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Spectral Properties of 2′-Oxygenated Flavones[1)]

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The ultraviolet, proton nuclear magnetic resonance and mass spectra of 56 flavones were measured, and the data were applied for the structure elucidation of 2′-oxygenated flavones.

Keywords——UV-spectrum; ^{1}H-NMR spectrum; mass spectrum; 2′-oxygenated flavone; acetylation shift

Recently, a number of reports have appeared on flavonoids possessing hydroxyl or methoxyl groups at the 2′ or/and 6′ position in ring B.[2)] In usual structural elucidation of flavonoids, hydroxyl groups are detected by the observation of bathochromic shifts in the ultraviolet (UV) spectra obtained with reagents such as $AlCl_3$, H_3BO_3, AcONa and NaOMe. To our knowledge, there is no reports on the spectral properties of 2′- or/and 6′-oxygenated flavone. We describe in this paper the proton nuclear magnetic resonance (^{1}H-NMR), UV and mass spectral (MS) properties of 2′- or/and 6′-oxygenated flavones.

The UV Spectra of 2′- or/and 6′-Hydroxyflavones in the Presence of NaOMe

A strong base, *e.g.* NaOMe or NaOH, can be used for the detection of 3- and/or 4′-hydroxyl groups. Mabry *et al.*[3)] reported that a 4′-hydroxyl group shows a large bathochromic shift of band I (about 40—65 nm) without a decrease in intensity, and a 3-hydroxyl group gives a 50—60 nm bathochromic shift of band I with a decrease in intensity. The reason for the large bathochromic shift and increase in intensity of band I in 4′-hydroxyflavones in the presence of NaOMe or NaOH may be as follows; in the case of base addition, 4′-hydroxyflavone is ionized[4)] and forms a stable planar structure as illustrated in Chart 1.

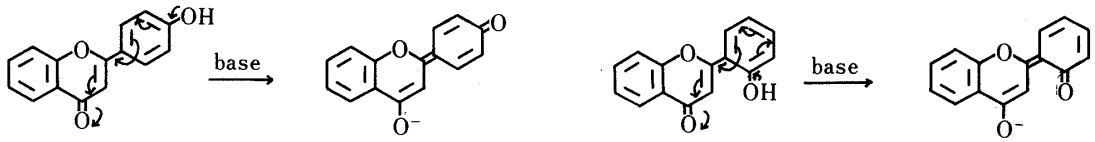

Chart 1

Similar large shifts would be expected for 2′-hydroxyflavones, since similar ionization must occur as shown in Chart 1. From experiments on apigenin[5)] and 2′,5,7-trihydroxyflavone,[6)] it has become apparent that the UV absorption bands of the two flavones are similar in wavelength, but the intensity of band I in the latter flavone is weak compared with that of apigenin. This difference may arise besause of torsion between rings B and C. The addition of NaOMe to both flavones in methanol produced large bathochromic shifts of band I of 55 and 64 nm, with increased intensity, but the degree of increment in the latter is smaller than that of apigenin. The difference can be explained as follows; apigenin can form a planar structure, but 2′- or 6′-substituted flavones cannot because of the substituent group. Experiments on some 2′-hydroxyflavones (**2**—**16**) showed that flavones possessing a 2′-hydroxyl group and lacking

TABLE I. UV Properties of Apigenin and 2′-Oxygenated Flavones

Flavone	λ_{max} nm (log ε)	
	MeOH	+NaOMe[a]
4′,5,7-OH (Apigenin)	268 (4.11) 333 (4.15)	275 (4.19) 388 (4.49)
2′,5,7-OH	267 (4.35) 335 (3.83)	273 (4.35) 399 (3.94)
2′,5,6′,7-OH	259 (4.23) 308 (4.00) 330sh (3.75)	266 (4.29) 335 (4.00)

a) Three drops of 2.5% NaOMe–MeOH solution were added.

a 6′-oxygenated group (**2**—**10**) produce large shifts of band I (about 60—80 nm). Further, flavones having a hydroquinone moiety (**11**—**13**) are decomposed. On the other hand, 2′-hydroxyflavones having a 6′-oxygenated group show different behavior. For example, 2′,5,6′,7-tetrahydroxyflavone[7] gave the UV spectral data shown in Table I. In this flavone, the band I peak is weak, and the shape of the UV curve is similar to that of flavanones. Furthermore, in the presence of NaOMe, flavones of this type (2′,5,6′,7-tetrahydroxyflavones, **15** and **16**) show small shifts of about 10—30 nm. The decrease in intensity of band I and the small shift may be accounted for by large torsion between rings B and C. Kimura *et al.*[2d] reported that the angle between rings B and C in 3′,5,6′,7-tetrahydroxy-2′,8-dimethoxyflavone (**15**) is about 60°, based on an X-ray analysis. Thus a large bathochromic shift upon addition of a strong base may be caused not only by a 4′-hydroxyl group, but also by a 2′-hydroxyl group except when a 6′-oxygenated group is present. Accordingly, it is difficult to distinguish between a 4′-hydroxyl group and a 2′-hydroxy group from the UV spectral data.

The H-3 Chemical Shifts in 2′- or/and 6′-Oxygenated Flavones

The H-3 proton appears near 6.3 ppm in ^{1}H-NMR spectra.[8] A number of reports[9] have appeared on the effects of ring A substitution on the H-3 proton, but the effects of ring B substitution on H-3 have not been reported. We examined this effect by using a flavone lacking substituents (**17**) and eighteen methoxyflavones (**18**—**35**) as model compounds (shown in Table III). Compound **17** give a singlet at 6.83 ppm. Among the monomethoxyflavones (**18**—**24**), only 2′-methoxyflavone (**22**) showed the H-3 signal at low field (7.10 ppm). On the other hand, six monomethoxyflavones except **22**, which lacks 2′- and 6′-oxygenated groups) showed the H-3 signal at high field (6.51—6.80 ppm) compared with that of **17**. Among **25**—**32**, similar low-field shifts are observed in the flavones which possess a 2′-methoxyl group (**28**—**32**). In this case, the H-3 proton appears at near 7.0 ppm. From this result, it seems that the chemical shifts appear at low field in the case of 2′-methoxyflavones lacking a substitutent at the 6′ position as compared with those of flavones which lack both 2′- and 6′-substituents. On the other hand, the H-3 chemical shifts of 2′,6′-oxygenated flavones exhibit different behavior. The chemical shift of H-3 of 2′,6′-dimethoxyflavone (**33**) appears at high field (6.4 ppm) compared with that of the flavone lacking 2′- and 6′-substituents. In polymethoxyflavones (**34**, **35**), similar high field shifts are observed, as shown in Table III. From examination of some 2′,6′-dioxygenated flavones, it has become apparent that the H-3 chemical shifts in flavones of this type appear at 6.10—6.40 ppm. Such shifts are observed not only with methoxyl groups, but also with other oxygenated groups, as shown in Tables IV and V.

The Acetylation Shifts of the H-3 Proton in 2′ or/and 6′-Oxygenated Flavones

Mabry and Markham[8] reported that the signals of the protons on the flavone nucleus

Table II. The Bathochromic Shift of Band I in the UV Spectra of 2′-Oxygenated Flavones in the Presence of NaOMe

Position of OH/OMe		Bathochromic shift (nm)[a]
4′	(1)	61
2′	(2)	60
2′/3′	(3)	80
2′/4′	(4)	65
2′,5,7/8	(5)[12]	73
2′,5,7/6	(6)[12]	60
2′/5,6,7,8	(7)	72
2′,5/6,7,8	(8)[13]	67
2′,5,7/4′,5′,6	(9)[14]	64
2′,5/3′,5′,6,7	(10)[15]	84
2′,5,5′/7,8	(11)[16]	dec.
2′,5,5′/6,7	(12)[16]	dec.
2′,5,5′/6,7,8	(13)	dec.
2′,5,7/6′	(14)	27
3′,5,6′,7/2′,8	(15)[17]	16
2′,5,6′,7/3′,8	(16)[17]	28

a) Measured in MeOH.

Table III. ^{1}H-NMR Chemical Shifts of H-3 of Methoxyflavones

Position of OMe		Chemical shifts[a] of H-3 (ppm)
None	(17)	6.83
5	(18)	6.60
6	(19)	6.78
7	(20)	6.51
8	(21)	6.80
2′	(22)	7.10
3′	(23)	6.74
4′	(24)	6.69
3′,4′	(25)	6.70
3′,5′	(26)	6.78
3′,4′,5′	(27)	6.73
2′,3′	(28)	6.96
2′,4′	(29)	7.08
2′,5′	(30)	7.12
2′,3′,4′	(31)	6.96
2′,3′,5′	(32)	6.96
2′,6′	(33)	6.40
2′,3′,5′,6′	(34)	6.46
2′,3′,4′,5′,6′	(35)	6.40

a) Measured in $CDCl_3$.

Table IV. The H-3 Chemical Shifts of 2′-Oxygenated Flavones

Flavone		Chemical shift of H-3 (ppm)
3		7.12[a]
4		7.10[a]
5		7.00[a]
6		7.05[a]
7		7.27[a]
8		7.19[a]
2′,3′,4′,5,5′,6,7-OMe[18]	(36)	6.95[b]
2′, 3′,4′,5,5′,7,8-OMe[18]	(37)	6.93[b]
2′-iso-OPr[c]	(38)	7.08[b]
2′,5,7-iso-OPr	(39)	6.90[b]

a) d_6-DMSO. b) $CDCl_3$. c) iso-OPr means isopropyloxy.

Table V. The Chemical Shifts of 2′- and 6′-Oxygenated Flavones

Flavone		Chemical shift of H-3 (ppm)
2′,5,6′,7-OH 3′,8-OMe	(16)	6.15[a]
2′,5,6′-OH 7,8-OMe	(40)	6.20[a]
4′,5,6′-OH 2′,3′,6,7-OMe	(41)[19]	6.38[a]
2′,4′,5-OH 3′,6,6′,7-OMe	(42)[19]	6.38[a]
2′,3′,4′,5,6,6′,7-OMe	(43)[18]	6.33[b]
2′,3′,4′,5,6,6′,7,8-OMe	(44)[18]	6.38[b]
2′,5,8-OMe 3′,6′,7-iso-OPr[c]	(45)	6.22[b]
2′,5,6′,7-iso-OPr	(46)	6.16[b]
5,7,8-OMe 2′,6′-OPr	(47)	6.18[b]
2′,5,8-OMe 6′,7-iso-OPr	(48)	6.15[b]
3′,5,8-OMe 2′,6′,7-iso-OPr	(49)	6.20[b]
2′,5,7,8-OMe 6′-iso-OPr	(50)	6.15[b]

a) d_6-DMSO. b) $CDCl_3$. c) iso-OPr means isopropyloxy.

ortho and *para* to acetoxyl groups are shifted downfield by about 0.3—0.5 ppm. However, there has been no examination of the acetylation shifts of the H-3 proton. As shown in Table VI, among three monohydroxyflavones (**1**, **2**, **51**), only the H-3 proton of **2** is shifted to high field by 0.51 ppm; the other two flavones show no shift. This result suggests that only a 2′-acetoxyl group affects H-3. This shift was already observed by Govindachari *et al.*,[10] but they simply presented the spectral data. We systematically examined the acetylation shifts. From observations of **3**, **4**, **7**, **8** and **52**, all 2′-hydroxylflavones which lack a 6′-oxygenated group show high-field shifts by about 0.39—0.72 ppm, as can be seen in Table VI. Nevertheless, 2′-

TABLE VI. The Acetylation Shifts of H-3 of 2′-Oxygenated Flavones

Flavone	Chemical shift of H-3[a] (ppm)	Acetylation shift[a] (ppm)
3′-OH (**51**)	6.78	−0.03
4′-OH (**1**)	6.78	−0.03
2	7.16	−0.51
3	6.99	−0.39
4	7.10	−0.51
7	7.27	−0.72
8	7.19	−0.67
2′,5-OH 3′,7,8-OMe (**52**)	7.20	−0.71
2′,6′-OH (**53**)	6.28	+0.02
2′-OH 6′-OMe (**54**)	6.30	−0.01

a) Measured in d_6-DMSO.

TABLE VII. The Intensities of $M^+ - 17$ and $M^+ - 31$ in the MS of 2′-Oxygenated Flavones

Flavone	$M^+ - 17$ (rel. int.)	Flavone	$M^+ - 31$ (rel. int.)
1	10	**26**	11
2	17	**22**	4
3	12	**28**	4
5	—	**30**	5
15	6	**31**	3
52	—	**32**	—
53	12	**33**	5
2′,3-OH (**55**)	100	2′,3′-OMe (**56**)	100

hydroxyflavones having a 6′-oxygenated group show no shift. Accordingly, this shift is specific for 2′-hydroxyflavones which lack a 6′-oxygenated group. By observing this shift, it is possible to differentiate between 2′-hydroxy-4′-methoxyflavone and 2′-methoxy-4′-hydroxyflavone, which cannot be distinguished simply from the UV spectral data.

The MS of Flavones Having 2′ or/and 6′-Oxygenated Group(s)

It was reported that $M^+ - 17$ and $M^+ - 31$ are characteristic fragments of 2′-hydroxy- and 2′-methoxyflavones.[8] As shown in Table VII, $M^+ - 17$ and $M^+ - 31$ are not specific to 2′-hydroxy- and 2′-methoxyflavones; for example, compound **1** gives a strong peak at $M^+ - 17$ compared with **5**, **15** and **52**, and the intensity of $M^+ - 31$ in **26** is stronger than those of **22**, **28**, **30**, **31**, **32** and **33**, which possess methoxyl groups at the 2′- or/and 6′-positions. Thus it is suggested that $M^+ - 17$ in 2′-hydroxyflavones and $M^+ - 31$ in 2′-methoxyflavones are not diagnostic. However, only 2′-oxygenated flavonols[11] *e.g.* **55** and **56**, give base peaks at $M^+ - 17$ and $M^+ - 31$.

In this paper, we describe the spectral properties of 2′-oxygenated flavones. The specific features probably arise because of the torsion between rings B and C. A study on the torsion angle of some 2′- or/and 6′-oxygenated flavones is in progress.

Experimental

The UV spectra were taken on a Hitachi 323 spectrometer and MS were obtained on a JEOL JMS-300 mass spectrometer at 70 eV. ^{1}H-NMR spectra were taken on a Hitachi R-20B instrument at 60 MHz; chemical shifts are given in δ values (ppm) with tetramethylsilane as an internal standard. The melting points were determined on a

Buchi melting point apparatus, and are uncorrected.

The melting points of flavones used for measurement were as follows: **1**, 240 °C; **2**, 218—220 °C; **3**, 235—236 °C; **4**, 244—246 °C; **5**, 287—289 °C; **6**, 253—254 °C; **7**, 192 °C; **8**, 230 °C; **9**, 285—287 °C; **10**, 260 °C (dec.); **11**, 265 °C; **12**, 267—269 °C; **13**, 234 °C; **14**, 252 °C (dec.); **15**, 252—254 °C; **16**, 163—164 °C; **17**, 98—99 °C; **18**, 144—145 °C; **19**, 162—164 °C; **20**, 99—101 °C; **21**, 200—202 °C; **22**, 102—103 °C; **24**, 158—159 °C; **25**, 140 °C; **26**, 135—136 °C; **27**, 174—176 °C; **28**, 80—83 °C; **29**, 93—94 °C; **30**, 108—110 °C; **31**, 90—91 °C; **32**, 112—113 °C; **33**, 147—148 °C; **34**, 132—133 °C; **35**, 97—98 °C; **36**, 83—84 °C; **37**, 117—118 °C; **40**, 281 °C; **41**, 220 °C (dec.); **42**, 130—132 °C; **43**, 113—114 °C; **51**, 240 °C; **52**, 210 °C (dec.); **53**, 248—249 °C; **54**, 207—208 °C; **55**, 220 °C; **56**, 145—146 °C. **38**, **39**, **46**, **47**, **48**, **49** and **50** were oils.

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