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Potential Inhibitors of Platelet Aggregation from Plant Sources, IV.* Platelet Anti-Aggregatory Components of *Cassiae Semen*

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Abstract—An activity guided fractionation of the methanol extract of *Cassiae Semen* yielded three platelet anti-aggregatory components, aurantio-obtusin, chryso-obtusin and emodin.

Keywords—*Cassiae Semen* · platelet anti-aggregatory activity · aurantio-obtusin · chryso-obtusin · emodin.

Activation of blood platelets within vessels causes platelet aggregation which is a crucial factor in the pathogenesis of various ischemic diseases. Hence inhibitors of platelet aggregation are of potential clinical utility. in the prevention of cardiovascular disease, cerebral ischemia or stroke and in post-operative situations following, for example, angioplasty, organ transplant and coronary artery by-pass surgery[1-9].

Various efforts have been given to look for inhibitors of platelet aggregation in recent years [10-15]. Crude plant extracts were also evaluated for the investigation of inhibitors of blood platelet aggregation[1,2]. The $CHCl_3$ soluble fraction prepared from the MeOH extract of *Cassia obtusifolia* (Linne) Endlicher (Leguminosae) is one of the solvent fractions showed strong inhibitory activities against ADP-, arachidonic acid(AA)- or collagen-induced platelet aggregation. Thus, the work proceeded to the activity guided treatments and fractionations of the $CHCl_3$ soluble fraction to identify the platelet anti-aggregatory components.

Cassiae Semen have been considered as one of the most important oriental medicine and employed for purgative, tonic, diuretic and antihypertensive purposes. Much attention have been given for decades to various phytochemical investigations of *Cassiae Semen* which had led to the isolation and identification of various anthraquinone, naphtopyrone, tetrahydroanthracene and naphthooxepin derivatives from the seeds of *C. obtusifolia* and related *Cassia* spp.[16-30]. After the reconfirmation of its inhibitory activity against induced platelet aggregation, the $CHCl_3$ soluble fraction was chromatographed over silica gel column yielding two sub-fractions with inhibitory activities. Comp. **1** and **2** were separated from the first active sub-fraction after the rechromatography over silica gel and comp **3** was obtained from the second active sub-fraction. The ^{1}H-NMR spectra of comp. **1** showed two aromatic protons at δ 7.88 and 7.61, four methoxyl groups at δ 4.02 ($3\times OCH_3$) and 3.98

* For the previous papers in the series, see reference 1~3.

Table I. Effects of comp. 1, 2 and 3 against ADP-, AA- or collagen-induced platelet aggregation

Compound[a]	Aggregating agents		
	ADP[b]	AA[c]	Collagen[d]
Controls			
PRP (without aggregating agents)	—[e]	—	—
PRP (with aggregating agents)	++	++	++
1	+	+	±
2	+	++	+
3	++	+	+

a) Concentration of compound 1 mg/ml, b) 7.5×10^{-7} g/ml of ADP, c) 6.5×10^{-5} g/ml of AA, d) 1×10^{-5} g/ml of collagen, e) The degree of platelet aggregation induced was judged as the following; —, no aggregation, ±, slight aggregation, +, intermediate aggregation, ++, as much aggregation as PRP plus aggregating agent alone.

and one proton at δ 6.70 which disappeared on acetylation of the compound. Comp. 2 showed, on its NMR spectra, four aromatic protons and one methyl group and gave trimethylated product with CH_2N_2. Comp. 3 gave on its NMR spectra two aromatic protons at δ 7.94 and 7.40, two methoxyl groups at δ 4.12 and 4.01 and one methyl protons at δ 2.39. On treatment with CH_2N_2, 3 gave dimethylated analog, however on acetylation with Ac_2O/pyridine yielded triacetylated product. 2 was identified as 1, 6, 8-trihydroxy-3-methylanthraquinone (emodin) by the direct comparison with an authentic sample (tlc, mmp, IR, ^{1}H-NMR) obtained from Sigma Chem. Co., U.S.A. 1 and 3 were identified as 1, 6, 7, 8-tetramethoxy-2-hydroxy-3-methylanthraquinone (chryso-obtusin) and 1, 7-dimethoxy-2, 6, 8-trihydroxy-3-methylanthraquinone (aurantio-obtusin) respectively with the comparison of various data of themselves and their derivatives with the literature values[17,20].

The inhibitory activities of 1, 2 and 3 against platelet aggregation induced by ADP, AA or collagen are tabulated in Table I. 1 was inhibitory against ADP, AA and collagen induced aggregations while 2 was inhibitory against ADP and collagen induced and 3 against AA and collagen induced platelet aggregations. However, the inhibitory activities were rather mild possibly due to their lower solubility (The compounds were added as suspensions to the test solution). It is also assumed that other components with potent inhibitory activities might also be present in the plant. Further investigations are in progress for the isolation of other active components.

Experimental

Melting points were determined on a Mitamura-Riken apparatus and are uncorrected. The IR spectra were taken on Perkin-Elmer 283 B spectrometer. NMR spectra were recorded on a Varian FT-80A instrument operating at 80MHz. Mass spectra were obtained on a Hewlett-Packard 5985B GC/MS system equipped with a direct inlet system and operating at 70 eV. ADP (adenosine 5′-diphosphate dicyclohexylammonium salt), AA (arachidonic acid sodium salt) and collagen (acid soluble, from calf skin) were purchased from Sigma Chemical Company.

Plant Material

Cassiae Semen were purchased from the crude drug market in Seoul, Korea and identified as the seeds of *Cassiae obtusifolia* by Prof. M. Takido of College of Science and Technology, Nihon University, Tokyo, Japan.

Extraction and Fractionation

The crushed seeds were first extracted with hexane and then extracted with refluxing MeOH for 6 hrs (three times.) The resulting MeOH extract was then partitioned between 9:1 MeOH $-H_2O$ and hexane. The MeOH-H_2O layer after concentration was partitioned again between $CHCl_3$ and H_2O. The $CHCl_3$ soluble fraction was chromatographed on a silica gel column eluting with $CHCl_3$-$CHCl_3$:MeOH(1:1) with gradually increasing MeOH proportion. The sixth and the eighth sub-fractions showed inhibitory activity against platelet aggregation. The sixth sub-fraction was chromatographed again on a silica gel column eluting with hexane-hexane:acetone (1:1) yielding comp. **1** and **2** The eighth sub-fraction yielded comp **3** on recrystallization with EtOAc.

Compound 1 (Chryso-obtusin)

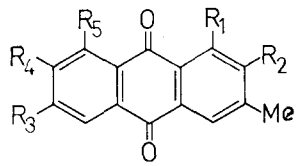
Mp 214~215°, recrystallized from EtOAc (yellow); IR ν_{max}^{KBr} 1670 cm^{-1}; ^{1}H-NMR($CDCl_3$) δ 7.88(1H, s, C_4-H), 7.61(1H, s, C_5-H), 6.70 (^{1}H, s, OH), 4.02(9H, s, 3×OCH_3), 3.98(3H, s, OCH_3), 2.38(3H, s, CH_3); MS *m/z* 358(M^+), 343(M^+-15).

Acetylation of 1 (1-a)

Comp **1** (5mg) was treated with the mixture of pryidine and acetic anhydride(0.5ml : 0.5ml) overnight under nitrogen and the mixture was concentrated yielding monoacetylated derivative **1-a.** ^{1}H-NMR($CDCl_3$) δ 7.91(1H, s, C_4-H), 7.58(1H, s, C_5-H), 4.02(3H, s, OCH_3), 3.99 (6H, s, 2×OCH_3), 3.95(3H, s, OCH_3), 2.40 (3H, s, $COCH_3$), 2.31(3H, s, CH_3).

Compound 2 (emodin)

Mp 253 ~ 255°; recrystallized from EtOAc (orange); IR ν_{max}^{KBr} 1630 cm^{-1}; ^{1}H-NMR($CDCl_3$-DMSO-d_6) δ 12.16(1H, s, OH), 12.03(1H, s, OH), 7.58(1H, s, C_4-H), 7.29(1H, d, *J*=2.3 Hz, C_5-H), 7.05(1H, s, C_2-H), 6.64(1H, d, *J*=2.3Hz, C_7-H), 2.44(3H, s, CH_3)



1 : R_2=OH, R_1, R_3, R_4, R_5=OMe
2 : R_2, R_4=H, R_1, R_3, R_5=OH
3 : R_2, R_3, R_5=OH, R_1, R_4=OMe

Methylation of 2 (2-m)

Comp **2** (5mg) was treated in EtOH solution with etheral CH_2N_2 and the trimethylated derivative **2-m** was obtained after the usual work-up. ^{1}H-NMR($CDCl_3$) δ 7.62(1H, s, C_4-H), 7.28(1H, d, *J*=2.3Hz, C_5-H), 7.07(1H, s, C_2-H), 6.72(1H, d, *J*=2.3Hz, C_7-H), 3.96 (6H, 2×OCH_3), 3.94(3H, OCH_3), 2.44(3H, s, CH_3).

Compound 3 (aurantio-obtusin)

Mp 262~265°; recrystallized from EtOAc(dark orange); IR ν_{max}^{KBr} 1653, 1625 cm^{-1}; ^{1}H-NMR($CDCl_3$) δ 13.33(1H, s, OH), 7.94(1H, s, C_4-H), 7.40(1H, s, C_5-H), 6.70(1H, s, OH), 6.41(1H, s, OH), 4.12(3H, s, OCH_3), 4.01(3H, s, OCH_3), 2.39(3H, s, CH_3).

Acetylation of 3 (3-a)

Ten mg of **3** was treated at room temp with the mixture of pyridine and acetic anhydride (1 ml:1 ml) and was worked-up as usual yielding triacetylated derivative **3-a.** mp 194°; ^{1}H-NMR ($CDCl_3$) δ 7.94(1H, s, C_4-H), 7.91(1H, s, C_5-H), 3.95(3H, s, OCH_3), 3.87(3H, s, OCH_3), 2.49(3H, s, $COCH_3$), 2.39(3H, s, $COCH_3$), 2.38(3H, s, $COCH_3$), 2.31(3H, s, CH_3).

Methylation of 3 (3-m)

Ten mg of **3** in EtOAc was treated with the excess etheral CH_2N_2 and the dimethylated derivative **3-m** was obtained after the usual work-up. ^{1}H-NMR($CDCl_3$) δ 13.11(1H, s, OH), 7.94(1H, s, C_4-H), 7.40(1H, s, C_5-H)), 4.03 (3H, s, OCH_3), 4.01(3H, s, OCH_3), 3.98(3H, s, OCH_3), 3.97(3H, s, OCH_3), 2.39(3H, s, CH_3).

Anti-platelet aggregation testing

The inhibitory activity testing against ADP, AA or collagen induced rat platelet aggregation were carried out by the modified smear method of Yun-Choi et al. described in the previous paper[31]. The degrees of measured platelet aggregation are shown in Table 1.

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