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Studies on Scutellariae Radix. VI.[1] Effects of Flavanone Compounds on Lipid Peroxidation in Rat Liver

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Compounds (I and II) which inhibited lipid peroxides formation (in *in vitro* experiments) were isolated together with various flavonoids from the roots of *Scutellaria baicalensis* Georgi. From the analytical and physical data, compounds I and II were identified as (2*S*)-2′,5,6′,7-tetrahydroxy-flavanone and (2*R*,3*R*)-2′,3,5,6′,7-pentahydroxy-flavanone, respectively.

Compound II inhibited the lipid peroxide formation by Fe^{2+} and ascorbic acid. Compounds I and II inhibited the lipid peroxide formation induced by adenosine diphosphate and reduced nicotinamide adenine dinucleotide phosphate in rat liver homogenate.

Keywords——(2*S*)-2′,5,6′,7-tetrahydroxy-flavanone; (2*R*,3*R*)-2′,3,5,6′,7-pentahydroxy-flavanone; *Scutellaria baicalensis* Georgi; lipid peroxide; flavanone

The Chinese crude drug consisting of the dried roots of *Scutellaria baicalensis* Georgi ("Ogon" in Japanese) is an important drug found in a large number of prescriptions in the Chinese system of medicine. According to ancient Chinese medicine and herbals, it is effective in treatments of suppurative dermatitis, diarrhea, inflammatory diseases and hyperlipemia.

In the previous paper,[2] we reported that Scutellariae Radix (ethyl acetate, methanol or water extract; wogonin, baicalein and baicalin) inhibited the lipid peroxide formation in rat liver induced by Fe^{2+} ascorbic acid and adenosine diphosphate (ADP).

In higher animals, lipid peroxides are known to injure the liver.[3] In the present study, we attempted to isolated the active substances (compounds I and II) from the ethyl acetate extract of the dried roots of *S. baicalensis*, and to examine the effects of these compounds on lipid peroxidation in rat liver.

Experimental

Melting point were determined on a Yamato MP-21 capillary apparatus and are uncorrected. Infrared (IR) spectra were measured on a Shimadzu IR-400 spectrometer. Ultraviolet (UV) spectra were obtained on a Shimadzu MPS-5000 machine. ^{1}H-Nuclear magnetic resonance (^{1}H-NMR) spectra were recorded in DMSO-d_6, $CDCl_3$ and D_2O on Hitachi R-22 (90 MHz) and Varian HA100d NMR spectrometers (100 MHz). Tetramethylsilane (TMS)was used as an internal standard and chemical shifts are reported in δ(ppm and Hz), s=singlet, d=doublet, br s=broad singlet, t=triplet, q=quartet, m=multiplet. Column chromatography was carried out using silica gel 60 (70—230 mesh, ASTM, Merck Co.) as the adsorbent.

Extraction and Isolation of Substances (Compounds I and II) inhibiting Lipid Peroxidation in Rat Liver (*In Vitro*)——The crushed drug (10 kg) was extracted with 4×20 l of EtOAc for 4 h under reflux. The EtOAc solution was concentrated to give a dark yellowish extract (210 g), which was fractionated into an MeOH-soluble fraction (160 g) and an MeOH-insoluble yellow powder (40 g). The MeOH-insoluble yellow powder was recrystallized from EtOAc and MeOH mixture to give baicalein. The MeOH-soluble extract (160 g) was chromatographed on a column of silica gel to afford several compounds (oroxylin A, wogonin, chrysin, skullcapflavone II, baicalein, baicalin, compounds I and II) as shown in Chart 1.

Compound I——Colorless prisms from *n*-hexane and EtOAc mixture, mp 240°C (dec.). Mg+HCl: orange-yellow. $FeCl_3$ reagent: dark green $[\alpha]_D^{22}$ +6.13 (c=1.012, MeOH). MS m/z 288 (M^+). *Anal.* Calcd for $C_{15}H_{12}O_6$: C, 62.50; H, 4.20; mol. wt., 288.25, Found: C, 62.54; H, 4.22. IR ν_{max}^{Nujol} cm^{-1}: 3450, 3200 (OH), 1640 (chelated C=O), 1610, 1517 (aromatic ring). UV λ_{max}^{EtOH} nm (log ε): 289 (4.21). ^{1}H-NMR (in DMSO-d_6)

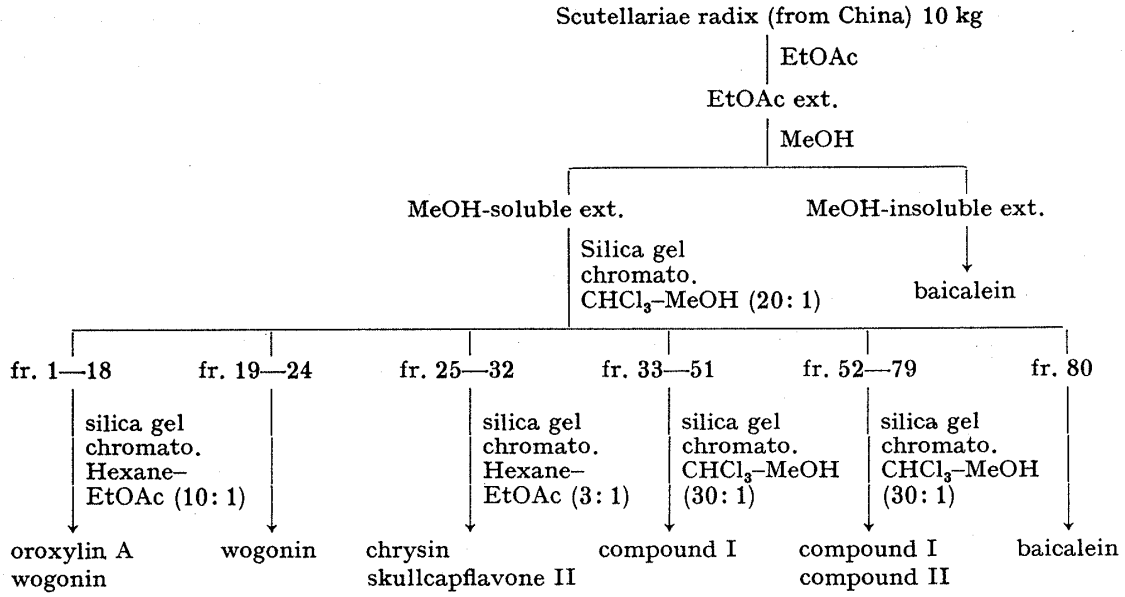


Chart 1

δ ppm: 2.35 (1H, d, J=4.0 Hz, C_3–H), 3.40 (1H, q, J=14.0, 17.0 Hz, C_3–H), 5.83, 5.87 (1H each, d, J=2.5 Hz, aromatic H), 5.84 (1H, q, J=14.0, 4.0 Hz, C_2–H), 6.32 (2H, d, J=9.0 Hz, aromatic H), 6.98 (1H, t, J=9.0 Hz, aromatic H), 9.48 (2H, s, OH×2, disappeared on addition of D_2O), 12.24 (1H, s, C_5–OH, disappeared on addition of D_2O). CD (c=0.126, MeOH) $[\theta]^{25}$ (nm): −29000 (284), +7800 (306), +9400 (327). Yield 2.5 g. From the physical data, it was concluded to be identical with an authentic sample of (2*S*)-2′,5,6′,7-tetrahydroxy-flavanone.[4]

Compound II——Colorless needles from MeOH, mp 221—225°C (dec.). Mg+HCl: pink. $FeCl_3$ reagent: dark green. ORD (c=1.03, MeOH) $[\phi]^{24}$ (nm): +448.4 (589), +543.1 (550), +732 (500), +991.7 (450), +193.6 (400). High resolution mass spectrum m/z: 304.0567 (M^+, $C_{15}H_{12}O_7$). IR ν_{max}^{Nujol} cm^{-1}: 3460, 3250 (OH), 1640 (chelated C=O), 1615, 1510 (aromatic ring). UV λ_{max}^{EtOH} nm (log ε): 292 (4.38), 326 sh (3.66); UV $\lambda_{max}^{EtOH(AlCl_3)}$ nm: 316, 376; UV $\lambda_{max}^{EtOH(AcONa)}$ nm: 296 sh, 329. CD (c=0.16, MeOH) $[\theta]^{20}$ (nm): −8000 (277), −1800 (299), +11000 (327). ^{1}H-NMR (in DMSO-d_6) δ ppm: 5.31, 5.62 (1H each, d, J=12.0 Hz, $C_{2,3}$–H), 5.19—5.98 (1H, br s, C_3–OH), 5.83, 5.90 (1H each, d, J=1.5 Hz, aromatic H), 6.34 (2H, d, J=8.5 Hz, aromatic H×2), 6. 96 (1, t, J=8.5 Hz, aromatic H), 9.58 (2H, br s, OH×2), 10.73 (1H, br s, OH), 12.11 (1H, s, C_5–OH). Yield 1.2 g.

II-Pentaacetate (IIa)——A solution of II (200 mg) in a mixture of Ac_2O (5 ml) and pyridine (2 ml) was allowed to stand at room temperature overnight. After the reaction mixture had been treated in the usual way, the product was recrystallized from MeOH to give colorless fine prisms, mp 108—110°C. High resolution MS m/z: 514.1129 (M^+, $C_{25}H_{22}O_{12}$). IR ν_{max}^{Nujol} cm^{-1}: 1750 (C=O), 1650 (chelated C=O), 1610, 1570 (aromatic ring). ^{1}H-NMR (in $CDCl_3$) δ ppm: 1.98 (3H, s, C_3–OAc), 2.24 (6H, s, OAc×2), 2.28 (3H, s, OAc), 2.38 (3H, s, OAc), 5.81, 6.08 (1H each, d, J=12.6 Hz, $C_{2,3}$–H), 6.57, 6.65 (1H each, d, J=2.5 Hz, aromatic H), 7.02 (2H, d, J=8.8 Hz, aromatic H×2), 7.42 (1H, q, J=8.8, 1.7 Hz, aromatic H). Yield 185 mg.

Animals——Young male Wistar King strain rats weighing 160 to 200 g were given water and standard laboratory diet *ad lib.* They were killed by decapitation and their livers were quickly removed.

Preparation of Liver Homogenate——Two grams of the liver tissue was cut into small pieces and then homogenized with 10 ml of 150 mM KCl Tris–HCl buffer (pH 7.2).

Estimation of Lipid Peroxide——In a glass test tube, 0.5 ml of liver homogenate (equivalent to 100 mg of liver tissue) was incubated with shaking for 1 h at 37°C in Tris-HCl buffer (pH 7.2) (0.2 ml)–4 mM $FeCl_2$ (0.1 ml)–6 mM ascorbic acid (0.2 ml) solution or Tris–HCl buffer (pH 7.2) (0.2 ml)–4 mM NADPH (0.1 ml)–40 mM ADP (0.1 ml) solution in the presence of compound I or compound II (100 μg/ml). After incubation, lipid peroxide values were estimated by the method of Yoden *et al.*,[5] and expressed as nmol per gram of wet liver tissue.

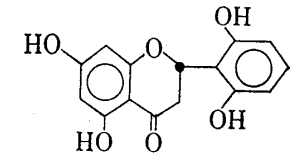
Results and Discussion

I) Determination of the Structure of Compounds I and II

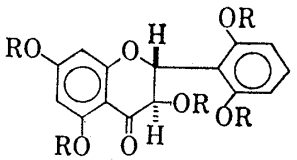
Compound I, colorless prisms, mp 240°C (dec.), exhibited a UV spectrum suggestive of the flavanone skeleton. Based on the IR, ^{1}H-NMR and analytical data, compound I concluded

to be identical with an authentic sample of (2*S*)-2′,5,6′,7-tetrahydroxy-flavanone isolated as an antibacterial substance from Scutellariae Radix.[4]

Compound II, colorless needles, mp 221—225°C (dec.), exhibited a UV spectrum similar to that of compound I[(2*S*)-2′,5,6′,7-tetrahydroxy-flavanone]. The UV spectrum of compound II showed a bathochromic shift on addition of $AlCl_3$ or AcONa. Therefore, the two hydroxyl group in II must be located at positions C-5 and C-7 in the A-ring of the flavanone skeleton. Acetylation of II afforded a pentaacetate (IIa), mp 108—110°C, $C_{25}H_{22}O_{10}$. In the ^{1}H-NMR spectrum, II showed signals based on an AB_2 pattern at 6.96 (1H, triplet, J=8.5 Hz) and at 6.34 (2H, doublet, J=8.5 Hz) in the aromatic region, identical with those of 2,6-dihydroxybenzoic acid and the B-ring of skullcapflavone II and (2*S*)-2′,5,6′,7-tetrahydroxy-flavanone (I). Therefore, the two hydroxyl groups in the B-ring of II can be placed at C-2′ and C-6′, respectively. II showed the presence of signals at 5.62, 5.31 (1H each, doublet, J=12.0 Hz, CH=CH, *trans*) which could be assigned to C_2–H and C_3–H, respectively. From these results, the structure of II was elucidated to be 2′,3,5,6′,7-pentahydroxy-flavanone. Gaffield[6] has reported that 3-hydroxy-flavanone, which has equatorial 2-aryl and 3-aryl substituents, exhibits a positive Cotton effect due to n-π^* transition (330 nm) and a negative Cotton effect in the π-π^* region (280—290 nm). The circular dichroism (CD) curve of II exhibited a negative Cotton effect at 277 nm (π-π^*) and a positive Cotton effect at 327 nm (n-π^*). It was concluded that the structure of II is (2*R*, 3*R*)-2′,3,5,6′,7-pentahydroxy-flavanone.



compound I



compound II and IIa
II: R=H; IIa: R=$COCH_3$

Fig. 1

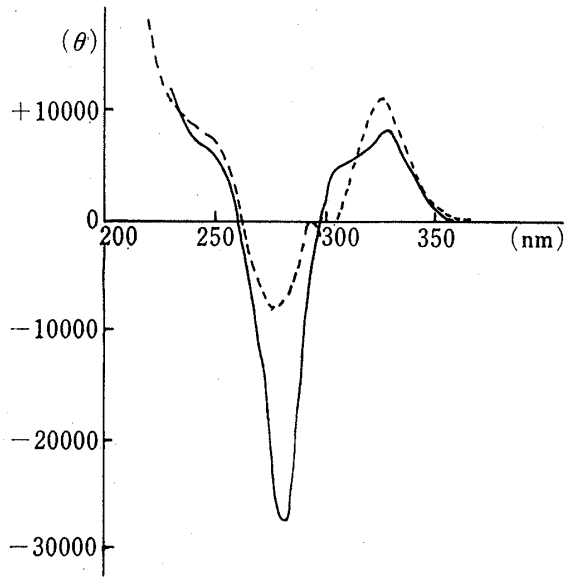


Fig. 2. CD curves of compounds I and II
——compound I, ·····compound II.

II) Effects of Compounds I and II on Lipid Peroxide Formation by $FeCl_2$–Ascorbic Acid and ADP–NADPH in Rat Liver Homogenate

As shown in Table I, compound II inhibited the lipid peroxide formation by $FeCl_2$ and ascorbic acid, but compound I had no effect. Compounds I and II also inhibited the lipid peroxide formation by ADP and NADPH.

We reported that oral administration of oxidized oil induced liver injury in rats, with elevation of serum GOT and GPT levels.[3] Compounds I and II isolated from the roots of Scutellariae Radix inhibited the lipid peroxide formation induced by Fe^{2+}-ascorbic acid and ADP–NADPH in rat liver homogenate, and may therefore prevent the destructive action of lipid peroxide on liver cells[7] by lowering the levels of lipid peroxide in the cells.

TABLE I.

a) Effects of Compounds I and II on $FeCl_2$–Ascorbic Acid-stimulated Lipid Peroxidation in Liver Homogenate

Additions (/ml reaction mixture)	LPO (nmol/g)	Significance
None	265.9±17.4[a)]	—
$FeCl_2$ (0.4 mM), AsA (1.2 mM)	1088.8±38.7	—
$FeCl_2$, AsA+Comp. I(100 μg/ml)	955.6±42.0	N.S.
$FeCl_2$, AsA+Comp. II (100 μg/ml)	335.2±11.4	$p<0.001$
$FeCl_2$, AsA+DL-α-tocopherol (100 μg/ml)	546.4±80.7	$p<0.001$

b) Effects of Compounds I and II on ADP–NADPH-stimulated Lipid Peroxidation in Liver Homogenate

Additions (/ml reaction mixture)	LPO (nmol/g)	Significance
None	267.3±16.3[a)]	—
ADP (4 mM), NADPH (0.4 mM)	414.3±16.0	—
ADP, NADPH+Comp. I (100 μg/ml)	314.0± 4.2	$p<0.001$
ADP, NADPH+Comp. II (100 μg/ml)	292.8±21.6	$p<0.001$
ADP, NADPH+DL-α-tocopherol (100 μg/ml)	216.4± 5.9	$p<0.001$

a) The results are means ± standard errors of 4—6 replicate experiments. The significance of differences from $FeCl_2$–ascrobic acid or ADP–NADPH is as indicated. N.S.: not significant. AsA: ascrobic acid.

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References and Notes

1) Part V: Y. Kimura, M. Kubo, K. Kusaka, M. Higashino, T. Tani, S. Arichi, and H. Okuda, *Chem. Pharm. Bull.*, **30**, 219 (1982).
2) Y. Kimura, M. Kubo, T. Tani, S. Arichi, and H. Okuda, *Chem. Pharm. Bull.*, **29**, 2610 (1981).
3) *a*) H. Okuda, *Igaku to Yakugaku*, **2**, 411 (1979); *b*) H. Ohminami, H. Okuda, T. Hamai, I. Kitagawa, M. Yoshikawa, S. Arichi, and T. Hayashi, *Eiyo to Shokuryo*, **34**, 105 (1981).
4) M. Kubo, Y. Kimura, T. Odani, T. Tani, and K. Namba, *Planta Medica*, **43**, 194 (1981).
5) K. Yoden, Y. Ito, and T. Tabata, *Yakugaku Zasshi*, **100**, 553 (1980).
6) W. Gaffield, *Tetrahedron*, **26**, 4093 (1970).
7) *a*) N. Shibata, T. Kinumaki, H. Okuda, and S. Fujii, *Arg. Biol. Chem.*, **37**, 1899 (1973); *b*) N.R. di Luzio and A.D. Hartman, *Fed. Proc.*, **26**, 1436 (1967); *c*) T.F. Salter and B.C. Sawyer, *Biochem. J.*, **123**, 823 (1971).