The first fractions afforded white crystals, which were recrystallized twice from methanol–chloroform giving 67 mg. of pure dehydro B (IX), m.p. 300.5–301°.

Anal. Calcd. for $C_{30}H_{44}O_2$: C, 82.51; H, 10.16. Found: C, 82.55; H, 10.14.

Alternatively, 105 mg. of F (X) was dissolved in 5 ml. of anhydrous pyridine and 0.3 g. of *p*-toluenesulfonylchloride was added. The reaction was left overnight at room temperature and water was added to the dark solution. The resulting precipitate was filtered, washed repeatedly with 20% sodium carbonate solution followed by water. The ditosylate was quite insoluble in most solvents and was not recrystallized, but appeared homogeneous on thin layer chromatography.

The crude tosylate was heated in a sealed tube at 100° for 1 hr. with 10 ml. of acetone and 250 mg. of sodium iodide. The tube was then cooled and the contents poured into water. The resulting precipitate was filtered and chromatographed on a short column of alumina using a gradient of solvents from benzene to ether. The first fractions afforded 19 mg. of a compound melting at 300–303° after two recrystallizations from methanol. This substance proved to be identical with dehydro B (IX) by mixture melting point determination and infrared comparison.

Acetylation of Sapogenin F.—A sample (100 mg.) of pure F (X) was acetylated with acetic anhydride–pyridine at room temperature. After 12 hr., water was added, the resulting precipitate filtered and repeatedly washed with water. Two recrystallizations from methanol–chloroform afforded in nearly quantitative yield F diacetate (XI), m.p. 222–226°, $[\alpha]_D$ −48°.

Anal. Calcd. for $C_{34}H_{50}O_6$: C, 76.61; H, 9.09. Found: C, 73.48; H, 9.23.

Oxidation of Sapogenin F.—The oxidation of 150 mg. of F (X) with Jones[2] reagent was performed as described before and the resulting compound (120 mg.) was repeatedly crystallized from various solvent mixtures but had a wide melting range above 150°. Diketo F (XII) had $[\alpha]_D$ +49° and showed only one large spot on chromatoplates in several solvents.

Anal. Calcd. for $C_{30}H_{42}O_4$: C, 77.21; H, 9.07. Found: C, 77.19; H, 9.13.

A portion (100 mg.) of diketo F (XII) was reduced and methylated as described before for the ketone VII. This procedure afforded bisdeoxy F methyl ester, m.p. 182–187°, $[\alpha]_D$ +81°, which was shown to be identical with deoxy B methyl ester (VIII) (methyl 3-deoxymachaerinate), m.p. 183–189°, $[\alpha]_D$ +81°, by mixture melting point determination and thin layer chromatography.

Sapogenin F Acetonide.—To a solution of 50 mg. of F (X) in 5 ml. of anhydrous acetone was added one drop of concentrated sulfuric acid and the reaction left overnight at room temperature. The course of the reaction can be followed conveniently by thin layer chromatography on silica gel using 3% ethyl acetate in benzene. Under these conditions, the unchanged sapogenin does not migrate, while the acetonide moves near the solvent front. The reaction mixture was concentrated and the acetonide precipitated by the addition of pentane. Attempted recrystallization normally resulted in reformation of the parent diol.

Acknowledgment.—Financial support for this cooperative study between the Instituto de Quimica Agricola and Stanford University has been provided by the Rockefeller Foundation and the National Institutes of Health (grant no. GM-06840) of the U. S. Public Health Service as well as by the Brazilian Conselho Nacional de Pesquisas. We are indebted to Drs. J. M. Wilson and H. Budzikiewicz for the mass spectra, to Dr. L. J. Durham for the n.m.r. spectra, and to Messrs. E. Meier and J. Consul for the microanalyses.

Anthocyanins and Related Compounds. II. Structural Transformations of Some Anhydro Bases

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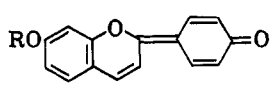
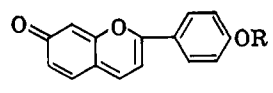
The spectra of anhydro bases derived from 4′,7-dihydroxyflavylium salts confirm a 7-keto structure for these compounds. At pH < 7 anhydro bases unsubstituted in the 3-positions rapidly hydrolyze to *cis*-2-hydroxychalcones. At about pH 9 and pH 12 spectral evidence suggests that anhydro bases derived from monohydroxyflavylium salts are hydrolyzed to yield *trans* and *cis* forms of ionized chalcones, respectively.

In aqueous solutions at pH > 4, anthocyanins and flavylium salts with at least one free hydroxyl in the 5-, 7-, 2′- or 4′- position lose a proton to form highly colored, labile anhydro bases. Since the pH of the cell sap of most plants[1] is in the range 4–6 it would seem probable that anhydro bases play a predominant role as reactive species in irreversible anthocyanin degradations in plant extracts. It is somewhat surprising, therefore, that the structures and transformation products of anhydro bases have not been more extensively investigated. Thus, although a 4′-keto structure of type I has been assigned arbitrarily to the anhydro bases derived from natural anthocyanins and other 4′,7-dihydroxyflavylium compounds,[2] experimental evidence in support of this structure has not been reported. Furthermore, while it is well established that in strongly alkaline (*e.g.*, sodium hydroxide) solutions anhydro bases are rapidly hydrolyzed to ionized chalcones,[3] the products formed at intermediate pH's are not, in many cases, known with certainty.

The anhydro base derived from a 4′,7-dihydroxyflavylium salt could conceivably arise by loss of a proton from either the 4′- or the 7-hydroxyl, resulting in structure I or II (R = H). Comparison of this



I



II

(1) K. Hayashi, in "The Chemistry of Flavonoid Compounds," T. A. Geissman, Ed., Pergamon Press Inc., New York, N. Y., 1962, p. 248.

(2) See reviews by F. Blank, *Botan. Rev.*, **13**, 241 (1947); *Handbuch d. Pflanzenphysiologie*, **10**, 300 (1958); N. Campbell in "The Chemistry of Carbon Compounds," Vol. IV^B, E. H. Rodd, Ed., Elsevier Publishing Co., Inc., New York, N. Y., p. 842; H. Kuhn and W. Sperling, *Experientia*, **16**, 237 (1960).

(3) A. Robertson and R. Robinson *J. Chem. Soc.* 1526 (1928); D. D. Pratt and R. Robinson, *ibid.*, 745 (1923).

anhydro base with anhydro bases derived from related monohydroxymethoxyflavylium salts, however, has now clearly indicated preferential removal of the proton from the 7-hydroxyl. While this investigation was in progress, Arora, Jain, and Seshadri[4] reached the same conclusion on the basis of methylation studies. These authors precipitated the anhydro base of 4′,7-dihydroxyflavylium chloride and attempted to determine the position of the remaining free hydroxyl by methylation *in aqueous potassium carbonate* solution. The 4′-methoxy anhydro base II (R = Me) was obtained (40%) and, therefore, structure II (R = H) was assigned to the original anhydro base. Evidence of this kind, however, is ambiguous since it is apparent that in alkaline solutions the *same* ionized anhydro base, a resonance hybrid of ionized forms I and II, will be obtained from either of these structures. Consequently, the position at which methylation occurs gives no information about the structure of the original hydroxy compound.

In the present investigation the anhydro bases were studied at pH's sufficiently low to exclude the possibility of ionization. It was found that in aqueous solutions at pH 5.6 the anhydro bases derived from 4′,7-dihydroxy- and 7-hydroxy-4′-methoxyflavylium salts are virtually identical in appearance (orange-red in dilute solutions), spectrum (λ_{max} 484 mμ, log ε 4.39, in each case), and rate of decomposition (thirty–thirty-five minutes for complete hydrolysis) (Fig. 1). Anhydro base I (R = Me) from 4′-hydroxy-7-methoxyflavylium chloride, on the other hand, decomposes within seconds at pH 5.6 (Fig. 2, C). At higher pH this anhydro base is more stable and its spectrum can be determined. Thus, at pH 6.4 and 6.9, I (R = Me) is an intense cerise-red and had a clearly defined λ_{max} at 506 mμ.

The anhydro bases derived from 4′,7-dihydroxy-3′-methoxyflavylium chloride at pH 5.7 and 7-hydroxy-3′,4′-dimethoxyflavylium chloride at pH 6.9 were similarly compared and found to be almost identical (λ_{max} 495 mμ, log ε 4.40; and λ_{max} 493 mμ, log ε 4.41, respectively). They differed distinctly in color and stability from the anhydro base (λ_{max} 529 mμ, log ε 4.60) derived from 4′-hydroxy-3′,7-dimethoxyflavylium chloride at pH 6.9. On the basis of these comparisons there can be no doubt that 4′,7-dihydroxyflavylium salts preferentially form anhydro bases of structural type II.

In Fig. 1 and 2 it will be noted that the products formed in the rapid decoloration of the anhydro bases have a well defined λ_{max} at 370 mμ. These hydrolysis products, therefore, are not colorless carbinol bases but the corresponding 2-hydroxychalcones.[5] This has been confirmed by the isolation of crystalline 2,2′-dihydroxy-3-methoxychalcone,[5] m.p. 179–180°, by hydrolysis of the anhydro base of 8-methoxy-2′-hydroxyflavylium chloride at pH 5.6.

As indicated, small increases in pH in the range 5.6–7.0 markedly stabilize anhydro bases; *e.g.*, complete hydrolysis of the anhydro base (λ_{max} 475 mμ) derived from 7-hydroxyflavylium chloride takes three hours at pH 6.4 but occurs in about twenty minutes at pH 5.6. For monohydroxyflavylium salts the λ_{max} of the derived anhydro bases remains constant as the pH is increased,

(4) S. K. Arora, A. C. Jain, and T. R. Seshadri, *J. Indian Chem. Soc.*, **39**, 285 (1962).

(5) See Part I., L. Jurd, *J. Org. Chem.*, **28**, 987 (1963).

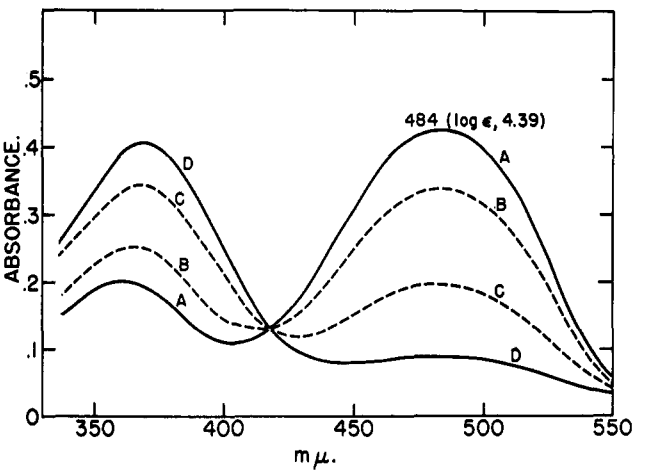


Fig. 1.—Spectrum of anhydro base from 4′,7-dihydroxyflavylium chloride (and from 7-hydroxy-4′-methoxyflavylium chloride (4.8×10^{-3} g./l.) at pH 5.6: (A) 1 min.; (B) 5 min.; (C) 1[illegible] min.; (D) 35 min.

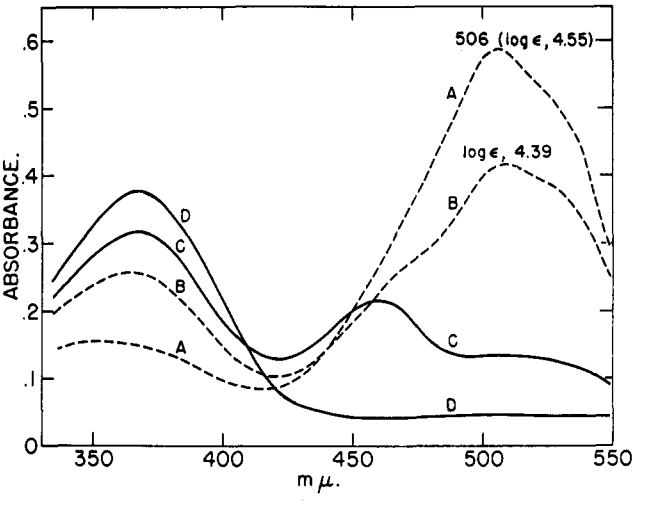


Fig. 2.—Spectra of the anhydro base from 4′-hydroxy-7-methoxyflavylium chloride (4.8×13^{-3} g./l.) taken at pH 6.9 (A), 6.4 (B), and 5.6 (C), after 1 min. D is the spectrum of the chalcone formed on decomposition of the anhydro base.

only the log ε value at the λ_{max} increases slightly as a result of the added stability. The λ_{max} of anhydro bases derived from di- and polyhydroxyflavylium salts, on the other hand, shift to longer wave lengths; *e.g.*, at pH 5.6, 6.4, and 6.9 the anhydro base of 4′,7-dihydroxyflavylium chloride changes from an orange-red to a cerise-red, and the λ_{max} shifts from 484 mμ to 489 mμ and 502 mμ, respectively. This bathochromic shift is due to the formation of significant and increasing quantities of the ionized anhydro base at pH 6–7 and illustrates the necessity of precise pH control in attempting to draw structural conclusions from comparisons of anhydro bases. At pH 8.0, conversion of 4′,7-dihydroxyflavylium chloride to the ionized anhydro base (λ_{max} 530 mμ, log ε 4.66) is complete. In more strongly alkaline solutions the absorbance at 530 mμ progressively decreases with the formation of a new peak at 482 mμ. At pH 12.0 quantitative hydrolysis of the ionized anhydro base into the fully ionized chalcone (λ_{max} 482 mμ, log ε 4.64) occurs on standing for a few minutes.

Transformations of Monohydroxyflavylium Salts at pH 9 and 12.—Due to resonance stabilization, the

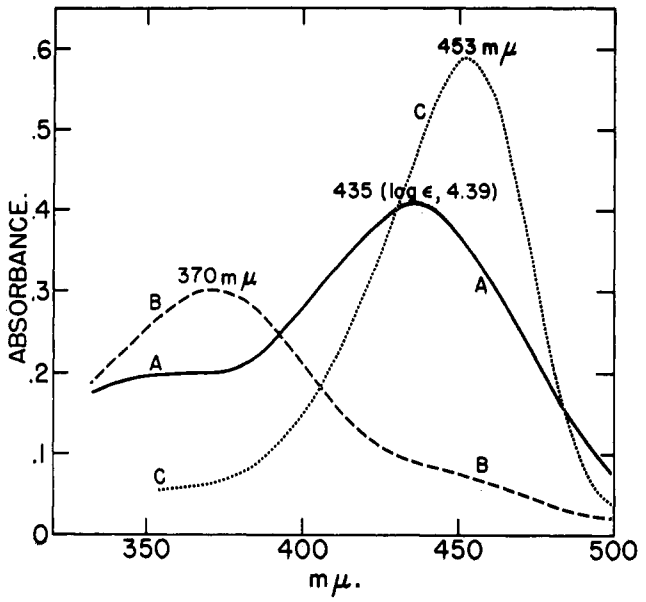


Fig. 3.—Spectrum of 4′-hydroxy-7-methoxyflavylium chloride (4.8×10^{-3} g./l.): (A) at pH 11.9; (B) the alkaline solution of A acidified to pH 1 and the spectrum taken at once; (C) the acid solution B taken after standing 2 hr.

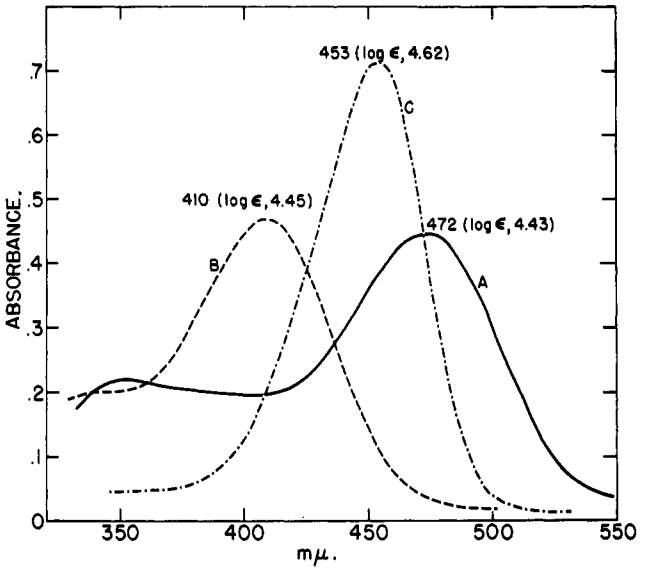


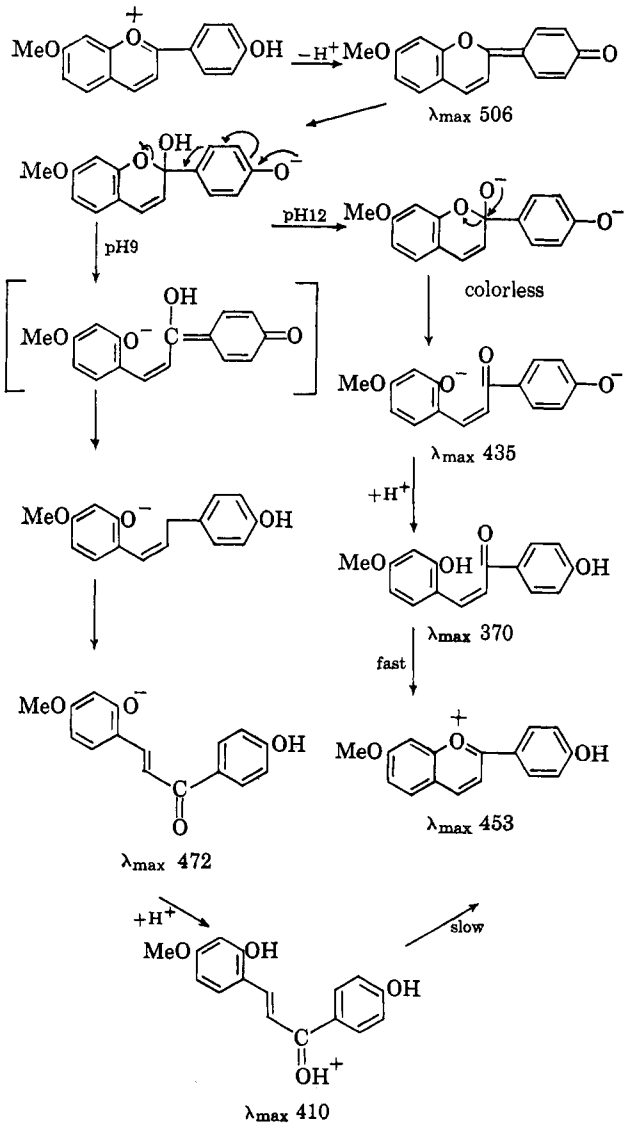
Fig. 4.—Spectrum of 4′-hydroxy-7-methoxyflavylium chloride (4.8×10^{-3} g./l.): (A) at pH 9.10; (B) the alkaline solution acidified to pH < 1 and spectrum taken at once; (C) the acid solution B after standing 24 hr.

hydrolysis of ionized anhydro bases in slightly alkaline solutions is relatively slow (twenty-four–forty-eight hours). Resonance stabilization of this type is not possible for anhydro bases derived from monohydroxyflavylium salts, and they decompose within seconds at about pH 9 to form yellow-orange compounds which differ distinctly from the products formed in more alkaline (pH 12) solutions.

For example, when 4′-hydroxy-7-methoxyflavylium chloride is adjusted to pH 11.9, the red solution (anhydro base) rapidly becomes colorless and then, on standing for an hour, intensely yellow. This yellow product, λ_{max} 435 mμ, log ε 4.39, is almost certainly the fully ionized *cis*-chalcone since, on acidification to pH < 1, the unionized chalcone (*cis*) initially formed (λ_{max} 370 mμ, log ε 4.26) rapidly regenerates the original

CHART I

STRUCTURAL TRANSFORMATIONS OF 4′-HYDROXY-7-METHOXYFLAVYLIUM CHLORIDE



flavylium salt (Fig. 3). The colorless intermediate first formed at pH 11.9 reforms the ionized anhydro base when the pH is lowered to 8. The colorless intermediate–chalcone change, however, is not reversible.

When 4′-hydroxy-7-methoxyflavylium chloride is diluted with buffers to give pH 9–10, the anhydro base is converted almost at once into a deeply yellow-orange product, λ_{max} 472 mμ, log ε 4.43, which at higher pH (12) becomes colorless and then forms the aforementioned ionized *cis*-chalcone (λ_{max} 435 mμ). The yellow-orange product is fairly stable at pH 9–10, about twenty-four hours being required for complete decomposition. When the yellow solution at pH 9–10 is acidified to pH < 1, an intensely lemon yellow compound, λ_{max} 410 mμ, log ε 4.45, is formed at once. On standing in the acid solution, this yellow product slowly reforms the original flavylium salt (λ_{max} 453 mμ, log ε 4.62) (Fig. 4). The yellow compound, λ_{max} 410 mμ, is not extracted from its acid solutions by nonpolar organic solvents. On the basis of these observations it is suggested that at pH 9–10 the anhydro base is primarily hydrolyzed to the mono-ionized *trans*

TABLE I

λ_{max} OF PRODUCTS FROM FLAVYLIUM SALTS AT pH 9 AND pH 12

Flavylium compounds	pH 8–10: Product λ_{max} mμ (log ε)	pH 8–10: Product on acidification λ_{max} mμ (log ε)	pH 12: Product λ_{max} mμ (log ε)	pH 12: Product on acidification λ_{max} mμ (log ε)
4′-Hydroxy-3′,7-dimethoxy	480 (4.43)	413 (4.45)	444 (4.48)	374 (4.37)
4′-Hydroxy-7-methoxy	472 (4.43)	410 (4.45)	435 (4.38)	370 (4.26)
7-Hydroxy-7-Hydroxy-3′,4′-dimethoxy	483 (4.68)	411 (4.48)	481 (4.53)	371 (4.26)
7-Hydroxy-4′-methoxy-	483 (4.70)	414 (4.44)	483 (4.56)	371 (4.28)
	483 (4.71)	413 (4.47)	481 (4.58)	370 (4.28)

chalcone. The complete reaction sequence postulated for 4′-hydroxy-7-methoxyflavylium chloride is summarized in Chart I.

7-Keto anhydro bases behave similarly. Thus, 7-hydroxy-4′-methoxyflavylium chloride, adjusted to pH 12.2, rapidly forms the ionized *cis*-chalcone, λ_{max} 481 mμ, log ε 4.58, which, on acidification to pH < 1, gives the unionized *cis*-chalcone, λ_{max} 370 mμ, log ε 4.28 (Fig. 5). At pH 9.9, however, the flavylium salt forms a yellow compound, λ_{max} 483 mμ, log ε 4.71, which differs distinctly from the ionized *cis*-chalcone, since on acidification to pH < 1 it immediately gives a bright lemon yellow compound, λ_{max} 413 mμ, log ε 4.47. On standing in the acid solution, this yellow product (protonated *trans*-chalcone) slowly regenerates the original flavylium salt (λ_{max} 457 mμ, log ε 4.65). Similar structural changes have been observed with the other flavylium salts listed in Table I. In each case the products formed at pH 9–10 and pH 12 are clearly distinguished by acidification to yield either yellow, protonated *trans*-chalcones (λ_{max} 410–414 mμ) or un-

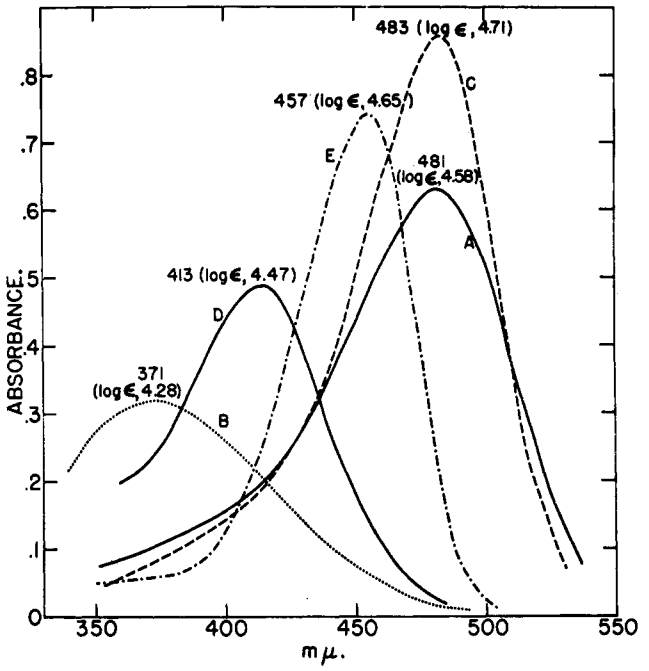


Fig. 5.—Spectrum of 7-hydroxy-4′-methoxyflavylium chloride (4.8×10^{-3} g./l.): (A) at pH 12.2; (B) acidification of A to pH < 1, spectrum taken at once; (C) at pH 10.0; (D) acidification of C to pH < 1, spectrum taken at once; (E) spectrum of B and D after standing 6 hr. and 71 hr., respectively.

ionized, almost colorless, *cis*-chalcones (λ_{max} 370–374 mμ).

Experimental

The flavylium salts used in this investigation are known compounds. They were prepared by Robinson's general methods, *vis.* acid condensation of the appropriate O hydroxyaldehydes and acetophenones in ethyl acetate or acetic acid solutions as described in Part I.[5]

The spectra of the flavylium salts used in this investigation were determined in buffered solutions[5] in 1-cm. silica cells.

The Synthesis of 5′-Deoxy-5′-S-(3-methylthiopropylamine)sulfoniumadenosine ("Decarboxylated S-Adenosylmethionine")

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5′-Deoxy-5′-S-(3-methylthiopropylamine)sulfoniumadenosine, the biological precursor of spermidine, has been synthesized by the condensation of 2′,3′-isopropylidene-5′-toluene-*p*-sulfonyladenosine and 3-thiopropylamine followed by removal of the acetonide group and subsequent methylation of the thioether. The corresponding (2-methylthioethylamine)- and (4-methylthiobutylamine)sulfoniumadenosine derivatives have been prepared in a similar way.

Although the specific biological function of the naturally occurring polyamines spermidine (1) and spermine (2) is not known, they have been shown to be

$NH_2(CH_2)_3NH(CH_2)_4NH_2$ (1) $\quad NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$ (2)

effective as growth factors for certain organisms and to exert a stabilizing effect on mitochondria and on DNA.[2-4] The path of biosynthesis of these compounds has been clarified by studies using labeled methionine and putrescine (1,4-diaminobutane) and the following scheme has been proposed on the basis of the labeling patterns in the various enzymatic products.[5]

(1) Inquiries should be addressed to the author at the Blood Program Research Laboratories, The American National Red Cross, Washington 6, D. C.

(2) For a full review on spermidine and spermine, see H. Tabor, C. W. Tabor, and S. M. Rosenthal, *Ann. Rev. Biochem.*, **30**, 579 (1961).

(3) H. Tabor, *Biochem. Biophys. Res. Commun.*, **4**, 228 (1961).

(4) H. Tabor, *Biochemistry*, **1**, 496 (1962).

(5) H. Tabor, S. M. Rosenthal, and C. W. Tabor, *J. Biol. Chem.*, **233**, 907 (1958).