Studies on the Constituents of *Scutellaria* Species. XIV.[1] On the Constituents of the Roots and the Leaves of *Scutellaria alpina* L.[2]

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From *Scutellaria alpina* L., two new flavones (I and II) were isolated, together with eighteen known flavonoids. The structures of I and II were shown to be 5,2′,6′-trihydroxy-6,7,8-trimethoxyflavone and 5,5″,6,6″,7,7″-hexahydroxy-8,8″-biflavone (8,8″-bibaicalein) on the basis of the chemical and spectral data. Compound I has already been synthesized.

Keywords *Scutellaria alpina*; Labiatae; flavonoid; flavone; biflavone; structure elucidation

Scutellaria alpina L. is a perennial herb of the family Labiatae, which is widely distributed in mountains of central and south-central Europe and lowlands of the Ukraine and south-central Russia.[3]

As regards the constituents of the plant, no work has been reported. As part of our studies on the flavonoid constituents of *Scutellaria* species, we have now examined this plant. As described in the experimental section, two new flavones (I and II) were isolated together with eighteen known flavonoids (III—XX) from the ethanol extracts of root and/or leaves of this plant, which was cultivated in Hokuriku University. The present paper deals with their structural determination.

Compound I was obtained from the root as yellow needles, mp 246—247 °C (dec.), $C_{18}H_{16}O_8$, 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. Diagnostic shifts in the ultraviolet (UV) spectrum suggested the presence of a hydroxyl at the C-5 position in the flavone nucleus.[4] The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of I showed the presence of three methoxyls (6H, 3.84 ppm; 3H, 4.02 ppm), two hydroxyls (2H, 9.99 ppm), one chelated hydroxyl (12.73 ppm) and one C-3 proton (6.35 ppm). In the aromatic region of the spectrum, the remaining three protons appeared as a doublet (2H, 6.46 ppm, J=8.0 Hz) and a triplet (1H, 7.16 ppm, J=8.0 Hz). These signals could be assigned to C-3′, 5′ and C-4′ protons, respectively, from their chemical shifts and coupling patterns. These findings indicated I to be a trimethyl ether of 5,6,7,8,2′,6′-hexahydroxyflavone.

The positions of the methoxyls were determined from the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum, in which the signals of carbons bearing the three methoxyls appeared downfield at 60.7, 61.5 and 61.8 ppm, respectively, indicating the three methoxyls to be di-*ortho* substituted by two substituents.[5] The methoxyls are, therefore, present at the C-6, C-7 and C-8 positions in the A-ring

From these results, the structure of I was determined to be 5,2′,6′-trihydroxy-6,7,8-trimethoxyflavone. Compound I has already been synthesized,[6] but this is the first report of its isolation from natural sources.

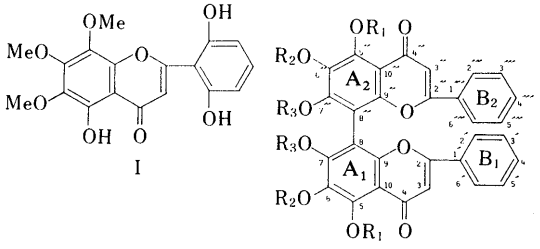
Compound II was obtained from the roots as yellow needles, mp >350 °C, Mg–HCl test (+). It gave the absorption bands of hydroxyl and conjugated carbonyl groups and benzene rings in the IR spectrum. The UV spectrum of II was characteristic of the flavone series, giving absorption maxima at 285 nm and 326 nm.[4] The fast atom bombardment mass spectrum (FAB-MS) of II showed the $[M+H]^+$ peak at m/z 539 as the base peak, although it failed to show the molecular ion peak in the electron impact (EI)-MS.

On methylation with CH_2N_2, II yielded a permethyl ether (IIa), mp 213—214 °C, $FeCl_3$ (−). The high-resolution (HR)-MS of IIa gave the molecular formula $C_{36}H_{30}O_{10}$ [m/z 622.1842 (M^+)]. Thus, the molecular formula of II was determined to be $C_{30}H_{18}O_{10}$.

The ^{1}H-NMR spectrum of II showed seven sets of signals in an integral ratio of 1:2:1:2:1:1:1. This suggests II to be a symmetric biflavone which consists of two monomeric halves of the same elemental composition, $C_{15}H_9O_5$. The ^{13}C-NMR spectra of both II and IIa showed signals which corresponded to just half the number of carbon atoms present in their respective molecular formulae. This is again in conformity with their symmetrical dimeric formulation.

The ^{1}H-NMR spectrum of II showed the presence of four hydroxyls (2H, 9.26 ppm; 2H, 10.17 ppm), two chelated hydroxyls (2H, 12.93 ppm) and two C-3/C-3″ protons (2H, 6.95 ppm). In the aromatic region of the spectrum, the remaining ten protons appeared as two triplets (4H, 7.38 ppm, J=7.3 Hz; 2H, 7.47 ppm, J=7.3 Hz) and a doublet (4H, 7.61 ppm, J=7.3 Hz) due to the B_1- and B_2-ring protons. These data suggested that all the hydroxyls were placed in the A_1- and A_2-rings. This was further supported by the ^{13}C-NMR spectrum of IIa, where the methoxyl carbon signals appeared downfield at 61.5, 61.7 and 62.4 ppm, indicating that these methoxyls were di-*ortho* substituted by two substituents.[5]

The locations of the hydroxyls were determined as follows. The presence of 5/5″-hydroxyls in II was apparent from the ^{1}H-NMR spectrum as mentioned above. In the



II : R_1=R_2=R_3=H
IIa : R_1=R_2=R_3=Me
IIb : R_1=R_2=Me, R_3=H

Fig. 1

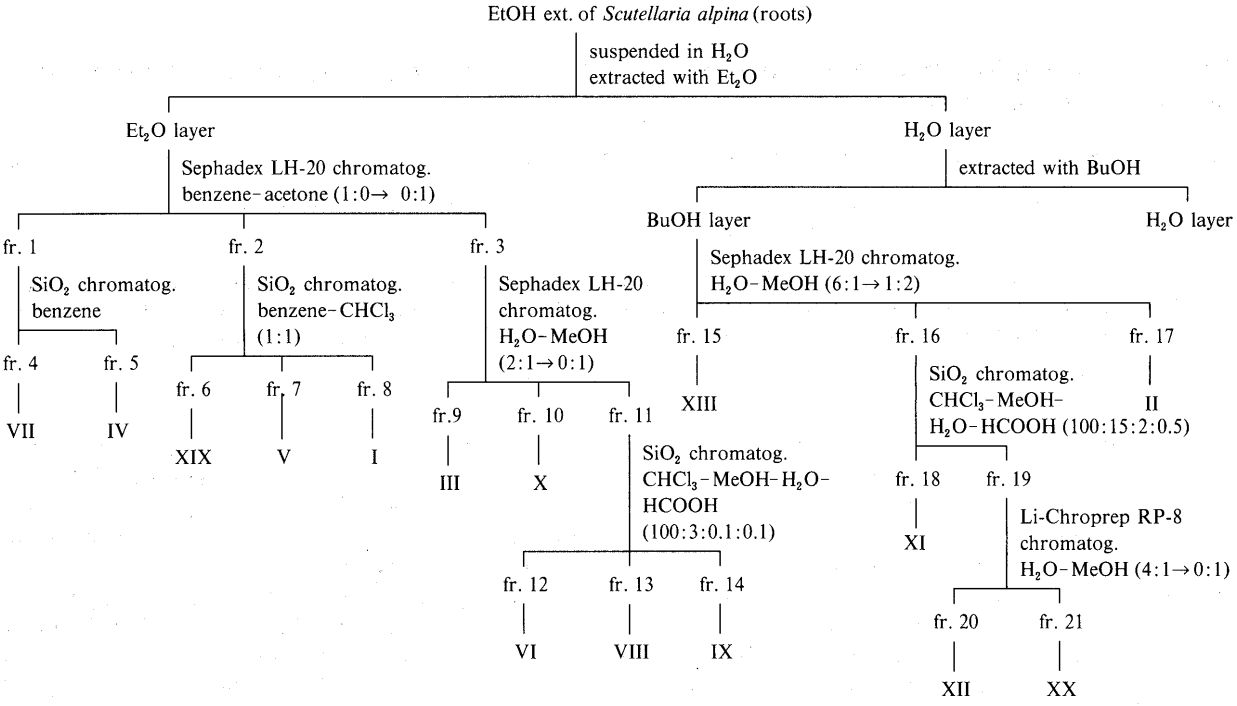


Chart 1

^{1}H-non-decoupling ^{13}C-NMR spectrum of II, the signal at 129.0 ppm was observed as a doublet (J=4.4 Hz), which was transformed into a singlet by irradiation of the chelated hydroxyl protons (12.93 ppm), indicating the presence of hydroxyls at the C-6/C-6″ positions.[7] The 7,7″-biflavone structure could be eliminated because it failed to explain the chemical shift (98.5 ppm) of C-7/C-7″, and the signal at 98.5 ppm was considered to be assigned to the C-8/C-8″ carbons having no hydroxyls. Compound II is, therefore, considered to be an 8,8″-dimer of 5,6,7-trihydroxyflavone. This was further supported by comparing the ^{13}C-NMR spectrum of II with that of 5,6,7-trihydroxyflavone (baicalein,[8] III). The C-8/C-8″, C-7/C-7″ and C-9/C-9″ signals of II were lower by 4.4 ppm, higher by 1.0 ppm and higher by 2.2 ppm, respectively, than the corresponding signals of III. Further, except for these three signals, the signals for all the carbon atoms of II appeared at essentially the same positions as those of the corresponding carbon atoms of III.

The structure of II was finally confirmed synthetically as follows. Oxidative coupling[9] of 5,6-di-*O*-methylbaicalein[10] in the presence of Ag_2O afforded a mixture of more than four compounds. The major compound, easily purified by chromatography, was the 8,8″- dimer of 5,6-di-*O*-methylbaicalein (IIb) as shown by MS, ^{1}H- and ^{13}C-NMR spectra. Compound IIb was methylated with CH_2N_2 to give IIa.

Thus, II was determined to be 5,5″,6,6″,7,7″-hexahydroxy-8,8″-biflavone(8,8″-bibaicalein). There is a pair of atropisomers in the structure II, but the circular dichroism (CD) spectrum revealed II to be a racemate.

Compounds III—XX are known flavones and were identified as baicalein (III),[8] wogonin (IV),[11] skullcapflavone II (V),[12] norwogonin (VI),[11] oroxylin A (VII),[11] scutevulin (VIII),[13] hispidulin (IX),[14] 5,7,4′-trihydroxy-8-methoxyflavone (X),[11] oroxylin A 7-*O*-glucuronide (XI),[15] wogonin 7-*O*-glucuronide (XII)[11] and baicalin (XIII)[8] (from roots);

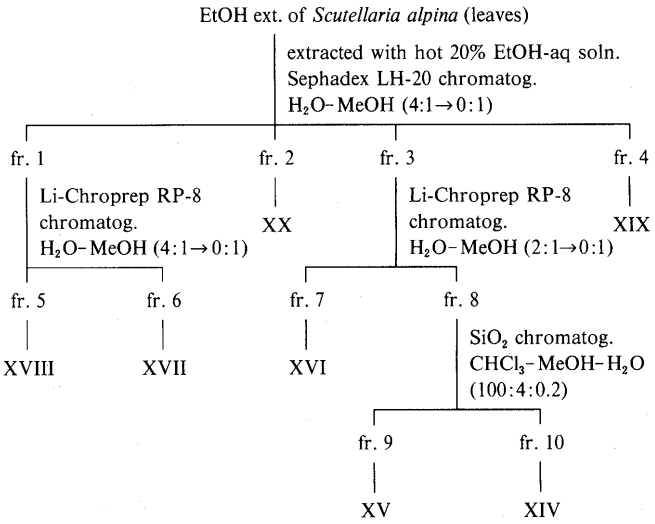


Chart 2

5,7,2′-trihydroxyflavone (XIV),[14] apigenin (XV),[16] scutellarein (XVI),[16] apigenin 7-*O*-glucuronide (XVII),[16] and scutellarin (XVIII)[16] (from leaves); chrysin (XIX),[16] and chrysin 7-*O*-glucuronide (XX)[16] (from both roots and leaves) by direct comparison with authentic samples.

Experimental

General Procedures The instruments were the same as described in the previous paper[1] except for the following. HR-MS were obtained with a JEOL JMS-DX-300 mass spectrometer. Thin layer chromatography (TLC) was carried out on Kieselgel 60F-254 (Merck) with the following solvent systems: $CHCl_3$–MeOH–H_2O–AcOH (100:5:0.2:2) (TLC-1), benzene–dioxane–AcOH (90:22:4) (TLC-2). Spots were detected by spraying dilute H_2SO_4 followed by heating.

Material *Scutellaria alpina* was cultivated in the botanical garden of Hokuriku University for two years, and harvested in September, 1987.

Extraction and Isolation As shown in Charts 1 and 2, fifteen compounds, I (15 mg), II (20 mg), III (60 mg), IV (40 mg), V (25 mg), VI

(10 mg), VII (20 mg), VIII (15 mg), IX (10 mg), X (10 mg), XI (25 mg), XII (50 mg), XIII (100 mg), XIX (30 mg) and XX (40 mg) were obtained from the roots (450 g) and seven compounds, XIV (10 mg), XV (20 mg), XVI (30 mg), XVII (20 mg), XVIII (40 mg), XIX (20 mg) and XX (30 mg), were obtained from the leaves (350 g).

I (5,2′,6′-Trihydroxy-6,7,8-trimethoxyflavone) Yellow needles (MeOH), mp 246—247 °C (dec.). EI-MS *m/z* (%): 360 (M^+, 80), 345 ($M^+ - CH_3$, 100), 211 ($C_9H_7O_6$, 40). HR-MS *m/z*: Found 360.0806, Calcd for $C_{18}H_{16}O_8$ (M^+) 360.0846; Found 345.0612, Calcd for $C_{17}H_{13}O_8$ ($M^+ - CH_3$) 345.0611. *Rf*: 0.41 (TLC-1), 0.48 (TLC-2). UV λ_{max}^{MeOH} nm (log ε): 270 (4.34), 315 (3.95), 355 sh (3.91); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 240 sh (4.37), 265 (4.29), 360 (4.01); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 280 (4.30), 298 sh (4.20), 335 (4.03), 404 (3.69); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 280 (4.30), 295 sh (4.23), 330 (3.97), 406 (3.65); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 269 (4.38), 315 (3.99), 355 sh (3.94). IR ν_{max}^{KBr} cm^{-1}: 3250, 3150 (OH), 1665 (conjugated CO), 1600 (arom. C=C). ^{1}H-NMR (100 MHz in DMSO-d_6): 3.84 (6H, s, $OCH_3 \times 2$), 4.02 (3H, s, OCH_3), 6.35 (1H, s, 3-H), 6.46 (2H, d, J= 8.0 Hz, 3′, 5′-H), 7.16 (1H, t, J=8.0 Hz, 4′-H), 9.99 (2H, s, 2′, 6′-OH), 12.73 (1H, s, 5-OH). ^{13}C-NMR (25 MHz in DMSO-d_6): 162.9 (C-2), 111.9 (C-3), 182.8 (C-4), 148.7 (C-5), 135.9 (C-6), 152.7 (C-7), 132.8 (C-8), 146.5 (C-9), 106.5 (C-10), 108.2 (C-1′), 157.0 (C-2′, 6′), 106.8 (C-3′, 5′), 132.3 (C-4′), 60.7, 61.5, 61.8 ($OCH_3 \times 3$).

II [5,5″,6,6″,7,7″-Hexahydroxy-8,8″-biflavone (8,8″-bibaicalein)] Yellow needles (MeOH), mp >350 °C. EI-MS *m/z* (%): 284 ($C_{16}H_{12}O_5$, 100). FAB-MS *m/z* (%): 539 (M^++H, 100). *Rf*: 0.12 (TLC-1), 0.25 (TLC-2). UV λ_{max}^{MeOH} nm (log ε): 221 (4.62), 241 (4.60), 285 (4.56), 326 (4.28); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 261 (4.64), 376 (4.19); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 228 (4.58), 253 (4.54), 275 (4.55), 295 sh (4.41), 320 (4.23), 388 (4.37); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 228 (4.58), 247 (4.62), 301 (4.47), 350 (4.35); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 261 (4.72), 376 (4.26); $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 261 (4.66), 376 (4.24). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1650 (conjugated CO), 1610, 1580 (arom. C=C). ^{1}H-NMR (400 MHz in DMSO-d_6): 6.95 (2H, s, 3/3″-H), 7.38 (4H, t, J=7.3 Hz, 3′, 5′/3‴, 5‴-H), 7.47 (2H, t, J=7.3 Hz, 4′/4‴-H), 7.61 (4H, d, J=7.3 Hz, 2′, 6′/2‴, 6‴-H), 9.26 (2H, br s, 6/6″-OH), 10.17 (2H, br s, 7/7″-OH), 12.93 (2H, s, 5/5″-OH). ^{13}C-NMR (100 MHz in DMSO-d_6): 162.7 (C-2/C-2″), 104.3 (C-3/C-3″), 182.3 (C-4/C-4″), 146.5 (C-5/C-5″), 129.0 (d, J=4.4 Hz, C-6/C-6″), 152.8 (C-7/C-7″), 98.5 (C-8/C-8″), 147.8 (C-9/C-9″), 103.9 (C-10/C-10″), 130.8 (C-1′/C-1‴), 125.7 (C-2′, 6′/C-2‴, 6‴), 128.9 (C-3′, 5′/C-3‴, 5‴), 131.7 (C-4′/C-4‴).

Methylation of II II (15 mg) was dissolved in 0.6 ml of *N*,*N*-dimethylformamide (DMF), then CH_3I (0.4 ml) and Ag_2O (100 mg) were added to the solution, and the reaction mixture was left for 14 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 using benzene–AcOEt (4 : 1) to give crude IIa, which was recrystallized from MeOH to give IIa (15 mg) as colorless needles, mp 213—214 °C. EI-MS *m/z* (%): 622 (M^+, 45), 607 ($M^+ - CH_3$, 100), 105 (C_7H_5O, 40). HR-MS *m/z*: Found 622.1842, Calcd for $C_{36}H_{30}O_{10}$(M^+) 622.1837; Found: 607.1649, Calcd for $C_{35}H_{27}O_{10}$ ($M^+ - CH_3$) 607.1602. *Rf*: 0.67 (TLC-1), 0.62 (TLC-2). UV λ_{max}^{MeOH} nm (log ε): 218 (4.71), 270 (4.58), 306 (4.48). No change was observed in the spectrum in the presence of NaOMe, NaOAc or $AlCl_3$. IR ν_{max}^{KBr} cm^{-1}: 1648 (conjugated CO), 1582 (arom. C=C). ^{1}H-NMR (400 MHz in $CDCl_3$): 3.87, 4.02, 4.13 (each 6H, each s, $OCH_3 \times 6$), 6.70 (2H, s, 3/3″-H), 7.29 (4H, t, J=7.3 Hz, 3′, 5′/3‴, 5‴-H), 7.39 (2H, t, J=7.3 Hz, 4′/4‴-H), 7.40 (4H, d, J=7.3 Hz, 2′, 6′/2‴, 6‴-H). ^{13}C-NMR (100 MHz in $CDCl_3$): 161.1 (C-2/C-2″), 107.8 (C-3/C-3″), 177.6 (C-4/C-4″), 153.7 (C-5/C-5″), 143.9 (C-6/C-6″), 156.5 (C-7/C-7″), 111.0 (C-8/C-8″), 151.4 (C-9/C-9″), 115.1 (C-10/C-10″), 131.1 (C-1′/C-1‴), 125.6 (C-2′, 6′/C-2‴, 6‴), 128.9 (C-3′, 5′/C-3‴, 5‴), 131.5 (C-4′/C-4‴), 61.5 (C-7/7″-O$\underline{C}H_3$), 61.7 (C-6/6″-O$\underline{C}H_3$), 62.4 (C-5, 5″-O$\underline{C}H_3$).

Synthesis of IIb Ag_2O (2 g) was added to a solution of 7-hydroxy-5,6-dimethoxyflavone (500 mg) in anhydrous benzene (50 ml), and the reaction mixture was heated under reflux on an oil bath for 4 h. After removal of the precipitate, the filtrate was evaporated to dryness and the residue was chromatographed on silica gel with a gradient of benzene–AcOEt (1 : 0→ 1 : 1) to give IIb as colorless needles (MeOH) (40 mg), mp 284 °C (dec.). EI-MS *m/z* (%): 594 (M^+, 45), 579 ($M^+ - CH_3$, 100), 105 (C_7H_5O, 80). HR-MS *m/z*: Found 593.9619 Calcd for $C_{34}H_{26}O_{10}$ (M^+) 594.1527; Found 579.0615, Calcd for $C_{33}H_{23}O_{10}$ ($M^+ - CH_3$) 579.1292. *Rf*: 0.42 (TLC-1), 0.17 (TLC-2). UV λ_{max}^{MeOH} nm (log ε): 218 (4.67), 235 (4.55), 268 (4.55), 317 (4.45); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 248 (4.66), 275 (4.65), 376 (4.27); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 218 (4.71), 235 (4.63), 268 (4.57), 317 (4.51); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 248 (4.67), 275 (4.63), 345 (4.17); $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 250 (4.67), 275 sh (4.63), 325 (4.30). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1640 (conjugated CO), 1588 (arom. C=C). ^{1}H-NMR (400 MHz in DMSO-d_6): 3.90, 3.94 (each 6H, each s, OCH_3), 6.78 (2H, s, 3/3″-H), 7.36 (4H, t, J=7.3 Hz, 3′, 5′/3‴, 5‴-H), 7.45 (2H, t, J=7.3 Hz, 4′/4‴-H), 7.56 (4H, d, J=7.3 Hz, 2′, 6′/2‴, 6‴-H), 10.45 (2H, br s, 7/7″-OH). ^{13}C-NMR (100 MHz in DMSO-d_6): 159.6 (C-2/C-2″), 107.1 (C-3/C-3″), 176.0 (C-4/C-4″), 151.7 (C-5/C-5″), 139.4 (C-6/C-6″), 155.1 (C-7/C-7″), 104.8 (C-8/C-8″), 151.7 (C-9/C-9″), 111.0 (C-10/C-10″), 131.0 (C-1′/C-1‴), 125.4 (C-2′, 6′/C-2‴, 6‴), 128.9 (C-3′, 5′/C-3‴, 5‴), 131.4 (C-4′/C-4‴), 61.4, 61.9 (C-5/5″, 6/6″-O$\underline{C}H_3 \times 4$).

Methylation of IIb IIb was methylated with CH_2N_2 to give a product which was identical (UV, IR, ^{1}H- and ^{13}C-NMR, mixed fusion) with IIa.

Identification of III—XX III (mp 255 °C, dec.), IV (mp 203 °C), V (mp 181 °C, dec.), VI (mp 253 °C, dec.), VII (mp 202 °C, dec.), VIII (mp 278 °C, dec.), IX (mp 291 °C), X (mp 300—302 °C, dec.), XI (mp 173—174 °C, dec.), XII (mp 270 °C, dec.), XIII (mp 230 °C, dec.), XIV (mp 284 °C), XV (350 °C), XVI (mp 345 °C, dec.), XVII (mp 227 °C, dec.), XVIII (mp 360 °C), XIX (mp 285 °C) and XX (mp 226 °C) were identified as baicalein,[8] wogonin,[11] skullcapflavone II,[12] norwogonin,[11] oroxylin A,[11] scutevulin,[13] hispidulin,[14] 5,7,4′-trihydroxy-8-methoxyflavone,[11] oroxylin A 7-*O*-glucuronide,[15] wogonin 7-*O*-glucuronide,[11] baicalin,[8] 5,7,2′-trihydroxyflavone,[14] apigenin,[16] scutellarein,[16] apigenin 7-*O*-glucuronide,[16] scutellarin,[16] chrysin[16] and chrysin 7-*O*-glucuronide,[16] respectively, by direct comparisons with authentic specimens (UV, IR, ^{1}H- and ^{13}C-NMR, mixed fusion).

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