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ANTHOCYANIDINS AND RELATED COMPOUNDS—XV
THE EFFECTS OF SUNLIGHT ON FLAVYLIUM SALT–CHALCONE EQUILIBRIUM IN ACID SOLUTIONS

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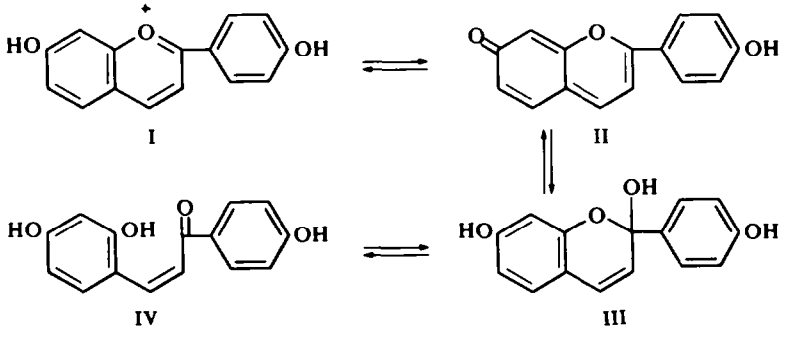
Abstract—The position of the equilibrium between flavylium salts and 2-hydroxychalcones in aqueous solutions is markedly effected by light. NMR and UV spectral data indicate that the chalcones formed at equilibrium have the *trans*-configuration. In sunlight these photoisomerize rapidly to *cis* 2-hydroxychalcones, which then cyclize in acid solutions to flavylium salts. In the absence of light the necessary, initial *trans* to *cis* isomerization is acid catalyzed and occurs readily only with those chalcones with stabilizing, electron-donating groups, e.g. methoxyl, at the 4 and/or 6 positions. Prolonged exposure of *cis* 2-methoxychalcones to light results in a photochemical demethylation with formation of the *cis* 2-hydroxychalcone or cyclic pseudo base.

THE OXIDATION and condensation reactions which are involved in irreversible destruction of natural anthocyanin pigments (polyhydroxyflavylium salts) are obscure. Decoloration which is reversible on acidification, however, is known to be due to hydrolytic changes which occur at the normal pH (pH 3–6) of plant extracts.[1] In connection with flavylium salt hydrolysis it was demonstrated some years ago[2,3] that salts, which lack a 3-substituent, form equilibrium mixtures with the corresponding 2-hydroxylchalcones, these chalcones being derived from unstable, intermediate anhydro bases and pseudo bases, e.g. I ⇄ IV.



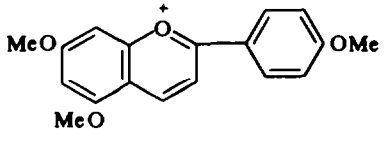
Identification of the equilibrium hydrolysis products as 2-hydroxychalcones was based on their absorption maxima (330–380 mμ) and the isolation of crystalline chalcones from two flavylium salts, viz. 2′-hydroxy-8-methoxy-flavylium chloride and 4′,8-dimethoxy-flavylium chloride IX. The stereochemistry of the chalcones formed at equilibrium in these aqueous solutions has not previously been investigated,

although because of the ease with which they *usually* recyclize (particularly those derived from 7-hydroxyflavylium salts of type I) they were represented[2] as *cis* chalcones.

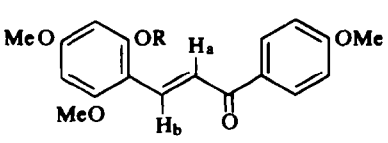
The existence of a flavylium salt–chalcone equilibrium in aqueous solutions has subsequently been confirmed by the extensive spectral, polarographic and kinetic studies of Timberlake and Bridle,[4] Nilsson,[5] Harper and Chandler,[6] and Kuhn *et al.*[7,8] Timberlake and Bridle, furthermore, made the significant observation that true equilibrium conditions for the flavylium–chalcone change are obtained only in complete darkness. Exposure of solutions to light resulted in a shift of equilibrium toward the flavylium salt form. These authors did not determine the photochemical transformations involved in producing shifts in the equilibrium. However, in this communication additional data is presented which appears to account satisfactorily for the pronounced differences in the ease with which two dissimilarly substituted flavylium salts interconvert with their related 2-hydroxychalcones in acid solutions in light and in the dark. Thus, of the four species known to be involved in attainment of flavylium equilibrium, viz. the flavylium cation, the anhydro base, the pseudo base, and the 2-hydroxychalcone, it seemed probable that photochemical and/or acid-catalyzed transformation of the chalcone is the factor which primarily governs a shift to the flavylium form. The experimental evidence, which fully supports this view, indicates that the 2-hydroxychalcones formed at equilibrium are all *trans* chalcones. In light these *trans* chalcones undergo an extremely facile photo-isomerization to *cis* chalcones which the rapidly cyclize (in acid solutions) by a non-photochemical process to flavylium salts. In the absence of light, on the other hand, the necessary, initial *trans* to *cis* isomerization is acid-catalyzed (and much slower than the photochemical isomerization) and occurs readily only with those chalcones which contain hydroxyl or methoxyl groups at the 4 and/or 6 positions, e.g. VIa, XVIa. In the absence of electron-donating groups in either of the positions, e.g. as in XIa, acid-catalyzed isomerization is extremely slow or does not occur, and appreciable flavylium salt formation in acid solutions in the dark is not observed.

Stereochemistry of 2-hydroxychalcones

A standard solution of 4',5,7-trimethoxyflavylium perchlorate V in 1% aq. HCl (λ_{max} 469 mμ, ε_{max} 37,200), diluted with aqueous buffer, pH 5·60, yields 2-hydroxy-4,6,4'-trimethoxychalcone VIa. In the absence of light at this pH conversion to the chalcone (λ_{max} 378 mμ, ε_{max} 22,500) is almost complete within 30 min. In light, however, an equilibrium is reached, containing approximately 60% flavylium salt and 40% chalcone. Exposure of the solution of the chalcone (obtained in the dark) to light results in partial recylization to the flavylium salt and formation of the same 60:40 equilibrium mixture. On a preparative scale the chalcone VIa crystallizes when a warm solution of V in buffered aqueous ethanol is cooled in the absence of



V



VIa, R = H
VIb, R = Me

light. This chalcone is identical with that synthesized by alkaline condensation of 2-hydroxy-4,6-dimethoxybenzaldehyde and 4-methoxyacetophenone. On methylation it gives a 2-O-methyl derivative, the NMR spectrum (Fig. 1) of which shows the ethylenic H_a and H_b protons as doublets at δ 7·83 and δ 8·24 respectively, with J = 16·0 c/s. The magnitude of J establishes the *trans* configuration VIb for this methyl derivative and, assuming no change in configuration during methylation, for the parent 2-hydroxychalcone.

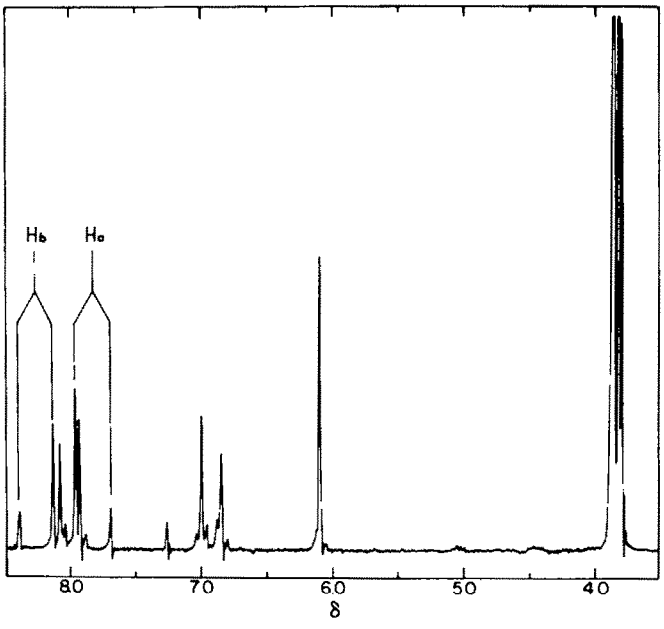


FIG. 1. 60 Mc spectrum in $CDCl_3$ of VIb; TMS as internal reference.

4′,7-Dimethoxyflavylium perchlorate XV and 4′,8-dimethoxyflavylium perchlorate IX, treated similarly with aqueous buffer solutions, also yielded the crystalline 2-hydroxychalcones. The ethylenic H_a and H_b protons in the 2-O-methyl derivatives XVIb and XIb of these chalcones appeared as doublets at δ 7·52, 8·06 and δ 7·58, 8·10 with J = 16·0 and 15·8 c/s respectively, and these chalcones, therefore, also have *trans* configurations.

Photochemical and acid-catalyzed transformations of trans *2-hydroxy-4,6,4′-trimethoxychalcone*

Dilution of a standard solution of VIa in alcohol with 1%aq. HCl results in its cyclization to the flavylium salt V. Cyclization occurs in both light and darkness, but the rate, as measured by the increase in absorbance at the λ_{max} of V, is unambiguously*

* In contrast to the pronounced effect of light on V–VIa interconversions Timberlake and Bridle[4] have reported that light has only a small and ill-defined effect on the equilibrium reactions of 5,7-dihydroxyflavylium salts.

more rapid in light than in the dark:

	% Conversion of VIa to V in 1% aq. HCl	
Time (min)	Light	Dark
5	92	—
10	99	—
30	100	39
120	—	71

Thus two distinct cyclization processes are involved.

When a solution of the *trans* chalcone VIa in 95% ethanol (λ_{max} 374 mμ, ε_{max} 31,200) is exposed to light, its spectrum rapidly and markedly changes (Fig. 2) with the formation of a new compound, λ_{max} 283 mμ (ε_{max} 15,500), with a weak, broad band at 380–400 mμ (ε_{max} 3000). Addition of a drop of HCl to this solution results in the immediate and quantitative formation of the flavylium salt V. Thus the photochemical transformation of VIa into the intermediate compound, λ_{max} 283 mμ, is the rate determining step in the observed rapid acid cyclization of VIa to V in light. This intermediate compound is unstable and it readily reverts to the *trans* chalcone VIa when light is excluded from its solution in alcohol (63% reconversion in 2 hr; 100% reconversion within 16 hr). Since photochemical *trans–cis* isomerization of ethylenic

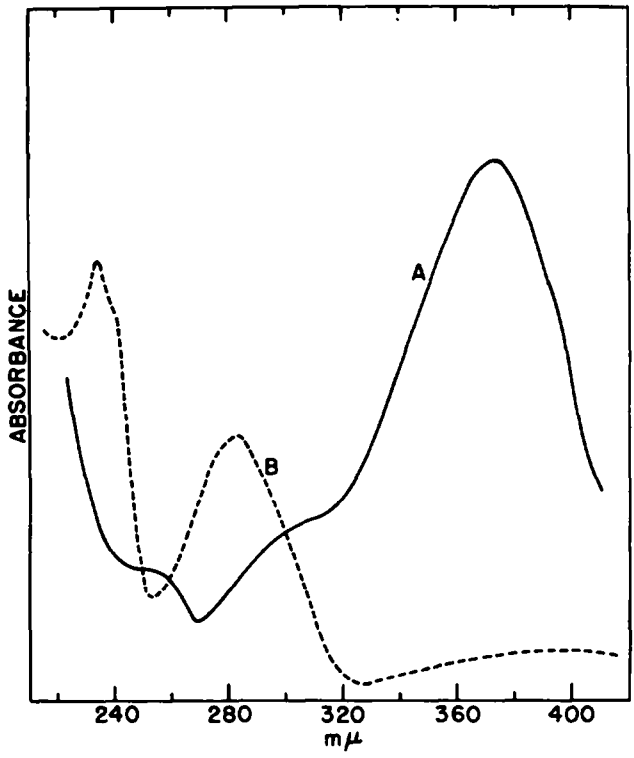
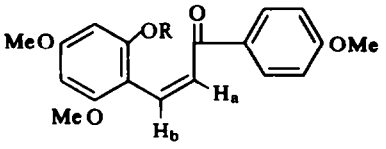
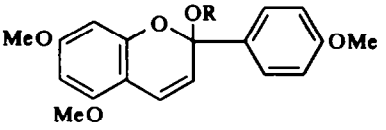


FIG. 2. UV spectrum in ethanol of (A) VIa, (B) VIa exposed to light for 30 min.

compounds is well-known, the intermediate photo product is considered to be the *cis* chalcone VIIa or a pseudo base VIII, formed by the facile, reversible cyclization (and possibly solvolysis) of VIIa.



VIIa, R = H
VIIb, R = Me



VIII, R = H or Et

A decision, based on unequivocal experimental evidence, between structure VIIa and VIII for the photo product has not been made, since attempts to isolate and crystallize the unstable product proved unsuccessful. Support for the assignment of either of these two structures for the photo product, however, was obtained by a parallel series of reactions on the 2-O-methyl derivative of the *trans* 2-hydroxychalcone.

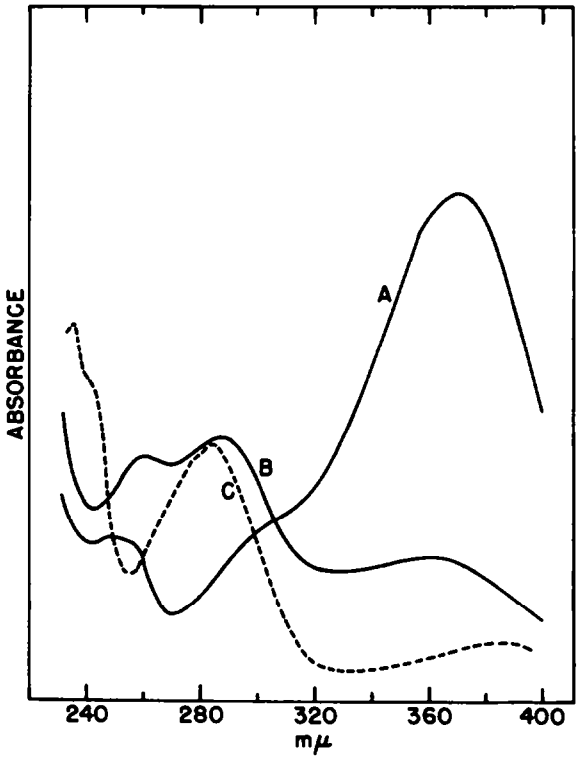


FIG. 3. UV spectrum in ethanol of (A) VIb, (B) VIb exposed to light 10 min, (C) VIb exposed to intermittent sunlight for 44 hr.

Thus, brief exposure to light of an alcoholic solution of VIb (λ_{max} 371 mμ, ε_{max} 29,500) resulted in its conversion to a similar intermediate (Fig. 3, 3B), λ_{max} 288 mμ, ε_{max} 15,350, with a weak, broad band at about 360 mμ. This photo product is stable in neutral alcohol in the dark and it was readily crystallized. It is a lower melting (m.p. 133–134°) isomer of VIb (m.p. 152°) and its NMR spectrum (Fig. 4) showed an upfield shift of the H_a and H_b protons to δ 6·46 and δ 6·92, with J = 13·0 c/s. The smaller magnitude of J and the lower melting point are in accord with the *cis* chalcone structure VIIb for

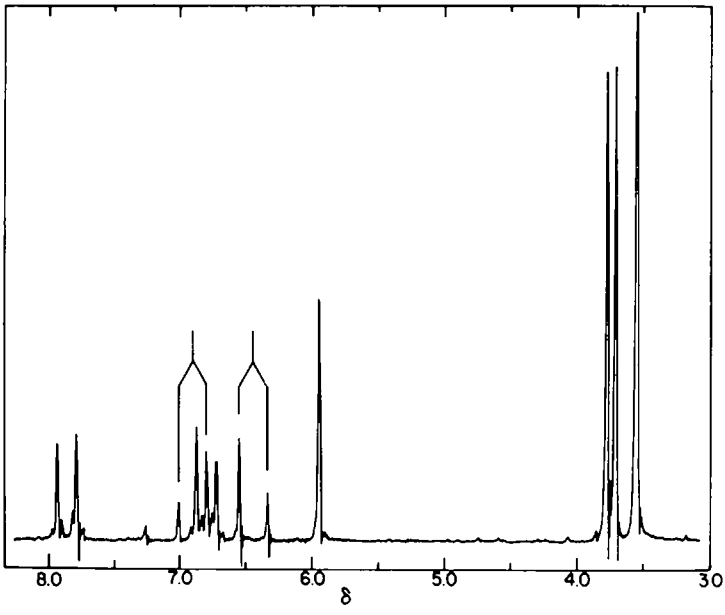
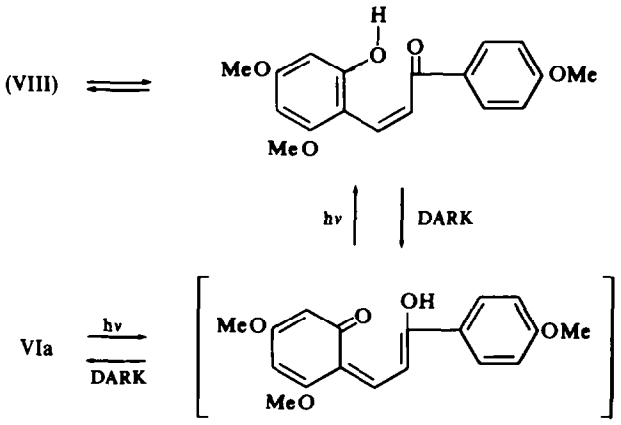


FIG. 4. 60 Mc spectrum in $CDCl_3$ of VIIb; TMS as internal reference.

this photo product. Confirmatory evidence for structure VIIb is provided by the methoxyl signals in the NMR spectrum. These show the presence of two identical methoxyl groups (δ 3·56) and two dissimilarly located methoxyl groups (δ 3·73 and δ 3·80). An alternate pseudo base methyl ether structure (VIII, R = Me) for this product is thereby excluded, since the signal of the alcoholic methoxyl group of this compound would be shifted upfield, e.g. the alcoholic methoxyl of XIX acetate appears at δ 3·30. VIIb is unstable in light and, as described below, prolonged exposure leads to photochemical demethylation of the 2-methoxyl group with formation of photo VIa.

As previously described, VIIb is stable in neutral alcoholic solutions in the dark, whereas the corresponding photo product from the 2-hydroxychalcone reverts to VIa. The instability of photo-VIa can be accounted for by an enolization process of the following type, which leads to the thermodynamically more stable *trans* isomer.

Enolization, however, is blocked by methylation of the 2-hydroxyl group and the *cis* chalcone is then stable:



Although the *cis* chalcone VIIb is perfectly stable in neutral ethanol solution in the absence of light, addition of a drop of acid results in its rapid isomerization (in the dark) to the *trans* isomer VIb (Fig. 5). This acid-catalyzed isomerization of

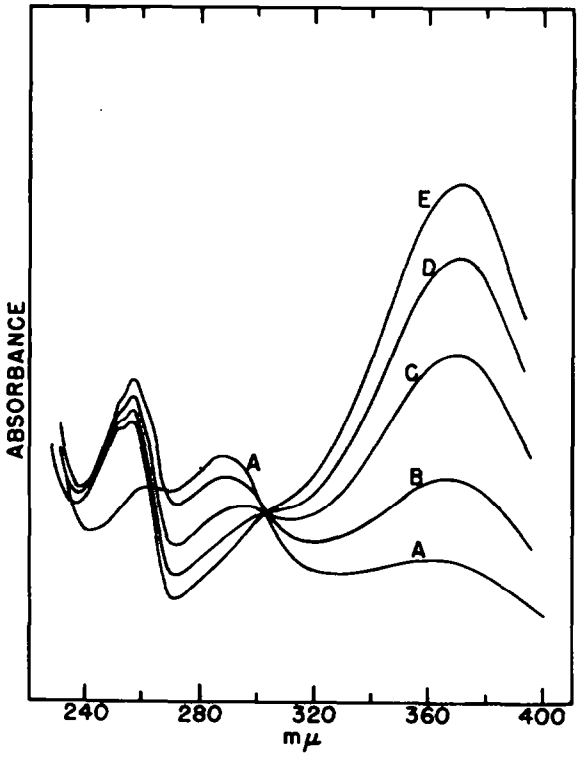
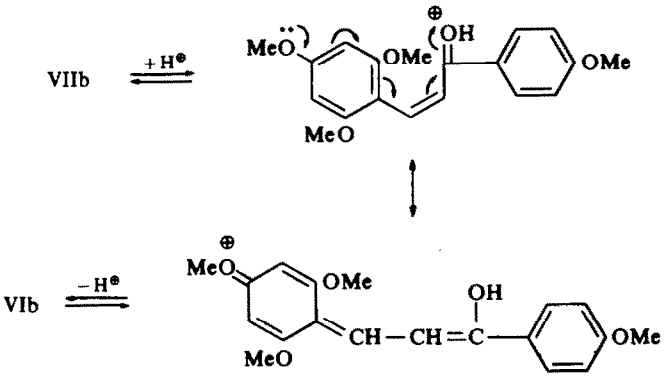


FIG. 5. UV spectrum in ethanolic HCl of VIIb. Spectrum measured immediately (A), after 4 min (B), after 12 min (C), after 26 min (D), after 1 hr (E).

2-methoxychalcones is dependent on the presence of additional methoxyl groups in the 4 and/or 6 positions, since *cis* 2,3,4′-trimethoxychalcone XIV, which lacks these groups, is stable on long standing in darkness in ethanolic HCl. Electron-donating groups in the 4 and 6 positions apparently facilitate acid isomerization by stabilizing the intermediate protonated chalcone:



The acid catalyzed isomerization VIIb → VIb suggests that the cyclization of *trans* 2-hydroxychalcones, such as VIa, to flavylium salts in acid solutions in darkness involves initial protonation of the *trans* chalcone and reversal of the above equilibrium by conversion of the unstable *cis* chalcone into the stable flavylium salt. On the basis of this interpretation it follows that the facility with which *trans* 2-hydroxychalcones cyclize in acid solutions without light should be dependent on the presence and number of stabilizing, electron-donating methoxyl groups in the 4 and/or 6 positions. Experimental support for this theory is provided by the following comparison:

	Time (hr)	Conversion to flavylium salt in EtOH–HCl (dark) %
VIa	0·5	39
	2·0	71
XVIa	0·5	22
	2·0	45
XIa	5·0	0

Thus, the 4,6-dimethoxychalcone VIa cyclizes at a distinctly greater rate than the 4-methoxychalcone XVIa, while XIa, which lacks a methoxyl group in either the 4 or 6 position, does not cyclize significantly on longer standing.

Equilibrium reactions of 4′,8-dimethoxyflavylium salts

The equilibrium reactions of flavylium salts which lack hydroxyl or methoxyl substituents in the 5 or 7 positions, e.g. IX, differ markedly from those of 7- and 5,7-substituted salts in a number of respects. Thus, when V and other 7-hydroxyflavylium

derivatives, e.g. I, are added to aqueous buffer at pH 3–6 and allowed to reach equilibrium with 2-hydroxychalcones, the intermediate formation of pseudo bases of type III cannot[2,6] be detected by spectral or polarographic measurements i.e. pseudo bases of this type are so unstable that their formation can only be inferred. When 4',8-dimethoxylflavylium perchlorate, λ_{max} 430 mμ, is diluted with aqueous buffer, pH 5·60, however the solution immediately becomes colorless with the formation of a compound with λ_{max} 263, 273 mμ (Fig. 6, A). This compound has been assigned[2] the pseudo

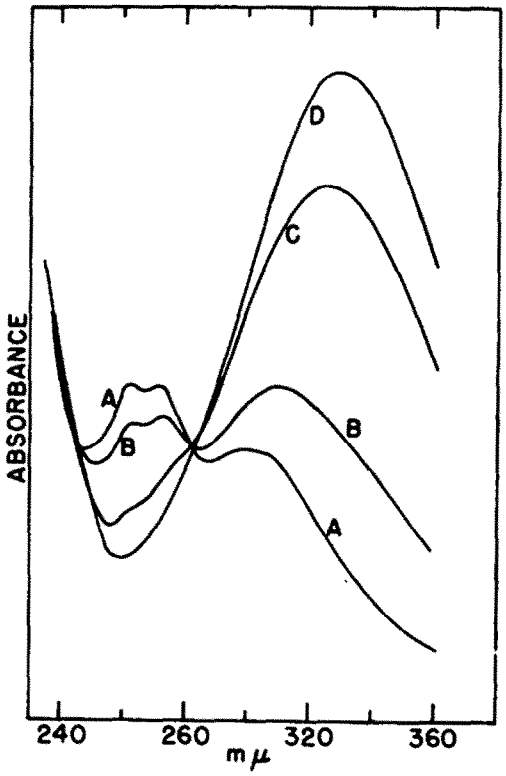
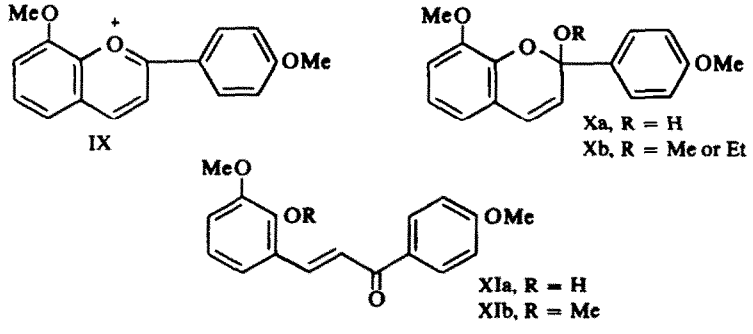
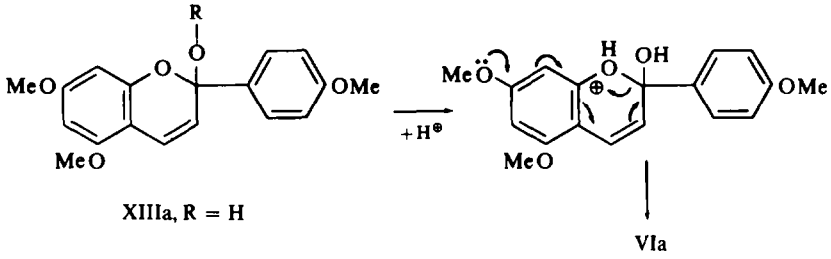


FIG. 6. Spectrum of IX in aqueous buffer, pH 5·60. Spectrum measured immediately (A), after 20 min (B), after 70 min (C), after 110 min (D).

base structure Xa. On standing, both in light and in the dark, the solution then becomes slightly yellow due to the relatively slow conversion of the pseudo base into the *trans*



chalcone XIa. In light at this pH the conversion of IX into XIa via the pseudo base Xa is quantitative. Thus the flavylium salt IX differs from V, which under the same conditions reaches a stable 60 : 40 equilibrium with its derived chalcone VIa. The difference in the behaviour of IX and V can be rationalized on the basis that IX is inherently less stable than V, in which the 5- and 7-methoxyl groups increase stability by extensive delocalization of the positive charge. On this basis, furthermore, the pseudo base Xa would be expected to be *more* stable than that formed from V, viz. XIIIa, since in the latter the methoxyl groups located at the 5 and 7 positions should facilitate ring opening by delocalization of the positive charge on an intermediate protonated pseudo base:



Like VIa, the *trans* 2-hydroxychalcone XIa forms the flavylium salt IX, when its solution in aqueous HCl is exposed to light, and the mechanism involved is similar. Thus, exposure of a solution of XIa in alcohol (λ_{max} 324 mμ) to light yields an inter-

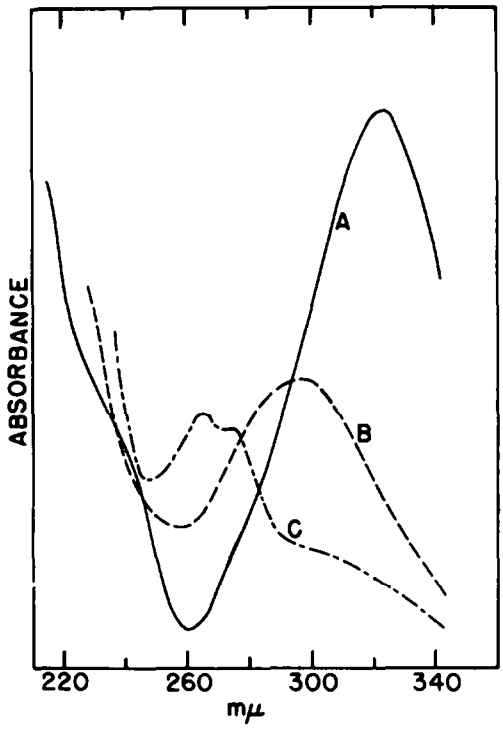
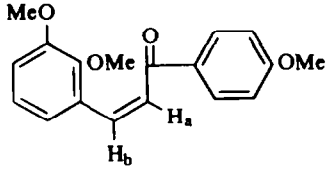


FIG. 7. UV spectrum in ethanol of (A) XIb, (B) XIb exposed to light for 20 min (C) XIb exposed to intermittent sunlight for 28 hr.

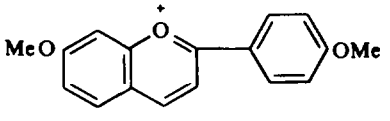
mediate, which is converted to the flavylium salt IX on addition of acid. The intermediate is unstable in neutral alcohol in the absence of light and reverts quantitatively to the *trans* chalcone. The 2-O-methyl derivative XIb of the *trans* chalcone, λ_{max} 323 mμ (ε_{max} 29,000), is converted rapidly into an isomer, λ_{max} 297 mμ (ε_{max} 15,400), when its alcoholic solution is exposed briefly to light (Fig. 7, B). The photo product was not crystallized. However, in accord with the *cis* chalcone structure XIV the NMR spectrum of the crude product showed the ethylenic H_a and H_b protons as doublets at δ 6·57 and δ 7·19, J = 13·0 c/s. The photo product XIV is stable in both neutral *and acidified* ethanol in the absence of light. The acid stability of XIV is in contrast with the



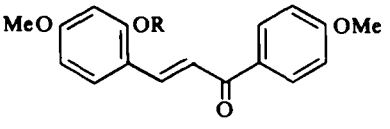
XIV

acid instability of VIIb and lends support to the proposal that acid catalyzed *trans* to *cis* isomerization with subsequent cyclization to flavylium salts occurs only with those 2-hydroxychalcones which contain electron-donating groups such as hydroxyl or methoxyl in the 4 and/or 6 positions.

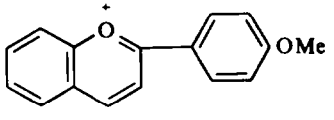
Although not yet studied in detail, it appears that the light and acid-catalyzed interconversions of 4′,7-dimethoxyflavylium XV and 4′-methoxyflavylium XVII salts and their corresponding chalcones XVI and XVIII are similar to those of V and IX respectively, and can be accounted for in terms of various proposals made in this communication. Two unexpected observations are noteworthy, viz. (1) the spectrum



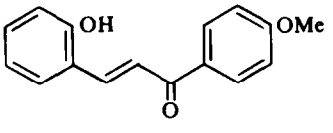
XV



XVIa, R = H
XVIb, R = Me



XVII



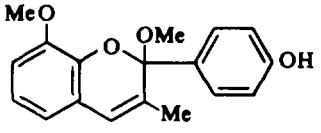
XVIII

of XVIa in alcohol does not change appreciably when it is exposed to light, although the 2-O-methyl derivative XVIb reacts normally, i.e. rapidly isomerizes to the *cis* chalcone, (2) dilution of an acid solution of XVII (λ_{max} 437 mμ) with aqueous buffer, pH 5·60, results in the immediate formation of a colorless compound, λ_{max} 283 mμ. This compound may be a pseudo base similar to Xa, but unlike Xa it is quite stable in light and in darkness and shows no tendency to undergo ring fission to XVIII.

Photochemical demethylation of 2-methoxychalcones

When spectroscopic solutions of the *trans* 2-methoxychalcones VIb and XIb in dilute ethanolic HCl are exposed to light they gradually become intensely yellow due to formation of the corresponding flavylium salts. Simple acid hydrolysis of the 2-methoxyl group is not the basis of this process, since the chalcones are completely stable in these acid solutions in the absence of light. Two photochemical steps are actually involved, viz. (1) isomerization of the *trans* to *cis* chalcone, and (2) photochemical demethylation of the 2-methoxyl group to yield a pseudo base or *cis* 2-hydroxychalcone.

Thus, exposure of an alcoholic solution of XIb to light rapidly yields the *cis* isomer XIV. On longer exposure (up to 28 hr) XIV is converted into a compound, whose spectrum (Fig. 7, C) is almost identical with that (Fig. 6, A) of the pseudo base Xa, formed from the flavylium salt IX at pH 5·60. Addition of acid to the photo product yields the flavylium salt and, therefore, it is considered to be a pseudo base methyl or ethyl ether, Xb, R = Me or Et. The free carbinol Xa is unlikely in view of its previously described instability (in aqueous solution). In support of the assigned pseudo base structure Xb for this photo product it is noteworthy that the spectrum of the authentic, crystalline pseudo base methyl ether XIX, prepared by treatment of 4'-hydroxy-8-methoxy-3-methyl-flavylium chloride with aqueous methanol, is identical with that of the photo product. Extinction coefficients are almost identical and both show the same characteristic double λ_{max} at 264 and 274 mμ.



XIX

VIb, similarly exposed to light in alcoholic solution, rapidly forms the *cis* chalcone VIIb and this, on prolonged exposure, is converted to a compound whose spectrum (Fig. 3, C) is identical with that of photo-VIa. Addition of acid to this solution immediately yields the flavylium salt V (75% overall yield). As indicated above, it is not known with certainty whether this photo product from VIa and VIIb is the *cis* 2-hydroxychalcone VIIa or a pseudo base VIII.

EXPERIMENTAL

4',5,7 Trimethoxyflavylium perchlorate V

A solution of 2-hydroxy-4,6-dimethoxy-benzaldehyde (18·2 g) and 4-methoxyacetophenone (15·0 g) in ethyl acetate (100 ml) and ethanol (20 ml) was cooled and saturated with HCl gas. The mixture was kept at 0° for 4 days and the red crystals of 4',5,7-trimethoxyflavylium chloride were collected. The crude chloride was recrystallized twice from glacial acetic acid–5% aq. perchloric acid to give *4',5,7-trimethoxyflavylium* perchlorate* as soft, orange-colored needles, which decomposed to a red solid at 234–236° and melt sharply at 258°. In 1% aq. HCl, λ_{max} 469, 322, 277 mμ (ε_{max} 37,200, 5900, 21,000; in ethanol–0·5% HCl, λ_{max} 477, 325, 278 mμ (ε_{max} 42,200, 6150, 20,500). (Found: C, 54·45; H, 4·39. Calc. for $C_{18}H_{17}O_8Cl$: C, 54·5; H, 4·32).

* Chloride and ferrichloride have been prepared previously by reaction of ethynyl *p*-methoxyphenyl ketone with phloroglucinol dimethyl ether[9] and by acid cyclization of the potassium salt of VIa.[10]

trans 2-*Hydroxy*-4,6,4'-*trimethoxychalcone* VIa

(a) The potassium salt of VIa has been prepared by Pratt *et al.*[10] and cyclized to V ferrichloride. Modification of their procedure yields crystalline VIa: A mixture of 2-hydroxy-4,6-dimethoxybenzaldehyde (2·0 g), 4-methoxyacetophenone (2·0 g), powdered KOH (2·0 g) and methanol (15·0 ml) was warmed for 3 hr and allowed to stand for 3 days. The dark solution, from which the K salt had partially separated, was diluted with water and benzene and the K salt of the product was collected. It was suspended in dilute aqueous acetic acid and the undissolved solid was recrystallized from methanol. VIa separated as golden-yellow needles, m.p. 164° (0·90 g). In alcohol λ_{max} 374 mμ (ε_{max} 31,200), inflections at 301, 255 mμ. (Found: C, 68·9; H, 5·78. Calc. for $C_{18}H_{18}O_5$: C, 68·8; H, 5·77).

The *acetate* of VIa, prepared by warming with acetic anhydride and pyridine, crystallized from acetone–methanol as yellow needles, m.p. 130–131°. (Found: C, 67·5; H, 5·56. Calc. for $C_{20}H_{20}O_6$: C, 67·4; H, 5·66). In ethanol, λ_{max} 355 mμ (ε_{max} 31,300).

A mixture of VIa (0·40 g), dimethylsulfate (2·0 ml), anhydrous potassium carbonate (4·0 g) and dry acetone (25 ml) was heated under reflux for 1·5 hr, concentrated, and diluted with water. The solid product, *trans* 2,4,6,4'-tetramethoxychalcone VIb, crystallized from methanol as yellow needles, m.p. 152° (0·34 g). In alcohol λ_{max} 371, 248–257 mμ (ε_{max} 29,500, 9510). (Found: C, 69·5; H, 6·10. Calc. for $C_{19}H_{20}O_5$: C, 69·5; H, 6·14).

(b) A solution of 4',5,7-trimethoxyflavylium perchlorate V (0·50 g) in warm ethanol (50·0 ml) was slowly diluted with aqueous buffer solution, pH 5·60 (100·0 ml). The filtered, warm solution was cooled to room temperature in the dark. After 2 hr the yellow, crystalline product was collected and recrystallized from methanol. VIa, m.p. and m.m.p. 164°, was obtained (0·36 g). (Found: C, 68·9; H, 5·78. Calc. for $C_{18}H_{18}O_5$: C, 68·9; H, 5·77).

Methylation of the above product (2·5 g) gave VIb as yellow needles, m.p. and m.m.p. 152° (2·2 g).

cis 2,4,6,4'-*Tetranethoxychalcone* VIIb

A solution of VIb (1·2 g) in methanol (150 ml) was exposed to sunlight for 2 days. Large, slightly yellow crystals, λ_{max} 360, 288, 260 mμ (cf. Fig. 3, B), separated (0·40 g). Recrystallized from acetone–methanol VIIb separated as slightly yellow prisms, m.p. 133–134°; m.m.p. with VIb, 120–122°. (Found: C, 69·6; H, 6·13. Calc. for $C_{19}H_{20}O_5$: C, 69·5; H, 6·14).

trans 2-*Hydroxy*-4,4'-*dimethoxychalcone* XVIa

(a) 50% Aq. KOH (3·0 ml) was added to a solution of 2-hydroxy-4-methoxybenzaldehyde (3·0 g) and 4-methoxyacetophenone (3·0 g) in warm ethanol (5·0 ml). After 48 hr dilute acetic acid was added and the product was collected. Recrystallized from methanol XVIa separated as yellow needles, m.p. 150° (1·30 g). (Found: C, 72·0; H, 5·76. Calc. for $C_{17}H_{16}O_4$: C, 71·8; H, 5·67).

The *acetate* of XVIa, prepared by brief heating with acetic anhydride and pyridine, separated from methanol as yellow plates. m.p. 135°. (Found: C, 69·9; H, 5·60. Calc. for $C_{19}H_{18}O_5$: C, 69·9; H, 5·56).

(b) Aqueous buffer, pH 5·45 (15·0 ml) was added to a warm solution of 4',7-dimethoxyflavylium perchlorate[11] (0·30 g) in ethanol (10·0 ml). On cooling in the absence of light yellow crystals separated (0·20 g). Recrystallized from methanol XVIa separated as yellow needles, m.p. and m.m.p. 150°. The *acetate* of the product crystallized as slightly yellow plates, m.p. and m.m.p. with XVIa acetate, 135°.

trans 2,4,4'-*Trimethoxychalcone* XVIb

A mixture of 2,4-dimethoxybenzaldehyde (7·0 g), 4-methoxyacetophenone (7·0 g), ethanol (100 ml) and 50% aq. KOH (25·0 ml) was maintained at 50–60° for 8 hr. Water was added and, after cooling, the solid product was collected. Recrystallized from methanol XVIb was obtained as yellow needles, m.p. 81–82° (11·5 g). (Found: C, 72·5; H, 5·99. Calc. for $C_{18}H_{18}O_4$: C, 72·5; H, 6·08).

4',8-*Dimethoxyflavylium perchlorate* IX

A solution of ortho-vanillin (30 g) and 4-methoxyacetophenone (30 g) in ethyl acetate (150 ml) and ethanol (45 ml)[12] was cooled in an ice-bath and saturated with HCl gas (10 min). After 24 hr the crystalline chloride was collected (60·0 g), washed with ethyl acetate, and dissolved in hot glacial acetic acid (300 ml) and 5% aq. $HClO_4$ (500 ml). The perchlorate crystallized on cooling. Recrystallized from acetic acid–aq. $HClO_4$ IX *perchlorate* separated as glistening, orange-red plates, m.p. 228–229°. (Found: C, 55·8; H, 4·16. Calc. for $C_{17}H_{15}O_7Cl$: C, 55·65; H, 4·13).

trans 2-*Hydroxy*-3,4'-*dimethoxychalcone* XIa

XIa was prepared[2] by hydrolysis of IX chloride and by alkaline condensation of ortho-vanillin and 4-methoxyacetophenone. Methylation[13] gave XIb.

4'-*Hydroxy*-2,8-*dimethoxy*-3-*methyl flav*-3-*ene* XIX

A solution of 4'-hydroxy-8-methoxy-3-methylflavylium chloride (2·0 g) in methanol (20·0 ml) and water (20·0 ml) was heated on a steam-bath for 5 min and allowed to cool. Almost colorless crystals separated (1·40g). Recrystallized from aqueous methanol XIV separated as glistening, colorless needles which rapidly became reddish on exposure to air When heated XIX becomes red at c 100° and melts to a deep red liquid at 138–140°. In alcohol, λ_{max} 274, 264 mμ (ε_{max} 12,000, 13,000). (Found: C, 72·6; H, 6·03; MeO—, 20·1. Calc. for $C_{18}H_{18}O_4$: C, 72·5; H, 6·08; 2 MeO—, 20·8).

The *acetate* of XIX, prepared by warming with acetic anhydride and two drops of pyridine, crystallized from methanol as colorless prisms, m.p. 101–102°. In alcohol, λ_{max} 273, 264 mμ (log ε 4·02, 4·05).

Spectral measurements

In the determination of spectra 1·0 ml aliquots of a standard solution of the flavylium salt (10^{-4} moles in 100·0 ml of solution in 1% aq. HCl or 95% ethanol containing 0·5% HCl) or chalcone (10^{-4} moles in 100·0 ml of solution in 95% ethanol) were diluted to 50·0 ml with 1% aq. HCl, citric acid–disodium hydrogen phosphate buffer (pH 5·60), or 95% ethanol as indicated in the text. Spectra were determined immediately and at intervals after standing at room temperature in darkness or after being exposed to light. Solutions were exposed to sunlight in Pyrex flasks. No attempt was made in this study to standardize the amount of light received; the measurements quoted in the text, therefore, can be considered only as relative approximations.

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