

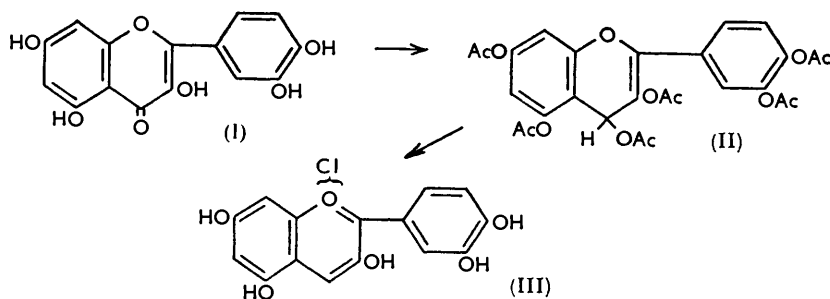
775. The Conversion of Flavonols into Anthocyanidins.

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Reductive acetylation of flavonols gives acetates of undetermined structure having one acetyl group less than would be expected. Hot mineral acid converts the acetates into the corresponding anthocyanidins smoothly and in good yield; hydrolysis with caustic alkalis at room temperature, followed by acidification in the cold, leads to a small quantity of anthocyanidin. This appears to be a useful general method for the production of anthocyanidins from flavonols.

An attempt was made to estimate keto-groups in mimosa and quebracho tannin extracts, by comparing the acetyl content of normal acetates and those made by reductive acetylation (zinc dust, sodium acetate, and acetic anhydride). An increase in acetyl content on reductive acetylation can be a useful indication of keto-groups in cases where the normal hydrazones, oximes, etc., are not formed. In the present case there was no increase of acetyl content although, as shown below, this is not necessarily evidence of the absence of keto-groups in these particular tannin extracts. The work however showed that reductive acetylation provides an improved method of converting flavonols into anthocyanidins and allows the isolation, as intermediates, of acetylated *leucoanthocyanidins*.

Quercetin (I) was therefore reductively acetylated with the expectation of producing 3:4:5:7:3':4'-hexa-acetoxyflav-2-en (II). This, on treatment with hydrogen chloride in propan-2-ol should give cyanidin (III) in a manner analogous to the conversion of flavan-3:4-diols¹ (the so-called *leucoanthocyanidins*) into anthocyanidins.



Direct reduction of quercetin to cyanidin has been reported by several workers. Willstätter and Mallison² employed mercury, magnesium, and hydrochloric acid at 35°: the yield was very low (~2%) by this procedure in this laboratory. Watson and Sen,³ and later Watson and Meek,⁴ reduced quercetin with sodium amalgam in ethanolic hydrochloric acid to a compound of uncertain nature. Malkin and Nierenstein⁵ treated quercetin with zinc dust (no sodium acetate) in boiling acetic anhydride and poured the resulting solution into hydrochloric acid: the product formed a deep-red solution when heated with ethanol and concentrated hydrochloric acid: the authors thought that the product was bimolecular. Mirza and Robinson⁶ also reported a two-stage conversion of flavonols into anthocyanidins involving reduction with lithium aluminium hydride in ether and treatment of the product with hydrochloric acid.

Smooth reductive acetylation of quercetin or its penta-acetate gave a good yield of

¹ Robinson and Robinson, *Biochem. J.*, 1933, **27**, 206.

² Willstätter and Mallison, *Sitzungsber. k. Akad. Wiss., Berlin*, 1914, 769.

³ Watson and Sen, *J.*, 1914, **105**, 395.

⁴ Watson and Meek, *J.*, 1915, **107**, 1576.

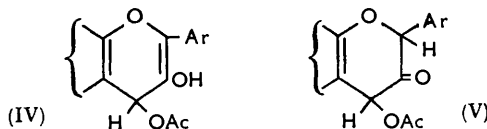
⁵ Malkin and Nierenstein, *J. Amer. Chem. Soc.*, 1930, **52**, 2864.

⁶ Mirza and Robinson, *Nature*, 1950, **166**, 997.

an orange-red product containing five acetyl groups instead of the expected six. Similarly, fisetin and k ampferol gave products containing one acetyl group less than expected. These coloured reduced acetates were all amorphous and appeared homogeneous on paper chromatography although purification failed to produce products with sharp melting points. The ultraviolet spectra of the reduced quercetin penta-acetates produced directly or from the normal penta-acetate were virtually identical and quite different from the spectra of quercetin or its normal penta-acetate (see Table). Treating the reduced acetates

	$\lambda_{\text{min.}}$ (m μ)	$E_{1\text{cm.}}^{1\%}$	$\lambda_{\text{max.}}$ (m μ)	$E_{1\text{cm.}}^{1\%}$
Quercetin	238, 284	470, 225	256, 371	750, 785
Quercetin penta-acetate	238, 270	385, 205	250, 298	432, 388
Reduced acetate of quercetin	—	—	Sh 258—274	165
Reduced acetate of penta-acetylquercetin...	—	—	Sh 260—272	120

with hydrogen chloride in propan-2-ol⁷ gave the anthocyanidins as expected. They were identified by the qualitative tests of Robinson and Robinson⁸ and by chromatography in the "Forestal" solvent⁹ against standard anthocyanidins. The overall yield of anthocyanidin was determined for the quercetin-cyanidin conversion by direct isolation of the cyanidin chloride by two different methods (57.5% and 24.3% respectively), while the yields for the other conversions were determined by spectrophotometric comparison with standard anthocyanidins¹⁰ at the wavelength of maximum optical density: quercetin \rightarrow cyanidin chloride 40.5%; k ampferol \rightarrow pelargonidin chloride 45.4%; fisetin \rightarrow fisetinidin chloride 10.9%. The low yield in the last conversion suggests that the presence of the hydroxyl group in the 5-position influences the yield beneficially and we have observed a similar result when reducing flavonols to 2:3-dihydroflavonols by sodium dithionite and sodium carbonate.¹¹



The structure of the reductively acetylated intermediates described above is not certain. To account for the colour it seems likely that they are acetyl derivatives (IV) of the anthocyanidins or acetates (V) of *pseudo*-bases, the two structures being tautomeric.

Deacetylation of the reduced acetate of quercetin with ethanolic potassium hydroxide at room temperature gave a dark green solution (cf. ref. 4), which changed immediately to a bright-red solution on acidification with cold hydrochloric acid. Extraction of this solution with *isopentyl* alcohol and chromatography of the extract showed that cyanidin had been formed, a result which seems to require the intermediates to have one of the above formul e.

The overall yield of anthocyanidins is the highest yet reported, and this technique appears to be a useful general method.

EXPERIMENTAL

Reductive Acetylation.—(a) *Quercetin.* Quercetin (1.0 g.) was refluxed for 1 hr. with zinc dust (1.0 g.) and anhydrous sodium acetate (0.5 g.) in acetic anhydride (20 ml.). A further 1.0 g. of zinc dust was then added and refluxing continued for another hour. The solution became deep red after 5 min. and remained so. It was finally filtered from the zinc, which was washed with a little glacial acetic acid. The combined liquids were poured into water (250 ml.), and the red solid which separated was filtered off, dissolved in the minimum volume

⁷ Hillis, *J. Soc. Leather Trades' Chemists*, 1954, **33**, 91.

⁸ Robinson and Robinson, *Biochem. J.*, 1931, **25**, 107.

⁹ Bate-Smith, *ibid.*, 1954, **58**, 122.

¹⁰ King and White, "Chemistry of the Vegetable Tannins—A Symposium," Soc. Leather Trades' Chemists, Croydon, 1956.

¹¹ Pew, *J. Amer. Chem. Soc.*, 1948, **70**, 3031.

of acetic acid, re-precipitated into water, and dried *in vacuo* (P_2O_5). It (1.331 g.) was a light orange-red substance, m. p. 80–100° (shrinking at 55–60°) (Found: C, 59.8; H, 4.6; Ac, 40.9. $C_{25}H_{22}O_{12}$ requires C, 58.2; H, 4.3; Ac, 41.9%).

Reductive acetylation of quercetin penta-acetate (m. p. 193°; Ac, 41.8%) (1.85 g.) as above yielded a buff-coloured substance, m. p. 80–100° (shrinking at 55–60°) (Found: Ac, 42.7%).

(b) *Robinetin*. The reduced acetate (1.2 g., from 1.0 g. of robinetin) was a red powder, m. p. 110°, shrinking at 95°.

(c) *Fisetin*. The reduced acetate (0.151 g. from 0.2 g. of fisetin) was a buff-coloured powder, m. p. 85–105° (shrinking at 75–80°) (Found: C, 62.6; H, 4.5; Ac, 34.8. $C_{23}H_{20}O_{10}$ requires C, 60.5; H, 4.4; Ac, 37.7%).

(d) *Kämpferol*. The reduced acetate (0.072 g. from 0.1 g.) was a pale orange-coloured solid, m. p. 92–100° (shrinking at 82°) (Found: C, 64.0; H, 5.0; Ac, 37.3%).

Conversion of the Reduced Acetates into Anthocyanidins.—(a) For qualitative work 10 mg. each of the reduced acetates were heated in sealed tubes with a 5 : 1 mixture (1 ml.) of 3N-hydrogen chloride and propan-2-ol (1 ml.) for 40 min. in a boiling-water bath. The products were chromatographed on Whatman No. 2 paper in acetic acid–water–concentrated hydrochloric acid (30 : 15 : 1). Standard anthocyanidin samples were run simultaneously for comparison.

For quantitative work larger amounts were used. The reaction mixtures were taken to dryness under reduced pressure in a stream of nitrogen at 60°. The crude products were then weighed and samples taken for spectrophotometry. The following yields of crude anthocyanidins were observed: quercetin reduced acetate (98.2 mg.) → cyanidin chloride (66.3 mg.); fisetin reduced acetate (58.4 mg.) → fisetinidin chloride (39.3 mg.); kämpferol reduced acetate (37.8 mg.) → pelargonidin chloride (29.3 mg.).

(b) *Spectrophotometry*. Measurements were carried out with a Unicam S.P. 500 spectrophotometer, on EtOH solutions. Crude anthocyanidins were dissolved in the acetic acid–water–hydrochloric acid solvent, and the absorption maxima compared with those of the authentic anthocyanidins. The anthocyanidin content of the crude products was calculated by direct proportion from the measured optical densities at the maxima. Individual results were as follows: cyanidin chloride (at 525 $m\mu$), 48.2% anthocyanidin in crude product, 40.5% overall yield; fisetinidin chloride (at 510 $m\mu$), 23.0% anthocyanidin in crude product, 10.9% overall yield; pelargonidin chloride (at 505 $m\mu$), 81.5% anthocyanidin in crude product, 45.4% overall yield.

Conversion of Quercetin Reduced Acetate into Cyanidin.—(a) *Isolation by chromatography*. Reductively acetylated quercetin (134.4 mg.) was converted into cyanidin as above; the product was taken to dryness and chromatographed in the above solvent on "Solka Floc" (powdered cellulose) (45 × 2 cm. diam.). The effluent corresponding to the broad red band of cyanidin was collected and, taken to dryness, gave chromatographically and spectrophotometrically pure cyanidin (62.5 mg., 57.5%).

(b) *Isolation by solvent extraction*. Reductively acetylated quercetin (208.5 mg.) was converted into the crude anthocyanidin as before, and the latter taken up in 1% w/v hydrochloric acid (25 ml.). The acid solution was extracted with ethyl acetate (3 × 25 ml.), these extracts being discarded, then with butan-1-ol (50 + 10 ml.). The combined butanol extracts were taken to dryness, yielding 40.6 mg. (24.5%) of chromatographically and spectrophotometrically pure cyanidin.

Deacetylation of Quercetin Reduced Acetate.—The reduced acetate (200 mg.) was kept overnight in a 2% solution (10 ml.) of potassium hydroxide in 50% aqueous ethanol, boiled previously to expel air, then saturated with nitrogen. A deep green solution was immediately formed and this became bright red immediately on acidification with 10% w/v hydrochloric acid at room temperature. The acidified solution was then diluted with water (35 ml.) and extracted with ethyl acetate (5 × 20 ml.). The aqueous residue, still bright red, was extracted with isopentyl alcohol (10 ml.), almost complete extraction of the colour being achieved. This extract was concentrated to 1 ml. Both extracts were chromatographed in the solvent system described above, cyanidin being found in the isopentyl alcohol extract.

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