Journal of Natural Products
Vol. 53, No. 3, pp. 630-633, May-Jun 1990

POTENTIAL INHIBITORS OF PLATELET AGGREGATION FROM PLANT SOURCES, V.[1] ANTHRAQUINONES FROM SEEDS OF *CASSIA OBTUSIFOLIA* AND RELATED COMPOUNDS[2]

HYE SOOK YUN-CHOI,* JAE HOON KIM,

Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea

and MICHIO TAKIDO

College of Pharmacy, Nihon University, Chiba 274, Japan

ABSTRACT.—Three anthraquinone glycosides, gluco-obtusifolin [**11**], gluco-chryso-obtusin [**15**], and gluco-aurantioobtusin [**13**], were found to be platelet anti-aggregatory constituents of seeds of *Cassia obtusifolia*. Various other anthraquinone analogues were also tested, and their structure-activity relationships are discussed.

Various plant extracts were evaluated (1,2) in an attempt to search for inhibitors of blood platelet aggregation, which is a crucial factor in the pathogenesis of various ischemic diseases (3–7). Cassiae Semen was one of the plant materials that showed inhibitory activities against ADP-, arachidonic-acid- (AA), or collagen-induced platelet aggregation. Three anthraquinones, aurantioobtusin [**12**], chrysoobtusin [**14**], and emodin [**8**], were reported as platelet anti-aggregatory constituents of Cassiae Semen (8). However, their inhibitory activities were rather mild, and it was assumed that other components with potent inhibitory activities might also be present in the plant.

Thus, further activity-guided fractionations of the extract of the seeds of *Cassia obtusifolia* (L.) Endlicher (Leguminosae) were undertaken, and three anthraquinone glycosides, gluco-obtusifolin [**11**], gluco-aurantioobtusin [**13**], and gluco-chrysoobtusin [**15**], with strong inhibitory activities against rat platelet aggregation were obtained. Various other structural analogues of 9,10-anthraquinones were also evaluated to investigate their structure-activity relationships.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on a Varian FT-80A instrument operating at 80 MHz. Mass spectra were obtained on a Hewlett-Packard 5985B equipped with a direct inlet system and operating at 70 eV. Melting points were determined on a Mitamura-Riken apparatus and are uncorrected. ADP (adenosine 5′-diphosphate dicyclohexylammonium salt), AA (arachidonic acid sodium salt), collagen (acid soluble, from calf skin), and β-glucosidase were purchased from Sigma Chemical Company, St. Louis. Various anthraquinones other than those separated from Cassiae Semen were purchased from either Sigma Chemical, U.S.A., or Carl Roth GmbH, West Germany.

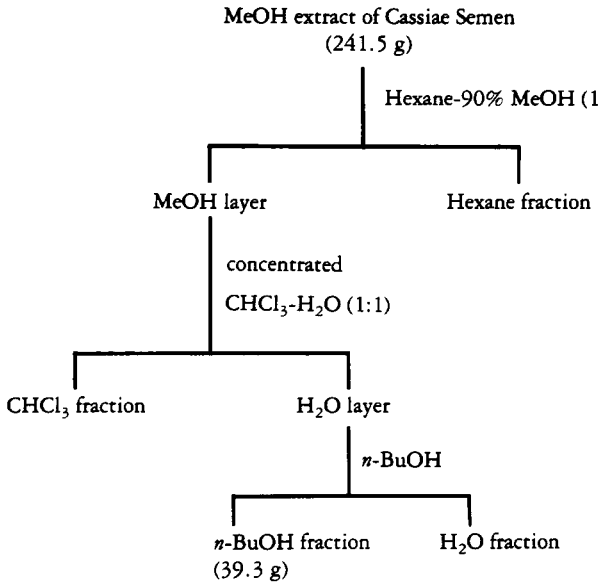
PLANT MATERIALS.—Cassiae Semen was purchased from the crude drug market in Seoul and identified as the seeds of *C. obtusifolia* by one of the authors (9), and the specimens were deposited at Natural Products Research Institute, Seoul National University, Seoul, Korea.

ACTIVITY-GUIDED ISOLATION OF ANTHRAQUINONES.—Cassiae Semen (3 kg) was first extracted with hexane and then with refluxing MeOH for 6 h three times. The resulting MeOH extract (241.5 g) was fractionated as shown in Scheme 1.

The active *n*-BuOH-soluble fraction (39 g) was chromatographed on a column of Si gel 60 (2 kg, particle size 0.063–0.020 mm, Merck). Elution with $CHCl_3$-Me_2CO (1:0→1:1) afforded 16 subfractions. Gluco-obtusifolin [**11**] (130 mg), gluco-chrysoobtusin [**15**] (30 mg), and gluco-aurantioobtusin [**13**] (70 mg) were obtained from the three active subfractions 11, 13, and 14, respectively. The glycosides and their

[1]For Part IV, see Yun-Choi *et al.* (8).

[2]A part of this report was presented at the International Symposium on New Drug Development from Natural Products, May 2–3, 1989, Seoul, Korea.



SCHEME 1. Fractionation of the MeOH extract of Cassiae Semen.

corresponding free anthraquinones, which were obtained by the enzymatic hydrolysis of the glycosides, were identified by direct comparison with the authentic samples previously isolated from the same plant by one of the authors (10–13).

ENZYMATIC HYDROLYSIS OF THE GLYCOSIDES.—A solution of each glycoside (10 mg) and β-glucosidase (10 mg) in 10 ml of H_2O was incubated at 37° overnight. The reaction mixture was extracted with EtOAc. After evaporation of the solvent, the residue was recrystallized.

Gluco-obtusifolin [**11**].—Mp 204° (from H_2O-saturated EtOAc); ms *m/z* $[M-162]^+$ 284, $[M-180]^+$ 266, $[M-208]^+$ 238; ir as reported by Takido and Takahashi (10); ^1H nmr (DMSO-d_6) δ 7.84 (1H, s, H-4), 7.70 (1H, d, $J=7.2$ Hz, H-6), 7.65 (1H, br, s, H-7), 7.31 (1H, dd, $J=7.2, 2.2$ Hz, H-5), 5.35–3.0 (11H), 3.88 (3H, s, OMe), 2.41 (3H, s, Me).

Gluco-chrysoobtusin [**15**].—Mp 226–228°; ms *m/z* $[M-162]^+$ 358, $[M-177]^+$ 343; ir and ^1H nmr as reported by Kitanaka *et al.* (11).

Gluco-aurantioobtusin [**13**].—Mp 238–240° (from H_2O-saturated EtOAc); ms *m/z* $[M-162]^+$ 330, $[M-180]^+$ 312; ir as reported by Takido and Takahashi (10); ^1H nmr (DMSO-d_6) δ 7.81 (1H, s, H-4), 7.18 (1H, s, H-5), 5.30–3.0 (11H), 3.84 (3H, s, OMe), 3.80 (3H, s, OMe), 2.36 (3H, s, Me).

Obtusifolin [**10**].—Mp 238° (from EtOAc); ms *m/z* $[M]^+$ 284, $[M-18]^+$ 266, $[M-46]^+$ 238; ir as reported by Takido (12); ^1H nmr ($CDCl_3$) δ 7.97 (1H, s, H-4), 7.73 (1H, d, $J=2.2$ Hz, H-7), 7.75 (1H, s, $J=7.8$ Hz, H-6), 7.25 (1H, dd, $J=7.8, 2.2$ Hz, H-5), 4.01 (3H, s, OMe), 2.40 (3H, s, Me).

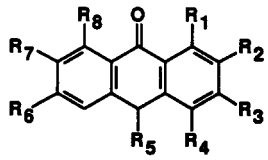
Chrysoobtusin [**14**] *and aurantioobtusin* [**12**].—Chrysoobtusin, mp 214°, and aurantioobtusin, mp 262°: all the data as reported by Yun-Choi *et al.* (8).

ANTI-PLATELET AGGREGATION TESTING.—The inhibitory activities of each compound against AA-, ADP- or collagen-induced rat platelet aggregation were screened by the modified smear method of Yun-Choi *et al.* (1). Each sample was first dissolved in EtOH and then homogenized with the addition of saline to give 1% of the final EtOH concentration.

RESULTS AND DISCUSSION

The platelet anti-aggregatory activities were found concentrated into $CHCl_3$-soluble and *n*-BuOH-soluble fractions when the MeOH extract was fractionated as shown in Scheme 1. The *n*-BuOH-soluble fraction was further subjected to activity-guided fractionations. Gluco-obtusifolin [**11**], gluco-aurantioobtusin [**13**], and gluco-chrysoobtusin [**15**] were identified as platelet anti-aggregatory components. Compound **13**, with two more oxygen functions (one OH and one OMe) than **11**, showed comparable

TABLE 1. Platelet Anti-aggregating Activities of 9,10-Anthraquinone Analogues.[a]



Compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Aggregating agents[b]		
									AA	ADP	collagen
1	OH	OH	H	H	=O	H	H	H	>0.25	>0.25	>0.25
2	OH	H	H	OH	=O	H	H	H	>0.25	>0.25	>0.25
3	OH	H	H	H	=O	H	H	OH	>0.25	>0.25	>0.25
4	OH	H	Me	H	=O	H	H	OH	>0.25	>0.25	>0.25
5	OH	H	CH_2OH	H	=O	H	H	OH	0.25	>0.25	0.25
6	OH	H	COOH	H	=O	H	H	OH	0.25	0.25	0.25
7	H	OH	H	H	=O	OH	H	H	>0.25	>0.25	>0.25
8[c]	OH	H	Me	H	=O	OH	H	OH	>0.25	>0.25	>0.25
9	OH	H	Me	H	=O	OMe	H	OH	0.25	0.25	>0.25
10	OMe	OH	Me	H	=O	H	H	OH	0.25	>0.25	0.25
11	OMe	Oglu	Me	H	=O	H	H	OH	0.05	0.25	0.1
12[c]	OMe	OH	Me	H	=O	OH	OMe	OH	>0.25	>0.25	>0.25
13	OMe	OH	Me	H	=O	Oglu	OMe	OH	0.05	0.05	0.1
14[c]	OMe	OH	Me	H	=O	OMe	OMe	OMe	0.25	0.25	0.25
15	OMe	Oglu	Me	H	=O	OMe	OMe	OMe	0.025	0.025	0.05
16	OH	H	Me	H	<H H	H	H	OH	>0.25	>0.25	>0.25
17	OH	H	CH_2OH	H	<H Oara	H	H	OH	>0.25	>0.25	>0.25

[a]Data show the minimum concentration (mg/ml) of each compound in which rat platelet aggregation was mostly inhibited, showing the degree of aggregation (±).

[b]Platelet-rich plasma (PRP) was pretreated with the test compound for 2 min before the addition of 6×10^{-6} g/ml AA, 1×10^{-6} g/ml ADP, or 6×10^{-6} g/ml collagen.

[c]Data for this compound are from Yun-Choi *et al.* (8).

inhibitory activities to those of **11** against AA- and collagen-induced aggregations; however, **13** was about 5 times more active than **11** against ADP-induced aggregation. Compound **15**, the fully *O*-methylated analogue of **13**, although having different positions for *O*-glu, was found to have approximately twice as much inhibitory activity as **13** against AA-, ADP-, and collagen-induced aggregations.

Various 9,10-anthraquinone analogues were also tested for their activities against AA-, ADP-, and collagen-induced platelet aggregation to investigate structure-activity relationships. From the limited analogues available (Table 1), it was suggestive that simple dihydroxy-, 3-methyldihydroxy-, and 3-methyl-trihydroxyanthraquinones (**1**–**4**, **7**, and **8**) were not suitable for anti-platelet activities. Replacement of the 3-methyl group with either a hydroxymethyl or carboxyl group (**5** and **6** vs. **4**) increased the inhibitory potencies. *O*-Methylation of the hydroxyl group produced a favorable influence on the inhibitory activities (**9** and **10** vs. **8**; **14** vs. **12**). An introduction of a glucose function at one of the free hydroxyls of **10**, **12**, and **14** yielded strong inhibitors, **11**, **13**, and **15**, against all three aggregation inducers tested, AA, ADP, and collagen. Moreover, compound **15** (gluco-chrysoobtusin), which is an analogue of **12** whose three hydroxyl groups are fully substituted by either methoxyl or *O*-glycosidyl functions, was found to be the most potent inhibitor of platelet aggregation among the anthraquinones tested. Introduction of a glucose function may potentiate the activity by

increasing the H_2O-solubility of each compound, because most of the tested compounds were introduced to the assay system as suspensions in 10% EtOH-saline (1% of the final EtOH concentration). Reduction of one of the quinone functions reduced activity as shown with **16** and **17** vs. **5**.

The inhibitory activities of the three most active compounds, **11**, **13**, and **15**, were compared in Table 2 with those of aspirin, a known inhibitor of platelet aggregation (1). Compounds **11** and **13** were as active as aspirin and **15** was about three times more active than aspirin against AA-induced platelet aggregation when compared with the molar concentrations. All three compounds, **11**, **13**, and **15**, were 5 to 50 times more potent than aspirin against ADP-induced aggregation and two to five times more active than aspirin against collagen-induced aggregation. Although extensive conclusions could not be made with only the present testing results, it was obvious that introduction of more hydroxyl groups and/or methylation or glycosylation of hydroxyl groups to 9,10-anthraquinones resulted in potent platelet anti-aggregatory activities.

TABLE 2. Inhibitory Activities of 9,10-Anthraquinone Glycosides and Aspirin Against Platelet Aggregation.[a]

Compound	Aggregating agent		
	AA	ADP	Collagen
11	0.05 (1.1×10^{-4})	0.25 (5.6×10^{-4})	0.1 (2.2×10^{-4})
13	0.05 (1.0×10^{-4})	0.05 (1.0×10^{-4})	0.1 (2.0×10^{-4})
15	0.025 (4.8×10^{-5})	0.025 (4.8×10^{-5})	0.05 (9.6×10^{-5})
Aspirin[b]	0.025 (1.4×10^{-4})	0.5 (2.8×10^{-3})	0.1 (5.6×10^{-4})

[a]Data show the minimum concentration of each compound in mg/ml and in M in parentheses. Other conditions are as shown in Table 1.

[b]Data for this compound are from Yun-Choi *et al.* (1).

ACKNOWLEDGMENTS

The authors express their appreciation to the Korea Science & Engineering Foundation for financial support (project number: 862-0302-004-2).

LITERATURE CITED

1. H.S. Yun-Choi, S.O. Kim, J.H. Kim, J.R. Lee, and H.-I. Cho, *J. Nat. Prod.*, **48**, 363 (1985).
2. H.S. Yun-Choi, J.H. Kim, and J.R. Lee, *Korean J. Pharmacogn.*, **17**, 19 (1986).
3. E. Genton, M. Gent, J. Hirsh, and L.A. Harker, *N. Engl. J. Med.*, **293**, 1174 (1975).
4. M.D. Verstraete, *Am. J. Med.*, **61**, 897 (1976).
5. H.J. Weiss, *N. Engl. J. Med.*, **298**, 1403 (1978).
6. N. Woolf, *Br. Med. Bull.*, **34**, 137 (1978).
7. J.R.A. Mitchell, *Br. Med. Bull.*, **34**, 103 (1978).
8. H.S. Yun-Choi, J.R. Lee, J.H. Kim, Y.H. Kim, and T.H. Kim, *Korean J. Pharmacogn.*, **18**, 203 (1987).
9. J. Iwasa, *Shoyakugaku Zasshi*, **12**, 57 (1958).
10. M. Takido and S. Takahashi, *Shoyakugaku Zasshi*, **17**, 43 (1964).
11. S. Kitanaka, F. Kimura, and M. Takido, *Chem. Pharm. Bull.*, **33**, 1274 (1985).
12. M. Takido, *Chem. Pharm. Bull.*, **6**, 397 (1958).
13. M. Takido, *Chem. Pharm. Bull.*, **8**, 246 (1960).
14. H.S. Yun-Choi, J.H. Kim, and J.R. Lee, *J. Nat. Prod.*, **50**, 1059 (1987).

Received 30 October 1989