Phytochemistry, 1964, Vol. 3, pp. 381 to 382. Pergamon Press Ltd. Printed in England

METHODS OF DISTINGUISHING BETWEEN FLAVONES AND FLAVONOLS

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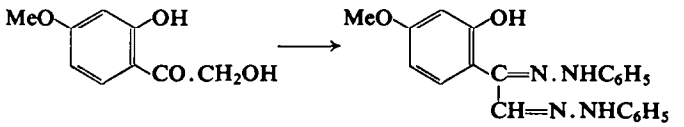
(*Received* 28 *August* 1963)

THE EARLIEST method to be used for distinguishing between flavones and flavonols was based on their different behaviour when subjected to aerial oxidation in alkaline solution.[1] However, although all flavonols are oxidized under these conditions whereas ordinary flavones are relatively stable, highly hydroxylated flavones also undergo decomposition. A similar drawback exists in the use of u.v. absorption spectra[2] for distinguishing between the two classes; the long wavelength band of flavones lies between 340–380 mμ and that of flavonols between 350–380 mμ, so there is a region of overlapping and no absolute distinction can be made.

The method of Balakrishna *et al.*[3] depends on the difference in the reactivity of the ketones obtained by alkali fission of the fully methylated flavonoid compounds. This method has now been modified and the resulting ketones demethylated with hydrobromic acid. In the case of flavonols this yields ω-hydroxyacetophenones (I),[4] which reduce Tollens' reagent and Fehling's solution and form osazones (II).[5,6] As the acetophenones resulting from flavones do not give these reactions, the method thus affords a clear distinction between the two types of flavonoid compound.



(I) (II)

Another difference between flavones and flavonols is found in their behaviour on treatment with sodium hydrosulphite; flavonols yield dihydroflavonols[7–9] whereas flavones are unaffected. Since dihydroflavonols (III) undergo isomeric change to pseudo base acetates (IV) when treated with acetic anhydride and sodium acetate and subsequent conversion into

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[2] L. JURD, *The Chemistry of Flavonoid Compounds* (Edited by T. A. Geissman), p. 110, Pergamon Press, Oxford, London, New York, Paris (1962).
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[4] S. K. GROVER, V. N. GUPTA, A. C. JAIN and T. R. SESHADRI, *J. Sci. Industr. Res. India* **19B**, 258 (1960).
[5] A. LEON, A. ROBERTSON, T. R. SESHADRI and R. ROBINSON, *J. Chem. Soc.* 2672 (1931).
[6] E. H. CHARLESWORTH, J. J. CHAVAN and R. ROBINSON, *J. Chem. Soc.* 372 (1933).
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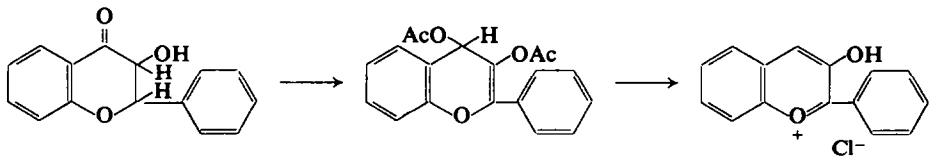
3-hydroxyflavylium salts (V),[10–12] which are not only deeply coloured but are also decolorized by ferric chloride,[5] flavonols can be readily distinguished from flavones.



(III) (IV) (V)

Reductive acetylation of the flavonoid with acetic anhydride and zinc dust in the presence of sodium acetate, followed by treatment with alcoholic hydrochloric acid,[12,13] can also be used. Flavonols give 3-hydroxyflavylium salts which lose colour with ferric chloride;[5] whereas the flavylium salts from flavones, being devoid of 3-hydroxyl, are not decolorized with ferric chloride.

All the above-mentioned tests can be employed on a semi-micro scale as described in the Experimental section.

EXPERIMENTAL

Method (I). The ketone (2 mg) obtained by the fission of the flavonol methyl ether was dissolved in the minimum quantity of glacial acetic acid and heated with an equal amount of aqueous hydrobromic acid (40%) for 1 hr on a boiling water bath. The resulting mixture was diluted with ice, extracted with ether and the ether solution washed with sodium bicarbonate solution and then by water. The ether was removed and the residue tested with Tollens' reagent, Fehling's solution, and phenyl hydrazine in acetic acid. Only ketones from flavonols gave a positive reaction.

Method (II). The flavonoid (10 mg) was dissolved in aqueous sodium carbonate (70 mg, 1 ml) and heated with sodium dithionite (200 mg) for 20 min on a boiling water bath. After cooling, the resulting mixture was acidified, exhaustively extracted with ethyl acetate and the ethyl acetate solution dried and evaporated. The residue left was refluxed with acetic anhydride (1 ml) and sodium acetate (5 mg) for 1 hr and the mixture poured on ice. It was extracted with ether, the ether evaporated and the residue refluxed with ethanol (3·5 ml) and concentrated hydrochloric acid (1·5 ml) for 1 hr, diluted with water (5 ml) and filtered. The filtrate was extracted with amyl alcohol and the amyl alcoholic solution was in turn extracted with 2% dilute hydrochloric acid (5 ml) after adding excess of light petroleum. Only flavonols yield a deep-coloured acid solution which, when treated with aqueous ferric chloride, fades rapidly and loses colour completely in 0·5 hr.

Method (III). The flavonoid (5 mg) was dissolved in acetic anhydride (1 ml) and refluxed with zinc dust (5 mg) and fused sodium acetate (5 mg) for 2 hr. The excess zinc was filtered off, washed with acetic acid and the filtrate poured on ice. The mixture was then extracted with ether, and the ether residue refluxed with alcoholic hydrochloric acid and the product treated in the same way as in Method II. Only in the case of flavonols did ferric chloride discharge the original deep-red colour.

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