

[Chem. Pharm. Bull.]
[28(9)2770-2778(1980)]

Studies on Antiatherosclerotic Agents.¹⁾ IX. Synthesis of 7-Ethoxycarbonyl-4-hydroxymethyl-6,8-dimethyl-1(2H)-phthalazinone (EG 626)

MASAYUKI ISHIKAWA, YUKUO EGUCHI, and AKIKO SUGIMOTO

Institute for Medical and Dental Engineering, Tokyo Medical and Dental University²⁾

(Received February 16, 1980)

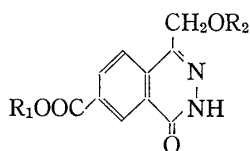
To enhance the biostability of the ester linkage of 7-ethoxycarbonyl-4-hydroxymethyl-1(2H)-phthalazinone, which has potent biological activities, the corresponding *tert*-butyl ester (**1e**) and 7-ethoxycarbonyl-4-hydroxymethyl-6,8-dimethyl-1(2H)-phthalazinone (EG 626) (**6**) were prepared. The synthesis and inhibitory effects on platelet aggregation and edematous arterial reaction of these and other closely related compounds were investigated. EG 626 appears to be a potentially effective antithrombotic and antiatherosclerotic agent.

Keywords—antithrombotic agent; antiatherosclerotic agent; inhibitor of platelet aggregation; inhibitor of edematous arterial reaction; 7-*tert*-butoxycarbonyl-4-hydroxymethyl-1(2H)-phthalazinone; 7-ethoxycarbonyl-4-hydroxymethyl-6,8-dimethyl-1(2H)-phthalazinone; EG 626; phthalazinol; cyclic AMP phosphodiesterase inhibitor; thromboxane A₂ antagonist

As part of our continuing search for more potent and safer antiatherosclerotic agents, we reported¹⁾ previously that 7-ethoxycarbonyl-4-hydroxymethyl-1(2H)-phthalazinone (**1b**) showed potent inhibitory activity both on platelet aggregation and edematous arterial reaction. The compound seemed worthy of further investigation, but it was found that it was susceptible to hydrolysis of the ester linkage, forming the corresponding carboxy compound, which was de-

void of biological activities. Since prolonged biological action is a prerequisite for antiatherosclerotic agents, further efforts were directed to the synthesis of derivatives of **1b** capable of providing adequate bioavailability without impairment of the pharmacological activities. This might be achieved by protection of the ester group of **1b** against hydrolytic biodegradation.

We first attempted to modify the ethyl ester (**1b**) to a bulky tertiary butyl ester. The acid (**1c**), obtained by hydrolysis and subsequent acetylation of **1a**, was reacted with isobutylene in the presence of conc. sulfuric acid for 10 hr at room temperature, providing the butyl



	R ₁	R ₂
1a	H	H
1b	C ₂ H ₅	H
1c	H	Ac
1d	<i>tert</i> -butyl	Ac
1e	<i>tert</i> -butyl	H
1f	isopropyl	H

Chart 1

ester (**1d**) in moderate yield. Alternatively, the latter compound could also be prepared by treatment of the above acid (**1c**) with thionyl chloride and subsequent refluxing of the resulting acid chloride with *tert*-butanol in the presence of dimethylaniline for 30 hr. Hydrolysis of **1d** with ethanolic potassium hydroxide at room temperature afforded the desired *tert*-butyl ester (**1e**). Unfortunately, biological testing of **1e** (inhibitory effects on platelet aggregation and edematous arterial reaction) showed less potent activities than those of the ester ethyl

1) Y. Eguchi, A. Sugimoto, and M. Ishikawa, *Chem. Pharm. Bull.*, **28**, 2763 (1980).

2) Location: 2-3-10 Surugadai, Kanda, Chiyoda-ku, Tokyo, Japan.

ester (**1b**). Furthermore, tests to assess the stability to alkaline solution or blood serum suggested that it was not a stable compound with adequate bioavailability.

Finally, we synthesized 7-ethoxycarbonyl-4-hydroxymethyl-6,8-dimethyl-1(2H)-phthalazinone (**6**), since the introduction of two methyl groups on sites adjacent to the ethyl ester of **1b** was expected to provide steric hindrance, rendering the compound less vulnerable to hydrolysis of the ethyl ester group. The synthesis of **6** was successfully carried out through the following pathways.

The Diels–Alder adduct³⁾ (**2a**) of ethyl isodehydroacetate and dimethyl acetylenedicarboxylate was hydrolyzed with alkaline solution to the phthalic acid (**2b**) and this in turn was treated with acetic anhydride to afford the phthalic anhydride (**3**). Treatment of the latter with excess malonic acid in pyridine at 80° to 85° for 6 hr afforded the 3-hydroxy-3-methylphthalide (**4**) in a yield of 60%. The structure of the phthalide (**4**) was determined by comparison of the final product (**6**) with a sample obtained through another, unambiguous synthetic route. The isomeric 5-ethoxycarbonyl-3-hydroxy-3,4,6-trimethylphthalide, assumed to be formed in about 20% yield, could not be crystallized, despite repeated column chromatography of the residue obtained from the mother liquor of the phthalide (**4**). Oxidation of **4** in an aqueous alkaline solution with potassium permanganate at room temperature, and subsequent treatment of the solution with hydrazine afforded the acid (**5a**), which in turn was esterified with ethanol and conc. sulfuric acid to provide the ester (**5b**) in 80% overall yield. Reduction of **5b** with sodium borohydride in ethanol gave the final product (**6**) in good yield; this was temporarily designated EG 626 (code number), and later phthalazinol.

The structure of **6** was determined by comparison with a sample prepared through the following synthetic route. Partial hydrolysis of the Diels–Alder adduct (**2a**) with 3% sodium hydroxide solution gave the monobasic acid (**7**) in nearly quantitative yield. Clearly the location of the carboxyl group of **7** should be the least hindered one, as depicted in Chart 2.

Treatment of **7** with thionyl chloride, and subsequent treatment of the resulting acid chloride with diazomethane in ethyl ether afforded the diazoketone (**8**). Decomposition of

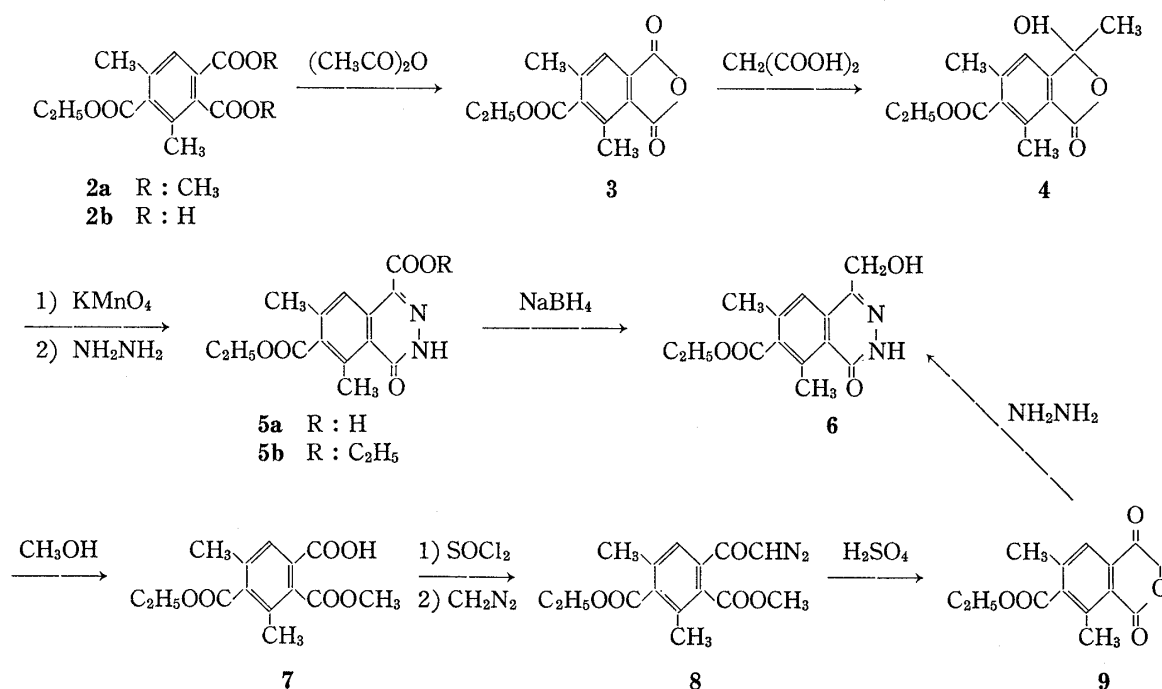


Chart 2

3) K. Alder and H.F. Rickert, *Chem. Ber.*, **70**, 1354 (1937).

the latter with dilute sulfuric acid gave the isochroman-1,4-dione (9). Refluxing of 9 with hydrazine hydrate in ethanolic solution for 2 hr and subsequent recrystallization of the product from ethanol-water afforded a compound which melted at 173–175°; the IR, Mass, and NMR spectra were identical with those of the sample obtained by the previous synthetic pathway.

Following the successful synthesis of 6, its chemical stability in alkaline solution was examined in a comparative study with 1b, 1f and 1e. Incubation of 1b and 1f with 0.3% sodium hydroxide solution resulted in nearly complete hydrolysis of 1b and 1f within 10 min, and while incubation of 1e led to partial hydrolysis, its half-life was approximately 1 hr and 30 min. In a test monitoring biodegradation, the compounds were incubated with rabbit serum; the results were compatible with those obtained in the alkaline hydrolysis test. In contrast to 1b, 1f and 1e, compound (6) was recovered unchanged in both experiments. These results are shown in Figs. 1 and 2.

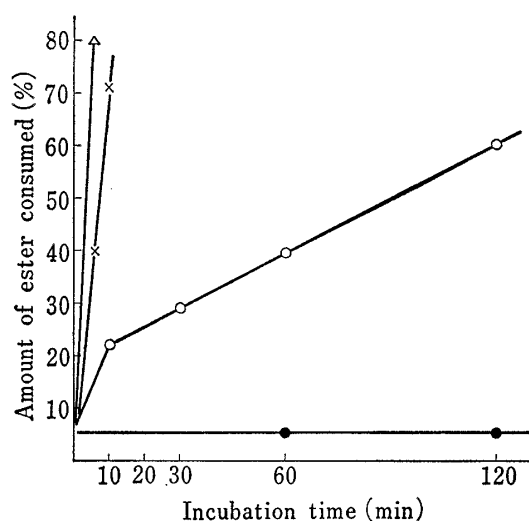


Fig. 1. Rate of Hydrolysis in 0.3% NaOH at 65°

Compounds: 5×10^{-6} mol/0.075 N NaOH (100 μ l)

○: 1e, △: 1b, ×: 1f, ●: EG 626.

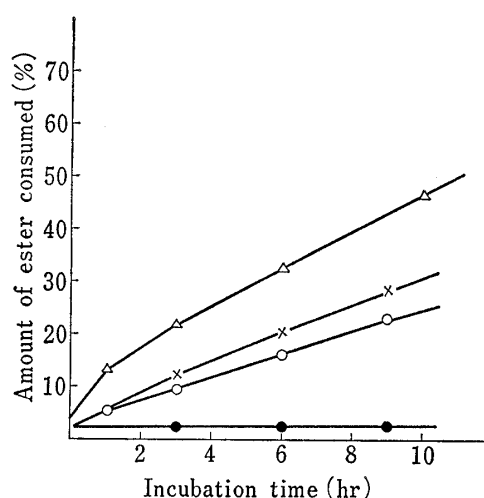


Fig. 2. Rate of Hydrolysis in Rabbit Serum at 35°

Compounds: 1.25×10^{-6} mol/Rabbit serum (100 μ l)

○: 1e, △: 1b, ×: 1f, ●: EG 626.

Next, the biological activities of 6 were examined. In inhibitory tests on platelet aggregation with rabbit platelet-rich plasma, using the optical density method of Born,⁴ 6 exhibited (at a final concentration of 1×10^{-4} M) a 68% inhibitory effect on the aggregation induced by 10 μ M ADP and 94% on that induced by 137 μ M arachidonic acid. The edematous arterial reaction⁵ induced by the intravenous administration of 10 μ g/kg of adrenaline was also strongly inhibited by 10 mg/kg of 6 given orally. Among this and related compounds which were tested, 6 showed the highest potency in the platelet aggregation and edematous arterial reaction tests. The higher biological activities of 6 compared to its prototype compound (1b) might be attributable to the increased lipophilicity of 6, but fixation of the oxygen atom of the carbonyl group of the compound in a perpendicular direction with respect to the plane of the phthalazinone nucleus might also contribute to better binding of the 6 molecule to receptor sites on target cells.

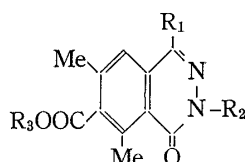
To clarify the structure-activity relationship, compounds closely related to 6 were prepared by conventional synthetic methods, and the biological activities were assayed. These results are summarized in Table I. Conversion of the ester moiety of 6 to the methyl ester

4) G.V. Born, *J. Physiol.* (London), **162**, 67P (1962).

5) T. Shimamoto and T. Sunaga, *Jap. Heart J.*, **3**, 581 (1962); T. Shimamoto, *J. Atheroscler. Res.*, **3**, 87 (1963).

(10) and isopropyl ester (11), which were prepared by the treatment of 12 with corresponding diazoalkanes, resulted in retention of a relatively high degree of activity, but the carboxyl compound (12) prepared by vigorous hydrolysis of 6 with alkali showed markedly decreased activity. Refluxing of 4 with hydrazine hydrate in ethanol afforded 13, and melting of 5a at 180—200° afforded the decarboxylated compound (14). Both compounds showed decreased activity, suggesting the pharmacological significance of the hydroxymethyl group of 6. Refluxing of 6 with N-bromosuccinimide in CCl₄ yielded a corresponding 4-formyl derivative, which in turn reacted with the Grignard reagent to give the secondary carbinol (15). The tertiary carbinol (16) was prepared from 5 by means of the Grignard reaction. Compounds 15 and 16, having a secondary hydroxymethyl group, exhibited decreased activity compared with 6. Refluxing of 6 with thionyl chloride and following the reaction of the product with nucleophilic reagents afforded 18 and 19. Among these compounds, together with the derivatives 20 and 21, no compound showed biological activities exceeding those of 6.

TABLE I.



No.	R ₁	R ₂	R ₃	Inhibition of platelet aggregation (%)		Inhibitory effect on EAR ^{a)}
				ADP (10 μM)	AA (137 μM)	
6	CH ₂ OH	H	Et	68	94	+++ ^{b)}
10	CH ₂ OH	H	Me	33	98	+
11	CH ₂ OH	H	CHMe ₂	47	98	++
12	CH ₂ OH	H	H	4	11	±
13	Me	H	Et	73	90	+
14	H	H	Et	30	80	—
15	CH(OH)Me	H	Et	57	87	+
16	C(OH)Me ₂	H	Et	5	52	+
18	CH ₂ CN	H	Et	65	92	—
19	CH ₂ NH ₂	H	Et	0	3	±
20	CH ₂ CH ₂ NH ₂	H	Et	13	9	±
21	CH ₂ CH ₂ OH	H	Et	31	59	+
22	CH ₂ OH	Me	Et	10	16	+

a) EAR (edematous arterial reaction) was induced by intravenous administration of 10 μg/kg of adrenaline.

b) +++: potent inhibition, ++: moderate inhibition, +: weak inhibition, —: no inhibition.

Investigations on the biological properties of 6 will next be described in brief.

Phthalazinol (6) had potent inhibitory effects on human platelet aggregation both in the primary and secondary stages, as induced by various aggregating agents such as ADP, arachidonic acid, adrenaline, and collagen.⁶⁾ The inhibitory effect of 6 on platelet aggregation may be a result of the inhibition of cyclic AMP phosphodiesterase. Reflecting the inhibitory effect on the edematous reaction of rabbit aorta, the compound was found to have inhibitory effects on atherosclerosis induced in rabbits by feeding a diet containing 1% cholesterol.⁷⁾ Based on experiments carried out with 6 and pyridinolcarbamate, Shimamoto proposed a new

6) T. Shimamoto, *Jap. Heart J.*, **16**, 76 (1975); T. Sano, T. Motomiya, H. Yamasaki, and T. Shimamoto, *Thromb. Haemostas.*, **37**, 329 (1977).

7) T. Shimamoto, *Blood Vessels.*, **15**, 170 (1978); M. Kobayashi, K. Moriya, T. Takahashi, M. Sakamoto, Y. Takashima, F. Numano, and T. Shimamoto, *Domyakukoka*, **5**, 233 (1977).

concept in the pathology of atherosclerosis. He postulated that the contraction of hyperactive endothelial cells is probably the key mechanism involved in atherogenesis, and that the anti-atherosclerotic effect of **6** is due to its relaxing properties in endothelial cells.⁸⁾

Concerning the biochemical mechanism underlying the pharmacological effects of **6**, this agent has a potent inhibitory effect on cyclic AMP phosphodiesterase.⁹⁾ As cyclic AMP plays an important physiological role, the potent inhibitory effect of **6** is of great interest. The pharmacological properties of the compound involving anti-platelet-aggregation could to some extent be due to its inhibitory effect on cyclic AMP phosphodiesterase. Secondly, it was found that **6** was a thromboxane A₂ antagonist,¹⁰⁾ without interfering with the synthesis of prostaglandins, including thromboxane A₂. Thromboxane A₂, a potent platelet aggregator and constrictor of blood vessels, was discovered in Sweden by Samuelsson's group¹¹⁾ and is assumed to be an important trigger or fatal events such as stroke, variant angina or myocardial infarction. Preventive properties of **6** against stroke and heart attack induced by thromboxane A₂ mixture were reported by Shimamoto.¹²⁾ In this connection, a paper¹³⁾ presented by Tanaka *et al.* described the potentiating activity of **6** on prostacyclin (PGI₂), a potent antiaggregator of platelets and dilator of blood vessels, which was discovered by Moncada *et al.*¹⁴⁾ Furthermore, interesting experiments in monkeys¹⁵⁾ have been reported by Alksne and Branson, in which the chronic vasospasm and arterial injury reaction produced by subarachnoid blood injection was entirely prevented by treatment with **6**.

Since **6** showed low toxicity in acute, subacute and long term toxicity tests in animals, clinical studies have been carried out in the neurological, angiological and cardiological fields.¹⁶⁾

Experimental

All melting points were determined in a capillary tube and are uncorrected. IR spectra were determined using a Hitachi model 285 spectrometer, UV spectra with a Hitachi model 323 spectrometer, and NMR spectra with a JEOL JUM-C-60HL machine. Mass spectra (MS) were recorded using a Hitachi RMU-7L spectrometer; in all cases direct sample insertion was carried out into the ion source. For spectroscopic data, the following abbreviations are used: d=doublet, m=multiplet, s=singlet, q=quintet, and t=triplet. Merck silica gel 60 was used for column chromatography.

4-Acetoxymethyl-7-tert-butoxycarbonyl-1(2H)-phthalazinone (1d)—A portion (1.2 g) of 4-acetoxymethyl-7-carboxy-1(2H)-phthalazinone (**1c**) was placed in a glass tube with 50 ml of dioxane, and the tube was cooled with dry ice-acetone. Isobutylene (12 ml) was introduced into the tube, and 4 ml of c. H₂SO₄ was added while stirring. After closing the tube, the contents were stirred for 10 hr at room temperature, then poured into 10% aq. Na₂CO₃ and extracted with EtOAc. The extract was washed with H₂O, dried over anhyd. MgSO₄ and concentrated. The residue was recrystallized from EtOH to give 760 mg of **1d**, mp 202–203°. *Anal.* Calcd for C₁₆H₁₈N₂O₅: C, 60.37; H, 5.70; N, 8.80. Found: C, 60.34; H, 5.73; N, 8.84. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1740, 1720, 1600. NMR (DMSO-*d*₆) δ : 1.62 (9H, s), 2.05 (3H, s), 5.28 (2H, s), 8.20 (2H, d, *d*, *J*=9 Hz, *J*=2 Hz), 8.75 (1H, d, *J*=2 Hz), 12.89 (1H, s).

- 8) T. Shimamoto, "Cardiovascular Disease," ed. by H.I. Russek, University Park Press., 1974, pp. 361–392; T. Shimamoto, "New Horizons in Cardiovascular Practice," ed. by H.I. Russek, *ibid.*, 1975, pp. 449–469; T. Shimamoto, H. Hidaka, K. Moriya, M. Kobayashi, T. Takahashi, and F. Numano, *Ann. N.Y. Acad. Sci.*, **275**, 266 (1976).
- 9) K. Adachi and F. Numano, *Jpn. J. Pharmacol.*, **27**, 97 (1977).
- 10) T. Shimamoto, Y. Takashima, M. Kobayashi, K. Moriya, and T. Takahashi, *Proc. Jpn. Acad.*, **52**, 591 (1976); S. Kaneko, Y. Takashima, M. Sakamoto, M. Nakajima, M. Shimizu, M. Ishikawa, Abstracts of Papers, The 99th Ann. Meeting of the Pharmac. Soci. of Japan, Sapporo, Aug. 1979.
- 11) M. Hamberg, J. Svensson, and B. Samuelsson, *Proc. Nat. Acad. Sci. USA.*, **72**, 2994 (1975).
- 12) T. Shimamoto, "Biochemical Aspects of Prostaglandins and Thromboxanes," ed. by N. Kharasch and J. Fried, Academic Press, 1977, pp. 189–197.
- 13) K. Tanaka, Y. Harada, and M. Katori, *Prostaglandins*, **17**, 235 (1979).
- 14) S. Moncada, R. Gryglewski, S. Banting, and J. Vane, *Nature*, **263**, 663 (1976).
- 15) M. Linder and J. Alksne, *Stroke.*, **9**, 472 (1978); J. Alksne and P. Branson, *ibid.*, **10**, 638 (1979).
- 16) T. Shimamoto *et al.*, Frontiers of Internal Medicine, 12th Int. Congr. Internal Med., Tel Aviv, 1974, Karger, Basel., 110 (1975); T. Shimamoto, H. Murase, and F. Numano, *Mech. Ageing Develop.*, **5**, 241 (1976).

Alternatively, 1d was Also Prepared by the Following Procedure.—A mixture of 2 g of **1c** and 20 ml of freshly distilled SOCl_2 was gently refluxed at 70–80° for 3 hr. Excess SOCl_2 was removed under reduced pressure, and 10 ml of anhyd. benzene was added to the residue. The benzene was removed under reduced pressure, and the residue was dissolved in 30 ml of abs. *tert*-butanol. Next, 2.2 ml of dimethylaniline was added with stirring and the mixture was refluxed for 30 hr in an apparatus protected from moisture. The solvent was evaporated off, and the resulting residue was mixed with aq. Na_2CO_3 solution and extracted with EtOAc. The EtOAc extract was washed successively with dil. HCl and H_2O , dried over anhyd. MgSO_4 and concentrated. The residue obtained was chromatographed on a silica gel column to give 1.2 g **1d**, melting at 202–203°. The spectroscopic data were identical with those of samples obtained by the foregoing procedure.

7-tert-Butoxycarbonyl-4-hydroxymethyl-1(2H)-phthalazinone (1e)—Compound **1d** (3.4 g) was added to a solution of 1.8 g of KOH in 150 ml of EtOH. The mixture was stirred at room temperature for 3 hr, saturated with carbon dioxide, and concentrated under reduced pressure, then the concentrate was poured into H_2O and extracted with EtOAc. The extract was dried over anhyd. MgSO_4 and concentrated. The residue was recrystallized from EtOH–*n*-hexane to give 2.8 g of **1e**, mp 270–272°. *Anal.* Calcd for $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_4$: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.86; H, 5.81; N, 10.09. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3500, 1730, 1660. MS m/e : 276 (M^+), 220. NMR ($\text{DMSO}-d_6$) δ : 1.61 (9H, s), 4.71 (2H, s), 8.27 (2H, m), 8.75 (1H, d, $J=2$ Hz), 12.79 (1H, s).

4-Ethoxycarbonyl-3,5-dimethylphthalic Acid Anhydride (3)—A solution of 4-ethoxycarbonyl-3,5-dimethylphthalic acid³⁾ in acetic acid anhydride was refluxed for 2 hr, then excess acetic acid anhydride was evaporated off under reduced pressure to afford an oil, which solidified on standing. The solid was recrystallized from ether–*n*-hexane to give **3** in 90% yield. mp 80–81°. *Anal.* Calcd for $\text{C}_{13}\text{H}_{12}\text{O}_5$: C, 62.90; H, 4.87. Found: C, 62.95; H, 4.77. MS m/e : 248 (M^+). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1840, 1770, 1750. NMR (CDCl_3) δ : 1.43 (3H, t, $J=7$ Hz), 2.50 (3H, s), 2.68 (3H, s), 4.48 (2H, t, $J=7$ Hz), 7.72 (1H, s).

6-Ethoxycarbonyl-3-hydroxy-3,5,7-trimethylphthalide (4)—A reaction mixture consisting of 7.8 g of **3**, 6.5 g of malonic acid dried by heating at 100° for 10 hr, and 7 ml of abs. pyridine was heated at 80–85° for 15 hr. After cooling, 10% aq. HCl was added, and the mixture was extracted with CHCl_3 . The organic layer was separated, washed with H_2O , and extracted with saturated NaHCO_3 . The aqueous phase was acidified with dil. HCl, and extracted with EtOAc. The EtOAc extract was dried over anhyd. MgSO_4 and concentrated. The residue was recrystallized from ether–*n*-hexane to give 5.4 g of **4**, mp 118–120°. *Anal.* Calcd for $\text{C}_{14}\text{H}_{16}\text{O}_5$: C, 63.62; H, 6.10. Found: C, 63.52; H, 6.14. NMR (CDCl_3) δ : 1.38 (3H, t, $J=7$ Hz), 2.36 (3H, s), 2.42 (3H, s), 4.31 (2H, q, $J=7$ Hz), 7.03 (1H, s).

The mother liquor of **4** was concentrated to give an oily residue, which was chromatographed on a silica gel column. After repeated chromatography, eluates with benzene– CHCl_3 (5:1) afforded a yellow oily residue, which showed a single spot on thin-layer chromatography. The IR, MS, and NMR spectra closely resembled those of **4**.

4,7-Bis(ethoxycarbonyl)-6,8-dimethyl-1(2H)-phthalazinone (5b)—A solution of 1.58 g of KMnO_4 in 80 ml of H_2O was added dropwise to a stirred solution of 1.32 g of **4** in 100 ml of 1% aqueous KOH solution. During addition of KMnO_4 , the temperature of the reaction mixture was maintained at 20–25° by external cooling. Stirring was continued for an additional 1.5 hr, the precipitated MnO_2 was filtered off, and the filtrate was saturated with carbon dioxide. Next, 5 ml of 80% hydrazine hydrate was added to the filtrate, and the solution was then heated at 70–80° for 2 hr. After cooling, the solution was acidified with dil. HCl, and the resulting precipitate was filtered and recrystallized from MeOH to give 1.1 g of 4-carboxy-7-ethoxycarbonyl-6,8-dimethyl-1(2H)-phthalazinone (**5a**), mp 216–218°. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 220, 298, 310, 322. The compound (**5a**) was refluxed with abs. EtOH and c. H_2SO_4 and work-up in the usual manner afforded **5b** mp 159–161° in 90–85% yield. *Anal.* Calcd for $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_5$: C, 60.32; H, 5.70; N, 8.80. Found: C, 60.28; H, 5.74; N, 8.77. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 220, 240, 299, 308, 323.

7-Ethoxycarbonyl-4-hydroxymethyl-6,8-dimethyl-1(2H)-phthalazinone (6)—A stirred solution of 500 mg of NaBH_4 in 200 ml of EtOH was treated portion-wise with 1.4 g of **5b** at –5–0°, and then a solution of 700 mg of CaCl_2 in 200 ml of EtOH was added dropwise. Stirring was continued for an additional 3 hr at the same temperature, and the reaction mixture was allowed to stand overnight at room temperature. The mixture was concentrated under reduced pressure, diluted with H_2O , and the pH was adjusted to 5 with acetic acid. The resulting precipitate was filtered off and recrystallized from EtOH– H_2O to give 1.3 g of **6**, mp 173–175°. *Anal.* Calcd for $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_4$: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.78; H, 5.80; N, 10.34. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 1730, 1650, 1600. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 218, 259 (8000), 292 (6200), 309 (5100), 321 (3800). NMR ($\text{DMSO}-d_6$) δ : 1.32 (3H, t, $J=7$ Hz), 2.32 (3H, s), 2.74 (3H, s), 4.40 (2H, q, $J=7$ Hz), 4.63 (2H, d, $J=5$ Hz), 5.38 (1H, broad), 7.82 (1H, s), 12.38 (1H, s).

4-Ethoxycarbonyl-2-methoxycarbonyl-3,5-dimethylbenzoic Acid (7)—A mixture consisting of 5 g of the Diels-Alder adduct (**2a**), 10 ml of 10% aq. KOH, and 50 ml of MeOH was refluxed for 4 hr. The solution was concentrated to 1/4 of the initial volume, diluted with H_2O , and the pH was adjusted to approximately 3–4 with dil. HCl. The resulting crystalline precipitate was filtered off, washed with H_2O , and air-dried to give 4.6 g of pure crystals of **7**, obtained by recrystallization from benzene–*n*-hexane. mp 103–105°. *Anal.* Calcd for $\text{C}_{14}\text{H}_{16}\text{O}_6$: C, 59.99; H, 5.75. Found: C, 59.87; H, 5.81. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3000, 1740, 1700.

7-Ethoxycarbonyl-6,8-dimethyl-isochroman-1,4-dione (9)—The compound **7** was refluxed gently with SOCl_2 , and excess SOCl_2 was distilled off under reduced pressure. The resulting residue was treated with an ethereal solution of excess CH_2N_2 . After evolution of N_2 gas, the solvent was distilled off and the residue thus obtained was chromatographed on a silica gel column. Concentration of the benzene- CHCl_3 (5:1) eluate and recrystallization from ether-*n*-hexane gave **8**, mp 78–79°, IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 2160, 1730. The latter compound (**8**) was added to a stirred mixture consisting of 15% aqueous solution of 2 ml of H_2SO_4 and 10 ml of dioxane at room temperature. The whole was stirred for 2 hr, and then the temperature of the solution was raised to 80–90° and this was maintained for 1 hr. The mixture was allowed to cool to room temperature, diluted with H_2O , and extracted with EtOAc. The extract was concentrated, and the residue was recrystallized from EtOH to afford **9** in 53% yield. mp 142–144°. *Anal.* Calcd for $\text{C}_{14}\text{H}_{14}\text{O}_5$: C, 64.11; H, 5.38. Found: C, 64.08; H, 5.41. NMR (CDCl_3) δ : 1.40 (3H, t, $J=7$ Hz), 2.39 (3H, s), 2.68 (3H, s), 4.33 (2H, q, $J=7$ Hz), 4.88 (2H, s), 7.72 (1H, s).

Alternative Preparation of 7-Ethoxycarbonyl-4-hydroxymethyl-6,8-dimethyl-1(2H)-phthalazinone (6)—A solution of 2 g of **9** and 4 ml of 80% hydrazine hydrate in 50 ml of EtOH was refluxed for 2 hr, then concentrated under reduced pressure, diluted with H_2O , and made slightly acidic with dil. HCl. The resulting precipitate was filtered off and recrystallized from EtOH- H_2O to give 1.2 g of **6**, mp 173–175°. The spectroscopic data were identical with those of a sample obtained by the foregoing procedure.

4-Hydroxymethyl-7-methoxycarbonyl-6,8-dimethyl-1(2H)-phthalazinone (10)—Esterification of 0.7 g of 7-carboxy-4-hydroxymethyl-6,8-dimethyl-1(2H)-phthalazinone (**12**) (mp 282–284° after recrystallization from MeOH), obtained by hydrolysis of **6** in 10% aq. NaOH at 80° for 10 hr, with an excess of CH_2N_2 in ethereal solution under H_2O cooling gave 0.3 g of colorless needles on recrystallization from MeOH, mp 203–205°. *Anal.* Calcd for $\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}_4$: C, 59.53; H, 5.38; N, 10.68. Found: C, 59.42; H, 5.43; N, 10.75. NMR ($\text{DMSO}-d_6$) δ : 2.31 (3H, s), 2.75 (3H, s), 3.83 (3H, s), 4.60 (2H, q, $J=5$ Hz), 5.31 (1H, t, $J=5$ Hz), 7.77 (1H, s), 12.27 (1H, s).

4-Hydroxymethyl-7-isopropoxyxycarbonyl-6,8-dimethyl-1(2H)-phthalazinone (11)—A water-cooled suspension of 0.7 g of **12** in 20 ml of ether was treated dropwise, with stirring, with an ethereal solution of excess 2-diazopropane prepared by the procedure described in *Org. Syn.*, **50**, 27 (1970). After stirring for an additional 2 hr, the solvent was evaporated off, and the residue was recrystallized from EtOAc-*n*-hexane to give 0.4 g of colorless needles (**11**), mp 177–179°. *Anal.* Calcd for $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_4$: C, 62.05; H, 6.25; N, 9.65. Found: C, 61.89; H, 6.21; N, 9.71. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 1730, 1660. NMR ($\text{DMSO}-d_6$) δ : 1.32 (3H, s), 1.41 (3H, s), 2.38 (3H, s), 2.75 (3H, s), 4.25 (1H, broad), 4.70 (2H, s), 5.27 (1H, m), 7.75 (1H, s), 11.95 (1H, s).

7-Ethoxycarbonyl-4,6,8-trimethyl-1(2H)-phthalazinone (13)—A solution of **4** and excess 80% hydrazine hydrate in EtOH was heated at 80° for 1.5 hr. Usual work-up of the mixture afforded **13**, mp 190–191° (recryst. from MeOH) in 90% yield. *Anal.* Calcd for $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_3$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.56; H, 6.27; N, 11.83. MS m/e : 260 (M^+), 231 ($\text{M}^+ - \text{C}_2\text{H}_5$). NMR (CDCl_3) δ : 1.39 (3H, t, $J=7$ Hz), 2.38 (3H, s), 2.47 (3H, s), 2.81 (3H, s), 4.42 (2H, q, $J=7$ Hz), 7.24 (1H, s), 10.60 (1H, s).

7-Ethoxycarbonyl-6,8-dimethyl-1(2H)-phthalazinone (14)—Crystals (700 mg) of **5a** were heated in a metal bath at 180–200° for 15 min, until the evolution of gas had subsided. The brown solid mass thus obtained was chromatographed on a silica gel column to give 360 mg of **14**, mp 171–172° (recryst. from EtOAc). *Anal.* Calcd for $\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.44; H, 5.69; N, 11.25. MS m/e : 246 (M^+), 217 ($\text{M}^+ - \text{C}_2\text{H}_5$), 201 ($\text{M}^+ - \text{OC}_2\text{H}_5$). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 257, 292, 309, 322. NMR (CDCl_3) δ : 1.50 (3H, t, $J=7$ Hz), 2.41 (3H, s), 2.90 (3H, s), 4.45 (2H, q, $J=7$ Hz), 7.40 (1H, s), 8.05 (1H, s), 11.48 (1H, s).

7-Ethoxycarbonyl-4-(1-hydroxyethyl)-6,8-dimethyl-1(2H)-phthalazinone (15)—A stirred mixture of 4.0 g of **6**, 3.0 g of NBS and 40 mg of benzoylperoxide in 100 ml of dry CCl_4 was refluxed at 70° for 2 hr. After cooling, the precipitate was filtered off, washed with H_2O , and then recrystallized from acetone- H_2O to give 3.2 g of 7-ethoxycarbonyl-4-formyl-6,8-dimethyl-1(2H)-phthalazinone, mp 213–214°. *Anal.* Calcd for $\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}_4$: C, 61.31; H, 5.15; N, 10.21. Found: C, 61.29; H, 5.11; N, 10.13. NMR ($\text{DMSO}-d_6$) δ : 1.25 (3H, t, $J=7$ Hz), 2.25 (3H, s), 2.62 (3H, s), 4.27 (2H, q, $J=7$ Hz), 8.61 (1H, s), 9.73 (1H, s), 13.27 (1H, s). An ethereal solution of 1.3 g of the formyl compound described above was treated dropwise with the Grignard reagent prepared from 1.5 g of methyl bromide and 400 mg of magnesium in ether, with stirring. The mixture was refluxed for 3 hr, decomposed with dil. H_2SO_4 and then extracted twice with ether. The extracts were combined, washed with H_2O , dried over anhyd. Na_2SO_4 and then concentrated. The residue was purified by column chromatography on silica gel employing EtOAc-*n*-hexane (1:1) as an eluent to give 800 mg of **15** in the form of colorless needles, mp 179–180° (recryst. from EtOH-*n*-hexane). *Anal.* Calcd for $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_4$: C, 62.05; H, 6.25; N, 9.65. Found: C, 62.18; H, 6.24; N, 9.72. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1735, 1650. NMR (CDCl_3) δ : 1.43 (3H, t, $J=7$ Hz), 1.59 (3H, d, $J=3$ Hz), 2.43 (3H, s), 2.80 (3H, s), 3.97 (1H, d, $J=3$ Hz), 4.46 (2H, q, $J=7$ Hz), 5.23 (1H, m), 7.71 (1H, s), 11.40 (1H, broad).

7-Ethoxycarbonyl-4-(1-hydroxy-1-methylethyl)-6,8-dimethyl-1(2H)-phthalazinone (16)—A stirred ethereal solution of 1.4 g of **5b** was treated dropwise with the Grignard reagent prepared from 1.5 g of methyl bromide and 400 mg of magnesium in ether. The mixture was refluxed for 10 hr. The reaction mixture was worked up in a conventional manner. The oily residue thus obtained was purified by column chromatography on silica gel. From the EtOAc-*n*-hexane (1:1) eluate, 210 mg of 4-acetyl-7-ethoxycarbonyl-6,8-dimethyl-1(2H)-phthalazinone was obtained as a by-product; this melted at 169–171° (recryst. from *n*-

hexane). Further elution with EtOAc-*n*-hexane (3:1) gave 890 mg of **16**, mp 162–163° (recryst. from EtOAc-*n*-hexane). *Anal.* Calcd for $C_{16}H_{20}N_2O_4$: C, 63.14; H, 6.62; N, 9.21. Found: C, 63.20; H, 6.58; N, 9.18. MS *m/e*: 304 (M^+), 289 ($M^+ - CH_3$), 275 ($M^+ - C_2H_5$). IR $\nu_{\max}^{KBr}$ cm^{-1} : 1735, 1715, 1660. NMR ($CDCl_3$) δ : 1.42 (3H, t, $J=7$ Hz), 1.73 (6H, s), 2.40 (3H, s), 2.76 (3H, s), 4.23 (3H, t, $J=7$ Hz), 4.48 (2H, q, $J=7$ Hz), 8.47 (1H, s), 11.20 (1H, s).

4-Chloromethyl-7-ethoxycarbonyl-6,8-dimethyl-1(2H)-phthalazinone (17)—A mixture of 5 g of **6** and 20 ml of $SOCl_2$ was refluxed for 2 hr. After removing excess $SOCl_2$ *in vacuo*, the residual solid was agitated with H_2O and stirred for 30 min. The solid was collected by filtration, washed with H_2O , and then recrystallized from acetone to give 4.1 g of **17** in the form of colorless needles, mp 187–189°. *Anal.* Calcd for $C_{14}H_{15}ClN_2O_3$: C, 57.04; H, 5.09; N, 9.50. Found: C, 57.10; H, 5.11; N, 9.71. NMR ($DMSO-d_6$) δ : 1.25 (3H, t, $J=7$ Hz), 2.30 (3H, s), 2.65 (3H, s), 4.25 (2H, q, $J=7$ Hz), 4.80 (2H, s), 7.65 (1H, s), 12.25 (1H, s).

4-Cyanomethyl-7-ethoxycarbonyl-6,8-dimethyl-1(2H)-phthalazinone (18)—Compound **17** (3.0 g) was added to a stirred solution of 1.5 g of NaCN in 25 ml of aq. MeOH (containing 5 ml of H_2O). The mixture was warmed at 60–70° for 1 hr and then concentrated under reduced pressure, and the concentrate was diluted with H_2O . The resulting precipitate was filtered off, air-dried, and dissolved in MeOH. After removal of insoluble material by filtration, and the filtrate was concentrated and allowed to stand at room temperature. The precipitate that formed was recrystallized from MeOH to give 1.1 g of **18**, mp 193–194°. *Anal.* Calcd for $C_{15}H_{15}N_3O_3$: C, 63.65; H, 5.30; N, 14.73. Found: C, 63.71; H, 5.23; N, 14.68. IR $\nu_{\max}^{KBr}$ cm^{-1} : 2250, 1730, 1640. NMR ($CDCl_3$) δ : 1.45 (3H, t, $J=7$ Hz), 2.48 (3H, s), 2.87 (3H, s), 3.98 (2H, s), 4.45 (2H, q, $J=7$ Hz), 7.40 (1H, s), 11.00 (1H, s).

4-Aminomethyl-7-ethoxycarbonyl-6,8-dimethyl-1(2H)-phthalazinone (19)—A mixture consisting of 1 g of **17**, 1 g of potassium phthalimide, and 10 ml of dimethylformamide was heated at 90–95° for 2 hr. The mixture was poured into ice-water, and the resulting precipitate was collected, washed with H_2O , and recrystallized from EtOH to afford 1.12 g of 7-ethoxycarbonyl-6,8-dimethyl-4-(N-phthaloylaminomethyl)-1(2H)-phthalazinone. The crystals (400 mg) thus obtained were dissolved in a mixture of 5 ml of 2*N* HCl and 5 ml of EtOH, and the solution was refluxed for 2 hr. After cooling, the precipitate was filtered off and washed with H_2O , and the filtrate was evaporated to dryness. The resultant residue was recrystallized from EtOH to afford 180 mg of the monohydrochloride of **19**. mp >200°. *Anal.* Calcd for $C_{14}H_{18}ClN_3O_3$: C, 53.94; H, 5.82; N, 13.48; Cl, 11.37. Found: C, 53.93; H, 5.63; N, 13.69; Cl, 11.41.

4-(2-Aminoethyl)-7-ethoxycarbonyl-6,8-dimethyl-1(2H)-phthalazinone (20)—A suspension of 200 mg of **18** and 80 mg of Pd-C (10%) in 12 ml of glacial acetic acid was hydrogenated under atmospheric pressure of H_2 . The absorption of the theoretical amount of H_2 was completed within 2 hr. After removal of the catalyst by filtration, the solvent was evaporated off under reduced pressure to give an oil, which solidified on standing. The solid was recrystallized from EtOAc to afford 80 mg of **20**, mp 143–144°. *Anal.* Calcd for $C_{15}H_{19}N_3O_3$: C, 62.26; H, 6.62; N, 14.52. Found: C, 62.19; H, 6.58; N, 14.60. MS *m/e*: 260 ($M^+ - C_2H_5$), 244 ($M^+ - OC_2H_5$). IR $\nu_{\max}^{KBr}$ cm^{-1} : 3400, 2900, 1720, 1650. UV $\lambda_{\max}^{EtOH}$ nm: 260, 295, 309, 320. NMR ($CDCl_3$) δ : 1.40 (3H, t, $J=7$ Hz), 2.40 (3H, s), 2.80 (3H, s), 3.05 (4H, broad), 4.45 (2H, q, $J=7$ Hz), 7.50 (1H, s).

7-Ethoxycarbonyl-4-(2-hydroxyethyl)-6,8-dimethyl-1(2H)-phthalazinone (21)—An ice-salt cooled solution of 700 mg of **20** in 10 ml of glacial acetic acid was treated dropwise with a solution of 500 mg of $NaNO_2$ in 2 ml of H_2O with vigorous stirring. When the evolution of the N_2 gas was complete, the reaction mixture was diluted with H_2O , made slightly alkaline with dil. K_2CO_3 , and then extracted with $CHCl_3$. The organic layer was separated, dried over anhyd. Na_2SO_4 and concentrated to give an oil, which showed two spots on thin layer chromatography. As the upper spot on the thin-layer chromatogram was assumed to be an acetylated product, the oil was again dissolved in a mixture of 4 ml of 10% aq. KOH and 20 ml of MeOH, and refluxed for 1 hr. The solvent was concentrated, and the concentrate was diluted with H_2O , acidified with dil. HCl, and then extracted with $CHCl_3$. The extract was washed with H_2O , dried over anhyd. $MgSO_4$ and concentrated to give an oil, which solidified while in the refrigerator. The solid was collected and recrystallized from EtOAc-*n*-hexane to afford 270 mg of **21**, mp 143–145°. *Anal.* Calcd for $C_{15}H_{18}N_2O_4$: C, 62.05; H, 6.25; N, 9.65. Found: C, 62.03; H, 6.19; N, 9.58. MS *m/e*: 290 (M^+), 261 ($M^+ - C_2H_5$), 245 ($M^+ - OC_2H_5$). IR $\nu_{\max}^{KBr}$ cm^{-1} : 3400, 1730, 1640. NMR ($CDCl_3$) δ : 1.40 (3H, t, $J=7$ Hz), 2.41 (3H, s), 2.79 (3H, s), 3.15 (2H, t, $J=5$ Hz), 4.10 (2H, t, $J=5$ Hz), 4.45 (2H, q, $J=7$ Hz), 7.45 (1H, s), 11.00 (1H, s).

7-Ethoxycarbonyl-4-hydroxymethyl-2,6,8-trimethyl-1(2H)-phthalazinone (22)—A mixture consisting of 500 mg of **6**, 3 ml of 10% NaOH, 1 ml of CH_3I , and 20 ml of EtOH was refluxed for 1.5 hr. After cooling, the reaction mixture was concentrated to 1/4 of the initial volume under reduced pressure. The concentrate was diluted with 20 ml of H_2O , acidified with dil. HCl, and then extracted with $CHCl_3$. The organic layer was washed with H_2O , dried over anhyd. $MgSO_4$ and concentrated, giving an oil. As the oil showed two spots on thin layer chromatography, the preparation was fractionated by column chromatography on silica gel. From the benzene-EtOAc (5:1) eluate, 80 mg of 7-ethoxycarbonyl-4-methoxymethyl-2,6,8-trimethyl-1(2H)-phthalazinone was obtained as by-product; this melted at 105–106° (recryst. from ether-*n*-hexane). Further elution with benzene-EtOAc (5:2) gave 320 mg of **22**, mp 144–145° (recryst. from EtOAc-*n*-hexane). *Anal.* Calcd for $C_{15}H_{18}N_2O_4$: C, 62.05; H, 6.25; N, 9.65. Found: C, 62.00; H, 6.23; N, 9.62. MS *m/e*: 290 (M^+), 261 ($M^+ - C_2H_5$), 245 ($M^+ - OC_2H_5$). NMR ($CDCl_3$) δ : 1.45 (3H, t, $J=7$ Hz), 2.40 (3H, s), 2.80 (3H, s), 3.40 (1H, t, $J=5$ Hz), 3.70 (3H, s), 4.45 (2H, q, $J=7$ Hz), 4.90 (2H, d, $J=5$ Hz), 7.55 (1H, s).

Stability in Alkaline Solution—A solution of 1.25×10^{-4} mol of a test compound in 500 μ l of DMSO was prepared. A 20 μ l aliquot of the solution (5.0×10^{-6} mol of the test compound) was placed in a test tube, and 10 μ l of DMSO and 100 μ l of 0.3% NaOH solution (7.5×10^{-6} mol) were added. The samples thus prepared were then incubated at 65° in a thermostated bath. Samples were taken from the bath at appropriate intervals, and 50 μ l of satd. aq. NaCl and 0.5 ml of EtOAc were added to each. The mixture was shaken vigorously on a vortex jar mixer for 2 min to extract the test compound remaining in the solution, and then the EtOAc layer was separated. The aqueous phase was re-extracted with 0.5 ml of EtOAc in the same manner, except that the shaking time was 1 min. The combined EtOAc extracts were evaporated to dryness, and the resulting residue was dissolved in EtOH for measurement of the optical density with a spectrophotometer. The quantity of the test compound was calculated by the conventional procedure.

Optical Data for the Test Compounds—1b) UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 252(6800), 262(6700), 306(7200). 1f) UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 251(7400), 261(7300), 307(7900). 1e) UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 252(6700), 261(6600), 306(7200). 6) UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 259(8000), 292(6200), 309(5100), 321(3800).

Stability in Rabbit Serum—Rabbits were anesthetized with pentobarbital sodium and blood samples collected through a cannula inserted into the carotid artery were allowed to stand at 4° for 1 hr. Serum was obtained by centrifugation for 20 min at 3000 rpm. Meanwhile, a solution of 1.25×10^{-5} mol of the test compound in 1000 μ l of DMSO was prepared. One ml of the serum obtained above was added to a 10 μ l aliquot (1.25×10^{-6} mol of the test compound), and the mixture was incubated at 35° in a thermostated bath. The incubated mixture was removed from the bath and after the addition of 2 drops of 0.3% NaOH solution, the mixture was extracted twice with 2 ml each of EtOAc by vigorous shaking on a vortex jar mixer for 2 min. The organic layer was separated, and the aqueous phase was re-extracted with 1 ml of EtOAc in the same manner, except that the shaking time was 1 min. The combined EtOAc extracts were evaporated to dryness, and the resulting residue was dissolved in EtOH for measurement of the optical density, using a spectrophotometer. The quantity of the test compound was calculated by conventional procedures.

Preparation of Rabbit Platelet-rich Plasma (PRP)—Rabbits were anesthetized with ethyl ether and blood samples were collected through a catheter inserted into the carotid artery. The samples were citrated with 3.8% aqueous sodium citrate solution (1 ml of citrate/9 ml of blood) and red blood cells were precipitated by centrifugation for 15 min at 150 g at room temperature. The supernatant thus obtained was used as PRP.

Platelet Aggregation Test—The optical density method of Born⁹⁾ was used to assess the ability of test compounds to inhibit platelet aggregation, as induced by aggregating agents. A silicone-treated cuvette containing 0.435 ml of the PRP sample was placed in an aggregometer (SIENCO, dual sample aggregation meter, model DP-274E) set at 37° and 1200 rpm, and a solution of the test compound (1×10^{-4} M final concentration) in 2.5 μ l of DMSO and a siliconized stirring bar were added. After preincubation for 3 min, 10 μ l of an aqueous solution of ADP ($10 \mu\text{M}$ final concentration) or 10 μ l of a aqueous solution of AA ($137 \mu\text{M}$ final concentration) was added to induce platelet aggregation. Inhibition of platelet aggregation by a test compound was calculated by dividing the maximum deflection in the optical density curve by that observed with the control (2.5 μ l of DMSO alone) and then multiplying by 100. Each compound was tested three times and the inhibitory percentage shown in Table I is the average value obtained from the three experiments.

Inhibitory Test on Edematous Arterial Reaction—Test compounds at doses of 10 mg/kg were administered orally to white New Zealand adult rabbits weighing 2.5 to 3 kg. The rabbits were challenged 1 hr after the administration of test compounds by intravenous administration of adrenaline at a dose of 10 $\mu\text{g/kg}$. The rabbits were sacrificed 30 min after the challenge, and the thoracic aortae were quickly excised, fixed in 5% glutaraldehyde solution, and stained with hematoxylin and eosin. The histochemical specimens thus prepared were examined microscopically. Assessment of the inhibitory effect of these test compounds was made by comparing the degree of edematous changes with the effects seen in placebo (potato starch)-treated rabbits. Each compound was tested three times and the inhibitory effect shown in Table I is the mean value obtained from the three experiments.

Acknowledgement We thank Yoshimi Takashima and Dr. Shigeru Kaneko for doing the platelet aggregation tests, Masahiko Kobayashi and Kinya Moriya for help with the edematous reaction tests, and M. Ohara, Kyoto University, for help in revising the manuscript.