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ANTHOCYANINS AND RELATED COMPOUNDS—V

FORMATION OF BISFLAVENYLIDENES FROM FLAVONES BY REDUCTIVE DIMERIZATION

B. J. BERGOT and L. JURD
Western Regional Research Laboratory, Albany, California[*]

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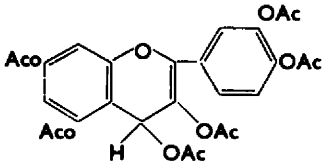
Abstract—Flavones, unsubstituted in the 3- and 5-positions, form yellow, dimeric bisflavenylidenes under the conditions of reductive acetylation. With mineral acids these bisflavenylidenes are monoprotonated to yield substituted 4-flavenylflavylium salts. Reductive acetylation of quercetin, on the other hand, gives a complex, amorphous mixture of acetates containing at least one colourless and three unidentified yellow compounds. The colourless product has been crystallised and yields cyanidin chloride with ethanolic hydrochloric acid. This colourless product is 3,3′,4′,5,7-pentaacetoxy-flav-3-ene admixed with a quantity of the isomeric flav-2-ene.

THE reductive acetylation of flavones and flavonols is an ill-defined reaction which has assumed greater significance since King and White[1] reported that anthocyanidins can be prepared in acceptable yields by further treatment of the crude reduction products with mineral acids. The reactions involved in these zinc reductions have aroused some controversy, primarily due to the fact that pure reduction products have rarely been separated and characterized. The latest investigation reported by Bayer and Krämer[2] prompts us to record our observations in this field.

Robertson and Robinson reductively acetylated rhamnetin,[3] quercetin,[4] tectochrysin and 7-hydroxy-4′-methoxyflavone[5] and obtained ochrous, amorphous products which, when treated with ethanolic hydrochloric acid, gave small quantities of the corresponding monomeric anthocyanidin and flavylium chlorides. Thus, they suggested that the reduction products probably contained acetates, e.g., I, of the carbinol bases of anthocyanidins, together with unidentified by-products derived from a pinacol type reduction. King and White,[1] however, reported that the crude reduction



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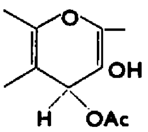

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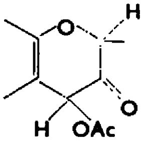

III

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[*] A laboratory of the Western Utilisation Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

[1] H. G. C. King and T. White, *J. Chem. Soc.* 3901 (1957).

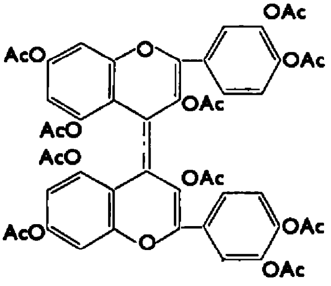
[2] E. Bayer and B. Kramer, *Chem. Ber.* 97, 1057 (1964).

[3] A. Robertson and R. Robinson, *J. Chem. Soc.* 2196 (1927).

[4] G. M. Robinson and R. Robinson, *Biochem. J.* 27, 206 (1933).

[5] A. Robertson, R. Robinson and M. Struthers, *J. Chem. Soc.* 1455 (1928).

products contained one less acetyl group than would be expected on the basis of I. Consequently, they proposed possible structures II and III. The formation of C_{15} reduction products was disputed by Malkin and Nierenstein[6] who claimed that the flavylium salts obtained from quercetin and rhamnetin by Robinson's procedure[3] contained only one-half of the expected chlorine content. They argued, therefore, that reductive acetylation gives bisflavenylidene compounds, e.g., quercetylene acetate IV, which with acids are converted into 4-flavenylanthocyanidins e.g. V.



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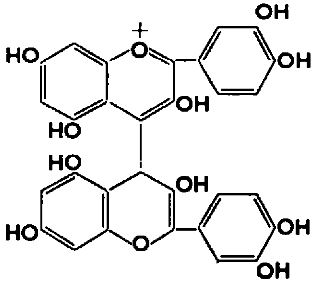
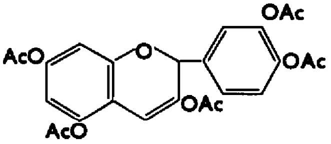


V

In 1959 Laumas and Seshadri[7] reported the important observation that reductive acetylation of quercetin and dihydroquercetin yielded the same product (m.p. 90–100°) and to this they assigned the flav-3-ene-3-ol acetate structure (VI). This product, however, was probably not completely pure for, apart from its m.p. range, Seshadri



VI

et al.[8] later pointed out that elemental analyses of crystalline products from the reduction of a number of flavonols and flavones indicated that these were mixtures of flav-3-enes of type VI and flavan-4-ol acetates. Finally, Bayer and Krämer[2] have now re-examined the reductive acetylation of quercetin (and simpler flavones) and Nierenstein's quercetylene hypothesis. They reported the isolation (in 60% yield) of a product whose mol. wt. is only one-half that of IV. Elemental analyses supported Seshadri's structure (VI) for this compound. Once again, however, it is clear that the physical properties reported for this compound, *viz.*, yellow-red powder, m.p. 100–140°, render its purity doubtful. Bayer and Krämer concluded that unlike other heterocyclic systems flavones do not undergo facile dimerization to bisflavenylidenes. In support of this conclusion they synthesised a number of model, authentic bisflavenylidenes by the action of copper and Raney nickel on thioflavones e.g., 7-methoxyflavone → VII → VIII (R=OMe, R_1=H).

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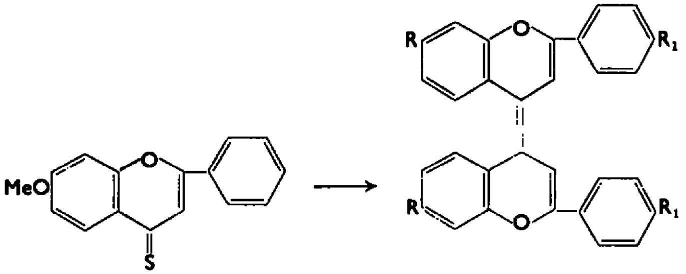
[6] T. Malkin and M. Nierenstein, *J. Amer. Chem. Soc.* **52**, 2864 (1930).

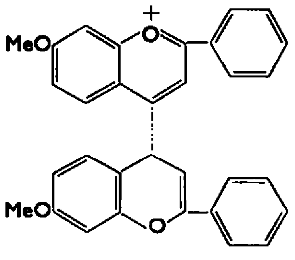
[7] K. R. Laumas and T. R. Seshadri, *Proc. Indian Acad. Sci.* **49A**, 47 (1959).

[8] H. G. Krishnamurty, V. Krishnamoorthy and T. R. Seshadri, *Phytochem.* **2**, 47 (1963).



VII VIII

In contrast to the observations of Bayer and Krämer we found that, under the conditions of reductive acetylation, flavones, unsubstituted in the 3- and 5-positions, readily formed crystalline bisflavenylidenes of the type synthesised by Bayer and Krämer from thioflavones. Thus, reduction of 7-methoxyflavone gave a yellow, crystalline compound, m.p. 223°. The mol. wt. and elemental analysis of this compound established its molecular formula as $C_{34}H_{24}O_6$. The bisflavenylidene structure (VIII, R=OMe, R_1=H) was further indicated by its intense absorption at 445 mμ and the formation of the 4-flavenylflavylium salt (IX) when solutions of the compound in chloroform–methanol were treated with hydrogen chloride. In support of structure IX, the absorbance of the bisflavenylidene hydrochloride at its λ_{max} (421 mμ, Log ϵ 4·45) is only about one-half that of 7-methoxyflavylium chloride at its λ_{max} (430 mμ, Log ϵ 4·62) as the same concentration (in g/l.; Fig. 1). The m.p. (224°) of Bayer and Krämer's bisflavenylidene (VIII, R=OMe, R_1=H) and the spectrum of its hydrochloride (λ_{max} 420, 306, 264 mμ) are in excellent agreement with those of the 7-methoxyflavone reduction product. Crystalline bisflavenylidenes were similarly obtained by reduction of 7-hydroxy-, 4′-hydroxy, 4′-methoxy and 7-hydroxy-4′-methoxyflavones. Bisflavenylidenes, however, were not detected in the reduction of 5-hydroxyflavone derivatives, *viz.*, apigenin, acacetin and apigenin trimethyl ether, or 7-hydroxy-3-methylflavone.



IX

In this investigation, thin layer chromatography on silicic acid showed the amorphous product formed by reductive acetylation of quercetin is not homogenous[1] but a mixture containing at least one colourless and three yellow-orange compounds. The colourless constituent(s) migrates rapidly on silicic acid columns and is thus easily separated. Thus obtained (30–35% yield) it crystallises readily from methanol as colourless needles which, as previously noted,[2,7] yield cyanidin on acid or alkaline hydrolysis. This colourless product (λ_{max} 273 mμ, Log ϵ 4·59) migrates as a single component on silicic acid in four different solvent systems and its C, H and acetyl analytical values are in excellent agreement with Seshadri's 3,3',4',5,7-pentaacetoxyflav-3-ene structure (VI). The homogeneity of this colourless product, however, is questionable since, after repeated recrystallization, it melts over an extended range (115–135°). On the basis of the analytical data it would seem most probable that this product is actually a mixture of flav-3-ene (VI) and the isomeric flav-2-ene.*

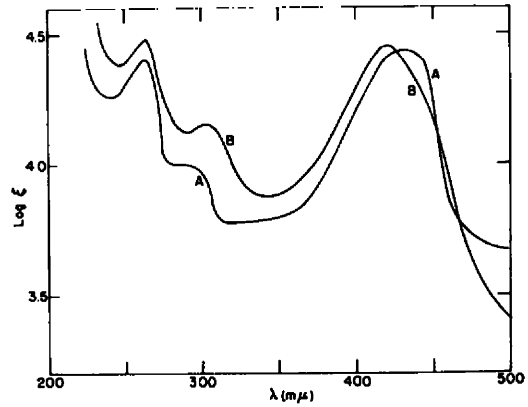


Fig. 1. Spectra in 0·05% ethanolic hydrochloric acid of (A) 7-methoxyflavylium chloride (4×10^{-3} g/l.) and (B) IX (8×10^{-3} g/l.).

Attempts to crystallize the yellow-orange reductive acetylation products of quercetin have not been successful. However, the presence of Nierenstein's quercetylene acetate cannot be unequivocally excluded since (1) the crude products do have the yellow colour typical of bisflavenylidenes and (2) acid hydrolysis of the total, crude reduction product gives, in addition to cyanidin, traces of a second, red anthocyanidin-like compound. Although this second compound tends to streak on paper chromatograms its R_F values (Table 1) distinctly differ from those of cyanidin.

Table 1. R_F Values of anthocyanidins from reductive acetylation of quercetin

Solvent	R_F Cyanidin (authentic)	R_F Red products from reductive acetylation of quercetin
Forrestal solvent	·53	·53
		·81
Water–acetic acid–conc. HCl	·25	·25
80:40:5, v/v		·64
Formic acid-3N HCl	·22	·22
1:1, v/v		·60

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* This conclusion has now been confirmed by the NMR spectrum (kindly determined by Dr. A. C. Waiss) of the product, which shows that it consists of a mixture of the flav-3-ene (vinyl proton, 4·07τ) and isomeric flav-2-ene (benzylic protons, 6·42τ). Integration of the absorption peaks indicates that the ratio of flav-3-ene and flav-2-ene is approximately 4 to 1.

EXPERIMENTAL

Reduction of flavones

General procedure. The flavone (2–10 g) was treated with Zn dust (1 part), anhydrous sodium acetate (1 part) and acetic anhydride (10 parts) and heated under reflux for 1 hr. More Zn dust (1 part) was added and refluxing was continued for an additional hr. Unreacted Zn was filtered and the filtrate cooled, whereupon the bisflavenylidene crystallized. It was collected and recrystallized from tetrahydrofuran–methanol.

4'-Hydroxyflavone (2·0 g) *yielded the bisflavenylidene* (VIII, R = H, R_1 = OAc) as yellow-orange needles, m.p. 263°, $\lambda_{\max}^{\mathrm{EtOH}}$ 430, 304, 253 mμ (0·60 g). (Found: C, 77·4; H, 4·63; CH_3CO-, 16·2. $C_{34}H_{24}O_6$ requires: C, 77·3; H, 4·58; 2 CH_3CO-, 16·3%).

4'-Methoxyflavone (2·0 g) gave the bisflavenylidene (VIII, R = H, R_1 = OMe) as yellow needles, m.p. 257°, $\lambda_{\max}^{\mathrm{EtOH}}$ 436, 293, 248 mμ (0·93 g). Unreacted 4'-methoxyflavone was isolated from the reaction filtrate. (Found: C, 81·2; H, 5·07; MeO-, 12·9. $C_{32}H_{24}O_4$ requires: C, 81·3; H, 5·12; 2 MeO-, 13·1%).

7-Hydroxyflavone (5·0 g) yielded the *bisflavenylidene* (VIII, R = OAc, R_1 = H) as yellow needles, m.p. 272–274°, $\lambda_{\max}^{\mathrm{EtOH}}$ 432, 295, 242 mμ (1·5 g). (Found: C, 77·3; H, 4·68; 2 CH_3CO-, 16·4. $C_{34}H_{24}O_6$ requires: C, 77·3; H, 4·58; 2 CH_3CO-, 16·3%).

7-Methoxyflavone (10.0 g) gave the bisflavenylidene (VIII, R = OMe, R_1 = H), yellow needles, m.p. 223°, $\lambda_{\max}^{\mathrm{EtOH}}$ 445, 306, 241 mμ (1·5 g). (Found: C, 81·3; H, 5·19; MeO-, 13·1; mol. wt. (osmometric[*] detn. in $CHCl_3$) 437. Calc. for $C_{32}H_{24}O_4$: C, 81·3; H, 5·12; 2 MeO-, 13·1%; mol. wt. 472).

7-Hydroxy-4'-methoxyflavone (3·0 g) gave the bisflavenylidene (VIII, R = OAc, R_1 = OMe) as yellow-orange needles, m.p. 248°, $\lambda_{\max}^{\mathrm{EtOH}}$ 429, 310, 253 mμ (0·95 g). (Found: C, 73·3; H, 4·80; MeO-, 10·5; CH_3CO-, 14·6; mol. wt. (in $CHCl_3$) 556. $C_{36}H_{28}O_8$ requires: C, 73·4; H, 4·80; 2 MeO-, 10·55; 2 CH_3CO-, 14·6%; mol. wt. 588).

7-Methoxy-4-(7-methoxyflav-2-enyl)flavylium chloride (IX). The bisflavenylidene (VIII, R = OMe, R_1 = H; 0·5 g) was dissolved in a mixture of $CHCl_3$ (50 ml) and methanol (10 ml) and treated with HCl gas for 1 hr. The mixture was concentrated to small volume and added to 10% HCl aq. The red solution deposited a red, crystalline solid on cooling (0·25 g). On recrystallization from $CHCl_3$–methanol–10% HCl aq, IX separated as orange-red needles, R_F ·98 (n-butanol sat. with 2N HCl), $\lambda_{\max}^{\mathrm{EtOH-0.5\%HCl}}$ 421, 305, 264 mμ, Log ε 4·45, 4·15, 4·18. (Found: C, 76·2; H, 5·07; MeO-, 11·8. Calc. for $C_{32}H_{25}O_4Cl$: C, 75·6; H, 4·92; 2 MeO-, 12·2%).

Reductive acetylation of quercetin

A mixture of quercetin (5 g), Zn dust (5 g), anhydrous sodium acetate (2·5 g) and acetic anhydride (100 ml) was heated under reflux for 1 hr, treated with more Zn dust (5 g) and refluxed for an additional hr. The filtered reaction mixture was added to water (1 l.) After 2 hr the precipitated gum was collected and warmed with glacial acetic acid (50 ml). A small quantity of undissolved solid was filtered. The filtrate was poured into water (500 ml) and the yellow-orange, anhydrous solid was collected (6·6 g). Thin layer chromatograms on silicic acid in ether of this crude product showed the presence of a colourless constituent (R_F ·59) and at least 3 yellow compounds (R_F ·23, ·14, ·05).

The crude mixture of acetates was dissolved in warm ethyl acetate (10·0 ml), diluted with ether (50 ml) and filtered. The filtrate was added to a column of silicic acid which was then eluted with ether until the yellow products began to appear in the eluate. The colourless elute was evaporated to a gum. This was dissolved in warm methanol (10·0 ml) and cooled. Colourless crystals separated (2·3 g). Repeatedly recrystallized from methanol and from ethyl acetate-Skellysolve F the product was obtained as colourless needles, m.p. 115–135°. (Found: C, 60·3; H, 4·49; CH_3CO-, 43·2. Calc. for $C_{25}H_{22}O_{11}$: C, 60·2; H, 4·45; 5 CH_3CO-, 43·15%).

On silicic acid the colourless crystalline product migrated as a single compound: R_F ·59 (ether), ·33 (ether–Skellysolve F, 2:1), ·60 (acetone–chloroform, 1:10), ·69 (methanol).

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[*] Mechrolab Vapour Pressure Osmometer Model 301A.