[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WELLESLEY COLLEGE]

The Flavonoid Constituents of Barley (*Hordeum vulgare*). II. Lutonarin[1]

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A glycoside which on hydrolysis yields luteolin derivatives that resemble the unusual flavones vitexin and saponaretin (apigenin derivatives) has been isolated from barley.

Previous work[2] on many genotypes of barley (*Hordeum vulgare*) has shown that the principal flavonoid constituent of the leaves is saponarin, a 7-glucoside which on hydrolysis yields an equilibrium mixture of saponaretin and vitexin. In addition, barley was shown to contain two other relatively important flavonoid compounds, the behavior of which on paper chromatography and on hydrolysis suggested similarity to but not identity with saponarin and its two aglycons. Vitexin and saponaretin have hydroxylated hexyl groups attached to the 8-position of apigenin. The structure of the side chain in vitexin has been recently postulated by two groups[3,4] to take the form of a hydroxylated tetrahydrofuran ring, but that of saponaretin is still a matter of speculation.[2,5]

Continued investigation by paper chromatographic and spectrographic methods of the compound originally designated as spot 0[2] has shown it to be a glycoside of a flavone related to luteolin. It has been named lutonarin. Unlike saponarin and the substance called spot 1, lutonarin is not present in all samples of developing barley. It has been obtained only from plants grown outdoors in soil and only at certain stages of growth. Like saponarin lutonarin yields two aglycons on hydrolysis, first lutonaretin and later lutexin, both of which are convertible to an equilibrium mixture of the two in hot acid solution. Lutonarin and particularly its aglycons are much less stable than saponarin and its aglycons, at least in the dilute solutions used in this work, for they often disappeared completely or produced on chromatography a vivid yellow band as yet unidentified. The compounds are relatively strong acids as they grew yellow on paper or in solution at a characteristically low *p*H. Spots on paper viewed in ultraviolet light in the presence of ammonia show a characteristic orange tint in comparison with the other flavonoids in barley and with luteolin.

That lutonarin and its two aglycons have luteolin-like nuclei is shown by the similarity of their ultraviolet absorption spectra with that of luteolin and the shifts in the spectra obtained with various diagnostic reagents (Table I). Evidence for the ortho dihydroxyl groups on the flavone nuclei include the shifts in the spectra, similar to those of luteolin, with aluminum chloride plus sodium acetate[6] and with boric acid plus sodium acetate[7,8] as well as the green coloration with ferric chloride obtained with a relatively concentrated solution of lutonarin. The unreliable behavior of the spectrum of lutonarin in the presence of sodium acetate resembles that of saponarin[2] and suggests that lutonarin is also a 7-glycoside.[9]

That the compounds possess hydroxylated side chains is postulated from the behavior of the aglycons on paper chromatography coupled with the co-occurrence of lutonarin with saponarin in barley. They run considerably slower than luteolin in organic solvents and much faster in aqueous solvents, facts suggesting greater polarity and possibility for hydrogen bonding. Since the spectra do not suggest further hydroxylation of the aromatic rings and since continued hydrolysis did not lead to the removal of another sugar group by cleavage of a glycosidic link, it seems logical to assume that the extra hydroxyl groups are on a side chain attached by a C—C bond as in vitexin and related compounds. Whether the side chains in the lutonarin series of compounds are the same as in the saponarin series cannot be postulated yet except on biogenetic grounds, but work has been commenced on this problem.

Comparison of lutonaretin and lutexin with orientin, isolated by Hörhammer *et al.* from *Polygonum orientale* and *Spartium junceum L.* and tentatively identified by them as a difficulty hydrolyzable glycoside of luteolin[7] leads to the interesting speculation that one of them is identical with orientin. The spectra, with the exception of the relatively unreliable sodium acetate shift,[2] are very similar, particularly in the case of lutexin (orientin, $\lambda_{max.}$

(1) This investigation was supported in part by a research grant (G-1830) from the National Science Foundation to which grateful acknowledgment is made.

(2) M. K. Seikel and T. A. Geissman, *Arch. Biochem. Biophys.*, **71**, 17 (1957).

(3) W. H. Evans, A. McGookin, L. Jurd, A. Robertson, and W. R. N. Williamson, *J. Chem. Soc.*, 3510 (1957).

(4) L. H. Briggs and R. C. Cambie, *Tetrahedron*, **3**, 269 (1958).

(5) R. C. Cambie, *Chem. & Ind. (London)*, **87** (1959).

(6) L. Jurd and T. A. Geissman, *J. Org. Chem.*, **21**, 1395 (1956).

(7) L. Hörhammer, H. Wagner, and F. Gloggengiesser, *Arch. Pharm.*, **291/63**, 126 (1958); L. Hörhammer, H. Wagner, and H. S. Dhingra, *Arch. Pharm.*, **292/64**, 83 (1959).

(8) L. Jurd, *Arch. Biochem. Biophys.*, **63**, 376 (1956).

(9) T. A. Geissman and U. Kranen-Fiedler, *Naturwissenschaften*, **43**, 226 (1956).

in alcohol 351, 257, with sodium acetate 381, 274, and with boric acid and sodium acetate, 377, 263 mμ).[10a]

Hörhammer mentions the possibility of a C—C bond for his compounds. That it would be possible to break such a bond to some extent under the conditions he used is in part substantiated by the ease with which vitexin was converted to apigenin by Briggs and Cambie.[4,10b]

Lutonarin represents the third type of flavonoid nucleus believed to possess a hydroxylated side chain. Vitexin and related compounds have been discovered lately in an increasing number of plants,[2] but the only other compound reported is the anthocyanin in *Spirodela oligorrhiza*.[11] Recently evidence has been obtained that *Oxalis cernua* contains the luteolin analog.[12]

EXPERIMENTAL

Paper chromatography. In general the methods of chromatography described in the first paper were used.[2] For assay of the relative amounts of the different flavonoids in different samples of plant material one dimensional chromatography of the standard aqueous extracts with BAW (equal volumes of butanol and 27% acetic acid, v/v) as the developing solvent was employed. For following the hydrolysis of the glycoside, 5, 15, and 30% aqueous acetic acid solutions were preferred as the developing solvents, the second giving the best separation of spots. Because the glycoside and its aglycons were extremely unstable in air at room temperature in anything but acid environments, the following precautions were found desirable in obtaining solutions of them by the method of paper chromatographic banding: (a) extracts were banded at once, (b) elutions were done in the presence of minute traces of hydrochloric acid vapor and generally in an atmosphere of nitrogen or carbon dioxide, and (c) extracts and eluates were stored in a refrigerator below 0°.

Ultraviolet absorption spectra. Methods described in the first paper were employed,[2,13] all elutions for spectra being done with 95% alcohol. In addition, the aluminum chloride–sodium acetate shift[8] was determined by adding excess solid sodium acetate to the solution on which the aluminum chloride shift had been determined, and the boric acid–sodium acetate shift[8] by adding to 3 ml. of alcoholic eluate in the spectrophotometer cell 0.75 ml. of saturated boric acid in 95% alcohol and excess solid sodium acetate and by checking the readings until the shift was complete.

Plant material. Barley was grown from commercial seed[2] and from the genotype Atlas 46.[2,14] Plant material containing lutonarin could be obtained only under certain cultural conditions which have not yet been completely worked out so each crop must be assayed as it grows. Only field grown crops contained it. It was obtained in a concentration equal to 70–100% of that of the saponarin in the following crops listed by place, month and age: Los Angeles, May, 2–3 weeks; Wellesley, June–July, 5.5 and 7 weeks; Wellesley, September 4–7 weeks. Younger material grown in Wellesley showed no lutonarin as follows: June–July, 4 and 5 weeks, August, 4–5.5 weeks. Highest concentrations were generally found just before the plants went to seed, but lutonarin disappeared after seeding. It can be developed in the plants after harvesting, but only in a concentration equal to 10–50% of the saponarin concentration, by drying at room temperature, at elevated temperatures or under ultraviolet light, or by standing in water in a refrigerator, the highest concentrations being obtained if the roots are still attached to the leaves.

Isolation and purification of lutonarin. Aqueous extractions of barley followed essentially the method described earlier.[2] For isolation work 10 g. of dried barley (from commercial seed) was extracted five times with 200 ml. of ether. The first two aqueous extracts (200 and 150 ml.), which removed

TABLE I

DATA ON LUTONARIN AND ITS AGLYCONS

	Luton-arin	Luton-aretin	Lutexin	Lute-olin
A. Color tests[a,b]				
NaOH	1Y			Y
H_2SO_4	1Y			Y
$FeCl_3$	G			G
$Pb(OAc)_2$	Y			Y
Alc. Mg-HCl	OPk			PkO
B. R_f values				
BAW[c]	0.39	0.66	0.54	0.86
5% HOAc	0.33	0.19	0.10	0.02
15% HOAc	0.59	0.40	0.19	0.07
30% HOAc	0.70	0.56	0.39	0.24
C. Colors on paper[b]				
Vis. light	1Y	C	C	C
Vis. + NH_3	Y	1Y	1Y	Y
U.V. light	P	P	P	P
U.V. + NH_3	bOY	BYO	YO	GY
D. Absorption spectra, λ_{max} mμ[d]				
95% alc.	353	354	352	352
			Tr. 295	Tr. 295
	270	270	269	Sh. 267
	260	259	258	259
+$AlCl_3$	390	388	388	389[e]
	Sh. 365	363	358	361
	Sh. 295	Sh. 295	297	
	278	279	276	275
		Sh. 266	Sh. 266	
+$AlCl_3$ + NaOAc	422	419	419	418[e]
	Tr. 329	Tr. 330	Tr. 332	329
	277	277	275	274
+H_3BO_3+ NaOAc	380	384	381	373.5[e]
	264	264	264	261
+NaOAc	[f]	410	400	382[e]
		Tr. 320		
		275	270	264.5
+$NaOC_2H_5$	413	415	414	413
		283	276	271

[a] On solutions. [b] C = colorless (perhaps due to low concentration), Y = yellow, P = purple, O = orange, G = green, Pk = pink, B = brown, l = light, p = pale, b = bright. [c] See text. [d] Tr. = barely discernible maximum in a minimum; Sh. = shoulder. [e] These spectra are in the literature, footnotes 6 and 8. [f] Once no change was observed in the spectrum except for a slight increase in absorption at long wave lengths; once the original peak plus one of equal intensity at 418 mμ were observed.

(10a) Footnote added in proof: Recent comparison of orientin (kindly furnished by Dr. Hörhammer) and lutexin by paper chromatography in ten solvent systems showed no separation of spots and hence offers further evidence that the two are identical.

(10b) This work cannot be repeated in this laboratory.

(11) T. A. Geissman and L. Jurd, *Arch. Biochem. Biophys.*, **56**, 259 (1955).

(12) T. A. Geissman, private communication.

(13) T. A. Geissman, J. B. Harborne, and M. K. Seikel, *J. Am. Chem. Soc.*, **78**, 825 (1956).

(14) Supplied by Dr. C. W. Schaller, Department of Agronomy, University of California, Davis, Calif.

most of the flavonoid material, were finally evaporated *in vacuo* to 25 ml. giving a solution approximately ten times as concentrated as the standard solution.[2] From this solution crude lutonarin was isolated by banding on Whatman #3 filter paper with BAW (see earlier) as the developing solvent and, after elution, was purified from traces of the other flavonoids by rebanding with 30% acetic acid, once for hydrolysis work, twice for spectral work. The color tests, chromatographic data and ultraviolet absorption spectral data obtained from the purified eluate are given in Table I.

Isolation and purification of lutonaretin and lutexin. Lutonarin was hydrolyzed by 1*N* hydrochloric acid as previously described,[2] the reaction being complete in 20–30 hr. At that time the ratio of lutonaretin (the aglycon which formed first) to lutexin was approximately 2:1, but refluxing for 4 days gave a 1:1 ratio. After the methanol had been volatilized, the reaction mixture was neutralized to *p*H 5 with solid sodium acetate. The two aglycons were separated by banding with 15% acetic acid and were eluted with 50% methanol. Each was purified by rebanding with BAW. Chromatographic and spectral data obtained on solutions of these compounds are given in Table I, and the results are compared with results on luteolin obtained simultaneously or recorded in the literature. Co-chromatography with luteolin showed separation of spots in all four solvents.

Interconversion of the aglycons. Solutions of both purified aglycons were subjected to the usual hydrolytic conditions[2] for approximately one day. Paper chromatographic studies of the resulting solutions with four solvents (Table I) showed that each aglycon had been converted in part into the other; the ratio of lutonaretin to lutexin was 1:1 from lutonaretin, 1:2 from lutexin.

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Alcoholysis of Alkyl Benzyl Esters of Phthalic Acid[1]

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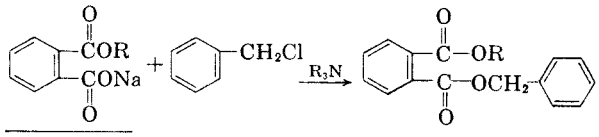
Alkyl benzyl phthalate esters of high purity are not readily prepared by simple conventional methods, especially when the alkyl group contains eight or more carbon atoms. However, a smooth procedure for the preparation of these unsymmetrical esters has been found, involving alcoholysis of the more easily prepared lower alkyl benzyl phthalates with higher molecular weight alcohols. In this alcoholysis reaction the benzyl group exhibits a surprising immobility toward displacement, even if the displacing alcohol has a boiling point higher than that of benzyl alcohol. A mechanistic theory is advanced to explain this immobility.

Methods of preparation of esters date back to the early days of organic chemistry, and one would anticipate little novelty in the reactions of alcohols and acids or in esterification techniques involving alcoholysis or acidolysis. Yet, in our study of the alcoholysis of alkyl benzyl *o*-phthalates, we have observed a behavior anomalous to classical description in that alcoholysis of unsymmetrical diesters does not unequivocally lead to displacement of the lower boiling alcohol by a higher boiling alcohol.

For some time we have been interested in the synthesis of unsymmetrical esters of phthalic acid, particularly those containing a benzyl moiety. These materials have utility in the plasticization of poly(vinyl chloride); this being particularly true of the higher alkyl benzyl phthalates. Preparation of some of these esters by conventional means can be extremely cumbersome. An obvious method of preparation for such esters would involve reaction between the sodium salt of the selected alkyl acid phthalate with benzyl chloride, in the presence of an amine catalyst.[2]



(1) Presented in part before the Division of Organic Chemistry at the 134th Meeting of the American Chemical Society, Chicago, Ill., September 1958.

(2) Reid, U. S. Patent **1,554,032** (September 15, 1925).

This method is not entirely feasible, however, with the higher alkyl benzyl phthalates, due to the gross insolubility of the sodium alkyl phthalate in many of the common organic solvents. Nonetheless, it is possible to obtain a small (30–40%) yield of the desired unsymmetrical ester if one utilizes the potassium salt of the acid ester and a several-fold excess of the alcohol used in preparation of the alkyl acid phthalate. Preparation of the lower alkyl benzyl phthalates does not result in this low yield, however. Thus, methyl, ethyl, or butyl benzyl phthalates can be prepared smoothly and in excellent yield from the sodium or potassium salt of the acid ester and benzyl chloride. The preparation of the unsymmetrical esters by use of benzyl alcohol and the aliphatic alcohol is also contraindicated, since, assuming equal rates of reaction of the two alcohols, one obtains a statistical mixture of the three possible esters (25% dialkyl phthalate, 50% alkyl benzyl phthalate, and 25% dibenzyl phthalate). Separation of the pure unsymmetrical ester from the other two components is often difficult.

It occurred to us that it should be possible to take advantage of the relative ease of preparation of the lower alkyl benzyl phthalates and utilize these materials as a starting point in the synthesis of the higher alkyl benzyl phthalates. It remained, therefore, only to displace the lower alkyl group, which should be easily carried out by the base-catalyzed alcoholysis with the desired alcohol. In the case of