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Flavonoids with Bradykinin Antagonistic Effects from Scutellariae Radix

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Abstract—Seven flavonoid components were isolated from fr. C of Scutellariae radix which showed antagonistic effects against bradykinin(BK). The results indicated that two oxygen functions (either -OH or $-OCH_3$) at 2′- and 6′-positions and/or an oxygen function at 6-position of flavone seemed to be favored for the BK inhibitory activities. Skullcapflavone-II(**IV**) which contains 6-OCH_3, 2′-OH and 6′-OCH_3 in the structure was the most active among the flavones tested.

Keywords—Scutellariae radix • bradykinin antagonistic • oroxylin A • wogonin • skullcapflavone-I • skullcapflavone-II • chrysin • 5, 2′, 6′ -trihydroxy-7, 8-dimethoxyflavone • baicalein

Bradykinin (BK, Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) is a potent inflammatory peptide whose generation in tissues and body fluids elicits numerous responses including vasodilation, edema, smooth muscle spasm, as well as pain and hyperalgesia. There are substantial evidences that BK and related kinins, such as kallidin, contribute to the inflammatory responses in acute and chronic diseases including allergic reactions, arthritis, asthma, sepsis, viral rhinitis, and inflammatory bowel diseases. BK is also a potent algesic agent, comparable in potency to substance P, and many times more potent than serotonin, histamine, or acetylcholine. In addition to its algesic and proinflammatory actions, BK exhibits a vasodilator action. And because of its ability to lower blood pressure, BK has been implicated in the pathogenesis of several shock syndromes, particularly septic or endotoxic shock.[1~4] Kinin receptors were originally classified according to the relative potencies of agonists in isolated vascular smooth muscle preparations and divided into two classes, B_1 and B_2, although there are recent evidences for the third BK receptor which may not fall into the typical B_1 or B_2 receptor classification.[5] B_1 receptors were better characterized until the discovery of the competitive B_2 receptor antagonists in 1985, however, B_2 receptors are far more ubiquitous than B_1 receptors and mediate most physiological effects of BK. Since the report by Stewart and Vavrek that substitution with D-Phe for L-Pro in position 7 of the BK sequence provided the ability to inhibit B_2 receptor-mediated effects, hundreds of analogues have been synthesized, and the structure-activity relationships of these antagonists were analysed.[2,3,6,7] With the aid of the specific antagonists of BK, the physiological, pharmacological, and pathological roles of BK are being exploited in various biological

systems. Antagonists of this type block the majority of the biological effects of BK that are mediated by B_2 receptors and, in primary studies, produced encouraging results in the relief of cold symptoms caused by the rhinovirus, in the suppression of pain from burns, and in the treatment of allergic asthma.[8~10] However, the BK antagonists with the BK analogous peptide structure, unfortunately, have very short half-lives *in vivo*. The availability of a stable and specific non-peptide BK antagonist could provide a possible new form of therapeutic potential.

Scutellariae Radix (the root of *Scutellaria baicalensis* Georgi, Labiatae) have been used for the treatment of inflammatory diseases, suppurative dermatitis, allergic diseases, fever *etc.* in Oriental traditional medicine. Various biological activities including antithrombotic, anti-allergic, anti-inflammatory activities were reported with the solvent extracts or with the constituents.[11~13] As regards the constituents, flavonoids are the major components reported and about 40 flavonoids (either in free form or as glycosides) have been identified from this plant so far.[12,14,15]

In the previous paper, we reported the fr. C (the flavonoid and neutral component-containing ether soluble fraction) prepared from the EtOAc extract of Scutellariae Radix showed antagonistic effects against bradykinin (BK) *in vitro* and *in vivo*.[16] Fr. C inhibited the BK-induced contractions in rat ileum and uterus. Oral administration of fr. C reduced the plasma extravasation induced by intradermal injection of BK in mice, decreased the numbers of writhing syndrome induced by the *i.p.* injection of 0.7% acetic acid and lowered the mortality caused by endotoxic shock.[16] The present paper deals with a study on the antagonistic effects against BK with various flavonoids obtained from Scutellariae Radix.

Experimental

General experimental procedure

Melting point was determined on a Mitamura-Riken apparatus and are uncorrected. ^{1}H-NMR spectra were obtained on a Varian FT-80A (80MHz) using TMS as an internal standard. MS spectra were determined on a Hewlett-Packard 5985B GC/MS System equipped with a direct inlet system.

Plant material and reagents

Scutellariae radix was purchased from a local crude drug market and identified as *Scutellaria baicalensis* Georgi by Prof. Hyung Joon Chi of Natural Products Research Institute, Seoul National University. BK (Bradykinin acetate salt) was purchased from Sigma Chemical Company.

Extraction and fractionation

The dried and chopped Scutellariae radix was extracted and fractionated as described in the previous paper.[16] Fr. C was chromatographed on a silica gel column eluting with $CHCl_3$ with gradually increasing concentration of MeOH affording seven flavonoids as shown in Scheme I. Compounds **I, II, III, IV, V, VI** and **VII** were directly compared with the authentic samples (mixed mp and TLC) and the spectral data were compared with the reported data.

Oroxylin A (I)

mp 200~201° (from benzene), MS, *m/z* 284 (M^+), 269(M^+-CH_3), 241(M^+-CH_3CO), 167, 139; ^{1}H-NMR(d_6-DMSO) δ : 3.76(3H, s, OCH_3), 6.62(1H, s, C_3-H), 6.93(1H, s, C_8-H), 7.5~8.2(5H, m, $C_{2'\sim6'}$-H), 12.89(1H, s, C_5-OH).[17]

Wogonin (II)

mp 207~209°(from mixture of EtOAc and $CHCl_3$), MS, *m/z* 284(M^+), 269(M^+-CH_3), 241 (M^+-CH_3CO), 167, 139; ^{1}H-NMR(d_6-DMSO) δ : 3.85(3H, s, OCH_3), 6.31(1H, s, C_3-H),

6.96(1H, s, C_6-H), 7.4~8.2(5H, m, $C_{2'\sim6'}$-H), 10.72(1H, brs, C_7-OH), 12.47(1H, s, C_5-OH).[17]

Skullcapflavone-I(III)

mp 254~255°(from benzene), MS, *m/z* 314 (M^+), 299(M^+-CH_3), 181, 153; ^{1}H-NMR(d_6-DMSO) δ : 3.82, 3.91(3H each, s, 2×OCH_3), 6.58(1H, s, C_3-H), 7.12(1H, s, C_6-H), 6.9~7.9(4H, m, $C_{3'\sim6'}$-H) 10.82(1H, s, $C_{2'}$-OH), 12.67(1H, s, C_5-OH).[17,19]

Skullcapflavone-II(IV)

mp 180~181°(from benzene), MS, *m/z* 374 (M^+), 359(M^+-CH_3), 211, 183; ^{1}H-NMR(d_6-DMSO) δ : 3.73, 3.77, 3.80, 3.98(3H each, s, 4×OCH_3), 6.32(1H, s, C_3-H), 6.60(2H, d, *J*=8.5Hz, $C_{3'}$ or $C_{5'}$-H), 7.30(1H, t, *J*=8.5Hz, $C_{4'}$-H), 10.10(1H, s, $C_{2'}$-OH), 12.50 (1H, s, C_5-OH).[17]

Chrysin(V)

mp 277~278°(from benzene), MS, *m/z* 254 (M^+), 152, 124; ^{1}H-NMR(d_6-DMSO) δ : 6.21 (1H, d, *J*=2.0Hz, C_6-H), 6.50(1H, d, *J*=2.0 Hz, C_8-H), 6.92(1H, s, C_3-H), 7.4~8.2(5H, m, $C_{2'\sim6'}$-H), 10.83(1H, s, C_7-OH), 12.79 (1H, s, C_5-OH).[18]

5,2′,6′-Trihydroxy-7,8-dimethoxyflavone(VI)

mp 234~237°(from EtOAc), MS, *m/z* 330 (M^+), 315(M^+-CH_3), 181, 153; ^{1}H-NMR(d_6-DMSO) δ : 3.72, 3.90(3H each, s, 2×OCH_3), 6.26(1H, s, C_3-H), 6.43(2H, d, *J*=8.1Hz, $C_{3'}$ or $C_{5'}$-H), 6.58(1H, s, C_6-H), 7.12(1H, t, *J*=8.1Hz, $C_{4'}$-H), 9.86(2H, s, $C_{2'}$ or $C_{6'}$-OH), 12.70(1H, s, C_5-OH).[14]

Baicalein(VII)

mp 264~265°(from EtOH), MS, *m/z* 270 (M^+), 168, 140; ^{1}H-NMR(d_6-DMSO) δ : 6.63 (1H, s, C_3-H), 6.94(1H, s, C_8-H), 7.5~8.2 (5H, m, $C_{2'\sim6'}$-H), 12.43(1H, s, C_5-OH).[17]

BK-induced contraction in the isolated ileum

Terminal ileum was isolated from male rats (Sprague-Dawley, 150~200g) and 2~3cm segment of ileum was suspended in 10ml tissue bath filled with modified Kreb's solution(composition, g/*l*; KH_2PO_4 0.16, KCl 0.35, $CaCl_2$ 0.25, $MgSO_4 \cdot 7H_2O$ 0.29, NaCl 6.9, $NaHCO_3$ 2.1, glucose 1.98) at 36°C and bubbled with 95% O_2-5% CO_2. After resting period of about 60 min, isotonic contractions were measured under a resting load of 1g and recorded on a kymograph. 0.1ml of test sample solution was added to the bath 1 min. prior to BK and the effect of the test compound was evaluated comparing with the contraction induced by BK alone.

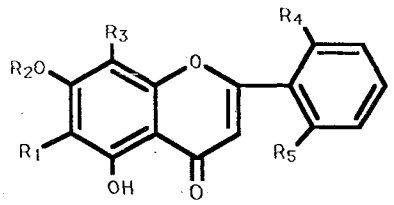
BK-induced contraction in the isolated uterus

Uterine preparations were obtained from virgin rats (Sprague-Dawrey, 150~200g) treated with diethyl stilbesterol (0.1mg/ml ethanol/kg, s.c.) 24hr before the experiments. An uterine strip was suspended in 10ml organ bath containing modified De Jalon's solution (composition, g/*l*; NaCl 9.0, KCl 0.42, $CaCl_2$ 0.045, glucose 0.5, $NaHCO_3$ 0.5, $MgCl_2$ 0.005) at 31°C and bubbled with 95% O_2-5% CO_2. And the tests were performed as with isolated ileum.

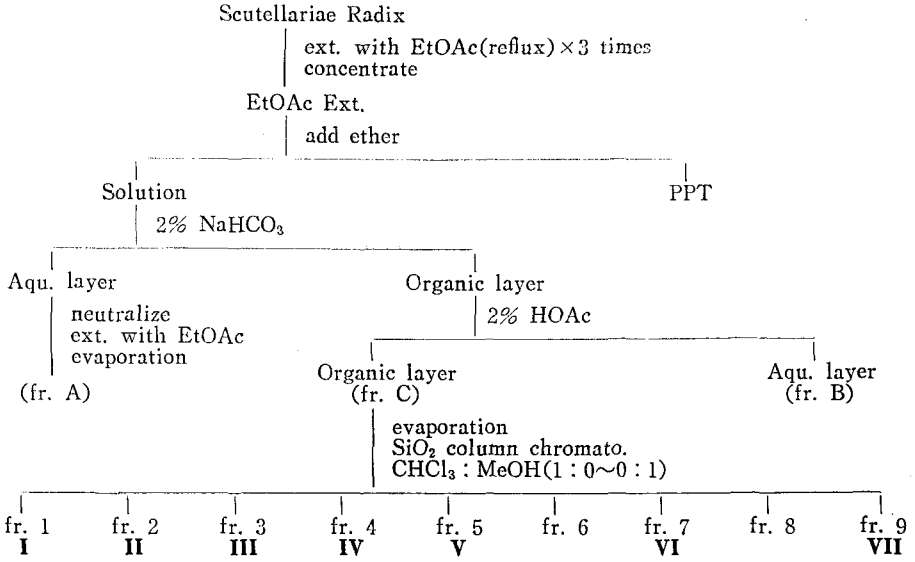
Results and Discussion

For the purpose of separating BK antagonistic components, fr. C, the active fraction, was subjected to a silica gel column chromatography affording seven flavonoids(**I, II, III, IV, V, VI,** and **VII**). The authors extracted several batches of Scutellariae radix (Scutellariae radix either cultivated in Korea or imported from China are marketed in the crude drug market in Seoul) to secure the various flavonoid components reported. **I, II** and **VII** could be obtained from all the batches extracted. The contents of **III, IV, V** and **VI** were variable and could not even be detected from some of the batches. The results of extraction, fractionation and separation

Table I. Effects of flavonoids **I-VII** against BK-induced contraction at isolated rat ileum and uterus



	R_1	R_2	R_3	R_4	R_5	conc: (μg/ml)	% inhibition	
							ileum	uterus
I oroxylin A	OCH_3	H	H	H	H	20	—	—
						40	23	34
II wogonin	H	H	OCH_3	H	H	20	63	—
						40	76	14
III skullcapflavone-I	H	CH_3	OCH_3	OH	H	20	18	—
						40	28	15
IV skullcapflavone-II	OCH_3	CH_3	OCH_3	OH	OCH_3	20	62	53
						40	93	90
V chrysin	H	H	H	H	H	20	11	—
						40	23	14
VI 5,2′,6′-trihydroxy-7,8-dimethoxyflavone	H	CH_3	CCH_3	OH	OH	20	14	14
						40	52	37
VII baicalein	OH	H	H	H	H	20	—	—
						40	42	32



Scheme I. Extraction, fractionation and separation of flavonoids from Scutellariae radix

of several batches of S. radix are summerized in Scheme I. **I-VII** were identified as oroxylin A, wogonin, skullcapflavone-I, skullcapflavone-II, chrysin, 5,2′,6′-trihydroxy-7,8-dimethoxyflavone and baicalein respectively (Table I) by the analysis of the NMR and MS data and with

the direct comparison of the mixed mp, TLC *etc.* with the authentic samples obtained from M. Takido of Nihon University in Japan.

The antagonistic effects of each flavonoids against BK were evaluated with the use of rat ileum and rat uterus and the results are summarized in Table I. The existence of at least two types of kinin receptors have been proposed and the B_1 receptor is thought to be induced by *de novo* synthesis. The BK-induced contraction of rat uterus is thought to be mediated by B_2 receptor, while rat isolated ileum contains both B_1 and B_2 receptors. The majority of actions of kinins were assumed to be mediated via B_2 receptors and the stimulation of B_2 receptor is associated with various pathophysiological conditions *e.g.* plasma extravasation, nociceptive stimulation *etc.* In isolated ileum, all of the seven flavonoids showed inhibitory effects against BK. At the concentration of 40μg/ml, **II, IV, VI** and **VII** showed 76%, 93%, 52% and 42% inhibition respectively and **II** and **IV** were observed to give more than 50% inhibition at the concentration of 20μg/ml. At isolated uterus, **IV** was most antagonistic against BK among the seven flavonoids showing 90% inhibition at 40μg/ml and 53% at 20μg/ml. **I, VI** and **VII** were also mild antagonistic(34%, 37% and 32% respectively at 40μg/ml) against BK. The results from the limited number of Scutellariae flavonoids indicated that the antagonistic effect at the B_2 receptor is favored by the two oxygen functions at R_4 and R_5(2′- and 6′-positions of flavonoid) as in the case of **IV** and **VI**. An oxygen function at R_1 (6-position of flavonoid) also seemed to be preferred for the B_2 antagonistic effect (**I** and **VII** vs **V**). The oxygen functions at R_1 and two oxygen functions at both R_4 and R_5 seemed to contribute to the concentration of electron density at the oxygen of C=O by either interferring the hydrogen bonding between C=O and C_5-OH or by resonance.

Conclusion

Seven flavonoid components were separated from fr. C of Scutellariae radix. The antagonistic effects against BK B_2 receptor seemed to be favored by an oxygen function (either -OH or $-OCH_3$) at 6- and/or two oxygen functions at 2′- and 6′- of flavone both of which contribute to the concentration of electron density at the oxygen of $_4$C=O. Skullcapflavone-II (**IV**) which contains 6-OCH_3, 2′-OH and 6′-OCH_3 in the structure was the most active among the Scutellariae flavonoids tested.

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