Note

Study on the Structure of
5,7,4'-Trihydroxyflavanones

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Received November 27, 1984

Currently, the position of C-alkylation at C-6 and C-8 in flavanones with hydroxy groups at C-5 and C-7 is elucidated by the various methods shown in Table I. In the course of studying 5,7,4'-trihydroxyflavanone (naringenin) derivatives,¹⁾ however, it was found that some of these methods do not help to unequivocally determine the 6- and 8-alkylnaringenins **1**, **2**, **3**, and **5**.

FIG. 1.

TABLE I. METHODS FOR DETERMINING
C-ALKYLATION AT C-6 AND C-8
IN FLAVANONES

(1)	Gibbs test ^{2,3)}
(2)	UV shift ⁴⁾
(3)	¹ H-NMR
	1. 5-OH ⁵⁾
	2. Gem-dimethyl of an isopentenyl group ⁶⁾
	3. Acetylation of 5-OH ⁷⁾
(4)	Cyclization
	1. Acid treatment after methylation of 7-OH ⁸⁾
	2. Acid treatment ⁹⁾
(5)	¹³ C-NMR
	1. Chemical shift ³⁾
	2. Long-range selective proton decoupling ^{1,10)}

Concerning (1) in Table I, 6-isopentenylnaringenin (**1**), 8-isopentenylnaringenin (**2**), and compounds **3** and **5** (Fig. 1.) are all positive to the Gibbs test,^{2,3)} which has been widely used for determining C-alkylation at C-6 and C-8 in 5,7-dihydroxyflavanones. This fact shows that the Gibbs test does not help to determine C-alkylation at C-6 and C-8 in 5,7,4'-trihydroxyflavanones.

Concerning (2) in Table I, it has been reported⁴⁾ that when an isopentenyl group is present *ortho* to a chelated hydroxy group, there is no AlCl₃-induced bathochromic shift in the UV spectra. According to our experiment, however, 6-isopentenylnaringenin (**1**) and **3**, each of which contains an alkyl group *ortho* to a chelated hydroxy group, begins to show a bathochromic shift some minutes after adding AlCl₃. This fact suggests that (2) requires care and promptness for determining C-alkylation in these compounds.

Concerning (3)-1 in Table I, it has been reported⁵⁾ that the position of an isopentenyl group influences the chemical shift of the chelated OH proton, and in compounds with an isopentenyl group at C-8, the corresponding signal was shifted more downfield than in compounds with an isopentenyl group at C-6 in ¹H-NMR. According to our experiment, however, the chemical shift of the chelated OH proton for compound **2** shifted more upfield than that of **1** in both CDCl₃ and acetone-*d*₆.

Concerning (3)-2 in Table I, it has been reported⁶⁾ that the signals of gem-dimethyl in an isopentenyl group collapse to a singlet if the isopentenyl group is at C-8, while compounds with an isopentenyl group at C-6 show separated methyl signals in ¹H-NMR. By this method, the structures of **1** and **2** can be correctly deduced from ¹H-NMR data in CDCl₃ and acetone-*d*₆, but not from those in CD₃OD, in which both **1** and **2** showed separated methyl signals.

Concerning (3)-3 in Table I, it has been reported that a diamagnetic shift of the peri-proton after acetylation of C-5 OH can be useful in determining C-alkylation at C-6 and C-8.⁷⁾ According to our experiment, **1** and **3** can be correctly deduced from this method, but it must be carefully used, because the change in chemical shift for the peri-proton of **1** after acetylation was small (about 0.08 ppm upfield shift in acetone-*d*₆ after acetylation).

Concerning (4)-1 and 2 in Table I, it was experimentally shown that the former method required care in determining C-alkylation, because some side-reactions occurred during methylation; in the latter method, treatment of **1** with some acids (formic acids) gave a low yield of **4**, from which **1** could be unequivocally deduced. Generally, chemical methods such as (3)-3, (4)-1 and (4)-2 would not be adequate for a small amount of naturally occurring compounds.

Concerning (5)-1 and 2 in Table I, the former method required a precise comparison with a parent compound to determine C-alkylation at C-6 or C-8 in flavanone derivatives, because the difference of chemical shifts between C-6 and C-8 was small (about 0.9 ppm). Compared with

the former method (and the other methods so far discussed), (5)-2 is more advantageous in determining the position of C-alkylation in 5,7-dihydroxyflavanone derivatives.

Thus, some methods in Table I are not fully reliable in determining C-6 and C-8 alkylation in 5,7-dihydroxyflavanone derivatives, and might possibly lead to the wrong conclusions.

EXPERIMENTAL

1. *Preparation of compounds 3 and 4.* 6-Iso-pentenylaringenin (1) (30 mg) in chloroform (15 ml) was treated with trifluoroacetic acid (=TFA, 0.2 ml) in the usual way to give a mixture of 3 and 4, which were separated by silica gel TLC (CHCl_3 -MeOH=9:1) to obtain 3 (11 mg) and 4 (13 mg).

Compound 3: mp 189.0~189.5°C (colorless needles, recrystallized from aq. HCO_2H ; lit.⁹⁾ 152~153°C). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 296 (19,900). $\lambda_{\text{max}}^{\text{MeOH} + \text{AlCl}_3}$ *: after 0 min, 296 (19,500), 310 (11,100); after five min, 296 (17,400), 298 (17,600), 310 (14,400); after ten min, 296 (15,900), 302 (16,700), 310 (15,400), after fifteen min, 296 (14,000), 310 (17,100); after twenty min, 318 (18,800). MS m/z : 340 (M^+ , 68%), 220 (40), 165 (100), 120 (66). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3450, 1640, 1580, 1520. $^1\text{H-NMR}$ δ (CDCl_3): 1.34 (6H, s, $2 \times \text{CH}_3$), 1.79 (2H, t, $J=6.8$ Hz, CH_2), 2.62 (2H, t, $J=6.8$ Hz, Ar- CH_2), 12.38 (1H, s, 5-OH). Gibbs test: sky blue.

Compound 4: mp 208~208.5°C (colorless needles, recrystallized from AcOEt-hexane; lit.⁹⁾ 266~268°C). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 286 (14,800). MS m/z : 340 (M^+ , 64%), 220 (50), 165 (100), 120 (59); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 1640, 1595, 1520. $^1\text{H-NMR}$ δ (acetone- d_6): 1.33, 1.35 (each 3H, 2s, $2 \times \text{CH}_3$), 1.77 (2H, t, $J=6.9$ Hz, CH_2), 2.60 (2H, t, $J=6.9$ Hz, Ar- CH_2).

2. *Preparation of compound 5.* 8-Isopentenylaringenin (2, 30 mg) in chloroform (15 ml) was treated with TFA (0.2 ml) in the usual way to obtain compound 5 (21 mg), mp 204.5~205°C (colorless needles, recrystallized from aqueous methanol; lit.⁹⁾ 157~158°C). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 294 (20,100). $\lambda_{\text{max}}^{\text{MeOH} + \text{AlCl}_3}$ nm: 320 (+26). MS m/z : 340 (M^+ , 100%), 220 (50), 165 (85), 120 (35). IR

$\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 1640, 1590, 1520, $^1\text{H-NMR}$ δ (CDCl_3): 1.33, 1.34 (each 3H, 2s, $2 \times \text{CH}_3$), 1.75 (2H, t, $J=7.0$ Hz, CH_2), 2.58 (2H, $J=7.0$ Hz, Ar- CH_2), 11.75 (1H, s, 5-OH). Gibbs test: sky blue.

Acknowledgments. The author wishes to express his thanks to Kirin Co. for permission to publish this work, and to Dr. Y. Shirataki, Josai University, for helpful discussions.

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* The behavior of 1 was similar to 3 in its UV spectrum.