The Synthesis of 5,2′-Dihydroxy-6,8-dimethoxyflavone and Its Isomers: A Revised Structure of Skullcapflavone I

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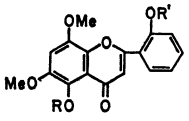
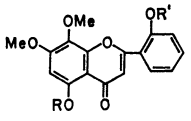
5,2′-Dihydroxy-6,8-dimethoxyflavone, which had been proposed as the structure of skullcapflavone I isolated from *Scutellaria baicalensis* Georgi, was synthesized from 2-hydroxy-3,5,6-trimethoxyacetophenone. However, the synthetic flavone was not identical with the natural flavone. Thus, two isomeric flavones, 5,2′-dihydroxy-7,8-dimethoxyflavone and 5,2′-dihydroxy-6,7-dimethoxyflavone, were prepared from the corresponding acetophenones; the structure of skullcapflavone I was confirmed to be 5,2′-dihydroxy-7,8-dimethoxyflavone.

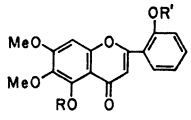
Skullcapflavone I has recently been isolated from roots of *Scutellaria baicalensis* Georgi, along with skullcapflavone II; its structure has been proposed as 5,2′-dihydroxy-6,8-dimethoxyflavone (**1**) on the basis of the spectroscopic data and degradative studies.[1] The properties of skullcapflavone I are not, however, consistent with those of 5,2′-dihydroxy-6,8-dimethoxyflavone (**1**) synthesized from 2-hydroxy-3,5,6-trimethoxyacetophenone (**7**). Therefore, two isomers of **1**, 5,2′-dihydroxy-7,8-dimethoxyflavone (**2**) and 5,2′-dihydroxy-6,7-dimethoxyflavone (**3**), were prepared in order to confirm the structure of skullcapflavone I; consequently, the properties of the former were thus found to be identical with those of skullcapflavone I. We wish now to report that the structure of natural skullcapflavone I is 5,2′-dihydroxy-7,8-dimethoxyflavone (**2**).



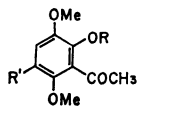
(**1**) R=R′=H
(**9**) R=Me, R′=H
(**10**) R=R′=Ac



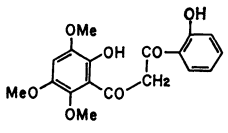
(**2**) R=R′=H
(**12**) R=Me, R′=$C_6H_5CH_2$
(**13**) R=Me, R′=H
(**14**) R=R′=Ac



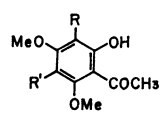
(**3**) R=R′=H
(**16**) R=Me, R′=H
(**17**) R=R′=Ac



(**4**) R=Me, R′=Ac
(**5**) R=Me, R′=OH
(**6**) R=Me, R′=OMe
(**7**) R=H, R′=OMe



(**8**)



(**11**) R=OMe, R′=H
(**15**) R=H, R′=OMe

1,3-Diacetyl-2,5,6-trimethoxybenzene (**4**)[2] was oxidized with 30% hydrogen peroxide in a mixture of acetic acid and concentrated sulfuric acid; the hydrolysis of the resultant compound gave 3-hydroxy-2,5,6-trimethoxyacetophenone (**5**). The acetophenone **5** was converted into the methyl ether (**6**) and was then demethylated with anhydrous aluminum chloride in ether to give 2-hydroxy-3,5,6-trimethoxyacetophenone (**7**). After the condensation of **7** with 2-benzyloxybenzoyl chloride, the debenzylated diketone (**8**) was obtained by the Baker-Venkataraman rearrangement. The compound **8** was cyclized with anhydrous sodium acetate in acetic acid to yield a 2′-hydroxyflavone derivative (**9**), which was then converted with anhydrous aluminum chloride in acetonitrile into 5,2′-dihydroxy-6,8-dimethoxyflavone (**1**) (mp 246.5—248 °C). The flavone **1** afforded the corresponding diacetate (**10**). The flavone **1** and the diacetate **10** should be consistent with skullcapflavone I and its diacetate respectively, but they were not identical with each other, as is shown in Tables 1 and 2. That is, in the NMR spectra of the acetate of skullcapflavone I and the diacetate **10**, it appears that there is a remarkable difference between the chemical shifts of protons on the A ring of the flavone nucleus, but that other proton signals show very similar chemical shifts. Therefore, it may be assumed that the structure of skullcapflavone I is the isomer of **1**, that is, **2** or **3**.

2′-Hydroxy-5,7,8-trimethoxyflavone (**13**) could not be prepared from the acetophenone (**11**)[3] *via* the corresponding diketone by the method described above. The condensation of the acetophenone **11** with 2-benzyloxybenzaldehyde in the presence of piperidine gave an oily chalcone derivative, which was then converted into a benzyloxyflavone derivative (**12**) by oxidative cyclization with selenium dioxide. The flavone **12** was debenzylated with palladium charcoal to give a hydroxyflavone derivative (**13**) and was then demethylated with anhydrous aluminum chloride in acetonitrile to afford the desired flavone **2** (mp 253—254 °C), which was subsequently converted into the diacetate (**14**). 5,2′-Dihydroxy-6,7-dimethoxyflavone **3** was also synthesized from the acetophenone (**15**)[4] *via* the corresponding chalcone by using the same method as was used in the case of the flavone **2**. The flavone **3** led to the diacetate (**17**).

The physical and spectral data of synthetic flavones and these acetates are listed in Tables 1 and 2. In Tables 1 and 2, the NMR and UV spectral data of skullcapflavone I and its diacetate are shown to be identical with those of the synthetic flavone **2** and the

Table 1. Mp and UV spectra of flavones[a)]

Compound	Mp/°C		λ_{max}/nm (log ε)
Skullcapflavone I[1)]	263—265	(EtOH)	271(4.40), 340(4.05)
	(266—267)[b)]	(EtOH–$AlCl_3$)	280(4.34), 342(4.05), 400(3.84)
Acetate[1)]	143—144		
1	246.5—248	(EtOH)	284(4.41), 321(4.10), 339(4.11)
		(EtOH–$AlCl_3$)	253(4.03), 287_i(4.29), 301(4.35), 339(4.21), 355_i(4.19)
		(EtOH–AcONa)	285(4.37), 319_i(4.02), 340(4.00), 411(3.77)
10	195—195.5	(EtOH)	269(4.47), 345(3.69)
2	253—254	(EtOH)	273(4.44), 343(4.11)
	(266.5—267.5)[b)]	(EtOH–$AlCl_3$)	282(4.39), 294_i(4.34), 345(4.11), 404(3.91)
		(EtOH–AcONa)	271(4.41), 343(4.01), 416(3.81)
14	135—136	(EtOH)	257(4.45), 303(4.13)
3	254—255	(EtOH)	248(4.12), 271(4.36), 339(4.26)
		(EtOH–$AlCl_3$)	254(4.09), 278_i(4.35), 285.5(4.44), 354(4.28)
		(EtOH–AcONa)	270.5(4.34), 337(4.15), 415(3.73)
17	145—146	(EtOH)	254(4.31), 301(4.28)

a) i: Inflection point. b) The melting points were measured with a Yanagimoto micro-melting-point apparatus in our laboratory.

Table 2. NMR spectra of flavones

Compound	Solvent	C_3–H	Arom H		OMe	OAc	OH
			A ring	B ring			
Skullcapflavone I[1)]	DMSO	6.55—7.90(6H, m)			3.82(3H, s)	—	10.79
					3.91(3H, s)	—	12.64
Acetate[1)]	$CDCl_3$	6.50(s)	6.69(s)	7.16—7.81(4H, m)	3.82(3H, s)	2.28(3H, s)	—
					3.91(3H, s)	2.40(3H, s)	—
1	DMSO	6.91—7.95(6H, m)			3.85(3H, s)	—	10.93
					3.93(3H, s)	—	12.16
10	$CDCl_3$	6.50(s)	6.90(s)	7.10—7.83(4H, m)	3.88(3H, s)	2.27(3H, s)	—
					3.94(3H, s)	2.43(3H, s)	—
2	DMSO[a)]	7.09(s)	6.54(s)	6.9—7.95(4H, m)	3.78(3H, s)	—	10.99
					3.88(3H, s)	—	12.86
14	$CDCl_3$[a)]	6.44(s)	6.64(s)	7.1—7.85(4H, m)	3.84(3H, s)	2.30(3H, s)	—
					3.92(3H, s)	2.40(3H, s)	—
3	DMSO	7.11(s)	6.90(s)	6.90—7.80(4H, m)	3.71(3H, s)	—	10.95
					3.89(3H, s)	—	12.99
17	$CDCl_3$	6.45(s)	6.80(s)	7.10—7.80(4H, m)	3.83(3H, s)	2.27(3H, s)	—
					3.93(3H, s)	2.46(3H, s)	—

a) The NMR spectra of 2 and 14 could be superimposed on those of the literature.[1)]

diacetate **14** respectively. The melting point of the flavone **2** was not depressed by admixture with the natural flavone, and the UV spectrum of the flavone **2** was also superimposable on that of the natural flavone. On the basis of these results, the structure of skullcapflavone I was confirmed to be 5,2′-dihydroxy-7,8-dimethoxyflavone **2**, which had previously been proposed as the structure of andrographin isolated from *Andrographis paniculata*.[5)] The flavone **2** has also been produced in differentiating tissue cultures of *Andrographis paniculata*.[6)]

Experimental

All the melting points were determined in glass capillaries and are uncorrected. The NMR spectra were measured with a JEOL PS-100 spectrometer (100 MHz), using tetramethylsilane as the internal standard (δ, ppm). The UV spectra were taken on a Hitachi 124 spectrophotometer.

2,3,5,6-Tetramethoxyacetophenone (6). 1,3-Diacetyl-2,5,6-trimethoxybenzene (**4**)[2)] (10 g) and concd sulfuric acid (5 ml) were dissolved in acetic acid (5 ml) below 10 °C, and to the solution with stirring was then added dropwise a mixture of 30% hydrogen peroxide (7 g) and acetic acid (10 ml) below 10 °C. The resulting mixture was subsequently stirred for 5 h at room temperature. After the addition of water to the reaction mixture, the mixture was extracted with ether, and the ethereal solution was washed with aqueous sodium carbonate and water. The solvent was evaporated from the ethereal solution to give crude 3-acetoxy-2,5,6-trimethoxyacetophenone.[7)] The acetophenone was hydrolyzed with 10% sodium hydroxide in methanol (50 ml) for 10 min; then, a crude phenol derivative (**5**) (6 g) was obtained by a usual treatment. A mixture of **5** (16 g) and dimethyl sulfate (20 g) was refluxed with anhydrous potassium carbonate (40 g) in anhydrous acetone (65 ml) for 5 h. After water had been added to the mixture, the solvent was removed, and then the residue was extracted with ether. The ethereal solution was washed with 10% aqueous sodium hydroxide and water, and dried. The solvent was removed, and the residue was distilled under reduced pressure to give an acetophenon (**6**) (15.5 g); it solidified at room temperature: bp 108 °C/0.25 mmHg; NMR ($CDCl_3$) δ 2.47 (3H, s, CH_3CO), 3.73 and 3.83 (each 6H, s, OCH_3), 6.51 (1H, s, Arom H). Found: C, 60.02; H, 6.78%. Calcd for $C_{12}H_{16}O_5$: C, 59.99; H, 6.71%.

2-Hydroxy-3,5,6-trimethoxyacetophenone (7). A mixture of **6** (5 g) and anhydrous aluminum chloride (5.2 g) in anhydrous ether (40 ml) was stirred for 5 h in an ice bath and then allowed to stand overnight in a refrigerator. The reaction mixture was poured into diluted hydrochloric acid, and the solvent was evaporated to afford a precipitate (**7**), which was subsequently recrystallized from methanol as yellow needles (2.6 g): mp 62—63.5 °C; NMR (DMSO) δ 2.41 (3H, s, CH_3CO), 3.63 (3H, s, OCH_3), 3.76 (6H, s, $OCH_3\times2$), 6.80 (1H, s, Arom H), 9.43 (1H, s, OH). Found: C, 58.48; H, 6.24%. Calcd for $C_{11}H_{14}O_5$: C, 58.40; H, 6.24%.

2-Hydroxy-3,5,6-trimethoxy-ω-(2-hydroxybenzoyl)acetophenone(8). A mixture of **7** (2 g) and 2-benzyloxybenzoyl chloride (3.5 g) was heated in the presence of pyridine (3.5 g) at 100 °C for 5 h, and then the reaction mixture was poured into a mixture of ice and hydrochloric acid. The mixture was extracted with ethyl acetate and treated by a usual method. The solvent was then evaporated, and the residue was dried sufficiently to give a crude ester (3.2 g). The ester was stirred in the presence of freshly powdered potassium hydroxide (3.5 g) in pyridine (10 ml) at 60 °C for 4 h, and then a mixture of ice and hydrochloric acid was added to the mixture. After the mixture had been extracted with ether and treated by a usual method, the ether was evaporated to yield a precipitate (**8**), which was subsequently recrystallized from ethyl acetate as yellow plates (300 mg): mp 140—140.5 °C. Found: C, 62.56; H, 5.35%. Calcd for $C_{18}H_{18}O_7$: C, 62.42; H, 5.24%.

2'-Hydroxy-5,6,8-trimethoxyflavone (9). A mixture of **8** (150 mg) and anhydrous sodium acetate (1 g) in acetic acid (6 ml) was heated for 4 h, and then water was added to the reaction mixture to give a precipitate (**9**), which was subsequently recrystallized from methanol as pale yellow needles (100 mg): mp 231—232 °C; NMR (DMSO) δ 3.66, 3.87, and 3.96 (each 3H, s, OCH_3), 6.9—7.9 (6H, m, Arom H). Found: C, 65.66; H, 4.94%. Calcd for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91%.

5,2'-Dihydroxy-6,8-dimethoxyflavone (1). A mixture of **9** (240 mg) and anhydrous aluminum chloride (0.5 g) in acetonitrile (3 ml) was heated at 80 °C for 4 h, and then 2% aqueous hydrochloric acid was added to the reaction mixture. The mixture was heated on a water bath for 30 min and then allowed to stand at room temperature to give a precipitate (**1**), which was subsequently recrystallized from methanol as yellow needles (200 mg); mp 246.5—248 °C. Found: C, 65.02; H, 4.66%. Calcd for $C_{17}H_{14}O_6$: C, 64.96; H, 4.49%.

The Diacetate (10) of 1. The flavone **1** was converted into a diacetate (**10**) by an acetic anhydride-pyridine method; it was subsequently recrystallized from methanol as colorless prisms; mp 195—196.5 °C. Found: C, 63.41; H, 4.67%. Calcd for $C_{21}H_{18}O_8$: C, 63.31; H, 4.55%.

2'-Benzyloxy-5,7,8-trimethoxyflavone (12). A mixture of 2-hydroxy-3,4,6-trimethoxyacetophenone (**11**)[3] (3.5 g) and 2-benzyloxybenzaldehyde (bp 153—155 °C/1 mmHg) (3.5 g) was refluxed in the presence of piperidine (4.5 g) in ethanol (20 ml) for 7 h; the solvent was then removed under reduced pressure, and the residue was extracted with ethyl acetate. The solution of ethyl acetate was washed with diluted hydrochloric acid and water, and dried. The solvent was evaporated to give a crude chalcone, which was subsequently refluxed in the presence of selenium dioxide (4 g) in 1-pentanol for 22 h. The reaction mixture was filtered out, and the filtrate was condensed sufficiently under reduced pressure. The residue was extracted with ethyl acetate, and the extract was washed with aqueous sodium carbonate solution and diluted hydrochloric acid, and dried. After the removal of the solvent, the residue was solidified in a small amount of ether and the solid was recrystallized from ethyl acetate as yellow prisms of **12** (2.6 g): mp 145.5—146.5 °C. Found: C, 71.52; H, 5.23%. Calcd for $C_{25}H_{22}O_6$: C, 71.76; H, 5.30%.

2'-Hydroxy-5,7,8-trimethoxyflavone (13). The flavone **12** (1.01 g) was hydrogenated over palladium on charcoal (10%; 190 mg) in methanol until the uptake of hydrogen ceased. The solvent was then removed under reduced pressure, and the residue was recrystallized from methanol to give yellow needles (**13**) (744 mg): mp >270 °C. Found: C, 65.82; H, 4.91%. Calcd for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91%.

5,2'-Dihydroxy-7,8-dimethoxyflavone (2). The flavone **13** (147 mg) was heated with anhydrous aluminum chloride (0.4 g) in acetonitrile (1 ml) at 80 °C for 2.5 h. The mixture was then heated with diluted hydrochloric acid (15 ml) for 3 min on a water bath to give a precipitate (**2**), which was subsequently recrystallized from methanol to give yellow needles (118 mg): mp 253—254 °C. Found: C, 64.69; H, 4.43%. Calcd for $C_{17}H_{14}O_6$: C, 64.96; H, 4.49%.

The Diacetate (14) of 2. The flavone **2** was converted into a diacetate (**14**) as colorless needles: mp 135—136 °C. Found: C, 63.08; H, 4.51%. Calcd for $C_{21}H_{18}O_8$: C, 63.31; H, 4.55%.

2'-Hydroxy-5,6,7-trimethoxyflavone (16). A mixture of 2-hydroxy-4,5,6-trimethoxyacetophenone (**15**)[4] (1.1 g) and 2-benzyloxybenzaldehyde (1.15 g) was refluxed in the presence of piperidine (1.2 ml) in ethanol (8 ml) for 7 h, and then the reaction mixture was worked-up in the same manner as in the case of **12** to give an oily chalcone. After the chalcone had been treated with selenium dioxide (1.5 g) in 1-pentanol for 20 h to afford an oily flavone, the oily compound was chromatographed over a polyamide column with methanol. The eluate containing the major product was again chromatographed over a silica-gel column with chloroform to give a yellow oily compound (1 g). This oily compound was treated with palladium on charcoal to give a precipitate (**16**), which was subsequently recrystallized from methanol as colorless prisms (550 mg): mp 241—242 °C. Found: C, 65.69; H, 4.85%. Calcd for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91%.

5,2'-Dihydroxy-6,7-dimethoxyflavone (3). A mixture of **16** (100 mg) and anhydrous aluminum chloride (0.3 g) in acetonitrile (1 ml) was heated at 70 °C for 2 h, and then the reaction mixture was worked-up in the same manner as in the case of **1** to give a precipitate, which was subsequently recrystallized from methanol as pale yellow needles (**3**) (52 mg): mp 254—255 °C. Found: C, 64.99; H, 4.40%. Calcd for $C_{17}H_{14}O_6$: C, 64.96; H, 4.49%.

The Diacetate (17) of 3. The flavone **3** was converted into a diacetate (**17**), which was then recrystallized from aqueous methanol as colorless plates: mp 145—146 °C. Found: C, 63.25; H, 4.46%. Calcd for $C_{21}H_{18}O_8$: C, 63.31; H, 4.55%.

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