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Studies on Nepalese Crude Drugs. V.[1] On the Flavonoid Constituents of the Root of *Scutellaria discolor* COLEBR. (1)[2]

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Four new flavanones (I—IV) and a new chalcone (V) were isolated from the root of *Scutellaria discolor* COLEBR., together with wogonin, norwogonin, 5,7,2′-trihydroxy-8-methoxyflavone, 5,7-dihydroxy-8,2′-dimethoxyflavone and wogonin 7-*O*-glucuronide. The structures of I—V were shown to be 2(*S*)-5,7-dihydroxy-8,2′-dimethoxyflavanone, 2(*S*)-7-hydroxy-5,8,2′-trimethoxyflavanone, (±)-5,2′-dihydroxy-7,8,6′-trimethoxyflavanone, (±)-5,2′-dihydroxy-6,7,6′-trimethoxyflavanone and 2′,4′-dihydroxy-2,3′,6′-trimethoxychalcone, respectively, based on spectral data and simple chemical modifications.

Keywords——*Scutellaria discolor*; Labiatae; flavone; flavanone; chalcone; strucure elucidation

Scutellaria discolor COLEBR. is an annual herb of the family Labiatae, which is widely distributed in Nepal, India and China. In Nepal, the plant is called "Nilo Butte Ghans," and its leaves are used as a folk remedy for colds, cuts and insect stings.[3] The dried whole herb of this plant is also a crude drug which is known as "Wa Er Cao" (挖耳草) in China and has been used as an antipyretic, antidotic and anti-inflammatory for the treatment of colds, gastroenteritis, tympanitis and other diseases.[4]

As regards the constituents of this plant, no work has been reported. As part of our studies on Nepalese crude drugs, the flavonoid constituents of this plant have now been examined. As described in the experimental section, four new flavonones (I—IV) and a new chalcone (V) were isolated together with five known flavones (VI—X) from the ethanol extract of the root of this plant, which was collected in Central Nepal. This paper deals with their structural identification.

Compound I was obtained as colorless needles, mp 208 °C (dec.), $C_{17}H_{16}O_6$, and was positive to the Mg–HCl test. The infrared (IR) spectrum gave absorption bands corresponding to hydroxyl and conjugated carbonyl groups and aromatic rings. The ultraviolet (UV) spectrum was characteristic of the flavanone series, and the diagnostic shifts strongly suggested the presence of a 5,7-dihydroxy system in I.[5] The flavanone nucleus was also confirmed by the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, in which the signals due to the C-3 and C-2 protons were observed as an ABX type at 2.74 ppm (1H, dd, J=3.3 and 17.0 Hz), 3.22 ppm (1H, dd, J=12.4 and 17.0 Hz) and 5.77 ppm (1H, dd, J=3.3 and 12.4 Hz). The ^{1}H-NMR spectrum further showed the presence of two methoxyls (3.65, 3.83 ppm), one hydroxyl (10.68 ppm) and one chelated hydroxyl (11.91 ppm). In the aromatic region of the spectrum, the remaining five protons occurred as a singlet (6.00 ppm, 1H), for the A-ring proton and two doublets (7.56 ppm, 1H, J=7.3 Hz; 7.09 ppm, 1H, J=7.40 Hz) and two multiplets (centered at 7.06 ppm, 1H; 7.40 ppm, 1H) for the B-ring protons.

Based on these findings, I was considered to be a 5,7-dihydroxyflavanone derivative with

one methoxyl in the A-ring at the C-6 or C-8 position, and another in the B-ring. The chemical shifts and splitting patterns of the B-ring protons suggest that the B-ring is substituted at the 2′-position by a methoxyl. The arrangement of substituents in the B-ring was also supported by the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum, in which the observed values were in agreement with the calculated values of the signals obtained by the use of 5,7,8-trimethoxyflavanone[6b] as a model compound, with addition of the substituent effects of methoxyl in a benzene ring (B-ring).[7]

The position of the remaining one methoxyl in the A-ring was determined by the long-range selective proton decoupling (LSPD) method[8] in the ^{13}C-NMR spectrum as follows. In the ^{1}H non-decoupling ^{13}C-NMR spectrum of I, the signal of the carbon attached to an isolated aromatic hydrogen was observed at 96.0 ppm in the form of a double doublet (J= 162.5 and 6.7 Hz), which changed to a doublet when the chelated hydroxyl proton at the C-5 position was selectively irradiated, indicating that the isolated aromatic proton was present at the position *ortho* to the chelated hydroxyl, *i.e.*, no substituent was present at the C-6 position.[8c] These results led us to conclude that the methoxyl in the A-ring is present at the C-8 position.

The oxidation pattern of I was further confirmed in the following way. Compound I was methylated by Kuhn's method[9] to give the dimethyl ether (Ia), mp 174 °C (dec.), $C_{19}H_{20}O_6$, which was dehydrogenated by the use of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dioxane[10] to afford a flavone corresponding to Ia. The flavone obtained was identical with 5,7,8,2′-tetramethoxyflavone prepared from skullcapflavone I (5,2′-dihydroxy-7,8-dimethoxyflavone)[11] by Kuhn's methylation.[9]

It is known that flavanones having 2(S)-configuration exhibit a positive Cotton effect due to n–π^* transition (~330 nm) and a negative Cotton effect due to π–π^* transition (270—290 nm) in the circular dichroism (CD) spectra.[12] The CD curve of I exhibited positive and negative maxima at 310 and 288 nm, respectively, which established the 2(S)-configuration.

From these results, the structure of I was determined to be 2(S)-5,7-dihydroxy-8,2′-dimethoxyflavanone.

Compound II was obtained as colorless needles, mp 214 °C (dec.), $C_{18}H_{18}O_6$, and was positive in the Mg–HCl test. It gave the absorption bands of hydroxyl and conjugated carbonyl groups and benzene rings in the IR spectrum. The ^{1}H-NMR spectrum of II showed the presence of three methoxyls (3.65, 3.73, 3.83 ppm) and a non-chelated hydroxyl (10.33 ppm). On methylation with CH_2N_2, II gave Ia. The bathochromic shift in the UV spectrum caused by addition of NaOAc suggested the presence of a free hydroxyl group at the C-7 position.[5] Compound II is, therefore, 7-hydroxy-5,8,2′-trimethoxyflavanone. Further confirmation was obtained as follows. In the ^{13}C-NMR spectrum of II, the signal pattern of the B-ring was almost identical with that of I. The carbon signals of one of the three methoxyls appeared downfield at 60.4 ppm, which indicated the methoxyl to be on the C-8 carbon, being di-*ortho*-substituted by two oxygen functions.[6] The absence of the characteristic signal due to the chelated hydroxyl proton in the ^{1}H-NMR spectrum of II indicates that the remaining methoxyl is on the C-5 carbon.

The 2(S)-configuration of II was confirmed in the same way as in the case of I.[12] Compound II was, therefore, determined to be 2(S)-7-hydroxy-5,8,2′-trimethoxyflavanone.

Compound III was obtained as pale yellow needles, mp 202 °C (dec.), $C_{18}H_{18}O_7$. It was positive to the Mg–HCl test, and gave the absorption bands of hydroxyl, conjugated carbonyl groups and benzene rings in the IR spectrum. The UV spectrum and diagnostic shifts suggested the presence of a hydroxyl at the C-5 position and the absence of a free hydroxyl at the C-7 position.[5] The ^{1}H-NMR spectrum of III showed the signals of three methoxyls (3.54, 3.76, 3.85 ppm), one hydroxyl (9.87 ppm), one chelated hydroxyl (12.14 ppm) and an ABX type for the C-2 (5.94 ppm) and C-3 protons (2.55, 3.90 ppm). These findings indicated III to

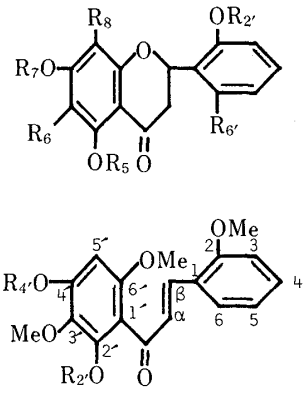
be a dihydroxytrimethoxyflavanone possessing a hydroxyl at the C-5 position.

In the aromatic region of the spectrum, the remaining four protons were observed as a singlet (1H, 6.22 ppm), a broad doublet (2H, 6.55 ppm, $J=8.3$ Hz) and a broad triplet (1H, 7.19 ppm, $J=8.3$ Hz). The former singlet could be assigned to the C-6 proton by the LSPD method[8] in the same manner as in the case of I. The latter three signals were assigned to the C-3′,5′ and C-4′ protons, respectively, from their chemical shifts and coupling patterns as well as the broadness of their signals, showing the presence of an unsymmetrical structure of the B-ring. This was further supported by the ^{13}C-NMR spectrum, in which the C-2′ and 6′ carbons were observed at 157.3 and 159.4 ppm, and the C-3′ and 5′ carbons at 109.1 and 102.8 ppm, respectively, as non-equivalent signals. The remaining two methoxyls are, therefore, located in the A-ring at the C-7 and C-8 positions. The arrangement of substituents in the A-ring was further confirmed by the ^{13}C-NMR spectrum, in which the carbon signals due to the A-ring of III were almost superimposable on those of 5-hydroxy-7,8,2′-trimethoxyflavanone (Ib) prepared from I by partial methylation with CH_2N_2. The CD spectrum revealed III to be a racemate.[12]

Thus, the structure of III was established as (±)-5,2′-dihydroxy-7,8,6′-trimethoxyflavanone.

Compound IV was obtained as colorless needles, mp 221 °C (dec.), $C_{18}H_{18}O_7$. It was positive to the Mg–HCl test, and gave the absorption bands of hydroxyl, conjugated carbonyl groups and benzene rings in the IR spectrum. The elemental composition and spectral characteristics indicated IV to be a dihydroxytrimethoxyflavanone having three oxygen functions in the A-ring, and two oxygen functions in the B-ring, like III. The presence of a chelated hydroxyl at the C-5 position was confirmed by the UV and ^{1}H-NMR spectra in the same manner as in the cases of I and III. Comparison of the ^{1}H- and ^{13}C-NMR spectra of IV with those of III showed that the chemical shifts and coupling patterns of the signals originating from the B-rings were consistent in both compounds, indicating that the B-ring of IV has the same structure as that of III. Therefore, the remaining two methoxyls must be placed in the A-ring. An isolated aromatic hydrogen in the A-ring was confirmed not to be present at the position *ortho* to the chelated hydroxyl at the C-5 position by the LSPD method.[8] The C-6 position is, therefore, substituted by a methoxyl. These results led to two possible structures, 5-hydroxy-6,7-dimethoxy and 5-hydroxy-6,8-dimethoxy, for the A-ring of IV. The ^{13}C-NMR spectrum supported the former structure, as follows. The carbon signal of one of the three methoxyls in IV appeared downfield at 60.0 ppm, and was assigned to the methoxyl on the C-6 carbon, being di-*ortho*-substituted by two oxygen functions in the former structure.[6] (Such a deshielded signal is expected not to be seen in the latter structure). The CD spectrum revealed IV to be a racemate.[12] From these results, the structure of IV was established as (±)-5,2′-dihydroxy-6,7,6′-trimethoxyflavanone.

Compound V was obtained as yellow needles, mp 134 °C (dec.), $C_{18}H_{18}O_6$. It gave a negative color reaction to Mg–HCl, and showed absorption bands assignable to hydroxyl, conjugated carbonyl groups and benzene rings in the IR spectrum. The UV spectrum was characteristic of the chalcone series, giving an absorption maximum at 368 nm, which was shifted bathochromically by adding $AlCl_3$, suggesting the presence of a 2′-hydroxyl in V.[5] The ^{1}H-NMR spectrum showed the presence of three methoxyls (3.68, 3.87, 3.91 ppm), one hydroxyl (10.47 ppm) and one chelated hydroxyl (14.04 ppm). Five of the remaining seven protons appeared in the aromatic region as a singlet (1H, 6.10 ppm) due to the A-ring proton, and a broad doublet (1H, 7.11 ppm, $J=7.3$ Hz), a broad double doublet (1H, 7.70 ppm, $J=$ 7.8 and 1.5 Hz) and two multiplets (each 1H, centred at 7.03 and 7.45 ppm) due to the B-ring protons. The signal pattern of the latter four protons suggests that the B-ring is substituted by one oxygen function at the C-2 position. The remaining four oxygen functions are, therefore, considered to be in the A-ring. An isolated aromatic hydrogen in the A-ring was confirmed



		R_5	R_6	R_7	R_8	$R_{2'}$	R_6
I	2(S)	H	H	H	OMe	Me	H
Ia	2(S)	Me	H	Me	OMe	Me	H
Ib	2(S)	H	H	Me	OMe	Me	H
II	2(S)	Me	H	H	OMe	Me	H
III	(±)	H	H	Me	OMe	H	OMe
IV	(±)	H	OMe	Me	H	H	OMe
		$R_{2'}$	R_4				
V		H	H				
Va		Me	Me				

Fig. 1

not to be present at the position *ortho* to the chelated hydroxyl at the C-2′ position by the LSPD method,[8] *i.e.*, the C-3′ position is substituted. Two protons due to H_α and H_β appeared equivalently at 7.96 ppm as a singlet (2H), which is unusual but has previously been observed in the spectra of 2′-hydroxy-4′,6′-dimethoxy- and 2′-hydroxy-3′,4′,6′-trimethoxychalcones.[6b] In the dimethyl ether (Va) of V obtained from V by Kuhn's methylation,[9] these protons (H_α, H_β) were observed at 6.97 and 7.55 ppm, each as a doublet with *trans* coupling ($J=16.1$ Hz).

In the mass spectrum (MS), the fragmentation pattern of V closely resembled that of II, strongly suggesting that V was the chalcone corresponding to II.[13] The relationship between II and V was confirmed by the fact that II was readily cleaved with 5% KOH–MeOH to give V. Hence V was determined to be 2′,4′-dihydroxy-2,3′,6′-trimethoxychalcone.

Compounds VI—X are known flavones and were identified as wogonin,[14] 5,7-dihydroxy-8,2′-dimethoxyflavone,[15a] norwogonin,[14b,15b] 5,7,2′-trihydroxy-8-methoxyflavone[15c,16] and wogonin 7-*O*-glucuronide,[17] respectively, by direct comparison with authentic samples.

Work on other flavonoids in this plant is in progress.

Experimental

General Procedures——All melting points were determined on a Yanagimoto micro melting point apparatus and are recorded uncorrected. UV spectra were determined with addition of diagnostic reagents by standard procedures[5] on a Hitachi recording spectrophotometer, type 323. IR spectra in KBr disk were run on a JASCO IR-A-2 spectrometer. NMR spectra were taken in DMSO-d_6 on a JEOL JNM-FX-100 spectrometer (^{1}H-NMR at 100 MHz and ^{13}C-NMR at 25 MHz), and chemical shifts are given in δ (ppm) with tetramethylsilane (TMS) as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). Electron impact-mass spectra (EI-MS) were taken on a JEOL JMS-DX-300 mass spectrometer. CD spectra were run on a JASCO J-20A atutomatic recording spectropolarimeter. Thin layer chromatography (TLC) was carried out on Kieselgel 60 F 254 (Merck) with the following solvent systems: $CHCl_3$–MeOH–H_2O–AcOH (100:4:0.2:0.1) (TLC-1), *n*-hexane–acetone–AcOH (60:40:0.1) (TLC-2), $CHCl_3$–MeOH–H_2O–HCOOH (25:8:1:3) (TLC-3), AcOEt–acetone–H_2O–AcOH (10:10:3:1) (TLC-4). Spots were detected by spraying of dil. H_2SO_4 followed by heating.

Extraction and Separation——The dried root (150 g) of *Scutellaria discolor* COLEBR., collected in Central Nepal in 1983, was extracted with boiling EtOH. The EtOH extract was concentrated to dryness to give a residue, which was suspended in H_2O and successively extracted with Et_2O, AcOEt and *n*-BuOH. The Et_2O layer was concentrated to dryness and the residue (3 g) was chromatographed on silica gel (500 g) using *n*-hexane–acetone (10:1→1:1) as an eluent to give seven fractions, fr. 1—7, in the order of elution. Fraction 1 gave I. Fraction 2 was rechromatographed on silica gel [solvent: benzene–$CHCl_3$ (1:1)] to give III, V and VI. Fraction 3 was subjected to rechromatography on silica gel [solvent: benzene–$CHCl_3$ (1:1)] to give IV and VII. Fraction 4, containing a mixture of two flavonoids, was subjected to repeated chromatography on silica gel [solvent: $CHCl_3$–MeOH–H_2O (100:2:0.1)] to give VIII and IX. Fraction 5 gave crude II, which was purified by chromatography on silica gel (solvent: $CHCl_3$) to give purified II. The AcOEt-soluble portion was concentrated and the residue (5.1 g) was chromatographed on silica gel (500 g) with a gradient of AcOEt–acetone–H_2O (20:5:1→5:5:1) as an eluent to give X. Yields: I (20 mg), II (40 mg), III (3 mg), IV (4 mg), V (40 mg), VI (500 mg), VII (200 mg), VIII (2 mg), IX (40 mg), X (300 mg).

I. (2(*S*)-5,7-Dihydroxy-8,2′-dimethoxyflavanone)——Colorless needles (MeOH), mp 208 °C (dec.). *Anal.* Calcd for $C_{17}H_{16}O_6$: C, 64.55; H, 5.10. Found: C, 64.73; H, 5.12. Mg–HCl (+). *Rf*: 0.59 (TLC-1), 0.38 (TLC-2). UV λ_{max}^{MeOH} nm (log ε): 241 (3.94), 293 (4.29), 342 (3.75); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 253 (3.85), 280 (3.61), 332 (4.49); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 242 sh (4.13), 280 sh (3.84), 317 (4.50), 390 (3.84); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 242 sh (4.18), 280 sh (3.90), 314 (4.50), 380 (3.86); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 255 sh (3.84), 280 (3.63), 332 (4.47); $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 293 sh (4.07), 333 (4.28). IR ν_{max}^{KBr} cm^{-1}: 3150 (OH), 1630 (conjugated CO), 1600 (arom. C=C). ^{1}H-NMR: 3.65, 3.83 (each 3H, each s, $-OCH_3 \times 2$), 2.74 (1H, dd, J=17.0, 3.3 Hz, *cis* 3-H), 3.22 (1H, dd, J=17.0, 12.4 Hz, *trans* 3-H), 5.77 (1H, dd, J=12.4, 3.3 Hz, 2-H), 6.00 (1H, s, 6-H), 6.98—7.13 (1H, m, 5′-H), 7.09 (1H, br d, J=7.4 Hz, 3′-H), 7.32—7.49 (1H, m, 4′-H), 7.56 (1H, br d, J=7.3 Hz, 6′-H), 10.68 (1H, s, 7-OH), 11.91 (1H, s, 5-OH). ^{13}C-NMR: 74.0 (C-2), 41.1 (C-3), 196.6 (C-4), 158.8 (C-5), 96.0 (C-6, $J_{(C-6)-(6-H)}$=162.5 Hz, $J_{(C-6)-(5-OH)}$=6.7 Hz), 160.1 (C-7) 128.7 (C-8), 154.7 (C-9), 101.8 (C-10), 126.6 (C-1′), 156.4 (C-2′), 111.4 (C-3′), 129.9 (C-4′), 120.7 (C-5′), 126.8 (C-6′), 55.7 (C-2′–O<u>C</u>H$_3$) 60.4 (C-8–O<u>C</u>H$_3$). MS *m/z* (%): 316 (M$^+$, 79), 182 ($C_8H_6O_5$, 100), 167 ($C_7H_3O_5$, 53). CD (c=0.005, MeOH) $[\theta]^{25}$ (nm): +15502 (310) (positive maximum), −59623 (288) (negative maximum).

i) Complete Methylation of I by Kuhn's Method:[9] CH_3I (0.2 ml) and Ag_2O (50 mg) were added to a solution of I (10 mg) in *N*,*N*-dimethylformamide (DMF) (0.3 ml), and the reaction mixture was left for 20 h with occasional shaking. Then $CHCl_3$ was added, and after removal of the resulting precipitate by filtration, the filtrate was evaporated to dryness. The residue was chromatographed on silica gel (10 g) using benzene–AcOEt (10 : 1) as an eluent to give crude Ia, which was recrystallized from MeOH to give Ia, colorless needles, mp 174 °C (dec.). *Anal.* Calcd for $C_{19}H_{20}O_6$: C, 66.27; H, 5.85. Found: C, 66.46; H, 5.87. $FeCl_3$ (−). *Rf*: 0.63 (TLC-1), 0.19 (TLC-2). UV λ_{max}^{MeOH} nm (log ε): 237 sh (4.06), 286 (4.19), 330 (3.71); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 238 (4.23), 284 (4.27), 329 (3.91). IR ν_{max}^{KBr} cm^{-1}: no OH, 1670 (conjugated CO), 1580, 1590 (arom. C=C). ^{1}H-NMR: 3.63, 3.91 (each 3H, each s, $-OCH_3 \times 2$), 3.83 (6H, s, $-OCH_3 \times 2$), 2.61 (1H, dd, J=16.3, 3.4 Hz, *cis* 3-H), 2.97 (1H, dd, J=16.3, 11.7 Hz, *trans* 3-H), 5.69 (1H, dd, J=11.7, 3.4 Hz, 2-H), 6.37 (1H, s, 6-H), 6.97—7.12 (1H, m, 5′-H), 7.08 (1H, br d, J=7.8 Hz, 3′-H), 7.30—7.51 (1H, m, 4′-H), 7.53 (1H, br d, J=7.8 Hz, 6′-H). ^{13}C-NMR: 73.7 (C-2), 43.8 (C-3), 188.4 (C-4), 157.4 (C-5), 90.4 (C-6), 158.4 (C-7), 130.2 (C-8), 156.0 (C-9), 105.6 (C-10), 126.9 (C-1′), 156.1 (C-2′), 111.3 (C-3′), 129.7 (C-4′), 120.7 (C-5′), 126.5 (C-6′), 55.6, 56.0, 56.1 (–O<u>C</u>H$_3 \times 3$), 60.3 (C-8–O<u>C</u>H$_3$). MS *m/z* (%): 344 (M$^+$, 55), 210 ($C_{10}H_{10}O_5$, 100), 195 ($C_9H_7O_5$, 40). CD (c=0.005, MeOH) $[\theta]^{25}$ (nm): +12189 (337) (positive maximum), −31150 (276) (negative maximum).

ii) Partial Methylation of I: MeOH–Et_2O (3 : 2) solution (5 ml) of I (7 mg) was treated with ethereal CH_2N_2 (1 ml) for a short time. After removal of the solvent, the residue was chromatographed on silica gel (10 g) using benzene–AcOEt (95 : 5) as an eluent to give crude Ib, which was recrystallized from MeOH to give Ib, colorless needles, mp 138 °C (dec.). *Anal.* Calcd for $C_{18}H_{18}O_6$: C, 65.44; H, 5.49. Found: C, 65.39; H, 5.50. $FeCl_3$ (+). *Rf*: 0.81 (TLC-1), 0.37 (TLC-2). UV λ_{max}^{MeOH} nm (log ε): 240 sh (3.97), 290 (4.22), 347 (3.59); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 316 (4.34), 404 (3.61); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 313 (4.33), 400 (3.62); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 250 (4.20), 348 (3.54). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650 (conjugated CO), 1620, 1590 (arom. C=C). ^{1}H-NMR: 3.62, 3.83, 3.86 (each 3H, each s, $-OCH_3 \times 3$), 2.78 (1H, dd, J=17.3, 3.0 Hz, *cis* 3-H), 3.25 (1H, dd, J=17.3, 12.7 Hz, *trans* 3-H), 5.78 (1H, dd, J=12.7, 3.0 Hz, 2-H), 6.26 (1H, s, 6-H), 6.98—7.14 (1H, m, 5′-H), 7.10 (1H, br d, J=7.9 Hz, 3′-H), 7.32—7.50 (1H, m, 4′-H), 7.55 (1H, br d, J=7.8 Hz, 6′-H), 12.02 (1H, s, 5-OH). ^{13}C-NMR: 73.9 (C-2), 41.2 (C-3), 197.1 (C-4), 159.1 (C-5), 93.1 (C-6), 161.2 (C-7), 129.2 (C-8), 153.8 (C-9), 102.4 (C-10), 126.4 (C-1′), 156.3 (C-2′), 111.4 (C-3′) 129.9 (C-4′), 120.7 (C-5′), 126.8 (C-6′), 55.6, 56.3 (–O<u>C</u>H$_3 \times 2$), 60.4 (C-8–O<u>C</u>H$_3$). MS *m/z* (%): 330 (M$^+$, 88), 196 ($C_7H_6O_5$, 100), 181 ($C_6H_3O_5$, 78). CD (c=0.009, MeOH) $[\theta]^{25}$ (nm): +6212 (310) (positive maximum), −34941 (284) (negative maximum).

iii) Dehydrogenation of Ia: A mixture of Ia (5 mg), DDQ (4.5 mg) and dioxane (3 ml) was refluxed for 6 h. The reaction mixture was concentrated *in vacuo* and chromatographed on silica gel [solvent: benzene–AcOEt–AcOH (100 : 20 : 0.1)] to give yellow needles (from MeOH), mp 187 °C, which were identical (UV, IR, ^{1}H- and ^{13}C-NMR, mixed fusion) with 5,7,8,2′-tetramethoxyflavone prepared from skullcapflavone I by Kuhn's methylation.[11]

II. (2(*S*)-7-Hydroxy-5,8,2′-trimethoxyflavanone)——Colorless needles (MeOH), mp 214 °C (dec.). *Anal.* Calcd for $C_{18}H_{18}O_6$: C, 65.44; H, 5.49. Found: C 65.58; H, 5.48. Mg–HCl (+)-*Rf*: 0.37 (TLC-1), 0.14 (TLC-2). UV λ_{max}^{MeOH} nm (log ε): 240 sh (4.05), 287 (4.23), 329 (3.89); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 255 (3.97), 330 (4.42); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 238 sh (4.24), 287 (4.32), 326 (3.95), 370 sh (3.69); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 238 sh (4.28), 287 (4.33), 322 (3.99), 370 sh (3.74); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 254 sh (3.96), 329 (4.39); $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 291 (4.08), 329 (4.17). IR ν_{max}^{KBr} cm^{-1}: 3150 (OH), 1640 (conjugated CO), 1580 (arom. C=C). ^{1}H-NMR: 3.65, 3.73, 3.83 (each 3H, each s, $-OCH_3 \times 3$), 2.59 (1H, dd, J=16.4, 3.4 Hz, *cis* 3-H), 2.95 (1H, dd, J=16.4, 12.2 Hz, *trans* 3-H), 5.68 (1H, dd, J=12.2, 3.4 Hz, 2-H), 6.17 (1H, s, 6-H), 6.97—7.12 (1H, m, 5′-H), 7.08 (1H, br d, J=7.3 Hz, 3′-H), 7.30—7.50 (1H, m, 4′-H), 7.55 (1H, br d, J=7.3 Hz, 6′-H), 10.33 (1H, s, 7-OH). ^{13}C-NMR: 73.8 (C-2), 43.8 (C-3), 188.1 (C-4), 157.0 (C-5), 93.5 (C-6), 157.3 (C-7), 129.3 (C-8), 156.7 (C-9), 104.8 (C-10), 127.1 (C-1′), 156.2 (C-2′), 111.3 (C-3′), 129.7 (C-4′), 120.7 (C-5′), 126.5 (C-6′), 55.6 (C-5,2′–O<u>C</u>H$_3$), 60.4 (C-8–O<u>C</u>H$_3$). MS *m/z* (%): 330 (M$^+$, 38), 196 ($C_9H_8O_5$, 100), 181 ($C_8H_5O_5$, 47). CD (c=0.005, MeOH) $[\theta]^{25}$ (nm): +5390 (330) (positive maximum), −26940 (280) (negative maximum). Methylation of II with CH_2N_2: II was methylated with CH_2N_2 in the same manner as in the case of I to give a product which was identical (UV, IR, ^{1}H- and ^{13}C-NMR, mixed fusion) with Ia.

III. ((±)-5,2′-Dihydroxy-7,8,6′-trimethoxyflavanone)——Pale yellow needles (MeOH), mp 202 °C (dec.). *Anal.* Calcd for $C_{18}H_{18}O_7$: C, 62.42; H, 5.24. Found: C, 62.61; H, 5.26. Mg–HCl (+). *Rf*: 0.63 (TLC-1), 0.29 (TLC-2). UV λ_{max}^{MeOH} nm (log ε): 241 sh (3.98), 290 (4.18), 347 (3.65); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 241 sh (4.27), 292 (4.21), 360 (3.93); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 222 sh (4.45), 315 (4.36), 390 (3.85); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 223 sh (4.43), 313 (4.36), 374 (3.89); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 290 (4.16), 350 (3.63); $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 290 (4.06), 350 (3.48). IR ν_{max}^{KBr} cm^{-1}: 3150, 3350 (OH), 1620 (conjugated CO), 1590 (arom. C=C). ^{1}H-NMR: 3.54, 3.76, 3.85 (each 3H, each s, –OCH_3 × 3), 2.55 (1H, dd, J=17.1, 3.2 Hz, *cis* 3-H), 3.90 (1H, dd, J=17.1, 13.5 Hz, *trans* 3-H), 5.94 (1H, dd, 13.5, 3.2 Hz, 2-H), 6.22 (1H, s, 6-H), 6.55 (2H, br d, J=8.3 Hz, 3′,5′-H), 7.19 (1H, br t, J=8.3 Hz, 4′-H), 9.87 (1H, s, 2′-OH), 12.14 (1H, s, 5-OH). ^{13}C-NMR: 71.5 (C-2), 39.3 (C-3), 198.1 (C-4), 159.2 (C-5), 92.6 (C-6, $J_{(C\text{-}6)-(6\text{-}H)}$=163.2 Hz, $J_{(C\text{-}6)-(5\text{-}OH)}$=7.4 Hz), 160.9 (C-7), 129.0 (C-8), 154.6 (C-9), 102.2 (C-10), 111.5 (C-1′), 157.3 (C-2′), 109.1 (C-3′), 130.5 (C-4′), 102.8 (C-5′), 159.4 (C-6′), 55.8, 56.2 (–OCH_3 × 2), 60.2 (C-8–O$\underline{C}H_3$). MS *m/z* (%): 346 (M^+, 63), 328 ($C_{18}H_{16}O_6$, 47), 313 ($C_{17}H_{13}O_6$, 100), 181 ($C_8H_5O_5$, 42).

IV. ((±)-5,2′-Dihydroxy-6,7,6′-trimethoxyflavanone)——Colorless needles (MeOH), mp 221 °C (dec.). *Anal.* Calcd for $C_{18}H_{18}O_7$; C, 62.42, H, 5.24. Found: C, 62.62; H, 5.25. Mg–HCl (+). *Rf*: 0.58 (TLC-1), 0.25 (TLC-2). UV λ_{max}^{MeOH} nm (log ε): 240 sh (4.16), 290 (4.24), 346 (3.53); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 238 sh (4.34), 292 (4.24), 352 (3.71); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 225 (4.46), 316 (4.40), 374 (3.82); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 227 (4.47), 315 (4.41), 370 (3.85); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 290 (4.23), 347 (3.54); $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 289 (4.11), 345 (3.41). IR ν_{max}^{KBr} cm^{-1}: 3200, 3400 (OH), 1630 (conjugated CO), 1590 (arom. C=C). ^{1}H-NMR: 3.64, 3.75, 3.81 (each 3H, each s, –OCH_3 × 3), 2.51 (1H, dd, J=17.1, 3.2 Hz, *cis* 3-H), 3.93 (1H, dd, J=17.1, 13.7 Hz, *trans* 3-H), 5.95 (1H, dd, J=13.7, 3.2 Hz, 2-H), 6.23 (1H, s, 8-H), 6.52 (2H, br d, J=8.3 Hz, 3′,5′-H), 7.17 (1H, br t, J=8.3 Hz, 4′-H), 9.87 (1H, s, 2′-OH), 12.06 (1H, s, 5-OH). ^{13}C-NMR: 71.4 (C-2), 38.9 (C-3), 198.5 (C-4), 159.3 (C-5), 129.5 (C-6), 160.6 (C-7), 91.9 (C-8), 154.4 (C-9), 102.4 (C-10), 111.2 (C-1′), 157.2 (C-2′), 108.9 (C-3′), 130.5 (C-4′), 102.6 (C-5′), 159.6 (C-6′), 55.7, 56.2 (–O$\underline{C}H_3$ × 2), 60.0 (C-6–O$\underline{C}H_3$). MS *m/z* (%): 346 (M^+, 51), 328 ($C_{18}H_{16}O_6$, 43), 313 ($C_{17}H_{13}O_6$, 100), 181 ($C_8H_5O_5$, 63).

V. (2′,4′-Dihydroxy-2,3′,6′-trimethoxychalcone)——Yellow needles (MeOH), mp 134 °C (dec.). *Anal.* Calcd for $C_{18}H_{18}O_6$: C, 65.44; H, 5.49. Found: C, 65.32; H, 5.48. Mg–HCl (−). *Rf*: 0.51 (TLC-1), 0.28 (TLC-2). UV λ_{max}^{MeOH} nm (log ε): 250 sh (3.89), 315 sh (4.13), 368 (4.44); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 298 (4.00), 398 (4.46); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 252 sh (4.08), 320 sh (4.06), 400 (4.55); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 251 sh (4.16), 320 sh (4.10), 396 (4.54); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 253 sh (3.94), 301 (4.01), 396 (4.45); $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 258 sh (3.87), 280 (3.90), 322 (3.95), 418 (4.47). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1650 (conjugated CO), 1610, 1580 (arom. C=C). ^{1}H-NMR: 3.68, 3.87, 3.91 (each 3H, each s, –OCH_3 × 3), 6.10 (1H, s, 5′-H), 6.96—7.11 (1H, m, 5-H), 7.11 (1H, br d, J=7.3 Hz, 3-H), 7.36—7.53 (1H, m, 4-H), 7.70 (1H, dd, J=7.8, 1.5 Hz, 6-H), 7.96 (2H, s, α,β-H), 10.47 (1H, br s, 4′-OH), 14.04 (1H, s, 2′-OH). ^{13}C-NMR (*: may be reversed): 123.4 (C-1), 158.1 (C-2*), 111.9 (C-3), 132.1 (C-4), 121.0 (C-5), 129.0 (C-6), 105.5 (C-1′), 157.4 (C-2′*), 129.2 (C-3′), 158.5 (C-4′*), 91.6 (C-5′), 159.1 (C-6′*), 127.8 (C-α), 137.4 (C-β), 192.7 (>C=O), 55.7, 56.0 (–O$\underline{C}H_3$ × 2), 59.9 (C-3′–O$\underline{C}H_3$). MS *m/z* (%): 330 (M^+, 67), 196 ($C_9H_8O_5$, 100), 181 ($C_8H_5O_5$, 55).

Methylation of V: V was methylated in the same manner as in the case of complete methylation of I to give Va (2,2′,3′,4′,6′-pentamethoxychalcone) as a pale yellow powder. $FeCl_3$ (−). *Rf*: 0.67 (TLC-1), 0.26 (TLC-2). UV λ_{max}^{MeOH} nm (log ε): 235 sh (4.06), 290 (3.95), 344 (4.15). No change was observed when the spectrum was determined in the presence of NaOMe or NaOAc or $AlCl_3$. IR ν_{max}^{KBr} cm^{-1}: no OH, 1640 (conjugated CO), 1590 (arom. C=C). ^{1}H-NMR: 3.71, 3.72, 3.74, 3.82, 3.89 (each 3H, each s, –OCH_3 × 5), 6.58 (1H, s, 5′-H), 6.89—7.11 (2H, m, 3, 5-H), 7.34—7.51 (1H, m, 4-H), 7.70 (1H, dd, J=7.8, 1.5 Hz, 6-H), 6.97 (1H, d, J=16.1 Hz, α-H), 7.55 (1H, d, J=16.1 Hz, β-H). ^{13}C-NMR (* may be reversed): 122.7 (C-1), 158.1 (C-2), 111.9 (C-3), 132.2 (C-4), 120.9 (C-5), 129.1 (C-6), 116.1 (C-1′), 150.8 (C-2′*), 135.5 (C-3′), 152.7 (C-4′*), 93.7 (C-5′), 154.7 (C-6′*), 128.6 (C-α), 139.1 (C-β), 193. 1 (>C=O).

Conversion of II to V: II (10 mg) was dissolved in 0.5% KOH–MeOH (3 ml) and the solution was refluxed for 30 min. The reaction mixture was acidified with dil. HCl and extracted with benzene. After removal of the solvent, the residue was chromatographed on silica gel using benzene to give yellow needles (7 mg) (from MeOH), mp 134 °C (dec.), which were identical with V by direct comparisons (UV, IR, ^{1}H- and ^{13}C-NMR, mixed fusion).

Identification of VI—X——VI (mp 203 °C), VII (mp 231 °C), VIII (mp 253 °C (dec.)), IX (mp 278 °C (dec.)) and X (mp 270 °C (dec.)) were identified as wogonin, 5,7-dihydroxy-8,2′-dimethoxyflavone, norwogonin, 5,7,2′-trihydroxy-8-methoxyflavone and wogonin 7-*O*-glucuronide, respectively, by direct comparisons with authentic specimens (UV, IR, ^{1}H- and ^{13}C-NMR, mixed fusion).

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