolysis of both (IX) and (X) gave 5,7-dihydroxy-3-(3,4-dihydroxybenzyl)-2-methylchromone (XI).

Experimental

Paper chromatograms of phenolic substances were run with butan-2-ol–acetic acid–water (69 : 2 : 29) and acetic acid–water (1 : 49), and the spots revealed by *p*-nitrobenzenediazonium fluoroborate according to Freeman's method.[11] Alcoholic aluminium chloride shows many of the dihydrochalcone derivatives as fluorescent spots in ultraviolet light. Those with two free hydroxyls *ortho* to the carbonyl group show a yellowish-green and those with one free *o*-hydroxyl a bluish-green fluorescence.

Extraction of the Leaf.—Fresh leaf of the appropriate species was boiled in ethanol (1 : 3 w/v) for a few minutes, and the mixture then cooled and homogenised. After 24 hr. the whole was filtered, and the solid residue extracted twice more with ethanol. The combined extracts were evaporated under reduced pressure to about one-tenth of their volume and any solid filtered off from the now aqueous concentrate, which was twice extracted with light petroleum to remove fat and chlorophyll.

Isolation of Phloretin 4′-Glucoside (II).—The aqueous concentrate from the leaf of *M. trilobata* was extracted three times with ethyl acetate; removal of the solvent in a vacuum left a brown solid which after several crystallisations from 40% methanol (1 : 10 w/v) gave very pale yellow crystals (0·5—1% of wt. of fresh leaves), collapsing to a pale cream powder when dried over P_2O_5, of *glucoside*, m. p. 166°, $[\alpha]_D^{20}$ −70° (2% in EtOH) (Found: C, 57·6; H, 5·6. $C_{21}H_{24}O_{10}$ requires C, 57·8; H, 5·5%). Zemplén and Bognár [6] record for their compound m. p. 170—173°, $[\alpha]_D^{14}$ −99·5° (in EtOH). A specimen prepared by Jorio [7] by their method had m. p. 162—169° and mixed m. p. with (II) 157—162°; the compounds do not seem to be identical.

Hydrolysis of Phloretin 4′-Glucoside (II).—Dilute hydrochloric acid, or emulsin, under the usual conditions gave equimolecular proportions of glucose and phloretin in almost theoretical yields. The sugar was identified by chromatography in three solvents and its identity confirmed by conversion into the β-penta-acetate, m. p. 132°. The aglycone was identified as phloretin, m. p. and mixed m. p. 264°, which with acetic anhydride and pyridine at room temperature for 2 hr. gave the tetra-acetate, m. p. and mixed m. p. 94—95° (from ethanol), and with acetic anhydride and sodium acetate under the conditions described by King and Robertson [12] gave 5,7-diacetoxy-3-4′-acetoxybenzyl-2-methylchromone, m. p. and mixed m. p. 173°.

Methylation of Phloretin 4′-Glucoside (II).—Treatment with dimethyl sulphate in the presence of acetone and potassium carbonate, in the conditions of Hergert, Coad, and Logan,[13] gave a gum, which was hydrolysed by 1% hydrochloric acid in hot 50% aqueous methanol to phloretin 4,2′,6′-trimethyl ether (yield *ca.* 10%), m. p. 144° after repeated crystallisation from aqueous ethanol. Its m. p. was undepressed when mixed with an authentic sample, m. p. 144°, prepared, not by the method of Johnson and Robertson [10] (they give 142°), but through the corresponding 4′-hydroxy-4,2′,6′-trimethoxychalcone (m. p. 196°) obtained from 4-hydroxy-2,6-dimethoxyacetophenone by an adaption of the method of King and Robertson.[14]

Acetylation of Phloretin 4′-Glucoside (II).—(*a*) Acetic anhydride and pyridine gave the *hepta-acetate* (70%), m. p. 137° (from ethanol) (Found: C, 57·9; H, 5·1. $C_{35}H_{38}O_{17}$ requires C, 57·6; H, 5·2%). (*b*) The glucoside (1 g.) was heated under reflux for 4 hr. with acetic anhydride (5 ml.) and anhydrous sodium acetate (0·5 g.). After separation in the usual manner *5-acetoxy-3-4′-acetoxybenzyl-2-methyl-7-(tetra-*O*-acetyl-β-D-glucosyloxy)chromone* (V) was obtained (55%), having m. p. 210° after several crystallisations from ethanol (Found: C, 59·1; H, 5·1. $C_{35}H_{36}O_{16}$ requires C, 59·0; H, 5·1%). Deacetylation and hydrolysis under reflux with 50%

[11] Freeman, *Analyt. Chem.*, 1952, **24**, 955.
[12] King and Robertson, *J.*, 1934, 403.
[13] Hergert, Coad, and Logan, *J. Org. Chem.*, 1956, **21**, 304.
[14] King and Robertson, *J.*, 1931, 1704.

aqueous methanol containing about 3% of hydrogen chloride gave 5,7-dihydroxy-3-4′-hydroxybenzyl-2-methylchromone (VI) (60%), m. p. 216° (from 50% methanol–water), undepressed when mixed with an authentic sample, m. p. 217°, from phloretin.[12]

Acetylation of Phloridzin.—The conditions described above for the acetylation of phloretin 4′-glucoside with acetic anhydride and sodium acetate gave only a very small yield of crystals. At 160° (sealed tube) a much better yield (25%) was obtained; crystallisation from ethanol gave *7-acetoxy-3-4′-acetoxybenzyl-2-methyl-5-(tetra-*O*-acetyl-β-*D*-glucosyloxy)chromone* (VII), m. p. 214° (softens at 203°) (Found: C, 58·8; H, 5·1. $C_{35}H_{36}O_{10}$ requires C, 59·0; H, 5·1%). Deacetylation and hydrolysis gave the hydroxychromone (VI), m. p. 217°.

Isolation of 3-Hydroxyphloretin 4′-Glucoside (III).—To the aqueous concentrate from leaf of *M. sieboldii arborescens*, about one-tenth of its volume of saturated salt solution was added, and the small dark precipitate discarded. The clear solution was nearly saturated with salt, and left overnight in the refrigerator. The precipitate of crude glucoside was filtered off, dried thoroughly, and crystallised several times from ethanol (1 : 10, w/v). The pale cream *3-hydroxyphloretin 4′-glucoside* (yield 1%, on fresh leaf) retained one molecule of water even when dried in vacuum at 100°. It melted, with browning, over the range 125—140°, $[\alpha]_D^{20}$ −58° (2% in EtOH) (Found: C, 53·6; H, 5·6. $C_{21}H_{24}O_{11},H_2O$ requires C, 53·6; H, 5·5%). Zemplén, Bognár, and Szegö [8] record their compound as crystallising with three molecules of water, completely removed in a vacuum at 100°; the anhydrous compound softened at 155° and melted completely at 221°. Their acetyl derivative was amorphous. No analysis was given for the glucoside or its acetate.

Hydrolysis of 3-Hydroxyphloretin 4′-Glucoside (III).—Dilute hydrochloric acid, or emulsin, gave equimolecular proportions of glucose (identified as the β-penta-acetate) and *3-hydroxyphloretin*, in almost theoretical yields. This compound, too, retained a molecule of water after vacuum-drying at 100°, and melted at 232° (Found: C, 58·4; H, 5·2. $C_{15}H_{14}O_6,H_2O$ requires C, 58·4; H, 5·2%). From the products of alkaline decomposition, β-(3,4-dihydroxyphenyl)propionic acid was isolated, having m. p. and mixed m. p. 138°. The structure of the aglycone was confirmed by methylation with dimethyl sulphate in the presence of potassium carbonate and acetone, which gave 2′-hydroxy-3,4,4′,6′-tetramethoxydihydrochalcone, m. p. 125°, identical with a sample kindly supplied by Professor A. Robertson.[14]

Zemplén, Bognár, and Szegö [8] record m. p. 224° for their specimen of 3-hydroxyphloretin; no other details are given, and a direct comparison has not been possible.

Acetylation of 3-Hydroxyphloretin.—(*a*) Acetic anhydride and pyridine gave the *penta-acetate* (65%), m. p. 78—79° (from ethanol) (Found: C, 60·2; H, 5·0. $C_{25}H_{24}O_{11}$ requires C, 60·0; H, 4·8%). (*b*) Acetic anhydride and sodium acetate, under the conditions used for phloretin 4′-glucoside, gave *5,7-diacetoxy-3-(3,4-diacetoxybenzyl)-2-methylchromone* (IX) (35%), m. p. 155° after several crystallisations from ethanol (Found: C, 62·5; H, 4·8. $C_{25}H_{22}O_{10}$ requires C, 62·4; H, 4·6%). Deacetylation gave *5,7-dihydroxy-3-(3,4-dihydroxybenzyl)-2-methylchromone* (XI) (77%), m. p. 226° (from aqueous methanol) (Found: C, 64·6; H, 4·7. $C_{17}H_{14}O_6$ requires C, 65·0; H, 4·5%).

Methylation and Hydrolysis of 3-Hydroxyphloretin 4′-Glucoside.—The method previously described gave 4′-hydroxy-3,4,2′,6′-tetramethoxydihydrochalcone (25%), m. p. 107° after repeated crystallisation from aqueous methanol and undepressed by an authentic sample prepared by the method of King and Robertson [14] through the corresponding chalcone.

Acetylation of 3-Hydroxyphloretin 4′-Glucoside.—(*a*) Acetic anhydride and pyridine gave the *octa-acetate* (60%), m. p. 152° (from ethanol) (Found: C, 56·6; H, 5·1. $C_{37}H_{40}O_{19}$ requires C, 56·4; H, 5·1%). (*b*) Acetic anhydride and sodium acetate (conditions as for phloretin 4′-glucoside) gave *5-acetoxy-3-(3,4-diacetoxybenzyl)-2-methyl-7-(tetra-*O*-acetyl-β-*D*-glucosyloxy)-chromone* (X) (55%), m. p. 228° (from ethanol) (Found: C, 57·7; H, 5·0. $C_{37}H_{38}O_{18}$ requires C, 57·7; H, 4·9%), which by deacetylation and hydrolysis gave the hydroxychromone (XI) already obtained from 3-hydroxyphloretin.

The author thanks Professor A. Robertson for a specimen of 2′-hydroxy-3,4,4′,6′-tetramethoxydihydrochalcone, and the Director of the Royal Botanic Gardens, Kew, for providing leaf of *Malus trilobata*.

Department of Agriculture and Horticulture, University of Bristol,
Research Station, Long Ashton, Bristol. [*Received, April 26th,* 1961.]