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Imidazole-Catalyzed Hydrolysis of Acetoxyflavones (1,2) Physical and Chemical Properties of Hydroxyflavones. III.

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A method for the hydrolysis of acetoxyflavones with catalytic amounts of imidazole in 60% ethanol or 60% methanol is described. The method has been applied to the acetates of monohydroxyflavones, polyhydroxyflavones, and the trihydroxyflavones naringenin and hesperetin. Partial deacetylation of quercitrin heptaacetate is described. The methanesulfonates of several monohydroxyflavones, and the stability of flavonol methanesulfonate in the imidazole-ethanol reagent are reported.

The catalytic properties of acetylcholine esterase are very important biologically. Demonstration of the location of an imidazole-like group at the active site of the enzyme (4) has stimulated research on catalysis by imidazole, including studies of imidazole-catalyzed hydrolysis of phenyl acetate (5). In the present paper, we report a purification procedure based on the catalytic activity of this nitrogen heterocycle, and its application to several hydroxyflavones, polyhydroxyflavones and hydroxyflavones. The stability of flavonol methanesulfonate (6) in the imidazole-containing reagent is described.

In general, acetates of hydroxyflavones, including those of polyhydroxyflavones, are readily prepared and purified. In contrast, the parent hydroxyflavone, especially one with several hydroxyl groups, is either insoluble in common solvents or is soluble only in high-boiling solvents. Crystallization procedures for direct purification of polyhydroxyflavones thus are often unsatisfactory. Imidazole appeared potentially useful in hydrolysis of acetoxyflavones as a projected purification method because of its very weak basicity and, especially, because it can be used in catalytic amounts, thus minimizing the possibility of secondary reactions after the hydrolytic step. A satisfactory procedure involves the use of a quantity of imidazole equal to 1/10th the weight of the acetoxyflavonoid derivative in 60% ethanol in water (volume/volume) at reflux temperature. With certain flavonoids, especially those that might be unstable unless they crystallize rapidly from the hydrolysis medium, the substitution of methanol for ethanol has been essential.

In order to establish the generality of the imidazole-catalyzed hydrolysis procedure, the acetates of all monohydroxyflavones have been prepared and subjected to deacetylation. The results are presented in Table I. Yields range from 82% to quantitative, and the melting points of seven of the eight hydroxyflavones obtained are either essentially the same as those previously reported or slightly higher.

The purification procedure has been extended to chrysin diacetate (I), apigenin triacetate (III), and quercetin pentaacetate (V), with results as shown in Table II. The ready obtainment of relatively pure quercetin is of interest, in view of its wide-spread natural occurrence and the difficulty of direct purification by crystallization because of its low solubility in common solvents. The method has been extended to morin pentaacetate (VII) with the result indicated in Table II. We have obtained VII in two modifications, the melting points of which are in reasonable agreement with those reported for the dimorphs of morin pentaacetate (7). The observation reported in Table II pertains to the hydrolysis of VII, m.p. 146-149°. Extension of the method to primetin diacetate (IX) was of limited utility with the imidazole-ethanol reagent, inasmuch as the product primetin (X) was received with a m.p. of 217-225°. However, when 60% methanol was employed as the solvent, primetin was obtained in a highly pure state (Table II).

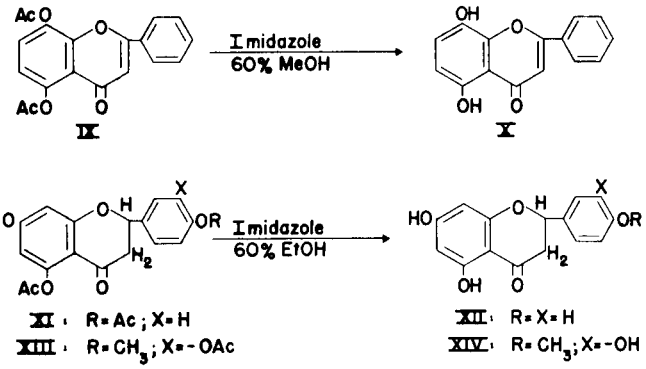
Application of imidazole-catalyzed hydrolysis to the triacetoxyflavones, naringenin triacetate (XI) and hesperetin triacetate (XIII), proceeds as shown in Table II. In the hydrolysis of XI and XIII, the known flavanones (XII and XIV) are expected products, rather than the isomeric chalcones, since the 5-hydroxyl group very probably stabilizes the flavanone ring system (8). However, imidazole-catalyzed hydrolysis does offer a new approach to the chalcone-flavanone equilibrium problem, as indicated by a preliminary study of 2,2'-diacetoxychalcone (9) in the imidazole-ethanol reagent (10). The catalytic activity of imidazole was demonstrated directly with XIII by omitting the nitrogen heterocycle from the hydrolysis medium. There was obtained 30% of starting material and an oily product which gave a positive ferric chloride test. Although the latter material obviously was a deacetylation product, it was very impure, and it is apparent that the imidazole is an effective and essential catalyst in the present procedure.

In flavonol glycosides such as quercitrin (XV), both phenolic and alcoholic hydroxyl groups are present, and there is a possibility of preferential solvolysis of the completely acetylated glycoside at the phenolic acetate groups. Deacetylation of quercitrin heptaacetate in methanol under imidazole-catalysis resulted in an amorphous product which, on

the basis of acetyl analysis, appears to be a quercitrin triacetate. In view of the demonstrated lability of all the acetoxyl groups in quercetin pentaacetate (V), the 3'-, 4'-, 5-, and 7-acetoxyl groups in quercitrin heptaacetate probably would be labile. Accordingly, it is likely that the three acetoxyl groups in the acetylated rhamnose moiety of quercitrin heptaacetate are the ones which have not been hydrolyzed under imidazole-catalysis. The infrared spectrum of the triacetate contains the expected ester and flavone carbonyl bands.

The attempted application of imidazole-catalyzed hydrolysis to an acetate, m.p. 82-85°, of racemic dihydroquercetin gave a red oily product which could not be crystallized or purified under any conditions employed. Inasmuch as a melting point of 149-149.5° recently has been reported (11) for racemic dihydroquercetin pentaacetate, it is very probable that our acetylation product was impure.

The methanesulfonates of 3'-, 4'-, 6-, and 7-hydroxyflavone have been prepared from the appropriate monohydroxyflavone and methanesulfonyl chloride in pyridine. 5-Hydroxyflavone was recovered unchanged from attempted mesylation in pyridine, an observation which can be correlated with the nonreactivity of quercetin 3, 3', 4', 7-tetramethyl ether under the same conditions (6). Properties and analyses for the mesylates are given in Table III. Possible demesylation of flavonol methanesulfonate (6) with the imidazole-ethanol reagent has been investigated. Flavonol methanesulfonate was recovered to the extent of approximately 80% from procedures utilizing either the catalytic or molar quantity of imidazole.



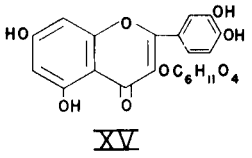


TABLE I

Monohydroxyflavones from Imidazole-Catalyzed Deacetylation of Monoacetoxyflavones (a)

Position of OH	Yield %	M.p., °C	Lit. m.p., °C
2'	87	245-246	249-250 (b)
3'	83 (c)	206-207	207-208 (b)
4'	93 (d)	269.5-271	269-270 (b)
3	89	170-171	169-170 (e)
5	93	157.5-158	156-157 (f)
6	98	235-236	231-232 (g)
7	100	244-244.5	240 (h)
8	82 (i)	250-252°	252.5-253.5° (i)

(a) M.p. in agreement (±1°) with literature value, except where otherwise indicated. (b) M. T. Bogert and J. K. Mareus, *J. Am. Chem. Soc.*, 41, 95 (1919). (c) Crude acetate, melting 77-93° (lit. (b) m.p., 97°), used in this experiment. (d) M.p. of acetate, 141-142° (lit. (b) m.p., 137°). (e) S. Kostanecki and W. Szabranski, *Ber.*, 37, 2820 (1904). (f) S. Sugasawa, *J. Chem. Soc.*, 1483 (1934). (g) S. Kostanecki, R. Levi and J. Tambor, *Ber.*, 32, 331 (1899). (h) I. Heilbron, *Dictionary of Organic Compounds*, Vol. 2, Oxford University Press, New York, 1953, p. 767. (i) M.p. of acetate, 155-157° (lit. m.p. 152°), D. Pillon, *Bull. soc. chim.* France, 39 (1955).

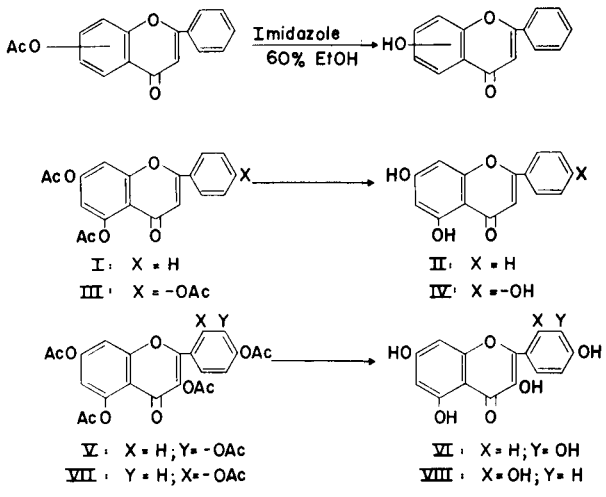


TABLE II

Flavonoid Substances Purified by Imidazole-Catalyzed Hydrolysis of the Acetate

Acetate Ordinal	Flavonoid Ordinal	Yield %	M.p. of Flavonoid, °C	Lit. m.p., °C
I (a)	II	81	286.5-287	274-275 (b)
III (c)	IV	98	350-352 (d)	352 (c)
V (e)	VI	91	308-311 (d)	313-314 (f)
VII (g)	VIII	99	295-296.5	302 (h)
IX (i)	X	60	230-231	230-231 (j)
XI (k)	XII	88	250-251	251 (l)
XIII (m)	XIV	97	225-229 (d)	233 (b)

(a) R. Robinson and K. Venkataraman, *J. Chem. Soc.*, 2347 (1926). (b) R. Seka and G. Prosche, *Monatsh.*, 69, 284 (1936). (c) M. Nakano, *J. Pharm. Soc. Japan*, 52, 341 (1932); *Chem. Abstr.*, 26, 4334 (1932). (d) Without recrystallization. (e) K. Freudenberg, *Ann.*, 433, 236 (1923). (f) St. v. Kostanecki, V. Lampe, and J. Tambor, *Ber.*, 37, 1402 (1904). (g) M.p. 146-149°, this study. (h) Reference 14. (i) By acetylation of X with Ac_2O-NaOAc and chromatographic purification of benzene solution on Florisil; m.p. and lit. m.p. 191-192°, J. Chopin and M. Chadenson, *Compt. rend.*, 250, 1864 (1960). (j) F. Mayer and A. H. Cook, The Chemistry of Natural Coloring Matters, Reinhold Publishing Corp., New York, 1943, p. 170. (k) T. A. Geissman and S. L. Friess, *J. Am. Chem. Soc.*, 71, 3894 (1949). (l) J. Shinoda and S. Sato, *J. Pharm. Soc. Japan*, 48, 117 (1928); *Chem. Zentr.*, 1929I, 244. (m) J. H. Looker and M. J. Holm, *J. Org. Chem.*, 25, 1829 (1960).

TABLE III

Methanesulfonic Esters of Monohydroxyflavones

Position of OSO_2CH_3	M.p., °C	Analyses % Carbon Calcd.	% Carbon Found	% Hydrogen Calcd.	% Hydrogen Found	% Sulfur Calcd.	% Sulfur Found
3	(a)						
6	181 (b)	60.75	60.58	3.82	3.76	10.13	10.30
7	160 (c)	60.75	61.16	3.82	4.11	10.13	10.08
3'	161 (d)	60.75	60.58	3.82	4.12	10.13	9.85
4'	167-168 (c)	60.75	61.23	3.82	4.23	10.13	9.92

(a) Reference 6. (b) Recrystallized once from methanol, twice from dilute ethanol, and once from 95% ethanol. (c) Recrystallized from ethyl acetate-cyclohexane. (d) Recrystallized five times from 95% ethanol.

EXPERIMENTAL (12)

Acetates of Hydroxyflavones.

References to the literature for the preparation of the acetates of the monohydroxyflavones have been cited previously (13). References to methods for acetylation of the other flavonoids are given in Table II. The acetylation method used for morin is described below.

Morin Pentaacetate.

Morin (1.0 g.) was acetylated by suspending it in 2.6 ml. of acetic anhydride, adding 3 ml. anhydrous pyridine, and permitting the resulting mixture to stand four hrs. at room temperature. The crude acetate was obtained as an oil, which solidified on standing overnight. Rather gummy solids were obtained upon attempted recrystallization of this solid from chloroform, benzene, benzene-*iso*octane, ethyl acetate-cyclohexane, or various alcohols. The crude acetate, melting 85-135° (14, 15), was extracted with anhydrous ether leaving a sticky insoluble solid which was discarded. Evaporation of the ether gave a white powder which, after drying in a vacuum desiccator, melted at 146-149°, with softening at lower temperatures (lit. (7) m.p., dimorphous, m.p.'s 146-147° and 115-116°). This fraction was used in the deacetylation experiment reported in Table II.

A preliminary purification of a sample of morin (L. Light and Co., Ltd.) by the procedure of Carruthers, Farmer and Laidlaw (14), with subsequent acetylation of the morin thus purified, gave a white product which could be purified from 95% ethanol. Filtration of this product, however, gave a gum. Addition of water to the filtrate gave a white product, which, upon further crystallization from 95% ethanol was obtained as a white powder, m.p. 113-117°. Possibly this fraction was the low melting dimorph previously reported (7).

Imidazole-Catalyzed Deacetylation of 8-Acetoxyflavone.

8-Acetoxyflavone (200 mg.), m.p. 155-157°, and 20 mg. imidazole were suspended in 12 ml. of 60% ethanol [ethanol-water 60:40 (v/v)]. The resulting mixture was heated under reflux for forty-seven hrs. Crystalline solid appeared in the boiling mixture. The hot reaction mixture was poured into a small beaker and permitted to stand two days. The crystalline 8-hydroxyflavone was collected by filtration and air-dried for one week, yield, 140 mg. (82%), m.p. 249-251.5°. Recrystallization from ethanol gave 8-hydroxyflavone, m.p. 250-252°. Application of the identical procedure (0.1 g. imidazole per gram of acetoxyflavone in 60 ml. of 60% ethanol) to quantities of other acetoxyflavones ranging from 325 to 550 mg. gave yields of the hydroxyflavone ranging from 83 to 100%, as shown in Table I.

Imidazole-Catalyzed Deacetylation of Hesperetin Triacetate.

In 30 ml. of 60% ethanol in water (v/v) were dissolved 500 mg. of hesperetin triacetate and 50 mg. of imidazole. The resulting solution was heated under reflux for twenty-four hrs. The heat source was removed and the reaction mixture evaporated to approximately 15 ml. under an air-jet. The solid resulting was collected on a sintered glass funnel and then suspended in a dilute hydrochloric acid solution (prepared by dissolving 0.2 ml. of conc. hydrochloric acid in 50 ml. of water), to remove any traces of imidazole. The insoluble hesperetin was collected by filtration and air-dried, yield 340 mg. (97%), m.p. 225-229°. Identical procedures were applied to chrysin diacetate (I), apigenin triacetate (III), quercetin pentaacetate (V), morin pentaacetate (VII), and naringenin triacetate (XI), with results as indicated in Table II.

Imidazole-Catalyzed Hydrolysis of Primetin Diacetate.

Primetin diacetate (0.5 g.) and 50 mg. imidazole were dissolved in aqueous methanol (from a mixture of 30 ml. methanol and 20 ml. water). The resulting solution was heated under reflux for 20 hrs., during which time a yellow crystalline substance separated. The crystalline solid was collected by filtration, washed with water and air-dried to give primetin in approximately 60% yield, m.p. 230-231°. The mixture m.p. with authentic primetin showed no depression.

Imidazole-Catalyzed Deacetylation of Quercitrin Heptaacetate.

Quercitrin heptaacetate (0.5 g.) was dissolved in 10 ml. of methanol and 50 mg. of imidazole added. The resulting solution was heated under reflux for twenty-four hrs. and then the methanol was evaporated under reduced pressure. The residue was washed with water and then extracted with ether. The dry ether extract was concentrated to a small volume and rapidly passed through a short Florisil (16) column. The ether percolate was concentrated to about 5 ml. and petroleum ether (b.p. 40-45°) added carefully until a yellow precipitate was obtained. The product was purified by several precipitations from ether (by addition of petroleum ether), and a yellow amorphous solid, m.p. 147° with softening at 135°, was obtained.

Anal. Calcd. for $C_{21}H_{17}O_{11}(COCH_3)_3$: CH_3CO, 22.47. Found: CH_3CO, 21.36.

The infrared spectrum (Nujol mull) showed bands at 1748 (ester CO), 1650 (flavone CO), and 1600 cm^{-1} (in-plane skeletal phenyl bands).

Mesylation of Monohydroxyflavones.

To a solution of 1.19 g. of 6-hydroxyflavone in 20 ml. of anhydrous pyridine was added 1.55 g. of methanesulfonyl chloride, b.p. 150-154° at 730 mm., in 2 ml. of pyridine. The tightly stoppered flask was permitted to stand in the freezing compartment of a refrigerator for forty-nine hrs. The mixture was poured into 200 ml. of ice-water and the resulting mixture permitted to stand one hour. The precipitated mesylate was collected by filtration, washed with water and air-dried, yield, 1.97 g. (102%). Recrystallization from methanol followed by two crystallizations from ethanol-water and a fourth from 95% ethanol gave analytically pure 6-mesyloxyflavone, m.p. 181° after drying *in vacuo* for seven hrs. at 80°. The other mesyloxyflavones were prepared by similar procedures; melting points and analyses are given in Table III.

Attempted Demesylation of Flavonol Methanesulfonate.

Flavonol methanesulfonate (258 mg.) and 25 mg. of imidazole in 17 ml. of 60% ethanol were heated under reflux for twenty-four hrs. Upon cooling, flavonol methanesulfonate (216 mg., 83%) crystallized and was collected by filtration and air-dried; the substance showed the characteristic photochromism (6). The recovered flavonol methanesulfonate was washed with 49 ml. of 5% sodium hydroxide. The insoluble solid was dried in a vacuum desiccator one week; recovery, 213 mg., m.p. 117-118°. In a subsequent study, essentially the same results were obtained, except that the recovered flavonol methanesulfonate melted 119-119.5° (lit. (6), 120-122°).

Reaction of 1 g. (1/316 mole) of flavonol methanesulfonate, dissolved as much as possible in 65 ml. of 60% ethanol, with 216 mg. (1/316 mole) of imidazole at reflux temperature for twenty-four hrs. gave a solution which gave an orange ferric chloride test. However, crystalline flavonol methanesulfonate was recovered in 76% yield. No other crystalline product proved isolable.

REFERENCES

(1) Taken in part from the Ph. D. thesis of Myron J. Holm, 1958, and the M. S. thesis of James L. Minor, 1961.

(2) This investigation was supported by a research grant (AI-01703) from the National Institute of Allergic and Infectious Diseases, Public Health Service.

(3) DuPont Postgraduate Teaching Assistant, 1956-57; Standard Oil of Indiana Foundation Fellow, 1957-58.

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(8) N. Narasimhachari and T. R. Seshadri, *Proc. Indian Acad. Sci.*, 27A, 223 (1948); *Chem. Abstr.*, 44, 1493 (1950).

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(10) There was obtained a product with a wide m.p. range, the infrared spectrum of which contained no ester carbonyl band. Thus deacetylation occurred, but also another reaction, presumably subsequent to hydrolysis of the acetoxyl groups. Acetylation of the reaction product gave a material, the weight of which after thorough drying was not as large as that required for the dihydroxychalcone diacetate. Two recrystallizations of this acetylation product from methyl acetate gave 2'-acetoxyflavanone, m.p. 146-147.5° (lit. (9) m.p., 148-148.5°).

(11) H. Aft, *J. Org. Chem.*, 26, 1958 (1961).

(12) Melting points are uncorrected and were observed in capillary tubes. The infrared measurements were carried out with a Perkin-Elmer Model 21 double-beam recording spectrophotometer.

(13) J. H. Looker and W. W. Hanneman, *J. Org. Chem.*, 27, 389 (1962).

(14) This melting range is in agreement with our previous observation (reference 6). However, in view of a subsequent report [(Mrs.) W. R. Carruthers, R. H. Farmer and R. A. Laidlaw, *J. Chem. Soc.*, 4442 (1957)] that morin obtained from L. Light and Co. (the source of morin in reference 6) contained small amounts of impurities, this acetylation product of necessity would have been impure.

(15) The m.p. range also may be attributable, in part, to the dimorphous character of morin pentaacetate. Rotenone is a well-known example of a dimorphous substance which sometimes gives indistinct melting points, even when pure (R. S. Cahn, *J. Chem. Soc.*, 1129 (1934)).

(16) A magnesium trisilicate analytical absorbent available from the Floridin Co., Inc.

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