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SHORT COMMUNICATION

THE UNSUITABILITY OF ETHANOL AS A SOLVENT FOR THE SPECTROSCOPIC DETECTION OF FUNCTIONAL GROUPS IN HYDROXYFLAVONES WITH ALUMINIUM CHLORIDE

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Abstract—$AlCl_3$ has proved useful as a chelatogenic reagent in the u.v. spectroscopic structure elucidation of flavonoids in alcohol solution. However, an apparent difference exists between ethanol and methanol solvents in their ability to stabilize aluminium complexes of flavonoids containing *ortho*-dihydroxyl groups. The present work shows that, in fact, there is no difference between anhydrous ethanol and methanol as solvents, but that the presence of traces of water in ethanol (but not methanol) strongly inhibits complex formation. Methanol is therefore recommended as solvent for the u.v. spectroscopic analysis of flavonoids.

The ability of $AlCl_3$ to chelate with certain sites on hydroxylated flavones, and thereby produce an u.v. spectral shift has proven useful for the elucidation of hydroxylation patterns in naturally occurring flavonoids.[1–5] The basis of the method is that the addition of an excess of $AlCl_3$ to an alcoholic (methanol or ethanol) solution of the flavone produces a bathochromic shift of the Band I (long wavelength) absorption if the flavone contains a free 3- or 5-hydroxyl group or an *ortho*-dihydroxyl system. The magnitude of this shift is characteristic of the type of binding site on the molecule (see Table 1).

Markham and Mabry[4] have demonstrated that complexes involving a flavone *ortho*-dihydroxyl grouping are aqueous acid unstable and that this characteristic can be used diagnostically both to distinguish them from, and to detect them in the presence of, com-

Table 1. Spectroscopic data for typical hydroxyflavones and their aluminium complexes in methanol[8]

Ligand	λ_{max} nm (log ϵ) Ligand	Complex	Bathochromic shift ($\Delta\lambda$) nm	Stoicheiometry (Ref. 8)
3-Hydroxyflavone	343 (4·24)	402 (4·32)	59	AlL_2*
5-Hydroxyflavone	335 (3·86)	396 (3·77)	61	AlL
3′, 4′-Dihydroxyflavone	341 (4·29)	380 (4·27)	39	AlL

* L = flavonoid anion.

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[1] L. Jurd and T. A. Geissman, *J. Org. Chem.* **21**, 1395 (1956).

[2] L. Hörhammer and R. Hänsel, *Arch. Pharm.* **285**, 438 (1952).

[3] L. Jurd, in *The Chemistry of Flavonoid Compounds* (edited by T. A. Geissman), p. 107, Macmillan, New York (1962).

[4] K. R. Markham and T. J. Mabry, *Phytochem.* **7**, 1197 (1968).

[5] L. Jurd, *Phytochem.* **8**, 445 (1969).

plexes involving the 3- or 5-hydroxyl-4-keto grouping. Recently Jurd[5] has reported an alternative method of distinguishing these two chelating systems based on his observation that complexes of the *ortho*-dihydroxyl type will form in absolute methanol, but not in absolute ethanol, while complexes of the 3- or 5-hydroxyl-4-keto type will form in both solvents. Although we routinely use methanol for the u.v. spectroscopy of flavonoids,[7] it has been our experience that $AlCl_3$ shifts could be obtained from 3- and 5-hydroxylated flavones and from 3′,4′-dihydroxyflavones irrespective of whether absolute ethanol or absolute metanol was used as solvent. Jurd's report,[5] however, prompted us to investigate the situation more fully.

The major difference between Jurd's technique[5] and our own[4] is that while we exclude water from the reactants, Jurd routinely uses an aqueous $AlCl_3$ reagent. For this reason we first studied the effect of water on aluminium-hydroxyflavone complex formation in both ethanol and methanol.

Figure 1 shows plots of the percentage of 3′, 4′-dihydroxyflavone (L), bound as an aluminium complex, versus mole fraction (m.f.) of various alcohols in alcohol–water mixtures. From these plots it is apparent that the amount of complex formed with any of the three alcohols is dependent on the proportion of water present. In particular, small amounts of water in ethanol (and 1-propanol) lead to extensive dissociation of the complex, whereas in methanol the effect of added water is small. These effects are probably due to the large changes in $\Delta H°$ and $\Delta S°$ for complex formation, which have been shown to occur during the variation in solvent composition in alcohol–water mixtures.[6] It is also significant that there is no difference between ethanol and methanol in their ability to stabilize the aluminium 3′,4′-dihydroxyflavone complex in the absence of water. This is evidenced by the fact that both the percentage of ligand involved in the complex, and the stoicheiometry of the complex formed (AlL),* are the same in both solvents.

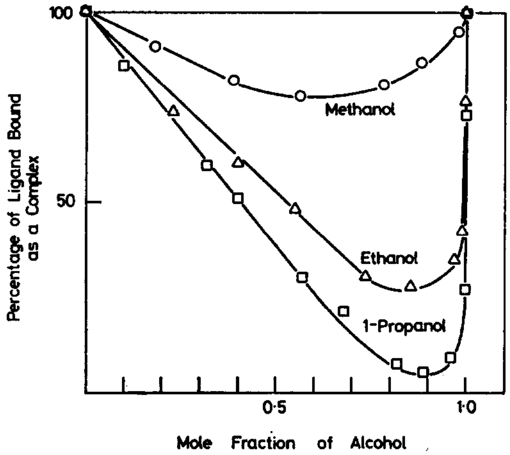

FIG. 1. EXTENT OF COMPLEX FORMATION IN ALCOHOL–WATER MIXTURES.

It appears from the data in Fig. 1 that the effect of water on aluminium complex formation of 3′,4′-dihydroxyflavone would be sufficient to account for the difference between Jurd's observations[5] and our own. Under Jurd's conditions of measurement the solution contains

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* Determined by the molar-ratio titration method (see Ref. 8).

[6] R. M. IZATT, D. EATOUGH and J. J. CHRISTENSEN, *J. Phys. Chem.* **72**, 2720 (1968), and references therein.

[7] T. J. MABRY, K. R. MARKHAM and M. B. THOMAS, *The Systematic Identification of Flavonoids*, Springer-Verlag, New York (1969).

about 2 per cent water (m.f. ethanol = 0·94). Thus only 30 per cent of the ligand present is complexed and the spectrum of the complex would appear merely as a shoulder on the side of Band I. The observation of Horowitz, reported by Jurd,[5] that a saturated solution of $AlCl_3$ in ethanol complexes with 3′,4′-dihydroxyflavone, may now be rationalized since sufficient $AlCl_3$ would be present under these conditions to completely dry the ethanol solution and thereby favour complex formation.

Since complex formation in ethanol is so sensitive to traces of water, ethanol is clearly unsuitable as a solvent to use in the $AlCl_3$ detection of *ortho*-dihydroxyl groups in flavonoids unless water is excluded both from the solvent and the reagent. Methanol, on the other hand, is ideal in that a satisfactory level of complex formation is obtained in the presence of any percentage of water. Methanol thus has the advantage that if the hydroxyflavone is glycosylated, water can be added to solubilize the compound without unduly affecting the $AlCl_3$ shift data. In addition, the presence of an *ortho*-dihydroxyl grouping in a hydroxyflavone may be readily and unambiguously distinguished from the presence of a 3- or 5-hydroxyl group by the relative acid stability of the two types of Al complexes.[4]

For the above reasons we strongly recommend the exclusive use of absolute methanol as a solvent for the spectroscopic analysis of hydroxyflavones.

It is interesting to note that the non-equivalence of methanol and ethanol as solvents for aluminium-hydroxyflavone complex formation is not limited to complexes of the *ortho*-dihydroxyl type. Porter and Markham[8] showed for 3-hydroxyflavone that the complex species favoured in *neutral methanol* is AlL_2 and in 0·1 M acidic methanol, Al_2L. However, Urbach and Timnick,[9] using the same technique, detected only Al_2L in *neutral ethanol.* Similarly[8] it was established that 5-hydroxyflavone forms the complex AlL in both *neutral methanol* and *neutral anhydrous ethanol* in the presence of equimolar amounts of $AlCl_3$. However, in 95 per cent ethanol under similar conditions no complex forms unless a 200-fold excess of $AlCl_3$ is added (see also Ref. 10).

EXPERIMENTAL

Spectra were determined on a Beckman DK-2A recording spectrophotometer.

Anhydrous methanol, ethanol, and 1-propanol were prepared by distillation of the alcohols from magnesium alkoxide according to the method of Vogel.[11] The percentages of ligand bound as a complex were determined from the relative absorbances at 342 nm (Band I position for 3′,4′-dihydroxyflavone) and 380 nm (Band I position for 1:1 complexes). In each solution the initial concentration of ligand was 3×10^{-5} M, and $AlCl_3$ was 15×10^{-5} M.

3′,4′-Dihydroxyflavone was prepared according to the method outlined in Ref. 8.

[8] L. J. Porter and K. R. Markham, *J. Chem. Soc.* c (1969), in press.

[9] F. L. Urbach and A. Timnick, *Analytical Chem.* **40**, 1269 (1968).

[10] K. Hayashiya, *Nippon Nogei Kagaku Kaishi* 33, 1063 (1959).

[11] A. I. Vogel, *Practical Organic Chemistry*, 2nd edition, Longmans, London (1951).