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TLC, UV AND ACIDIC TREATMENT IN THE DIFFERENTIATION OF 5,6- AND 5,8-DIHYDROXYFLAVONES, 3-METHOXYFLAVONES AND FLAVONOLS

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Abstract—UV and TLC techniques constitute easy procedures to distinguish between 5,6-dihydroxy-7,8-dimethoxy- and 5,8-dihydroxy-6,7-dimethoxyflavonoids, that are difficult to characterize by NMR and classical UV techniques. The acidic treatment of the original products to obtain Wessely–Moser isomers, useful for comparison purposes, yielded novel demethylated products. UV and MS data of 5,8,4′-trihydroxy-6,7,3′-trimethoxyflavone, 5,6,8,3′,4′-pentahydroxy-7-methoxyflavone and 5,6,8,4′-tetrahydroxy-7,3′-dimethoxyflavone are presented for the first time.

In the last few years several naturally occurring 5,6-dihydroxy-7,8-dimethoxyflavones[1,2] and 5,8-dihydroxy-6,7-dimethoxyflavones[3–5] have been isolated and characterized. The identification of these new flavonoid compounds tetrasubstituted on A-ring is not possible by the classical techniques for identification of flavonoids.[6] Thus, the location of the hydroxyl groups was not possible from NMR data in the case of thymonin (**3**),[1] and NMR studies either with solvent induced shifts[7] or with lanthanide shift reagents[8] are not as useful. The gossypetone reaction[9] was used as evidence to elucidate the structure of thymonin[1] but colour reactions are generally doubtful and they should be used with great caution as they may lead to erroneous conclusions, in particular when minute amounts of compound are available.

As we reported recently,[5] chromatographic and UV criterion could be used for distinguishing and characterizing these new flavonoid compounds.

In this work, in order to establish some rules for the characterization of these new flavonoid compounds tetrasubstituted on A-ring, we have examined the UV–methanol data of flavones trisubstituted on A-ring to establish differences to distinguish between 5,6-dihydroxy- and 5,8-dihydroxyflavonoids, and we also isolated and identified from natural sources the flavones thymusin (**1**), thymonin (**3**) and leucanthogenin (**5**), and to complete the family, the isomeric flavones isothymusin (**4**), isothymonin (**6**) and isoleucanthogenin (**2**) were obtained by means of acidic treatment and Wessely–Moser rearrangement.[10] In addition, the acidic treatment rendered the novel demethylated compounds demethylthymusin (**7**), demethylleucanthogenin (**8**) and demethylthymonin (**9**), that have been characterized too.

Differentiation of the isomeric 5,8-dihydroxy-6,7-dimethoxy- and 5,6-dihydroxy-7,8-dimethoxyflavones has been attempted by means of the classical chromatographic and UV procedures, and some new rules for distinguishing between 5,6-dihydroxy- and 5,8-dihydroxyflavones, 3-methoxyflavones and flavonols by means of comparison of their UV spectra in methanol have been established.

The obtaining of the Wessely–Moser isomers[10] and comparison of the UV and R_f values with those of the original product constitutes an easy technique for the characterization of flavonoids with free hydroxyl groups at C-6 or C-8, in particular in the cases when only trace amounts of flavonoids are available for analysis.

RESULTS AND DISCUSSION

UV spectral differentiation of 5,6-dihydroxy- and 5,8-dihydroxyflavones, 3-methoxyflavones and flavonols trisubstituted on A-ring

Distinction among 6- or 8-substitution in flavonoids trisubstituted on A-ring and differentiation between methoxyl or hydroxyl substitution at C-6 is possible by UV study after addition of $AlCl_3$ and $AlCl_3 + HCl$,[11,12] but distinction between methoxyl or hydroxyl at C-8 is not well established.[12,13]

The flavonoid compounds may be easily identified by their spectra in methanol since the majority exhibited an A maximum in the long UV range (band I) between 325 and 400 nm, and a second maximum at shorter wavelength (band II) between 230 and 295 nm. These two bands can be split into two maxima or a maximum and a shoulder or an inflection (BIa, BIb, BIIa and BIIb) (Fig. 1). In some cases it is possible to distinguish a supplementary maximum, shoulder or inflection (band III) between 295 and 325 nm.

Previous reports established that the position of the BII maximum is affected by the introduction of substituent(s) at position(s) 6 and/or 8. Thus, hydroxylation at C-6 produces a higher bathochromic shift than hydroxylation at C-8.[13,14] Moreover, the ratio of A band I/A band II (A = absorbance) constitutes a criterion for distinguishing the substituted position on A-ring. So, 8-substitution particularly decreases this value whereas 6-substitution increases it.[13]

Useful information to differentiate among 5,6-dihydroxy- and 5,8-dihydroxyflavonoid compounds trisubstituted on A-ring can be gained by comparison of their UV spectra in methanol (Tables 1 and 2). The comparative analysis of isomeric couples suggests the following characteristic differences.

(1) The 5,8-dihydroxyflavonoids exhibit a BIII

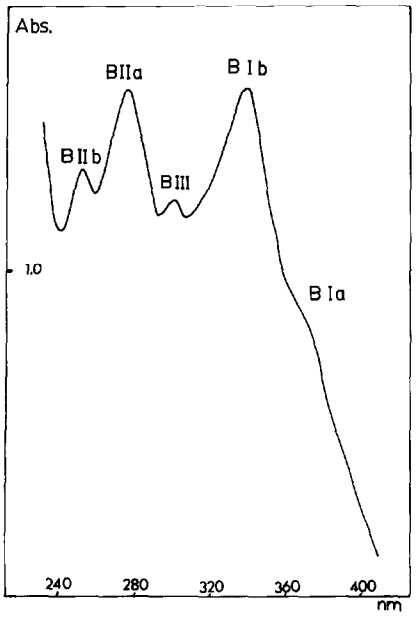


Fig. 1. Absorption bands observed in the UV spectra of flavonoid compounds dissolved in methanol.

between 295 and 325 nm (maximum, shoulder or inflection) which is absent in the spectra of 5,6-dihydroxyflavonoids.

(2) The 5,8-dihydroxyflavonols in all cases and the 5,8-dihydroxy-4′-monosubstituted flavones show a split in BI. Only 5,8-dihydroxy-7,4-dimethoxyflavone does not conform to this rule. The 5,6-dihydroxyflavonoids exhibit unsplit BI.

(3) The 5,8-dihydroxyflavonoids substituted on B-ring show a BI relative absorbance[13] lower than 1.00, being this value for BII or in the case of isoscutellarein derivatives (5,7,8,4′-tetrahydroxyflavone) for BIII. The 5,6-dihydroxyflavonoids show BI with a relative absorbance of 1.00.

(4) Flavonoids with a hydroxyl at C-8, generally show shorter wavelength values for BI and BII maxima than their isomeric compounds with hydroxyl groups at C-6.

UV differentiation of 5,6-*dihydroxy*-7,8-*dimethoxy- and* 5,8-*dihydroxy*-6,7-*dimethoxyflavones*

The UV values in methanol and after addition of aluminium chloride and plus hydrochloric acid obtained for the 5,6-dihydroxy-7,8-dimethoxy- and 5,8-dihydroxy-6,7-dimethoxyflavones available are shown in Table 3.

The differences observed in the methanol spectrum of 5,6-dihydroxy- and 5,8-dihydroxyflavonoids, are also found in these flavones tetrasubstituted on A-ring. Thus, a BIII is observed in 5,8-dihydroxy-6,7-dimethoxyflavones, and this band is absent in the isomeric 5,6-dihydroxy-7,8-dimethoxyflavones. Moreover, the BI and BII maxima of the former isomers appear at shorter wavelengths than the latter isomers. A split in BI is also observed in the 5,8,4′-trihydroxy-6,7-dimethoxyflavone (BIa 370 nm) as could be expected. Finally, the 5,8-dihydroxy-6,7-dimethoxyflavones with the exception of isothymonin (**6**), exhibited a relative absorbance for BI lower than 1.00 meanwhile the 5,6-dihydroxy isomers showed a BI with a relative absorbance of 1.00.

The spectral shifts relative to methanol spectrum observed after addition of $AlCl_3$ + HCl suggest that BIb can be related to C-6 substitution, being this shift 2 nm higher in 6-hydroxy than in 6-methoxy isomers, meanwhile BIa can be associated to the substituent at C-8, being more than 15 nm higher in 8-hydroxy than in 8-methoxy isomers (Table 4). The accurate location of this BIa (shoulder or inflection) is often difficult, because this is of low absorbance in the majority of 8-substituted flavones.

The shape of the $AlCl_3$ + HCl spectra of 6-hydroxy-8-methoxy compounds are characterized by two principal absorption bands, the BIb and the BIIa. On the other hand, the spectra of 6-methoxy-8-hydroxy compounds exhibit three or more bands between the BIIa and BIb (Fig. 2). This is useful for distinguishing between the two classes of isomeric flavones.

It is remarkable that the 5,8-dihydroxyflavones decompose much more quickly than the 5,6-dihydroxyflavones. This decomposition is increased in alkaline media, and it is clearly evidenced in the NaOMe–UV spectra, by the decrease in BI absorbance with time.

Chromatographic behaviour of 6-*hydroxy- and* 8-*hydroxyflavones*

Differences in the chromatographic behaviour on cellulose TLC (or PC) with 30 or 60% HOAc of flavones with free hydroxyl groups at C-6 or C-8 have been found, and this is an easy procedure to distinguish between them. Thus, 6-hydroxyflavonoids show lower R_f values than the isomeric 8-hydroxyflavones in these chromatographic systems (Table 5). This is of interest in the identification of Wessely–Moser products[10] obtained from acidic treatment of flavonoid compounds. As could be expected,[26] the contribution to the R_f values of the hydroxyl groups is more important than the contribution of methoxyls. Introduction of methoxyls on A-ring increased the R_f values, but introduction of a methoxyl on B-ring decreased them.

Acidic treatment of the flavones tetrasubstituted on A-ring

The naturally occurring flavones thymonin (**3**), thymusin (**1**) and leucanthogenin (**5**) were submitted to acidic treatment as described in the Experimental section, in order to obtain the Wessely–Moser isomers[10] isothymonin (**6**), isothymusin (**4**) and isoleucanthogenin (**2**), that completed the family of flavones tetrasubstituted on A-ring. In addition, the demethylated products demethylthymonin (**9**), demethylthymusin (**7**) and demethylleucanthogenin (**8**) were also obtained.

The presence of hydroxyl group at the C-8 position in flavones has a predominant effect in favouring the formation of the 6-hydroxy isomer in A-ring trisubstituted flavones,[10] and in fact, 8-hydroxyluteolin (**14**), isoscutellarein (**13**) and 8-hydroxychrysoeriol (**15**) rendered almost exclusively 6-hydroxyluteolin (**17**), scutellarein (**16**) and nodifloretin (**18**), respectively, upon acidic treatment.

In A-ring tetrasubstituted flavones, we found that the 6-hydroxy-8-methoxyflavones thymusin (**1**) and thymonin (**3**) rendered upon acidic treatment only a small

Table 1. UV spectra in methanol of 5,6-dihydroxyflavones, 3-methoxyflavones and flavonols trisubstituted on A-ring. The relative absorbance[13] is presented for the λ_{max} for each compound using the highest peak as 100% (1.00) of each spectrum. OR = methoxyl; OS = glycosyl; OH = hydroxyl; s = shoulder; i = inflection

SUBSTITUTION PATTERN								REFERENCES	BI				BIII	BII			
3	5	6	7	8	3'	4'	5'		BIa	BIb			BIII	BIIa		BIIb	
--	OH	OH	OH	--	--	--	--	6	--	323	(0.55)		--	274	(1.00)	247s	(0.55)
--	OH	OH	OS	--	--	--	--	6	--	315	(0.57)		--	278	(1.00)	244	(0.37)
--	OH	OH	OH	--	--	OH	--	13	--	336	(1.00)		--	284	(0.86)	--	
--	OH	OH	OR	--	--	OH	--	13	--	335	(1.00)		--	285	(0.86)	--	
--	OH	OH	OH	--	--	OR	--	13	--	335	(1.00)		--	286	(0.83)	--	
--	OH	OH	OR	--	--	OR	--	13	--	331	(1.00)		--	286	(0.89)	--	
--	OH	OH	OH	--	OH	OH	--	5	--	346	(1.00)		--	283	(0.79)	254s	(0.82)
--	OH	OH	OR	--	OH	OH	--	13	--	345	(1.00)		--	284	(0.75)	256s	(0.69)
--	OH	OH	OH	--	OR	OH	--	15	--	342	(1.00)		--	283	(0.80)	242s	(0.80)
--	OH	OH	OH	--	OH	OR	--	13	--	342	(1.00)		--	284	(0.78)	242s	(0.80)
--	OH	OH	OR	--	OR	OH	--	16	--	343	(1.00)		--	282	(1.00)	272s	(--)
--	OH	OH	OR	--	OR	OR	--	16	--	340	(1.00)		--	280	(0.90)	270s	(--)
OH	OH	OH	OH	--	OH	OH	--	17	--	360			--	272s		258	
OR	OH	OH	OR	--	--	--	--	3	--	358			--	274		--	
OR	OH	OH	OR	--	--	OH	--	18	--	339			--	277		--	
OR	OH	OH	OH	--	--	OH	--	19	--	340			--	281		--	
OR	OH	OH	OH	--	OH	OH	--	19	--	350			--	270s		258	
OR	OH	OH	OR	--	OH	OH	--	13	--	348	(1.00)		--	279	(0.82)	259	(0.84)
OR	OH	OH	OH	--	OR	OH	--	20	--	349	(1.00)		--	280	(0.69)	257s	(0.66)
OR	OH	OH	OR	--	OR	OH	--	13	--	352	(1.00)		--	281	(0.74)	256s	(0.67)
OR	OH	OH	OR	--	OH	OR	OR	13	--	339	(1.00)		--	281	(0.85)	240s	(0.77)

Table 2. UV spectra in methanol of 5,8-dihydroxyflavones, 3-methoxyflavones and flavonols trisubstituted on A-ring. Values and abbreviations are as for Table 1

SUBSTITUTION PATTERN								REFERENCES	BI				BIII		BII			
3	5	6	7	8	3'	4'	5'		BIa		BIb		BIII		BIIa		BIIb	
--	OH	--	OH	OH	--	--	--	6	--		364s	(0.10)	305	(0.50)	281	(1.00)	--	
--	OH	--	OS	OH	--	--	--	6	--		342s	(0.15)	315s	(0.22)	274	(1.00)	247	(0.31)
--	OH	--	OH	OH	--	OH	--	21	364s	(0.56)	330s	(0.88)	305	(1.00)	280	(0.95)	--	
--	OH	--	OS	OH	--	OH	--	21	366i	(0.38)	330s	(0.77)	306	(1.00)	280	(0.96)	--	
--	OH	--	OH	OH	--	OR	--	13	360s		328		302		281		--	
--	OH	--	OS	OH	--	OR	--	22	--		330s		303		281		--	
--	OH	--	OH	OH	OH	OH	--	23	--		340	(0.93)	302s	(0.82)	280	(1.00)	256	(0.82)
--	OH	--	OS	OH	OH	OH	--	5	--		341	(0.96)	298s	(0.78)	285	(1.00)	254	(0.91)
--	OH	--	OH	OH	OR	OH	--	15	--		332	(0.87)	318s	(0.78)	280	(1.00)	252s	(0.69)
--	OH	--	OS	OH	OR	OH	--	15	--		337	(1.00)	298	(0.80)	276	(1.00)	252	(0.90)
--	OH	--	OS	OH	OH	OR	--	24	--		335		298		278		255	
OH	OH	--	OH	OH	OH	OH	--	6	385	(0.75)	339	(0.64)	309	(0.46)	276	(0.87)	261	(1.00)
OH	OH	--	OH	OH	--	OH	--	17	386		328		310s		277		--	
OH	OH	--	OS	OH	OH	OH	--	6	385	(0.70)	343	(0.56)	307s	(0.31)	279s	(0.77)	261	(1.00)
OR	OH	--	OR	OH	--	OH	--	13	368s		326		306		278		--	
OR	OH	--	OR	OH	OH	OH	--	13	378s	(0.61)	343	(0.81)	302s	(0.68)	278	(1.00)	262	(0.92)
OR	OH	--	OR	OH	OR	OH	--	11	365s		339		305		277		257	
OR	OH	--	OR	OH	OH	OR	OH	25	372s		328s		302		278		--	

Table 3. UV spectra in methanol and after adding $AlCl_3$ and $AlCl_3$ + HCl of 5,6-dihydroxy-7,8-dimethoxy- and 5,8-dihydroxy-6,7-dimethoxyflavones and of their demethyl derivatives (7–9). Values and abbreviations are as for Table 1.

	MeOH					$AlCl_3$						
						$AlCl_3$+HCl						
COMP.	BIa	BIb	BIII	BIIa	BIIb	BIa	BIb	BIII	BIII	BIIa	BIIb	
1	--	334	--	297	--	445s(0.15)	370 (1.00)	--	313 (0.85)	290i(0.52)	--	--
		(1.00)		(0.96)		410s(0.13)	359 (1.00)	--	311 (0.89)	287i(0.63)	--	--
2	--	344	--	288	252	423 (1.00)	381s(0.45)	--	304 (0.81)	274 (0.75)	--	--
		(1.00)		(0.78)	(0.60)	410i(0.23)	367 (1.00)	314i(0.72)	302 (0.81)	--	258s(0.71)	--
3	--	343	--	289	250	450i(0.10)	378 (1.00)	--	303 (0.66)	--	259 (0.49)	238s(0.70)
		(1.00)		(0.87)	(0.53)	410i(0.23)	365 (1.00)	315i(0.62)	300 (0.73)	--	257 (0.68)	237s(0.80)
4	370i	330s	306	288s	--	450s(0.20)	357 (1.00)	323 (0.98)	--	289 (0.77)	--	--
	(0.29)	(0.76)	(1.00)	(0.81)		420s(0.27)	353 (0.83)	319 (1.00)	--	286 (0.83)	--	--
5	--	341	298i	284	254	432 (1.00)	--	314 (0.68)	--	285s(0.82)	275 (0.90)	--
		(0.98)	(0.94)	(1.00)	(0.80)	430s(0.24)	362 (1.00)	328 (0.79)	308 (0.84)	289 (0.84)	260 (0.84)	--
6	--	339	315	285	251s	450s(0.23)	366 (1.00)	327 (0.70)	310s(0.68)	290 (0.69)	258 (0.64)	238i(0.89)
		(1.00)	(0.85)	(0.93)	(0.72)	425s(0.23)	359 (1.00)	324 (0.84)	308 (0.81)	291 (0.78)	257s(0.80)	237i(1.00)
7	--	335s	304	--	--	468 (0.17)	372 (0.93)	320 (1.00)	--	--	--	--
		(0.69)	(1.00)			417s(0.57)	366 (0.89)	--	311 (1.00)	--	--	--
8	--	336	--	291	250i	407 (1.00)	--	319 (0.86)	--	270s(0.83)	--	--
		(0.89)		(1.00)	(0.79)	400i(0.74)	370 (0.93)	336s(0.90)	--	299 (1.00)	--	--
9	--	337	318s	293	251i	480s(0.15)	380 (1.00)	312 (0.80)	--	--	259s(0.55)	239s(0.78)
		(1.00)	(0.91)	(0.95)	(0.67)	406s(0.40)	366 (1.00)	--	304 (0.88)	--	256s(0.66)	238s(0.76)

Table 4. Spectral shifts relative to methanol spectra in BIa and BIb after addition of $AlCl_3$ + HCl of 5,6-dihydroxy-7,8-dimethoxy- and 5,8-dihydroxy-6,7-dimethoxyflavones

6-OH-8-OMe ISOMERS								6-OMe-8-OH ISOMERS							
5	6	7	8	3'	4'	ΔBIb	ΔBIa	5	6	7	8	3'	4'	ΔBIb	ΔBIa
OH	OH	OR	OR	--	OH	25	76	OH	OR	OR	OH	--	OH	23	90
OH	OH	OR	OR	OH	OH	23	66	OH	OR	OR	OH	OH	OH	21	99
OH	OH	OR	OR	OR	OH	22	67	OH	OR	OR	OH	OR	OH	20	86

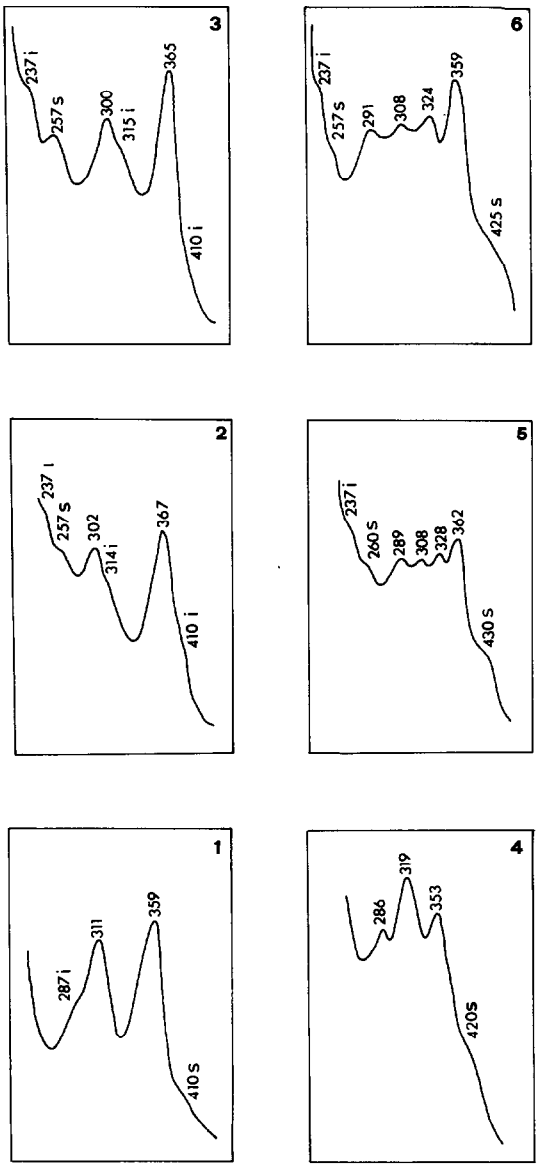
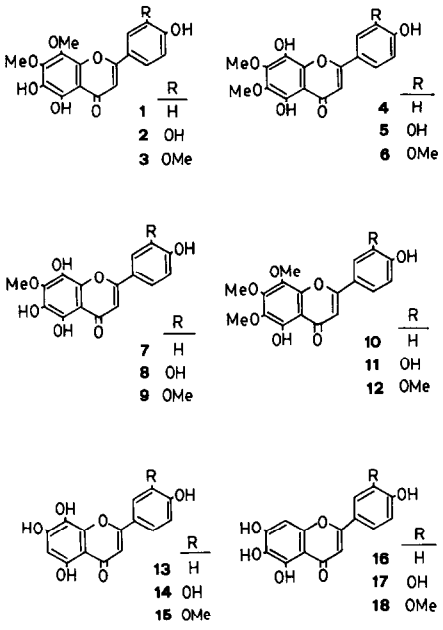


Fig. 2. Shape of UV spectra after addition of $AlCl_3$ + HCl for the 5,6-dihydroxy-7,8-dimethoxy-(1–3) and 5,8-dihydroxy-6,7-dimethoxyflavones (4–6) available.

amount (*ca* 10%) of the isomeric 6-methoxy-8-hydroxyflavones isothymusin (**4**) and isothymonin (**6**), and rather important quantities (*ca* 30%) of the demethylation products (**7**, **9**), meanwhile the 6-methoxy-8-hydroxyflavone leucanthogenin (**5**) yielded a small amount (*ca* 10%) of the isomeric 6-hydroxy-8-methoxyflavone isoleucanthogenin (**2**) and *ca* 30% of the demethylation product (**8**).



These results suggested that in the acidic conditions used in this work, demethylation from the tetra-substituted A-ring flavones is more favoured than the opening of the pyrone ring to give the isomeric change, and that the 6-hydroxy-8-methoxyflavones exhibited the same activation for the isomeric change than the 6-methoxy-8-hydroxyflavones.

Stronger acidic treatment of the 5-hydroxy-6,7,8-trimethoxyflavones sideritoflavone (**11**), xanthomicrol (**10**) and 8-methoxycirsilineol (**12**), yielded after 48 hr of treatment a very small amount (*ca* 2–5%) of the 6-demethyl products isoleucanthogenin (**2**), thymusin (**1**) and thymonin (**3**).

Table 5. TLC of 6-hydroxy- and 8-hydroxyflavones on cellulose F254 (Merk) with 30 and 60% HOAc

SUBSTITUTION PATTERN 5 6 7 8 3' 4'	TRIVIAL NAME	Nº	30%HOAc (Rf)	60%HOAc (Rf)
OH -- -- OH -- --	primetin	-	0.83	--
OH OH -- -- -- --	--	-	0.81	--
OH -- OH OH OH OH	hypolaetin	14	0.12	0.34
OH OH OH -- OH OH	6-hydroxyluteolin	17	0.09	0.30
OH -- OH OH OR OH	--	15	0.16	0.41
OH OH OH -- OR OH	nodifloretin	18	0.13	0.37
OH -- OH OH -- OH	isoscutellarein	13	0.19	0.44
OH OH OH -- -- OH	scutellarein	16	0.16	0.40
OH OR OR OH -- OH	isothymusin	4	0.34	0.72
OH OH OR OR -- OH	thymusin	1	0.23	0.62
OH OR OR OH OR OH	isothymonin	6	0.28	0.69
OH OH OR OR OR OH	thymonin	3	0.19	0.58
OH OR OR OH OH OH	leucanthogenin	5	0.23	0.61
OH OH OR OR OH OH	isoleucanthogenin	2	0.13	0.44

The above demethylation process is useful for synthetic purposes. Demethylation by means of hydriodic acid has been described previously as an essential stage in the synthesis of flavones,[10] the demethylation of 5-methoxyflavones to give 5-hydroxy compounds being easy.[27]

Characterization of the new demethylated products obtained by acidic treatment

The acidic treatment of the naturally occurring flavones (**1**, **3**, **5**) yielded in an appreciable amount the new demethylated products (**7**–**9**). These compounds showed lower R_f values than the parent flavones, as expected for the substitution of a methoxyl by a hydroxyl group, and decomposed quickly in solution giving a reddish colour.

The NMR techniques for the characterization of these new demethylated products were unsuccessful, thus, EIMS, UV and derivatization techniques were used.

The UV spectra in MeOH of the three demethylated compounds, showed the existence of free hydroxyl groups at C-5 and C-8, by the presence of a BIII, and the remarkable decomposition in alkaline media (as described above). The presence of free hydroxyls at C-5 and C-6, was also demonstrated by the bathochromic shift observed in the $AlCl_3$ + HCl spectra (BIb).[6,13] Free hydroxyls at C-4' were evidenced in all cases, by means of the NaOMe spectra in which a batochromic shift with an increase in intensity was observed.[6] The UV spectra in NaOMe and in NaOAc, suggested the existence of substituted hydroxyls at C-7. This was corroborated by the lack of Wessely–Moser rearrangement in these compounds, that demonstrated the same substitution pattern at C-6 and C-8 positions.

The EIMS of the underivatized compounds evidenced $[M]^+$ ions that were the base peak of the spectra, that were in accordance with flavone compounds with four hydroxyl and one methoxyl (**7**), five hydroxyl and one methoxyl (**8**) and four hydroxyl and two methoxyl (**9**) groups. The relative abundance of $[M-H]^+$ (25–35%) and $[M-Me]^+$ ions, and the presence of important $[M-H_2O]^+$ peaks (characteristic of polyhydroxylated flavones), supported the existence of free hydroxyls at C-6 and C-8 positions, as well as the existence of a methoxyl group at C-7. The Retro-Diels–Alder fragments ($[A_1-H]^+$ and $[B_1]^+$) characterized the substitution pattern on A- and B-rings of the flavone nucleus (Fig. 3). The substitution patterns were confirmed by diazomethane methylation that yielded the permethylated derivatives (tangeretin and nobiletin), identified by chromatographic comparisons against authentic samples.[28]

These results are in accordance with a stronger ether

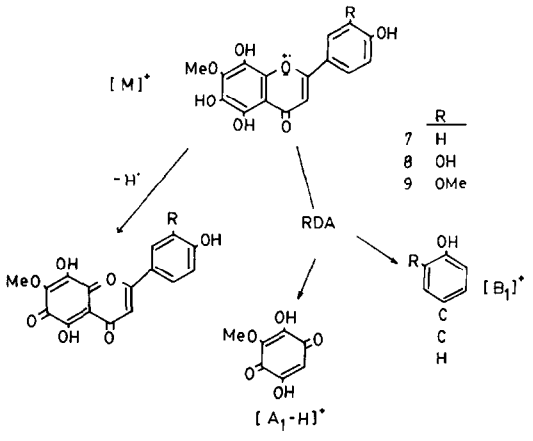


Fig. 3. EIMS fragmentation of demethylated flavones obtained by acidic treatment.

bond at the C-7 position, that at C-6 or C-8 positions, and this agrees with results obtained for the EIMS of permethylated flavonoid glycosides, in which the stronger glycosidic bond (that yield molecular ions with higher relative abundances) is located on the hydroxyl at the C-7 position.[29,30]

These new demethylated flavones constitute a new group of A-ring tetrasubstituted flavonoids, bearing three hydroxyl and one methoxyl group.

CONCLUSION

As we described above, the complete characterization of 5,8-dihydroxy-6,7-dimethoxy- and 5,6-dihydroxy-7,8-dimethoxyflavones is difficult by the classical NMR and UV techniques. In this report we have established that chromatographic and UV comparisons of the original product with its acidic-treatment isomer, allow to ascertain if the free hydroxyl group can be located at the C-6 or C-8 position in the parent flavonoid. This procedure is also useful in the structural determination of flavonoid compounds trisubstituted on A-ring, in particular when only minute amounts of compounds are available for analysis.

Furthermore, the acidic treatment carried out to obtain the Wessely–Moser isomers, yielded an important amount of the demethylated products in the case of 5,6-dihydroxy-7,8-dimethoxy- and 5,8-dihydroxy-6,7-dimethoxyflavonoids, but this acidic treatment only rendered a small quantity of demethylated products from the 5-hydroxy-6,7,8-trimethoxyflavonoids. This can be particularly useful for synthetic purposes.

EXPERIMENTAL

Flavones. Thymusin (**1**) and thymonin (**3**) were isolated from *Thymus membranaceus*[2] and *Thymus vulgaris*, respectively, and leucanthogenin (**5**) was obtained by enzymic hydrolysis (β-D-glucosidase) from its 8-β-D-glucoside isolated from *Sideritis leucantha*.[5] The isomeric compounds isothymonin (**6**), isothymusin (**4**) and isoleucanthogenin (**2**) were obtained by acidic treatment and Wessely–Moser rearrangement.[10]

Acidic treatment. The acidic treatment was carried out to obtain the Wessely–Moser isomers and the demethylation products. This was achieved by addition of 2 ml of 4 N HCl aq to 1 ml of a methanolic soln of the flavonoid compounds, and by heating (100°) for 4 hr in a stoppered screw tube. The MeOH was removed (N_2 flow) and the aqueous phase extracted with EtOAc. Flavonoids were isolated from this extract by preparative PC on Whatman No. 1 with 30% HOAc or 60% HOAc.

Characterization of demethylated products. The isolated demethylated products were permethylated by CH_2N_2, and compared by TLC on silica gel with EtOAc, $CHCl_3$–EtOAc–Me_2CO (5:4:1 and 5:1:4)[28] against nobiletin (5,6,7,8,3′,4′-hexamethoxyflavone) and tangeretin (5,6,7,8,4′-pentamethoxyflavone).

Demethylthymusin (**7**); cellulose TLC 60% HOAc (R_f 0.41); EIMS, *m/z* (rel. abund.), 316 $[M]^+$ (100), 315 $[M-H]^+$ (26), 301 $[M-Me]^+$ (18), 298 $[M-H_2O]^+$ (27), 197 $[A_1-H]^+$ (7), 183 (10), 155 (8), 118 $[B_1]^+$ (5).

Demethylthymonin (**9**); cellulose TLC 60% HOAc (R_f 0.39); EIMS, *m/z* (rel. abund.), 346 $[M]^+$ (100), 345 $[M-H]^+$ (29), 331 $[M-Me]^+$ (30), 328 $[M-H_2O]^+$ (30), 197 $[A_1-H]^+$ (8), 183 (9), 155 (9), 148 $[B_1]^+$ (7).

Demethylleucanthogenin (**8**); cellulose TLC 60% HOAc (R_f 0.29); EIMS, *m/z* (rel. abund.), 332 $[M]^+$ (100), 331 $[M-H]^+$ (35), 317 $[M-Me]^+$ (14), 314 $[M-H_2O]^+$ (36), 197 $[A_1-H]^+$ (12), 183 (5), 155 (4), 134 $[B_1]^+$ (5).

UV spectra were recorded on a Pye–Unicam SP-8 100 spectrophotometer by standard procedures.[6] The mass spectra were taken on a Hewlett–Packard 5993 GC–MS instrument, by direct inlet of samples (70 eV, ion source temperature 240°, probe temperature 280–300°).

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