FLAVONOIDS FROM THE LEAVES OF *KALMIA LATIFOLIA*[1]

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ABSTRACT.—The ethanolic residue from the leaves of *Kalmia latifolia* was divided into five partition fractions, of which the ethyl acetate-solubles were separated by chromatography. A total of twelve flavonoids were characterized by physical and chemical methods. Five of the seven dihydrochalcones are new natural products, namely, phloridzin 2"-acetate (**12**), 4-*O*-methylphloridzin (**14**), 3-hydroxyphloretin (**21**), 3-hydroxyphloridzin (**20**) and 3-hydroxyphloridzin 2"-acetate (**23**). The other two dihydrochalcones are phloretin (**3**) and phloridzin (**1**). Also isolated were eriodictyol (**24**), quercetin (**25**), hyperin (**26**), guaijaverin (**27**) and (+)-catechin (**28**), known compounds but not previously recorded from this source. The acetylation products of phloretin (**3**) obtained under different reaction conditions were studied and led to clarification of the literature. The compounds formed are 2',4,4'-triacetylphloretin (**4**), 2',4,4',6'-tetraacetylphloretin (**5**), 2',4,4',6'-tetraacetylphloretin enolacetate (**6**) and 5,7-diacetoxy-3-(4'-acetoxybenzyl)-2-methylchromone (**7**). The triacetylphloretin reported on hydrolysis of phloridzin (**1**), heptaacetate, is actually the monoacetate, 4-acetylphloretin (**8**).

The leaves of *Kalmia latifolia* L. (Ericaceae) gave an ethanolic extract that inhibited the feeding of gypsy moth larvae (*Lymantria dispar* L.) (1). Fractionation studies directed towards isolation of the active constituents resulted in division of the crude extract into five partition fractions, of which the so-called ethyl acetate soluble was the largest and most active (2). However, separation of this material by column chromatography showed that the phenolics, which make up over 93% of the fraction, were not responsible for the antifeeding properties. From the nonphenolic material ten grayanoid diterpenes were obtained, some of which were deterrent, and a report of this work has been made (2). The isolation and identification of the phenolic constituents is the subject of this communication.

Already described from this source are the dihydrochalcone glucosides phloridzin (**1**) (3) and asebotin (**2**) (4), but the claim for the latter has been disputed (5). A reinvestigation of the three recognized *Kalmia* species, *K. latifolia*, *K. angustifolia* and *K. polifolia*, has revealed phloridzin (**1**) to be present only in the first-named species, with asebotin (**2**) in the second and neither in the third (3, 6). More recently, phloretin (**3**), the aglycone of phloridzin and 2',6'-dihydroxy-4-methoxyacetophenone as well as asebotin (**2**), were described from *K. latifolia* (7). Our work has uncovered seven dihydrochalcones—including phloridzin (**1**) and phloretin (**3**)—three flavonols (two as glycosides), a flavanone and a flavan-3-ol.

RESULTS AND DISCUSSION

The ethyl acetate partition fraction (2) on separation by chromatography on silicic acid provided column fractions that were further purified on a column of Sephadex LH-20, followed by crystallization. A summary of the compounds ob-

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tained and the order of elution is given in table 1. The dihydrochalcones are considered first.

Phloridzin (**1**) was identified from spectral data and was confirmed by direct comparison with an authentic sample. Phloretin (**3**) was identified from spectral data and comparison of physical properties with literature values (8). The preparation of the acetate derivative for the purpose of confirmation led to the discovery of inconsistencies in the literature. For example, phloretin tetraacetate is variously reported to have a melting point between 93.5–4.5° (8, 10 and 11) and

TABLE 1. Results of chromatography of the ethyl acetate solubles on a silicic acid column.[a]

Fr.	Tube No.	Residue Wgt. g	Compounds	R_f[b]	Fr.	Tube No.	Residue Wgt. g	Compounds	R_f[b]
A	1–19	0.38	nonphenolics		H	71–92	0.45	guaijaverin (**27**)	0.13
B	20–21	0.12	eriodictyol (**24**)	0.48				3-hydroxyphloridzin 2"-acetate (**23**)	0.12
C	22–25	0.23	phloretin (**3**)	0.42					
D	26–35	1.54	phloretin (**3**)		I	93–157	6.02	phloridzin (**1**)	0.11
			quercetin (**25**)	0.38				hyperin (**26**)	0.07
E	36–41	0.46	3-hydroxyphloretin (**21**)	0.30	J	158–186	0.50	hyperin (**26**)	
			4-*O*-methylphloridzin (**14**)	0.25	K	187–271	2.56	(+)-catechin (**28**)	0.12
F	42–53	1.17	4-*O*-methylphloridzin (**14**)					3-hydroxyphloridzin (**20**)	0.06
G	54–70	0.84	phloridzin 2"-acetate (**12**)	0.19	L	272–500	7.60	residue (not studied)	

[a]For details, see the Experimental section.
[b]Tlc R_f values with Solvent System A.

also at 165° (9). Our material, obtained after several days treatment with acetic anhydride in pyridine at room temperature, melted at 169° and exhibited an ^{1}H-nmr spectrum not in accord with a tetraacetate structure. First, there was absorption for five acetate methyls (four singlets with one of double intensity); and, second, the expected four-proton methylene pattern for the α- and β-protons was not present. Instead there was present a one-proton triplet and a two-proton doublet, with both patterns located at lower field positions. The structure of this pentaacetate will be considered in detail later. Acetylation for a shorter period of time (24 hrs) produced three compounds which were separated chromatographically. The most polar component was the pentaacetate of the longer reaction time.

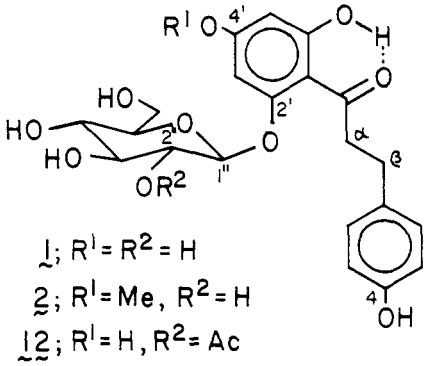
The other two products were both obtained crystalline from methanol and showed essentially the same melting point. The least polar, mp 94–5°,[5] possessed spectral properties of a triacetate with two peaks (one of double intensity) in the ^{1}H-nmr spectrum for three acetates. The AA'BB'-type quartet in the aromatic region along with a pair of doublets (J 2.5 Hz) for the *meta* protons and the four-proton methylene multiplet indicated no skeletal rearrangement during acetylation. A deuterium oxide exchangeable proton at 12.86 ppm for an intramolecular hydrogen-bonded phenolic group (12) and the dissimilar chemical shifts for the *meta* protons (H–3' and H–5') required that the phloroglucinol ring be unsymmetrically substituted. Therefore, the compound must be 2',4,4'-triacetylphloretin (**4**). The mass and infrared spectra were consistent with this assignment.

The acetate of intermediate polarity, mp 93–4°,[5] exhibited in the ^{1}H-nmr spectrum two singlets in the acetate region, each integrating for six protons. The

[5]A mixture (1:1) melting point of the triacetate **4** and the tetraacetate **5** was 75–84°.

aromatic region still contained the AA'BB'-type quartet for the protons of the disubstituted phenyl ring, but the *meta* protons of the phloroglucinol ring formed a two-proton singlet as would be expected for a symmetrically substituted ring. This and other spectral data are in agreement with the structure 2',4,4',6'-tetraacetylphloretin (**5**). Since the triaacetate **4** and the tetraacetate **5** have nearly identical melting points, one wonders which products were recorded in the literature.

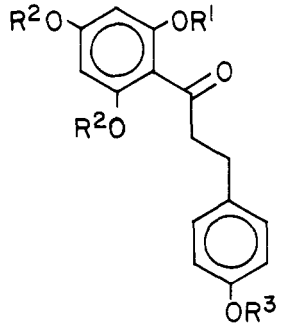
Returning to the pentaacetate, mp 169°, the structure (*Z*)-2',4,4',6'-tetraacetylphloretin enolacetate (**6**), was assigned on the following evidence. The ^{1}H-nmr spectrum showed four peaks (one of double intensity) for five acetates, a



1; $R^1 = R^2 = H$

2; $R^1 = Me$, $R^2 = H$

12; $R^1 = H$, $R^2 = Ac$



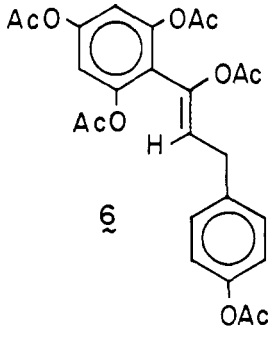
3; $R^1 = R^2 = R^3 = H$

4; $R^1 = H$, $R^2 = R^3 = Ac$

5; $R^1 = R^2 = R^3 = Ac$

8; $R^1 = R^2 = H$, $R^3 = Ac$

9; $R^1 = H$, $R^2 = Me$, $R^3 = Ac$



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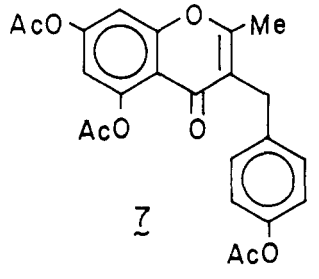

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one-proton triplet at 5.51 ppm and a two-proton doublet at 3.41 ppm, both patterns with *J* 7.6 Hz. These were assigned to vinylic and allylic (benzylic) protons, respectively. Since the ir spectrum lacked absorption for an aromatic ketone, the spectral data could be accommodated by an enol acetate function as in **6**. The two bulky phenyl units were placed *trans*, on the basis that the *trans* enol would be sterically favored over the *cis* enol. The ^{13}C-nmr spectrum[6] contained

[6]A study of the ^{13}C-nmr properties of phloretin (**3**) and its derivatives is underway and the results will be published separately.

only one carbon in the methylene-methine region, at 32.1 ppm, and appears as a triplet under off-resonance decoupling conditions. Irradiation of the two-proton doublet at 3.41 ppm collapsed the pattern at 32.1 ppm to a singlet, and irradiation of the one-proton triplet at 5.51 ppm converted an off-resonance-appearing doublet at 123.6 ppm to a singlet. The C-α and C-β carbons were located in this matter and provide support for the enol acetate structure **6**. It is clear that the phloretin acetate of the literature (9) with reported mp 165° must be formulated as the pentaacetate **6**.

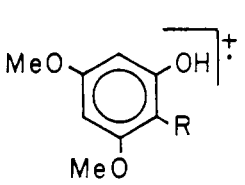
The acetylation products considered were formed under relatively mild conditions, and no cyclized compound was produced, as is reported with acetic anhydride and sodium acetate at 100° or higher (13). The product under these conditions is 5,7-diacetoxy-3-(4'-acetoxybenzyl)-2-methylchromone (**7**), which, incidently, has a melting point around 170°. The other physical properties can allow for easy differentiation from the pentaacetate **6**. In our hands, the pentaacetate **6** is obtained as the major product at steam bath temperature (see Experimental).

One last phloretin acetate recorded in the literature (14) needs clarification. Treatment of phloridzin (**1**) heptaacetate with hydrobromic acid gave a crystalline substance, mp 188–9°, considered to be 2',4,4'-triacetylphloretin (**4**). Since the melting point was significantly higher (by 94°) than our triacetate, the original structure proposed appeared in doubt, although the existence of polymorphs was a possibility. Repeating the hydrolysis as stated (14) gave a crystalline product, mp 195.5–7.0° in reported yield, but the spectral data were inconsistent with a triacetate. The ^{1}H-nmr spectrum contained a single three-proton singlet for an acetate at 2.23 ppm. The remainder of the spectrum was as expected for the phloretin system, with the additional note that the *meta* protons, H-3' and H-5', formed a two-proton singlet. Thus the phloroglucinol ring must be symmetrically substituted, and requires that the acetate be placed at C-4 or C-4'. The C-4 position appeared more likely since the chemical shift of the phloroglucinol *meta*-protons was not different from that of phloretin (**3**), while the AA'BB' pattern of the disubstituted aromatic system was moved upfield about 0.25 ppm. The mass spectrum supported the monoacetate formula of $C_{17}H_{16}O_6$ but did not indicate clearly the location of the acetate.

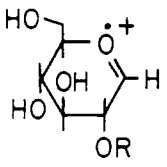
Treatment of the monoacetate **8** with diazomethane produced 4-acetyl-2',4'-dimethylphloretin (**9**), whose structure was determined from spectral data. The phenolic group at 13.99 ppm in the ^{1}H-nmr spectrum requires an intramolecular hydrogen-bonded location, and the two very intense peaks in the mass spectrum at *m/e* 181 and 154 for fragments **10** and **11**, respectively, place the methyl ethers in the phloroglucinol ring. Therefore, the acetate must be located in the benzylic ring, and the product formed by hydrobromic acid cleavage of phloridzin heptaacetate is 4-acetylphloretin (**8**).

A new glycoside, mp 117–20°d, was isolated, which from spectral and chemical studies was assigned the structure phloridzin 2''-acetate (**12**). In the ^{1}H-nmr spectrum, the expected peaks for the aromatic protons of a phloretin unit were present, such as the AA'BB' "quartet" for the disubstituted phenyl protons and a pair of doublets for the *meta* protons of the phloroglucinol ring. In addition, a three-proton singlet for an acetate was present at 2.02 ppm and a one-proton "triplet" at 5.07 ppm was designated for the proton on the acetate-bearing carbon. Since acetylation afforded phloridzin heptaacetate, only location of the acetate was needed to make a unique structural assignment for the glycoside. The chemical ionization mass spectrum with an intense peak at *m/e* 205, for

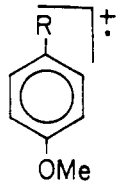
fragment **13**, placed the acetate on the glucose moiety. The second carbon (C–2") was favored, since the glycoside was resistant to hydrolysis by emulsin. Phloridzin (**1**), on the other hand, was cleaved. Also, the anomeric proton doublet at 5.39 ppm and the one-proton triplet, both with J 8.3 Hz, have peak intensities skewed towards each other, indicating a coupling relationship. From the pattern and coupling constant value of the proton on the acetate-bearing carbon, the acetate could be placed at either carbons 2", 3" or 4"; but only at C–2" would coupling to



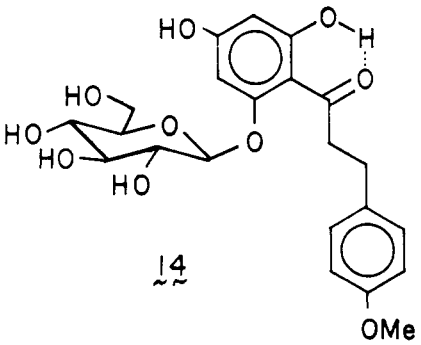
10, R = CO
11, R = H



13, R = Ac
15, R = H



16, R = CH=CH$_2$
17, R = CH$_2$



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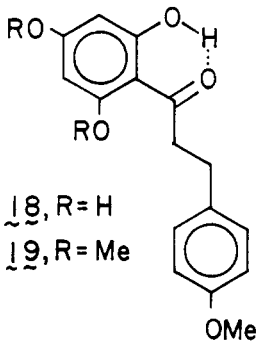
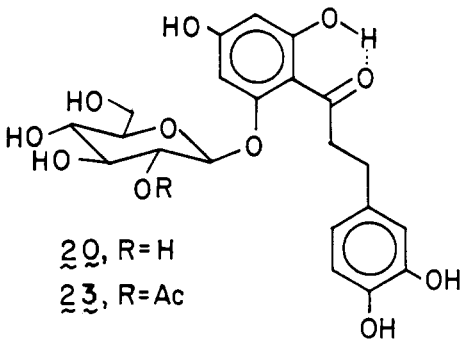
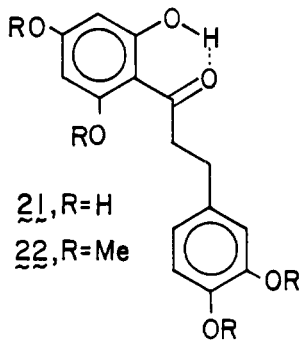


18, R = H
19, R = Me



20, R = H
23, R = Ac



21, R = H
22, R = Me

the anomeric proton occur. Irradiation at the anomeric proton frequency in a double resonance experiment at 270 MHz collapsed the triplet to a sharp doublet and supported the location of the acetate at C–2".

A methoxy-containing glycoside **14**, mp 198–201°, was isolated which has physical properties different from asebotin (**2**), mp 147.5°, (15 and references therein). Its ^{1}H-nmr spectrum has features similar to that of phloridzin (**1**)

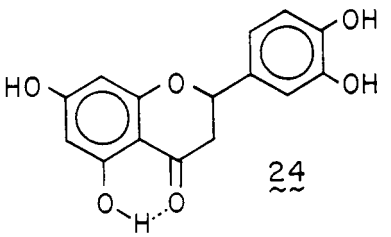
with the exclusion of the sharp three-proton singlet at 3.75 ppm for the methoxy. Elemental analyses support the formula $C_{22}H_{26}O_{10}$, and the mass spectrum, although not showing a molecular ion peak, contains fragment ions consistent with a methoxyphloridzin structure. In particular, the spectrum under chemical ionization conditions exhibits the base peak at *m/e* 289, which is the protonated methoxy phloretin fragment, and an intense peak at *m/e* 163 for the hexose fragment **15**. Accurate mass measurement of fragment ions *m/e* 134 and 121 is in agreement with formulas $C_9H_{10}O$ and C_8H_9O, respectively. These fragments with structures **16** and **17** require that the methoxy group be located at C–4. Acid hydrolysis of glycoside **14** gave an aglycone, mp 197–8°, identified as 4-*O*-methylphloretin (**18**) (16) from spectral features and comparison of physical properties with those in the literature, and glucose, identified chromatographically. Since a similar cleavage was readily accomplished by the enzyme emulsin, the glucosidic linkage must be β. Of the two locations, C–2' or C–4', for glucose attachment, the former was favored. First, the 4'-glucoside is known (16) and has mp 69–70° (hydrated) and 110° (anhydrous). Second, methylation of glucoside **14** followed by hydrolysis afforded in good yield 2',4,4'-trimethylphoretin (**19**), identical with the trimethyl ether prepared from phloridzin (**1**) in the same manner. The new glycoside is therefore 4-*O*-methylphloridzin (**14**).

The most polar new glycoside, mp 171–3°, was identified as 3-hydroxyphloridzin (**20**). The ^{1}H-nmr spectrum revealed similarities to that of phloridzin (**1**); for example, the benzylic C–β protons and the C–α protons each appear as triplets (the latter partially obscured by the sugar protons), and the sugar protons were little changed in pattern or location. The pair of doublets for H–3' and H–5' of the phloroglucinol ring underscored an unsymmetrical substitution. The clear difference between the two spectra concerned the other aromatic protons. Instead of the four-proton AA'BB' 'quartet", the new compound exhibited a three-proton ABC pattern typical of a 1,2,4 substituted benzene. The additional substituent must be a hydroxyl, since peracetylation yielded an octaacetate, and elemental analyses of the glycoside supported the formula $C_{21}H_{24}O_{11}$ with one more oxygen than phloridzin (**1**). Hydrolysis by acid gave glucose and 3-hydroxyphloretin (**21**) (17). The aglycone is also present in the leaves of *K. latifolia* (see Experimental). The point of attachment of glucose to the dihydrochalcone moiety must be at C–2', since methylation of the glucoside followed by acid hydrolysis yielded 2',4,4'-trimethyl-3-methoxyphloretin [or 3-(3,4-dimethoxyphenyl)-2'-hydroxy-4',6'-dimethoxypropiohenone] (**22**) (18). Hydrolysis by emulsin requires a β-linkage for the glucose. Sieboldin (4'-glucosyl 3-hydroxyphloretin), mp 125–40°, is a position isomer of 3-hydroxyphloridzin and has been reported from *Malus sieboldii* (8).

Another acetate-containing glucoside was identified as 3-hydroxyphloridzin 2''-acetate (**23**) from analysis of spectral data and the preparation of the peracetylated product, identical with 3-hydroxyphloridzin (**20**) octaacetate. Double irradiation studies at 270 MHz located the acetate on the second carbon of the glycosyl unit. The anomeric proton as a doublet at 5.38 ppm (J 8 Hz) collapses to a singlet when the triplet at 5.10 ppm with the same coupling constant is irradiated. The reverse irradiation experiment caused the triplet to change to a doublet.

Besides the seven dihydrochalcones discussed, five known flavonoids were also obtained. *Racemic* eriodictyol (**24**) (19) was identified by comparison of its physical properties and those of its acetate derivative with the values reported in

the literature (20). Quercetin (**25**) (21) and hyperin (**26**) (22) were identified from physical data and by direct comparison with known samples. The latter compound was also hydrolyzed and the products quercetin (**25**) and galactose were identified by comparison with known compounds. Guaijaverin (quercetin-3-α-*L*-arabofuranoside or fenicularin) **27** (23, 24) was characterized from spectral properties, comparison of physical data with those in the literature, and by direct comparison of the hydrolysis products, quercetin (**25**) and arabinose, with known samples. (+)-Catechin (**28**) (25), was identified from physical properties and by direct comparison with an authentic sample. The pentaacetate and tetramethyl derivatives were also directly compared with known materials.



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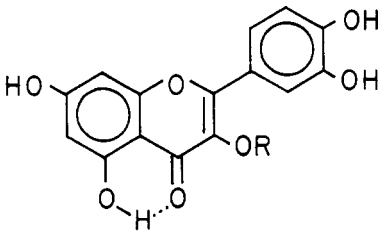
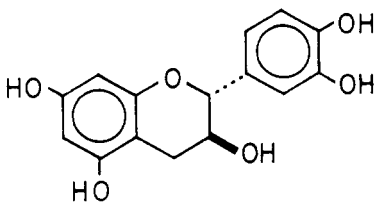


25, R = H
26, R = Gal
27, R = Ara



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EXPERIMENTAL[7]

CHROMATOGRAPHY OF THE ETHYL ACETATE SOLUBLE FRACTION.—The ethyl acetate solubles (25 g) designated as Fraction F in reference 2 were chromatographed on 2.4 kg of silicic acid containing 13.5% water. The column was poured as a slurry in solvent system A. The sample was dissolved in 30 ml of methanol, adsorbed onto 100 g of silicic acid, and dried in a rotary evaporator for 3 hrs, then adjusted to a weight containing 13.5% water. The mixture was applied to the top of the column and eluted with solvent system A. After 1.6 liters of effluent emerged, 85 ml fractions were mechanically collected and evaporated at reduced pressure. The residues were weighed and examined by tlc on silica gel G with solvent system A, then pooled to provide fractions containing one major constituent.

Further purification of silicic acid column fractions was performed on a column of 100 g of Sephadex LH-20 poured as a slurry in methanol. The sample was placed on the column in methanol and eluted with the same solvent. After passage of the hold-up volume (190 ml), 2.5 ml fractions were mechanically collected, evaporated, and the residues examined by tlc. Similar fractions were combined and yielded the crystalline products reported below. The order of emergence of the compounds from the silicic acid column is the order followed in their presentation and is summarized in table 1.

ERIODICTYOL (**24**).—Both pooled silicic acid column Fractions 20–21 and 22–25 after chromatography on Sephadex LH-20 gave subfractions which afforded crystalline eriodictyol (**24**)

[7]The conditions and instruments employed, the source of plant material and its extraction, plus the partitioning of the extract residue are given in reference 2. Solvent systems: A) chloroform-methanol-water (60:15:4, lower phase), and B) benzene-ethyl acetate (4:1). Hyperin and phloridzin were purchased from Pfaltz and Bauer, Inc., Stamford, Conn., and (+)-catechin from Sigma Chemical Co., St. Louis, Mo.

as needles (117 mg, 2 x 10^{-2}% of dried leaves) from methanol: mp 268° d [lit. value (19) mp 265–6°]; $[\alpha]_D$ 0° (*c* 0.47, MeOH); uv λ max 324 nm (sh, log ε 4.19), 289 (4.66) and 227 (sh, 4.71) and with flavonoid diagnostic reagents (20) NaOMe 323, 245; $AlCl_3$ 375, 309; $AlCl_3$/HCl 375, 309; NaOAc 325, 289 sh; and NaOAc/H_3BO_3 335 sh, 290, 231; which agree with those reported (p. 217 of reference 20); ir (KBr) ν max 3370, 1640, 1608, 1160 and 900 cm^{-1}; ^{1}H-nmr (90 MHz, acetone-d_6) δ 2.75, 3.14 and 5.38 [ABX analyzed, H-3 (2H) and H-2, *J* 17, 13, 4], 5.96 (s, H-6 and H-8), 6.87 and 7.04 (ABC pattern as 2s with the second peak of double intensity, H-2', H-5' and H-6'), and 12.17 (s, H-bonded OH, lost with D_2O).

Acetylation of eriodictyol (**24**, 30 mg) with acetic anhydride (0.4 ml) in pyridine (0.5 ml) at ambient temperature for 2 days gave, after crystallization from methanol, 23 mg of eriodictyol tetraacetate: mp 141–2° [lit. value (19) 136–7°]; $[\alpha]^{25}_D$ 0° (*c* 1.4, $CHCl_3$); uv λ max 315 nm (log ε 3.59) and 261 (3.88); ir ($CHCl_3$) ν max 1775, 1692, 1618 and 1175–1195 and 900 cm^{-1}; ^{1}H-nmr (60 MHz, chloroform-d) δ 2.29 (s, 3 Ac), 2.37 (s, Ac), 2.75–3.05 and 5.46 (ABXm, H-3 (2H) and H-2), 6.53 and 6.78 (ABq, H-6 and H-8, *J* 2.5), and 7.2–7.3 (ABCm, H-2', H-5' and H-6').

PHLORETIN (**3**).—The pooled silicic acid column Fractions 22–25 and 26–35, after separation on a Sephadex LH-20 column, gave subfractions that crystallized from aqueous methanol as fine needles (296 mg, 5 x 10^{-2}%) of phloretin (3): mp 263° d [lit. value (8) mp 264°]; uv λ max 286 nm (log ε 4.35), 225 (4.38) and with NaOMe 321 (4.46); ir (KBr) ν max 3250, 1605, 1515, 1165, 1075 and 825 cm^{-1}; ^{1}H-nmr (90 MHz, acetone-d_6) δ 2.7–3.5 (AA'BB'm, H-α and H-β), 5.93 (s, H-3' and H-5'), and 6.6–7.2 (AA'BB'q, H-2, H-3, H-5 and H-6); and ms (ei) *m/e* 274 (67%, M^-, $C_{15}H_{14}O_5$), 153 (100), 120 (64) and 107 (60), and (ci) *m/e* 275 (100%, M+1).

ACETYLATION OF PHLORETIN (**3**) WITH ACETIC ANHYDRIDE AND PYRIDINE.—A) *Reaction Time-24 Hours*: A 28 mg sample of phloretin (**3**) was treated for 24 hrs with 0.4 ml of acetic anhydride in 0.6 ml of pyridine at ambient temperature. Ice (2 g) was added to the reaction mixture, followed by extraction with chloroform. The chloroformic phase was washed with 1% hydrochloric acid, 1% sodium bicarbonate solution, and water then dried over anhydrous sodium sulfate. The residue left after evaporation of the chloroform showed three spots on tlc with solvent system B at R_f 0.31, 0.38 (major) and 0.44. Chromatography on a column of silicic acid (8 g) with benzene-ethyl acetate (9:1) as solvent and analysis of 5 ml effluent fractions yielded the three products.

The first eluted compound (R_f 0.44), phloretin 2',4,4'-triacetate (**4**), crystallized from methanol as prisms (9 mg): mp 94–5°; uv λ max 324 nm (sh, log ε 3.03) and 259 (3.54) with $AlCl_3$ a shift to 285 nm; ir ($CHCl_3$) ν max 1775, 1635, 1370, 1220, 1193, 1177 and 1132 cm^{-1}; ^{1}H-nmr (90 MHz, chloroform-d) δ 2.23 (s, Ac), 2.29 (s, 2 Ac), 2.9–3.4 (m, 4H, H-α and H-β), 6.49 and 6.68 (2d, H-3' and H-5', *J* 2.5), 6.9–7.3 (AA'BB'q, H-2, H-3, H-5 and H-6) and 12.86 (s, OH, lost in D_2O); and ms *m/e* 400 (18%, M^+, $C_{21}H_{20}O_8$), 382 (3), 358 (35), 340 (4), 316 (31), 274 (8), 256 (17), 237 (4), 195 (24), 153 (100), 120 (100), 107 (50), 78 (12) and 43 (35).

The second eluted product (R_f 0.38), phloretin tetraacetate (**5**), crystallized from methanol as robust rhomboids, mp 93–4°; uv λ max 313 (sh, log ε 2.92), 246 (sh, 3.77) and 220 (end, 4.27; ir ($CHCl_3$) ν max 1775, 1700, 1615, 1502, 1420, 1367, 1225, 1190, 1180, 1123, 1047 and 1020 cm^{-1}; ^{1}H-nmr (90 MHz, $CDCl_3$) δ 2.13 and 2.28 (2s, 4 Ac), 2.95–3.10 (m, 4H, H-α and H-β), 6.92 (s, H-3' and H-5'), 6.99 and 7.22 (AA'BB'q, H-2, H-3, H-5 and H-6, J_{AB} 9.6); and ms *m/e* 442 (4%, M^+, $C_{23}H_{22}O_9$), 400 (8), 382 (4), 316 (9), 298 (3), 279 (1), 237 (8), 195 (10), 153 (14), 120 (12), 107 (6), 78 (100) and 43 (32).

The third product (R_f 0.31), phloretin tetraacetate enolacetate (**6**), is described below as the product of the longer time acetylation.

B) *Reaction Time-96 Hours*: Acetylation of 30 mg of phloretin (**3**) as described in part A, but for 96 hrs, gave after the same work-up (less chromatography) a quantitative yield of 2',4,4',6'-tetraacetylphloretin enolacetate (**6**) as large needles: mp 169°; uv λ max 312 (log ε 2.5) and 236 (sh, 4.14); ir ($CHCl_3$) ν max 1770, 1620, 1510, 1483, 1427, 1372, 1225, 1195, 1127, 1048 and 1023 cm^{-1}; ^{1}H-nmr (90 MHz, $CDCl_3$) δ 2.08, 2.15 (double intensity), 2.25 and 2.28 (4s, 5 Ac), 3.41 (d, 2H, H-β, *J* 7.6) 5.51 (t, H-α, *J* 7.6), 6.85 (s, H-3' and H-5') and 6.9–7.3 (AA'BB'm, H-2, H-3, H-5 and H-6); and ms *m/e* 484 (7%, M^+, $C_{25}H_{24}O_{10}$), 440 (16), 424 (19), 400 (21), 382 (42), 358 (37), 340 (45), 316 (54), 298 (49), 274 (12), 256 (81), 237 (18), 195 (25), 166 (12), 153 (38), 120 (40), 107 (47) and 43 (100).

ACETYLATION OF PHLORETIN (**3**) WITH ACETIC ANHYDRIDE AND SODIUM ACETATE.—A) *At Steam Bath Temperature*: A mixture of 50 mg of phloretin (**3**), 100 mg of freshly fused sodium acetate, and 1 ml of acetic anhydride was heated on a steam bath for 10 hrs. After evaporation of the mixture at reduced pressure, the residue was treated with 5 g of ice. A heavy oil separated, which was taken up in chloroform by extraction (3 x 5 ml). Evaporation of the water-washed (4 x 5 ml) chloroform phase left a colorless liquid that crystallized directly. Recrystallization from ethanol gave 55 mg of 2',4,4',6'-tetraacetylphloretin enolacetate (**6**), mp 169°, identical (mp, ^{1}H-nmr and ir) with the product obtained with acetic anhydride and pyridine at room temperature for several days.

B) *At Reflux Temperature*: A mixture of 200 mg of phloretin (**3**), 400 mg of freshly fused sodium acetate and 4 ml of acetic anhydride was refluxed for 11 hrs. Evaporation of the

mixture under reduced pressure, followed by mixing of the residue with 10 g of ice separated a dark brown oil, which was taken up in chloroform (3x 5 ml). The chloroform extract was washed with water (4 x 5 ml) and evaporated. The residue was dissolved in 5 ml of benzene-ethyl acetate (8:2) and passed into a column of silicic acid (2 g). Elution with the same solvent gave 357 mg of a yellow residue that crystallized from ethanol to give 5,7-diacetoxy-3-(4'-acetoxybenzyl)-2-methylchromone (**7**), mp 171–2° [lit. values, mp 173° (3), 170° (13)]; uv λ max 296 nm (log ε 3.91) and 230 (4.44); ir ($CHCl_3$) ν max 1775, 1763, 1640, 1625, 1505, 1433, 1370 and 1190 cm^{-1}; ^{1}H-nmr (90 MHz, $CDCl_3$) δ 2.27, 2.33 (double intensity) and 2.43 (3s, 3 Ac and Me), 3.82 (s, 2H, benzylic), 6.79 and 7.16 (2d, H-6 and H-8, *J* 2.2), 6.95 and 7.19 (AA'BB'q, J_{AB} 8.9); and ms *m/e* 424 (6%, $C_{23}H_{20}O_8$ requires 424), 382 (55), 340 (67), 298 (100), 153 (36), 145 (15), 120 (13) and 107 (12).

CLEAVAGE OF PHLORIDZIN (**1**) HEPTAACETATE (ZEMPLEN'S ACETATE).—The procedure of Zemplen *et al.* (14) was followed exactly. A solution of 45 mg of phloridzin heptaacetate in 0.1 ml of chloroform at 0° was mixed with 0.1 ml of a glacial acetic acid solution saturated with hydrogen bromide at 0° and kept at ambient temperature for 3 hrs. After dilution of the reaction mixture with 1.2 ml of chloroform, 1.5 g of ice was added while the mixture was stirred vigorously. A yellow precipitate collected at the interface. The aqueous phase was removed, and the chloroform layer with the precipitate was treated with ice (1 g), stirred well, and the water wash removed. This was repeated seven times, then the whole mixture was filtered by suction. The precipitate was washed with water, then with chloroform, and sucked dry. The precipitate (15 mg, 78%) was crystallized from acetone-water (3:2, 0.15 ml) to give 4-acetylphloretin (**8**) as colorless plates: mp 195.5–7.0° [lit. (14) value, mp 188–9°]; ir (nujol) ν max 3510, 3350, 1730, 1625 (sh), 1595 and 1230 cm^{-1}; ^{1}H-nmr (90 MHz, acetone-d_6) δ 2.23 (s, 3H, Ac), 2.8–3.1 (m, 2H, β-H), 3.3–3.5 (m, 2H, α-H), 5.94 (s, 2H, H-3' and H-5'), 7.02 and 7.31 (AA'BB'q, 4H, J_{AB} 8.6); and ms *m/e* 316 (23%, M^+, $C_{17}H_{16}O_6$), 274 (39), 256 (14), 195 (3), 168 (12), 153 (100), 120 (74) and 107 (53).

Methylation of 15 mg of the monoacetate **8** in methanol with excess ethereal diazomethane for 2 hrs at −5° gave a residue that was crystallized from methanol to give 12 mg of 4-acetyl-2',4'-dimethylphloretin (**9**): mp 116–7°; ir ($CHCl_3$) ν max 1758, 1623, 1595, 1220, 1190, 1160, 1115 and 820 cm^{-1}; ^{1}H-nmr (90 MHz, $CDCl_3$) δ 2.29 (s, Ac), 2.9–3.1 (m, 2H, β-H), 3.2–3.4 (m, 2H, α-H), 3.82 and 3.83 (2s, 2 MeO), 5.92 and 6.08 (2d, *J* 2, H-3' and H-5'), 7.00 and 7.24 (AA'BB'q, 4H, J_{AB} 8.7) and 13.99 (s, OH at C-6', lost with D_2O); and ms *m/e* 344 (19%, M^+, $C_{18}H_{20}O_6$), 302 (5), 284 (4), 181 (100, $C_9H_9O_4$), 154 (56, $C_8H_{10}O_3$), 149 (10), 131 (12), 120 (21), and 107 (11).

QUERCETIN (**25**).—The pooled silicic acid column Fraction 26–35 after separation on a Sephadex LH-20 gave a subfraction that from methanol formed microcrystalline needles (29 mg, 5 x 10^{-3}% yield) of quercetin (**25**): mp 317° d [lit. value (21) mp 316–8°]; uv λ max 372 nm (log ε 4.34), 300 (sh, 3.89), 256 (4.33), and with flavonoid diagnostic reagents (see p. 126 of reference 20) NaOMe 332; $AlCl_3$ 456, 330, 273; $AlCl_3$/HCl 426, 363, 305, 268; NaOAc 390, 323, 275; and NaOAc/H_3BO_3 387, 305 sh, 260; ir (KBr) ν max 3370, 1655, 1613 and 1510 cm^{-1}; ^{1}H-nmr (90 MHz, acetone-d_6) δ 6.27 and 6.52 (2d, H-6 and H-8, *J* 2), 6.99 (d, H-3', *J* 9), 7.70 (dd, H-2', *J* 2, 9), 7.83 (d, H-6', *J* 2) and 12.16 (s, OH on C-5). Direct comparison (tlc, uv, ir and ^{1}H-nmr) of the isolated sample with quercetin obtained on hydrolysis of hyperin showed them to be the same.

3-HYDROXYPHLORETIN (**21**).—The pooled silicic acid column Fraction 36–41 was chromatographed on Sephadex LH-20 to give a subfraction that crystallized as tiny prisms (62 mg, 10^{-2}% yield) and was identified as 3-hydroxyphloretin (**21**): mp 228–9° d [lit. value (17) 224°]; uv λ max 325 nm (sh, log ε 3.49), 287 (4.20) and 225 (4.19); ir (KBr) ν max 3300–3400, 1630, 1603, 1520, 1445 and 897 cm^{-1}; and ^{1}H-nmr (90 MHz, acetone-d_6) δ 2.82 and 3.33 (AA'BB' 2t, H-α and H-β, J_{AB} 7.6), 5.93 (s, H-3' and H-5'), 6.5–6.8 (ABCm, H-2, H-3 and H-6). Direct comparison (tlc and mixture mp) of 3-hydroxyphloretin (**21**) with the aglycone of 3-hydroxyphloridzin (**20**) showed them to be the same.

4-*O*-METHYLPHLORIDZIN (**14**).—The pooled silicic acid column Fractions 36–41 and 42–53 were further separated on a column of Sephadex LH-20 to give a subfraction that crystallized from methanol to give 160 mg (3 x 10^{-2}% yield) of 4-*O*-methylphloridzin (**14**): mp 198–201° d; $[\alpha]^{28}D$ −46° (*c* 0.54, MeOH); uv λ max 325 nm (sh, log 3.75), 285 (4.34) and 224 (4.45); ir (KBr) ν max 3380, 1650, 1600, 1513, 1273, 1235, 1185, 1077, 895 and 827 cm^1; ^{1}H-nmr (90 MHz, acetone-d_6) δ 2.92 (t, 2H, H-β, *J* 7), 3.4–4.0 (m, H-α and glucosyl protons), 3.75 (s, MeO), 5.10 (m, anomeric proton), 6.02 and 6.27 (2d, H-3' and H-5', *J* 2), 6.87 and 7.22 (AA'BB'q, H-2, H-3, H-5 and H-6, J_{AB} 9); and ms (ei) no molecular ion but *m/e* 300 (3%), 288 (52, $C_{16}H_{16}O_5$), 270 (7), 269 (8), 153 (43, $C_7H_5O_4$), 134.0736 (45, $C_8H_{10}O$ requires 134.0732), 121.0659 (76, C_8H_9O requires 121.0653), 73 (45) and 60 (100), and (ci, *i*-butane) no quasimolecular ion but *m/e* 289 (100%, $C_{16}H_{17}O_5$), 163 (47, $C_8H_{11}O_5$), 145 (28, $C_8H_9O_4$), 127 (7, $C_6H_7O_3$), 121 (4), and 85 (12).

Anal. Calcd for $C_{22}H_{26}O_{10}$: C, 58.66; H, 5.82. Found: C, 58.57; H, 5.93%.

4-*O*-METHYLPHLORIDZIN (**14**) HEXAACETATE.—Acetylation of 30 mg of 4-*O*-methylphloridzin (**14**) by 0.4 ml of acetic anhydride in 0.5 ml of pyridine for 48 hrs gave, after the usual work-up,

the amorphous peracetylated product. Its ir spectrum contains peaks at ν max ($CHCl_3$) 1760, 1705, 1615, 1515, 1370, 1235–1195, 1123, 1067, 1047 and 900 cm^{-1}; and the ^1H-nmr spectrum showed peaks at δ (60 MHz, chloroform-d) 2.02 (double), 2.03, 2.08 (double) and 2.27 (4s, 6 Ac), 3.77 (s, MeO), 6.71 and 6.82 (2d, H-3' and H-5', J 2), 6.80 and 7.16 (AA'BB'q, H-2, H-3, H-5 and H-6, J_{AB} 9).

HYDROLYSIS OF 4-*O*-METHYLPHLORIDZIN (**14**).—A) *With Aqueous Acid*: A 20 mg sample of 4-methylphloridzin (**14**) was heated in 1.5 ml of 2 N HCl on a steam bath for 45 min. Upon cooling, the reaction mixture deposited a crystalline product which was collected and recrystallized from aqueous methanol to give 14 mg of 4-*O*-methylphloretin (**18**) as large needles: mp 197–8° [lit. (16) value, mp 196°]; uv λ max 284 nm (log ϵ 4.00) and 225 (4.1); ir (KBr) ν max 3320, 3260, 1643, 1617, 1518, 1280, 1240 and 1210 cm^{-1}; and ^1H-nmr (90 MHz, acetone-d_6) δ 2.8–3.5 (AA'BB'm, 4H, H-α and H-β), 3.75 (s, MeO), 5.94 (s, H-3' and H-5'), 6.82 and 7.19 (AA'BB'q, H-2, H-3, H-5 and H-6, J_{AB} 9).

The filtrate from the hydrolysis reaction was evaporated to dryness and the residue examined by ascending paper chromatography on Whatman #1 with ethyl acetate-pyridine-water (12:5:4) and *p*-anisidine hydrochloride as detecting reagent. The one spot (R_f 0.12) corresponded to the glucose standard of the known sugars, arabinose, galactose, glucose, rhamnose and xylose that were cochromatographed.

B) *With Emulsin*: A 1 mg sample of 4-*O*-methylphloridzin (**14**) was dissolved in 0.3 ml of 0.5 M pH 5 sodium acetate buffer and treated with 3 mg of emulsin at 38° for 36 hrs. Passage of the hydrolysis mixture through a small column of polyamide and analysis of the effluent by paper chromatography showed glucose to be present.

METHYLATION AND HYDROLYSIS OF 4-*O*-METHYLPHLORIDZIN (**14**).—A solution of 4-*O*-methylphloridzin (56 mg) in 10 ml of anhydrous acetone containing 0.5 g of potassium carbonate was heated under reflux, and 0.5 ml of dimethyl sulfate was added. After refluxing for 10 hrs, the mixture was filtered and the precipitate was washed with acetone. The residue remaining after evaporation of the combined acetone solution was heated with 5 ml of 2 N HCl on the steam bath for 45 min. After cooling, the reaction mixture was extracted with chloroform and the chloroformic residue was crystallized from methanol to give 26 mg (75% yield) of phloretin 2',4,4'-trimethyl ether as large prisms, mp 110°. The ir, uv, and ^1H-nmr spectra were identical with those of phloretin trimethyl ether obtained from phloridzin by the same procedure.

PHLORIDZIN 2"-ACETATE (**12**).—The silicic acid column Fraction 54–70 was further chromatographed on Sephadex LH-20. One subfraction was crystallized from water to give 430 mg (8 x 10^{-2}% yield) of phloridzin 2"-acetate (**12**) as glistening needles: mp 117–20° d; $[\alpha]^{25}D$ −48° (*c* 0.54, MeOH); uv λ max 284 nm (log ϵ 4.28) and 223 (4.37); ir (KBr) ν max 3380, 1730, 1623, 1593, 1510, 1230, 1200, 1065 and 890 cm^{-1}; ^1H-nmr (90 MHz, acetone-d_6) δ 2.02 (s, Ac), 2.8–3.4 (AA'BB'm, H-α and H-β), 3.5–4.0 (complex m, H-3", H-4", H-5" and H-6"), 5.07 (t, H-2", J 8.3), 5.39 (d, H-1", J 8.3), 6.02 and 6.21 (2d, H-3' and H-5', J 2), 6.72 and 7.12 (AA'BB'q, H-2, H-3, H-5 and H-6, J_{AB} 9), and 13.55 (s, OH at C-6', lost with D_2O); and ms (ci-isobutane) *m/e* 275 (100%, $C_{15}H_{14}O_5$), 205 (56, $C_8H_{13}O_6$, **13**), 187 (4, $C_8H_{11}O_5$), 169 (3, $C_8H_9O_4$), 163 (5, $C_6H_{11}O_5$), 145 (26, $C_6H_9O_4$), 127 (17, $C_6H_7O_3$) and 107 (8, C_7H_7O). At 270 MHz the ^1H-nmr spectrum (in methanol-d_4) exhibited peaks at δ 2.03 (s, Ac), 2.84 (t, X of ABX_2, 2H, H-β, J 7), 3.15 (dt, A of ABX_2, 1H, H-α, J 7, 18), 3.33 (dt, B of ABX_2, 1H, H-α, J 7, 18), 3.45–4.0 (m, 5H, glucosyl), 5.05 (dd, H-2", J 8, 9), 5.31 (d, H-1", J 8), 5.96 and 6.13 (2d, H-3' and H-5', J 2), 6.67 and 7.06 (AA'BB'q, H-2, H-3, H-5 and H-6, J_{AB} 8).

Anal. Calcd for $C_{23}H_{26}O_{11}\cdot 2H_2O$: C, 53.69; H, 5.87. Found: C, 54.10; H, 5.95.

ACETYLATION OF PHLORIDZIN 2"-ACETATE (**12**).—A 30 mg sample of phloridzin 2"-acetate was treated with 0.25 ml of acetic anhydride and 0.3 ml of pyridine for 2 days at ambient temperature. The crude acetate (37 mg) was crystallized from methanol to give 30 mg of phloridzin heptaacetate, mp 88–9°, identical (ir, uv, ^1H-nmr, mp and mixture mp) with the product obtained from phloridzin (**1**) on peracetylation.

HYDROLYSIS OF PHLORIDZIN 2"-Acetate (**12**).—Treatment of 30 mg of phloridzin 2"-acetate (**12**) with 3 ml of 2 N HCl on a steam bath for 45 min, followed by cooling produced a crystalline precipitate. Recrystallization of the collected precipitate from water gave a product that had a decomposition mp of 265°. It was identified as phloretin (**3**) by direct comparison (uv, ir, tlc and mixture mp) with a known sample. Examination of the residue from the aqueous acid solution by paper chromatography [see hydrolysis of 4-methylphloridzin (**14**)] showed the presence of glucose.

GUAIJAVERIN (**27**).—The silicic acid column Fraction 71–94 was chromatographed on Sephadex LH-20, and a subfraction was crystallized from acetone to give 70 mg (10^{-2}% yield) of yellow microcrystals of guaijaverin (**27**): mp 256° [lit. value (23, 24) mp 256°]; $[\alpha]^{28}D$ −58° (*c* 0.53, MeOH); uv λ max 358 nm (log ϵ 4.25), 295 (sh, 3.97), 266 (sh, 4.30), 256 (4.36), and with flavonoid diagnostic reagents (20) NaOMe 406, 328, 270; $AlCl_3$ 437, 364, 304 (sh), 274; $AlCl_3$/HCl 404, 360 (sh), 298, 268; NaOAc 388, 323, 272; and NaOAc/H_3BO_3 376, 294, 260; ir (KBr) ν max

3370, 1655, 1500, 1200 and 900 cm^{-1}; ^{1}H-nmr (90 MHz, acetone-d_6) δ 3.4–4.0 (m, 5H, arabinosyl unit), 5.16 (d, H-1", J 6.4), 6.19 and 6.38 (2d, H-6 and H-8, J 2), 6.86 (d, H-3', J 8), 7.57 (dd, H-2', J 2.5, 8) and 7.74 (d, H-6', J 2.5).

HYDROLYSIS OF GUAIJAVERIN (**27**).—A solution of guaijaverin (0.8 mg) in 1.5 ml of 2 N HCl was heated on the steam bath for 45 min, and on cooling the aglycone crystallized. Recrystallization from hot water gave a product whose melting point (317°), ir spectrum, and uv spectra with flavonoid diagnostic reagents was the same as those of quercetin (**25**). The hydrolysis filtrate gave a residue on evaporation, which cochromatographed with arabinose under conditions described under 4-methylphoridzin (**14**) hydrolysis.

3-HYDROXYPHLORIDZIN 2"-ACETATE (**23**).—From a second subfraction obtained by chromatography of silicic acid column Fraction 71–92 on Sephadex LH-20, crystals as tiny needles (450 mg, 2 x 10^{-2}% yield) formed from aqueous methanol. These were characterized as 3-hydroxyphloridzin 2"-acetate (**23**): mp 134–5°; $[\alpha]^{25}D$ −47° (c 0.55, MeOH); uv λ max 284 nm (log ϵ 4.26) and 220 (4.36); ir (KBr) ν max 3395, 1730, 1630, 1605, 1238, 1202, 1097 and 898 cm^{-1}; ^{1}H-nmr (90 MHz, acetone-d_6, D_2O exchanged) δ 2.05 (s, Ac), 2.81 (m, 2H, H-β), 3.1–4.1 (m, 7H), 5.09 (t, H-2", J 8), 5.37 (d, H-1", J 8), 6.02 and 6.27 (2d, H-3' and H-5') and 6.5–6.8 (m, H-2, H-3 and H-6) with a peak at 13.57 (lost in D_2O) for C-6' hydroxyl. At 270 MHz the ^{1}H-nmr spectrum (acetone-d_6, D_2O exchanged) showed the following peaks at δ 2.07 (s, Ac), 2.82 (t, X of ABX_2, 2H, H-β, J 7), 3.12 (dt, A of ABX_2, 1H, H-α, J 18, 7), 3.34 (dt, B of ABX_2, 1H, H-α, J 7, 18), 3.5–4.0 (m, 5H, glucosyl), 5.10 (dd, H-2", J 8, 9), 5.38 (d, H-1", J 8), 6.03 and 6.27 (2d, H-3' and H-5', J 2), 6.59 (dd, H-2, J 2, 8), 6.73 (d, H-3, J 8) and 6.79 (d, H-6, J 2).

ACETYLATION OF 3-HYDROXYPHLORIDZIN 2"-ACETATE (**23**).—A 10 mg sample of 3-hydroxyphloridzin 2"-acetate (**23**) was treated with 0.1 ml of acetic anhydride in 0.2 ml of pyridine for 2 days at ambient temperature. The product was crystallized from methanol to give 11 mg of 3-hydroxyphloridzin octaacetate as large rhombic crystals, mp 130–2°. The product was identical (mixture mp, ir, and ^{1}H-nmr) with the peracetylated product of 3-hydroxyphloridzin (**20**).

PHLORIDZIN (**1**).—Chromatography of silicic acid column Fraction 93–157 on Sephadex LH-20 afforded a fraction that crystallized from water to give 6 g (0.9% yield) of phloridzin as glistening needles: mp 117–8°, resolidification and then 155°; $[\alpha]^{24}D$ −49° (c 0.58, MeOH) [lit. values (26), mp 110.5°, $[\alpha]D$ −52.4° (abs. EtOH)]; uv λ max 285 nm (log ϵ 4.27), 223 (4.37); ir (KBr) ν max 3400, 1630, 1602, 1517, 1455, 1205 and 1075 cm^{-1}; ^{1}H-nmr (90 MHz, acetone-d_6) δ 2.89 (t, 2H, H-β, J 7.5), 3.3–4.0 (m, 8H, glucosyl and H-α), 5.11 (m, H-1"), 6.03 and 6.27 (2d, H-3' and H-5', J 2), 6.73 and 7.12 (AA'BB'q, H-2, H-3, H-5 and H-6, J_{AB} 8) and 13.70 (s, OH at C-6', lost in D_2O); ms no molecular ion but m/e 274 (17%, $C_{15}H_{14}O_5$), 166 (16), 163 (2, $C_6H_{11}O_5$), 153 (12, $C_7H_5O_4$), 144 (7), 126 (35), 120 (17, C_8H_8O), 107 (40, C_7H_7O), 98 (21), 73 (50) and 60 (100). Direct comparison (mixture mp, ir and ^{1}H-nmr) of the isolated compound with authentic phloridzin (**1**) showed them to be the same.

PHLORIDZIN (**1**) HEPTAACETATE.—A 69 mg sample of phloridzin (**1**) was treated with 0.5 ml of acetic anhydride in 0.5 ml of pyridine for one day at ambient temperature. The product was crystallized from methanol to give the heptaacetate (98 mg): mp 90–2°; $[\alpha]D$ −36° (c 0.32, MeOH); uv λ max 265 nm (log ϵ 3.67); ir ($CHCl_3$) ν max 1760, 1700, 1615, 1370 and 1195–1230 cm^{-1}; and ^{1}H-nmr (chloroform-d) δ 2.01 (double intensity), 2.04, 2.07, 2.08 and 2.27 (triple intensity) (5s, 7 Ac), 2.8–3.2 (m, 4H), 3.90 (m, 1H), 4.15–4.3 (m, 2H), 4.95–5.35 (m, 4H), 6.71 and 6.81 (2d, H-3' and H-5', J 2), 6.98 and 7.26 (AA'BB'q, H-2, H-3, H-5 and H-6, J_{AB} 8).

Anal. Calcd for $C_{35}H_{38}O_{17}\cdot 2H_2O$: C, 54.83; H, 5.52. Found: C, 54.98; H, 5.74%.

HYPERIN (QUERCETIN 3-GALACTOSIDE) (**26**).—Rechromatography of the silicic acid column Fractions 93–157 and 158–186 on Sephadex LH-20 gave a fraction that crystallized from water. The microneedle-like crystals showed the following properties: mp 236–7°; $[\alpha]^{24}D$ −8.6° (c 0.49, MeOH), $[\alpha]^{21}D$ −69° (c 0.68, Pyr) [lit. values (22) mp 230–3°, $[\alpha]D$ −84° (Pyr)], uv λ max 360 nm (log ϵ 4.27), 300 (sh, 3.98), 270 (sh, 4.30) and 258 (4.36), and with flavonoid diagnostic reagents (see p. 128 of ref. 20) NaOMe 408, 327, 271; $AlCl_3$ 434, 333, 303 sh, 275; $AlCl_3$/HCl 402, 365 sh, 300, 269; NaOAc 397, 324, 272; and NaOAc/H_3BO_3 376, 297, 261; ir (KBr) ν max 3425, 3300, 1650, 1603, 1360, 1200 and 990 cm^{-1}; ^{1}H-nmr (90 MHz, acetone-d_6) δ 3.4–3.95 (m, 6H, galactosyl), 5.16 (d, H-1", J 8), 6.20 and 6.40 (2d, H-6 and H-8, J 2), 6.86 (d, H-3', J 8), 7.58 (dd, H-2', J 2, 8) and 7.84 (d, H-6', J 2). Direct comparison (ir, uv and specific rotation) of the isolated compound with a known sample of hyperin ($[\alpha]^{21}D$ −59.7° (c 0.63, Pyr), $[\alpha]^{25}D$ −8.2° (c 0.55, MeOH)) showed them to be the same. Furthermore, acid hydrolysis as described for guaijaverin gave quercetin (**25**) as the aglycone and galactose as the sugar. Both compounds were identified by direct comparison (ir and uv for the former and paper chromatography for the latter) with authentic samples.

3-HYDROXYPHLORIDZIN (**20**).—Rechromatography of silicic acid column Fraction 187–271 on Sephadex LH-20 gave a fraction that crystallized from water to give 1.4 g (0.2%) of 3-

hydroxyphloridzin (**20**) as tiny needles. The physical properties are: mp 171–3°, $[\alpha]^{25}D$ −40° (*c* 0.47, MeOH); uv λ max 285 nm (log ε 4.36) and 222 (4.39); ir (KBr) ν max 3380, 1625, 1603, 1450, 1200 and 1070 cm^{-1}; ^1H-nmr (90 MHz, acetone-d_6) δ 2.83 (t, 2H, H-β, *J* 7), 3.49 (t, 2H, H-α, *J* 7), 3.5–4.1 (m, 6H, glucosyl), 5.14 (m, H-1"), 6.02 and 6.27 (2d, H-3' and H-5', *J* 2.5), 6.60 (dd, H-2, *J* 2, *J* 2, 8), 6.73 (d, H-3, *J* 8), 6.81 (d, H-6, *J* 2) and 13.74 (s, OH at C-6', lost in D_2O).

Anal. Calcd for $C_{21}H_{24}O_{11}\cdot 2H_2O$: C, 51.64; H, 5.78. Found: C, 51.86; H, 5.90.

3-HYDROXYPHLORIDZIN (**20**) OCTAACETATE.—A 62 mg sample of 3-hydroxyphloridzin (**20**) was treated with 1 ml of acetic anhydride in 1 ml of pyridine at ambient temperature for one day. After the usual workup, the product was crystallized from methanol to give the octaacetate: mp 130°, uv λ max 273 nm (log ε 3.81); ir ($CHCl_3$) ν max 1765, 1710, 1620, and 1185–1230 cm^{-1}; ^1H-nmr (90 MHz, chloroform-d) δ 2.01 (double intensity), 2.04, 2.07, 2.08 and 2.27 (triple intensity) (5s, 8 Ac), 2.7–3.2 (m, 4H), 3.90 (m, 1H), 4.15–4.3 (m, 2H), 4.9–5.3 (m, 4H), 6.71 and 6.81 (2d, H-3' and H-5', *J* 2), 7.0–7.2 (m, 3H, H-2, H-3 and H-6).

HYDROLYSIS OF 3-HYDROXYPHLORIDZIN (**20**). —A) *With Aqueous Acid*: A solution of 3-hydroxyphloridzin (53 mg) in 4 ml of 2N HCl was heated for 45 min on a steam bath. On cooling, a crystalline precipitate (27 mg) formed that was collected and recrystallized from aqueous methanol. The product was identified as 3-hydroxyphloretin (**21**), mp 228–9° d [lit. value (17) mp 224°] by direct comparison with the aglycone isolated separately from *K. latifolia*.

B) *With Emulsin*: A 1 mg sample of 3-hydroxyphloridzin in 0.3 ml of 0.1M sodium acetate buffer, pH 5.0, was treated with 1 mg of emulsin at 38° for 36 hrs. The hydrolysis product was identified as glucose by paper chromatography as described for the hydrolysis product of 4-*O*-methylphloridzin (**14**).

METHYLATION AND HYDROLYSIS OF 3-HYDROXYPHLORIDZIN (**20**).—To a solution of 3-hydroxyphloridzin (**20**, 50 mg) in 10 ml of anhydrous acetone was added 0.5 g of potassium carbonate and 0.5 ml of dimethyl sulfate, and the mixture was refluxed for 12 hrs. The reaction mixture was worked up, hydrolyzed, and further processed as described for the same reaction sequence applied to 4-*O*-methylphloridzin (**14**). The methylated aglycone, 3-methoxy-2',4,4'-trimethylphloretin (**22**) was crystallized from methanol: mp 126–7° [lit. value (18) mp 127°] uv λ max 286 nm (log ε 4.26); ir ($CHCl_3$) ν max 2835, 1620, 1590, 1515, 1260, 1200–1240, 1160, 1115, 1025 and 820 cm^{-1}; ^1H-nmr (60 MHz, chloroform-d) δ 2.7–3.5 (m, 4H, H-α and H-β), 3.82, 3.84, 3.86 and 3.88 (4s, 4 MeO), 5.91 and 6.07 (ABq, H-3' and H-5', *J* 2.5), 6.78 (br s, ABC pattern, 3H, H-2, H-3 and H-6) and 13.75 (s, OH at C-2', lost with D_2O).

(+)-CATECHIN (**28**).—The silicic acid column Fraction 187–271, on further separation on a column of Sephadex LH-20, gave a fraction that crystallized from water to give 0.56 g (7×10^{-2}%) of (+)-catechin (**28**) as needles: mp 174–5° and $[\alpha]D$ ±0° (*c* 0.63, MeOH) [lit. values (25) mp 176–7°, $[\alpha]^{25}D$ ±0° (*c* 2.0, EtOH)]. Direct comparison (ir and ^1H-nmr) of the isolated sample with authentic (+)-catechin showed them to be identical. Also, comparison (ir and ^1H-nmr) of the pentaacetate and the tetramethyl derivatives with known compounds confirmed the starting material to be (+)-catechin. The pentaacetate showed $[\alpha]D$ +27° (*c* 5.2, $CHCl_3$) [lit. value (25) $[\alpha]^{25}D$ +39.1° ($CHCl_3$)].

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