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A PROCEDURE FOR THE ULTRAVIOLET SPECTRAL DETECTION OF *ORTHO*-DIHYDROXYL GROUPS IN FLAVONOIDS

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Abstract—A spectroscopic procedure using anhydrous $AlCl_3$ and $AlCl_3/HCl$ is described for the detection of *ortho*-dihydroxyl groups in flavonoids. This method complements the sodium acetate–boric acid method which is commonly used for this purpose.[1, 2]

Aluminium chloride in the presence of less than 0·008 N HCl has previously been used as a reagent for detecting *ortho*-dihydroxyl groups in anthocyanins.[3] Furthermore, Harborne[4] in 1954 suggested that $AlCl_3$ might be used for recognizing *ortho*-dihydroxyl groups in other flavonoids. Later, Jurd and Geissman[5] established the pH requirements for the formation of $AlCl_3$/3′,4′-*ortho*-dihydroxyl group complexes when they found that the presence of NaOAc was necessary for complex formation. The acidic conditions (i.e. the presence of acid or water) normally used for the detection with $AlCl_3$ of 3- or 5-hydroxyl groups in flavonols were found to prevent $AlCl_3$/*ortho*-dihydroxyl group complex formation.

Our current investigations, all of which are consistent with the previous observations, have clarified the different effects of anhydrous $AlCl_3$ and $AlCl_3/HCl$ on the u.v. spectra of flavonoids (with the exception of anthocyanins) and have established that the comparison of the $AlCl_3/HCl$ spectrum with the one obtained with anhydrous $AlCl_3$ alone permits the recognition of *ortho*-dihydroxyl groups in the B-ring of flavones, flavonols, chalcones and aurones as well as the detection of free 3- or 5-hydroxyl groups. Moreover, our data indicate that *ortho*-dihydroxyl groups in the A-ring, which do not involve the hydroxyl group hydrogen-bonded to the carbonyl function, can also be detected.

We first distinguished the different effects of $AlCl_3$ and $AlCl_3/HCl$ in the course of the structure determination of a number of 5-desoxyflavones and 5-desoxyflavonols isolated from *Baptisia lecontei*.[6] It was observed that the addition of a few drops of unacidified 5 per cent methanolic $AlCl_3$ to a methanol solution of 3′,4′,7-trihydroxyflavone 7-*O*-rhamnoglucoside (I, R = rhamnoglucosyl) caused a 40 nm bathochromic shift in the long wavelength peak; significantly, this shift was completely reversed on the addition of dilute aqueous

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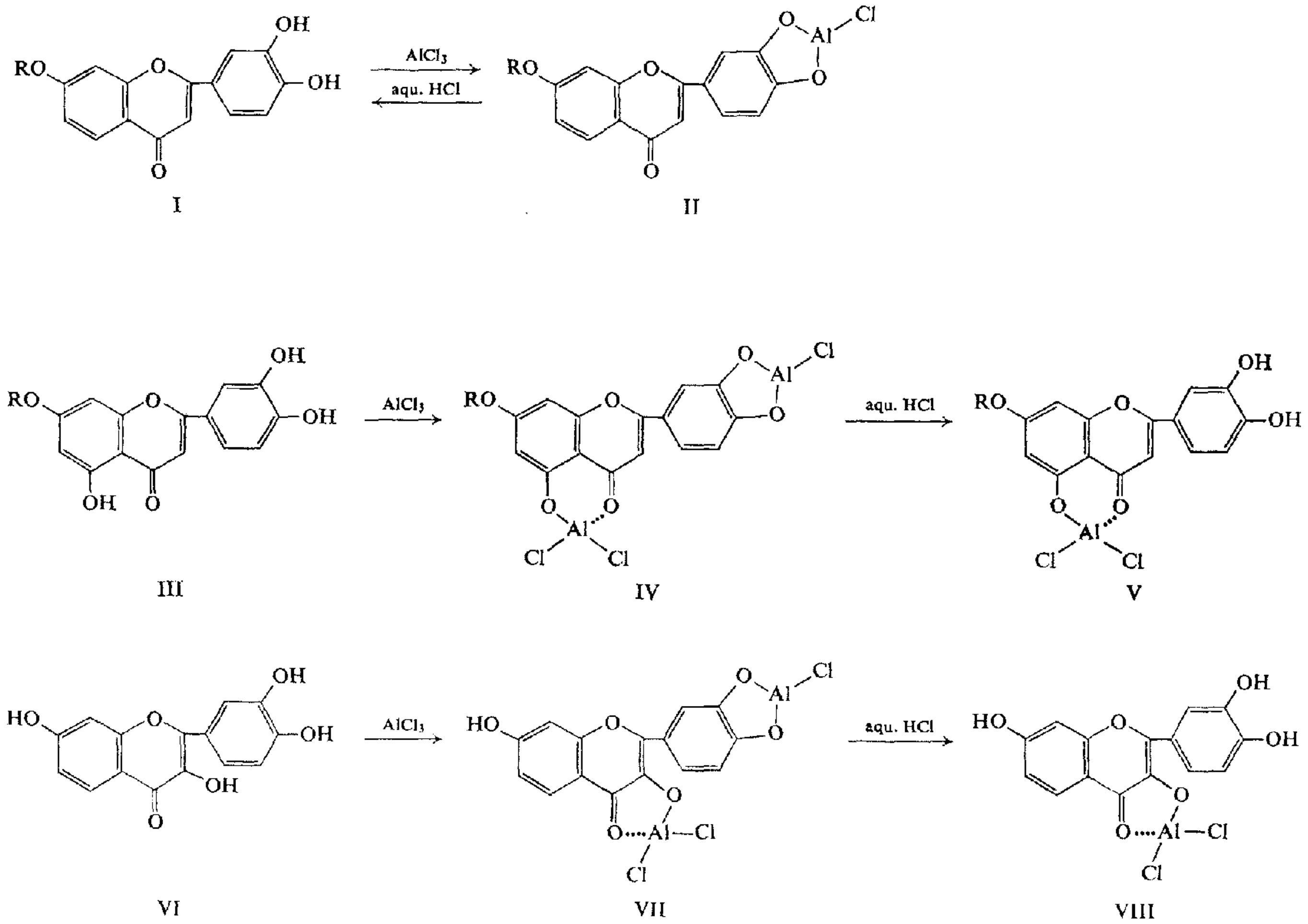

FIG. 1. THREE SCHEMES ILLUSTRATING THE TYPES OF COMPLEXES[5, 7] THAT $AlCl_3$ CAN FORM WITH FLAVONES AND FLAVONOLS IN THE PRESENCE OR ABSENCE OF ACID. IN I–V, R=RHAMNOGLUCOSYL.

HCl. When a 5-hydroxyl group is also present in the flavonoid, e.g. luteolin 7-*O*-rhamnoglucoside (III, R=rhamnoglucosyl), the 40 nm shift (observed for I) is additive to that recognized as characteristic of a free 5-hydroxyl group,[1] thus resulting in an 80 nm bathochromic shift; the 80 nm shift is reduced to 40 nm on the addition of aqueous HCl. In each of these cases an acid labile complex is formed between the *ortho*-dihydroxyl system and the AlCl$_3$ (see II and IV), and with the luteolin derivative, the addition of acid still leaves the 5-hydroxy-4-keto AlCl$_3$ chelate[1] (V) intact. In a similar manner, the 93 nm bathochromic shift observed in the long wavelength peak of 3′,4′,7-trihydroxyflavonol (VI, fisetin) on the addition of anhydrous AlCl$_3$, is reduced with acid to 63 nm, the shift expected[1] for the 3-hydroxy-4-keto AlCl$_3$ chelate (VIII). All of the above observations can be accounted for by the reactions shown in Fig. 1.

A comprehensive spectral analysis of 175 flavonoids[2] has established the applicability of the AlCl$_3$, AlCl$_3$/HCl reagent for the detection of *ortho*-dihydroxyl groups in flavonoids. Table 1 presents the relevant results of the survey.

TABLE 1. THE U.V. SPECTRA OF AlCl$_3$ COMPLEXES OF FLAVONOIDS CONTAINING *ortho*-DIHYDROXYL GROUPS*

Flavonoids with B-ring *ortho*-dihydroxyl groups	λ_{max} (nm) for long-wavelength band MeOH, AlCl$_3$	 MeOH, AlCl$_3$/aqμ. HCl	$\Delta\lambda$
Flavones			
3′,4′-Dihydroxyflavone	375	340	35
3′,4′,7-Trihydroxyflavone	370	340	30
3′,4′,7-Trihydroxyflavone 7-*O*-rhamnoglucoside	378	340	38
Luteolin	424	388	36
Luteolin 7-*O*-glucoside	425	387	38
Luteolin 7-*O*-rutinoside	427	392	35
Orientin	424	388	36
Isoorientin	423	387	36
Lucenin-1	426	386	40
Flavonols			
3,3′,4′-Trihydroxyflavone	465	425	40
Fisetin	455	424	31
Fisetin 3-*O*-glucoside	377	342	35
Quercetin	454	424	30
Quercetin 7-*O*-rhamnoside	455	425	30
Quercetin 3-*O*-galactoside	435	405	30
Quercitrin	429	399	30
Rutin	431	400	31
Quercetin 3,7-*O*-diglucoside	437	402	35
Quercetin 3-*O*-glucoside 7-*O*-rhamnoside	435	400	35
Quercetin 3-*O*-glucoside 7-*O*-rutinoside	438	403	35
Quercetin 3-methyl ether	437	404	33
Rhamnetin	449	422	27
Robinetin	445	425	20
Patuletin	453	424	29
Patuletin 3-*O*-glucoside	436	400sh	36
Patuletin 3-*O*-rutinoside	431	399sh	32
Patulitrin	456	424	32
Gossypetin†	490	445	45
Gossypin	450	442	8
Gossypitrin	473	449	24
Myricetin	445	427	18

	λ_{max} (nm) for long-wavelength band		
Flavonoids with B-ring *ortho*-dihydroxyl groups	MeOH, $AlCl_3$	MeOH, $AlCl_3$/aqμ. HCl	$\Delta\lambda$
Chalcones			
3,4-Dihydroxychalcone	410	363	47
2′,3,4-Trihydroxychalcone	512	453	59
2′,3,4,4′-Tetrahydroxychalcone	483	431	52
Aurones			
3′,4′-Dihydroxyaurone	455	410	45
3′,4′,6,7-Tetrahydroxyaurone (Maritimetin)	457	407	50
Leptosidin	443	402	41
Flavonoids with A-ring *ortho*-dihydroxyl groups			
Flavones and flavonols			
Baicalein	373	347	26
Gossypetin†	490	445	45
Isoflavones			
Texasin (6,7-dihydroxy-4′-methoxyisoflavone)	340	321	19
6-Hydroxygenistein	352	329sh	23
Flavanone			
5,6,7-Trihydroxyflavanone	326	315	11
Aurones			
6,7-Dihydroxyaurone	433	409	24
6,7,3′,4′-Tetrahydroxyaurone†	457	407	50

* The flavonoids in each class are listed in order of increasing oxygenation.

† These compounds contain both A- and B-ring *ortho*-dihydroxyl groups which produce an additive effect on the MeOH/$AlCl_3$ spectra.

EXPERIMENTAL

All spectra were measured on a Beckman DB-G spectrophotometer equipped with a Sargent model SLR recorder, using spectroscopic grade methanol as reference.

Preparation of Reagent Stock Solutions

Aluminium chloride ($AlCl_3$). Five g of fresh anhydrous reagent grade $AlCl_3$ were added cautiously to spectroscopic methanol (100 ml). The solution was stored in a glass container with a tightly fitting plastic stopper.

Hydrochloric acid (HCl), Conc. reagent grade HCl (50 ml) was mixed with water (100 ml) and stored in a glass-stoppered bottle.

Procedure for Spectra Determination

A solution of the flavonoid in methanol was prepared such that the optical density of the major absorption peak was in the region 0·6 to 0·8. *The* $AlCl_3$ *spectrum* was measured immediately after the addition of three drops of the $AlCl_3$ stock solution to about 2·5 ml of the above methanolic solution of the flavonoid. *The* $AlCl_3$/HCl *spectrum* was recorded immediately after the further addition of three drops of the HCl stock solution.

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