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Studies on Antiatherosclerotic Agents. VIII.[1] Synthesis and Structure-Activity Relationship of 4-Hydroxymethyl-1(2H)-phthalazinone Derivatives

YUKUO EGUCHI, AKIKO SUGIMOTO, and MASAYUKI ISHIKAWA

Institute for Medical and Dental Engineering, Tokyo Medical and Dental University[2]

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4-Hydroxymethyl-1(2H)-phthalazinone derivatives were synthesized and assayed for their inhibitory effects on platelet aggregation and edematous arterial reaction. Among the tested compounds, 7-ethoxycarbonyl-4-hydroxymethyl-1(2H)-phthalazinone showed the highest potency in these pharmacological tests. The structure-activity relationship of phthalazinone derivatives and related compounds is discussed.

Keywords——structure-activity relationship; antithrombotic agent; antiatherosclerotic agent; inhibitor of platelet aggregation; inhibitor of edematous arterial reaction; 7-ethoxycarbonyl-4-hydroxymethyl-1(2H)-phthalazinone; cyclic AMP phosphodiesterase inhibitor; relaxing agent for hyperactive arterial endothelial cells

The current trend in work on the chemotherapy of atherosclerosis is to develop compounds which will regulate cholesterol and triglyceride metabolism and thereby reduce the risk of vascular-related disorders, such as atherosclerosis, angina pectoris, coronary thrombosis and cerebral accidents.

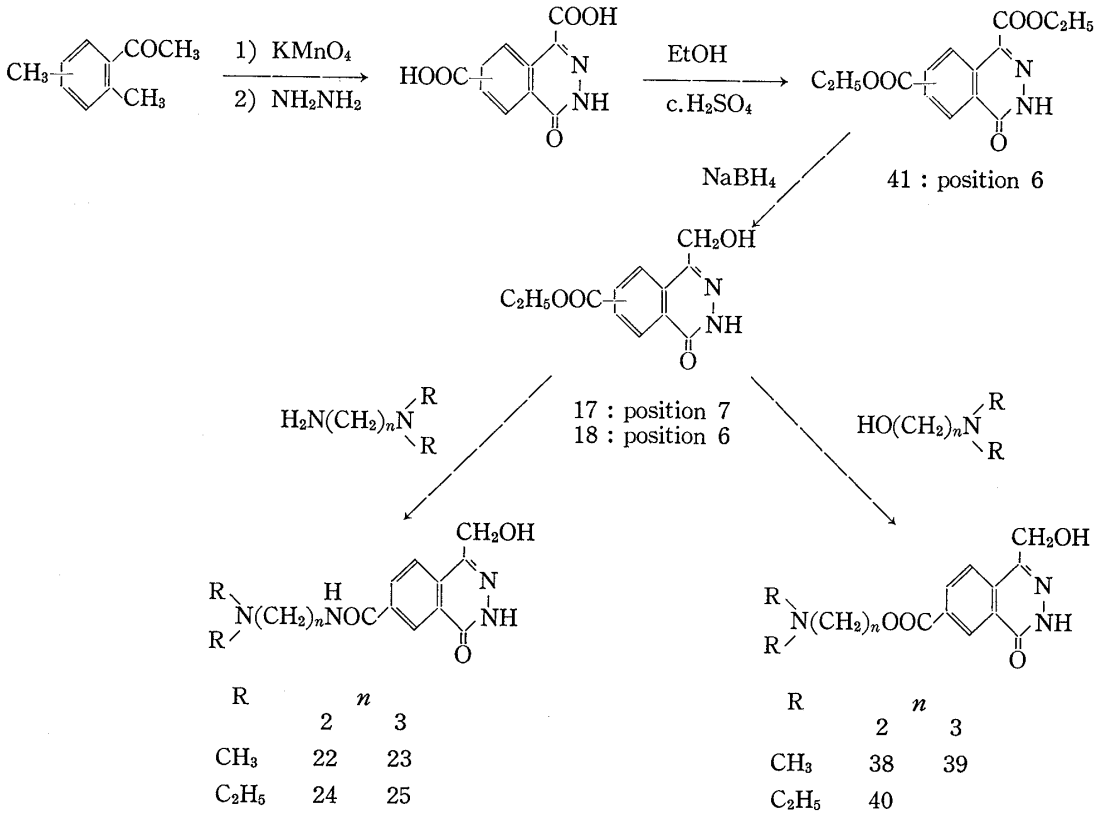
Since the discovery of clofibrate in 1962 by Thorpe,[3] this compound has been used as an hypolipidemic agent in many industrialized nations, and other drugs affecting lipid metabolism have been available for many years. Nevertheless, an epidemiologic study-the Coronary Drug Project,[4] involving over 5000 patients given a placebo or treated with clofibrate, nicotinic acid, D-thyroxine, or estrogens-did not provide positive results. Thus, the preventive effect of drugs affecting lipid metabolism is open to question, and the pathogenesis of atherosclerosis and the role of plasma lipids in the development of the atherosclerotic lesions remain to be elucidated.

In 1962 Shimamoto and collaborators[5] found that oral administrations of atherogenic substances such as cholesterol, animal fats or saturated fatty acids, or the intravenous administration of adrenaline or angiotensin II, induced edematous changes in the arterial wall, and such changes were assumed to be an early stage in the development of atherosclerosis. They termed the biological response on the arterial wall "edematous arterial reaction," and Shimamoto and one of the present authors (M. I.) began to search for a compound capable of preventing the edematous arterial reaction, assuming that such a compound could be of great value as an antiatherosclerotic agent. In 1963 2,6-pyridinedimethanol bis(N-methylcarbamate) (pyridinolcarbamate) was found to have inhibitory effect both on the edematous arterial reaction and on experimental atherosclerosis induced by cholesterol loading in rabbits.[6]

1) M. Ishikawa and Y. Eguchi, *Reports Inst. Med. Dent. Eng.*, **11**, 55 (1977).
2) Location: *2-3-10 Surugadai, Kanda, Chiyoda-ku, Tokyo, Japan.*
3) J.M. Thorpe and M.S. Waring, *Nature*, **194**, 948 (1962).
4) Coronary Drug Project, Research Group, *J. Amer. Med. Assoc.*, **220**, 996 (1972); **226**, 652 (1973); **231**, 360 (1975).
5) T. Shimamoto and T. Sunaga, *Jap. Heart J.*, **3**, 581 (1962); T. Shimamoto, *J. Atheroscler. Res.*, **3**, 87 (1963).
6) T. Shimamoto, F. Numano, and T. Fujita, *Amer. Heart J.*, **71**, 216 (1966); F. Numano, *Jap. J. Med.*, **5**, 307 (1966).

Since Shimamoto *et al.* reported that pyridinolcarbamate is clinically effective in the treatment of atherosclerotic diseases,[7] the drug has been prescribed as an antiatherosclerotic agent, both in Japan and in European countries. Impressive clinical improvement was seen in patients given pyridinolcarbamate, yet the drug is apparently not a hypolipidemic agent or a peripheral vasodilator in the ordinary sense. Instead of lowering the lipids levels in the blood, pyridinolcarbamate apparently exerts a preventive effect on edematous reactions in the arterial wall, and thereby normalizes the disturbed metabolism of the atherosclerotic arterial wall.

As a part of our studies in search of even more effective and safer antiatherosclerotic agents, we found that 4-carbamoyloxymethyl-1(2H)-phthalazinone derivatives and related compounds showed activity comparable to that of pyridinolcarbamate in the edematous arterial reaction test.[8] During further studies on these compounds, we found that 4-hydroxymethyl-1(2H)-phthalazinone (**1**)[8b] itself, which had been used as the starting compound for the synthesis of the carbamoyloxymethyl derivative, was fairly active. Since few investigations on phthalazinone derivatives have been reported in the field of medicinal chemistry, our attention was focused on compound (**1**) and further study on its derivatives was undertaken. Herein, we report on the structure-activity relationship of 4-hydroxymethyl-1(2H)-phthalazinone derivatives and describe the synthesis of some compounds related to **1**.



R	n = 2	n = 3
CH_3	22	23
C_2H_5	24	25

R	n = 2	n = 3
CH_3	38	39
C_2H_5	40	

Chart 1

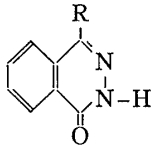
The pharmacological activities assessed in this study were inhibitory effects on platelet aggregation and preventive effects on the edematous arterial reaction. In the platelet aggregation test, ADP and arachidonic acid were used as aggregating agents, because ADP is

7) T. Shimamoto, F. Numano, T. Fujita, T. Ishioka, and T. Atsumi, *Asian Med. J.*, **8**, 825 (1965).
8) *a*) M. Ishikawa, Y. Eguchi, and A. Sugimoto, *Reports Inst. Med. Dent. Eng.*, **9**, 145 (1975); *b*) M. Ishikawa, Abstracts of Papers, The 1st symposium on Med. Chem., Tokyo, Nov. 1979.

thought to play a primary role in platelet aggregation and arachidonic acid is considered to induce platelet aggregation after being converted to thromboxane A_2,[9] an extremely potent platelet aggregator and one of the substances related to variant angina pectoris, heart attack, and cerebral disorders.

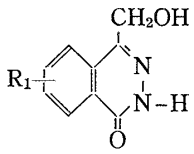
4-Hydroxymethyl-1(2H)-phthalazinone derivatives, the biological activities of which are shown in Table I, were prepared by the methods reported previously.[8] Among the derivatives having a functional group, such as a carboxy (**3**), ethoxycarbonyl (**4**), acetoxymethyl (**6**), or disubstituted aminomethyl (**8—12**) group in place of the hydroxymethyl group of **1**, none exhibited marked activity, although the latter compounds had fairly potent inhibitory activity in the platelet aggregation test. To examine the effect of substituents of the benzene ring on the activity, some compounds having a halogen atom (**14**, **15**, **29**), methyl (**26**), or methoxy (**13**, **28**) group were synthesized and assayed. While these moieties did not markedly affect the activity of the parent compound (**1**), an ester group substituted at position 7 of the phthalazinone nucleus was found to potentiate considerably the activity of **1** in the platelet aggregation test and edematous reaction test. This compound, 7-ethoxycarbonyl-4-hydroxymethyl-1(2H)-phthalazinone (**17**) was prepared by selective reduction of 4,7-bis-(ethoxycarbonyl)-1(2H)-phthalazinone with sodium borohydride in an ethanolic solution.[8a] Presumably, the electron-withdrawing effect of the C=N double bond made the ester at position 4 more susceptible to the reduction. Synthesis of the isomeric compound (**18**) having an ester group at position 6 was carried out by an analogous route, although the sodium borohydride reduction of the diester (**41**) was only possible under carefully controlled conditions in the presence of $CaCl_2$. Without $CaCl_2$, the only isolable product was the 4,6-bis(hydroxymethyl) derivative (**16**). As the activity of the compound (**18**) was decreased significantly compared with the ester (**17**), our attention was directed to the further modification of **17**. Treatment of **17** with methyl iodide in an alkaline medium afforded **30**, which showed

Table I. Biological Activity

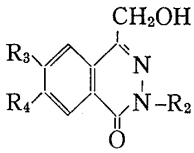


No.	R	Inhibition of platelet aggregation (%)		Inhibitory effect on EAR[a]
		ADP (10 μM)	AA(137 μM)	
1*	CH_2OH	9	13	++[b]
2*	CH_2OCONH_2	5	NT	++
3*	CO_2H	0	NT	±
4*	CO_2Et	0	NT	±
5*	$CO(CH_2)_3NMe_2$	4	NT	±
6*	CH_2OAc	3	NT	±
7	$C(Me)_2OH$	9	NT	±
8**	$CH_2N(Me)_2$	11	NT	±
9**	$CH_2N(Et)_2$	18	NT	±
10**		25	NT	±
11**		19	NT	−
12**		1	NT	−

9) M. Hamberg, J. Svensson, and B. Samuelsson, *Proc. Nat. Acad. USA.*, **72**, 2994 (1975).



	R_1			
13***	7-CH_3O	2	6	+
14***	7-Cl	4	9	+
15***	7-Br	0	12	+
16	6-CH_2OH	24	16	+
17***	7-CO_2Et	50	30	++
18	6-CO_2Et	20	20	+
19***	7-CO_2H	17	3	±
20***	7-$CONH_2$	18	11	±
21***	7-$CONHNH_2$	9	NT	±



No.	R_2	R_3	R_4	Inhibition of platelet aggregation (%) ADP (10 M)	Inhibition of platelet aggregation (%) AA (137 M)	Inhibitory effect on EAR[a]
22	H	H	$CONH(CH_2)_2N(Me)_2$	4	8	±[b]
23	H	H	$CONH(CH_2)_3N(Me)_2$	6	NT	−
24	H	H	$CONH(CH_2)_2N(Et)_2$	7	7	−
25	H	H	$CONH(CH_2)_3N(Et)_2$	19	NT	−
26***	H	Me	Me	11	NT	+
27***	Me	H	H	8	14	±
28***	Me	H	OMe	5	18	±
29***	Me	H	Br	5	10	±
30	Me	H	CO_2Et	22	35	+
31*	Ph	H	H	18	NT	±
32***	Ph	H	CO_2Et	11	NT	±
33	H	H	CO_2Me	13	10	−
34	H	H	CO_2n-Bu	69	68	+
35	H	H	CO_2i-Pr	56	91	+
36	H	H	CO_2i-Bu	43	68	+
37	H	H	$CO_2(CH_2)_2Cl$	64	72	+
38	H	H	$CO_2(CH_2)_2N(Me)_2$	20	10	+

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.
c) NT: not tested. *: reference 16*a*, **: reference 16*b*, ***: reference 8*a*.

a decreased inhibitory effect on platelet aggregation induced by ADP. Amide derivatives (**20**—**25**) of **17** showed markedly decreased activites. Modificafication of the ester moiety of **17** to methyl (**33**), isopropyl (**35**), *n*-butyl (**34**), isobutyl (**36**), and β-chloroethyl (**37**) ester enhanced the activity, except in the case of the methyl ester (**33**). In view of the decreased activity of **33**, the enhanced activity of (**34**—**37**) may be due to increased lipophilicity compared to **17**. The compounds (**38**—**40**), prepared by the introduction of a disubstituted amino group into the carbon chain of the ester moiety of **17** by means of ester exchange, showed rather decreased activities.

Thus, compound (**17**) appeared to be worthy of further investigation as a potential antithrombotic and antiatherosclerotic agent. In addition to the pharmacological activities described above, compound (**17**), designated as EG 467 (code number), showed[10] an inhibitory effect on the enhancement of platelet aggregability and adhesiveness induced by the administration of epinephrine, cholesterol, or angiotensin II in animal experiments. Morphological changes in the platelets were also suppressed[11] by **17**. Based on various experiments carried out with **17** and pyridinolcarbamate, Shimamoto[12] proposed a new concept concerning the key mechanism of atherogenesis. He suggested that atherogenic substances induce activation of the contractile proteins of hyperactive endothelial cells, thus widening the spaces at intercellular junctions and permitting the infiltration of *beta*-lipoproteins into the subendothelial layer. The compound (**17**) and pyridinolcarbamate presumably exhibit antiatherosclerotic activity through their relaxing effect on the contracted endothelial cells, thus protecting the integrity of the endothelial layer from atherogenic reactions. Meanwhile, it was found[13] that **17** competitively inhibited platelet cyclic AMP phosphodiesterase, an enzyme considered to regulate the cellular concentration of cyclic adenosine 3′,5′-monophosphate. The inhibitory effect of **17** on the enzyme is considered to be the biochemical mechanism responsible for the inhibitory effect of the compound on platelet aggregation and contraction of arterial endothelial cells.

Experimental

All melting points were determined in a capillary tube and are uncorrected. IR spectra were determined with a Hitachi model 285 spectrometer, UV spectra with a Hitachi model 323 spectrometer, and NMR spectra with a JEOL JUM-C-60HL spectrometer. For spectroscopic data, the following abbreviations are used: d=doublet, m=multiplet, s=singlet, q=quintet, and t=triplet.

4-(1-Hydroxy-1-methylethyl)-1(2H)-phthalazinone (**7**)——An ice-water cooled suspension of 2.5 g of 4-ethoxycarbonyl-1(2H)-phthalazinone (**4**) in 50 ml of THF was treated dropwise with the Grignard reagent prepared from 1 g of magnesium, 5.8 g of methyl bromide, and 100 ml of abs. ethyl ether, with stirring. The reaction mixture was then refluxed for 2 hr, acidified with dil. H_2SO_4 solution, and extracted with EtOAc. The combined EtOAc extracts were washed with H_2O, dried over anhyd. $MgSO_4$ and evaporated to dryness. The resulting residue was recrystallized from EtOH to give 2 g of **7**, mp 212—213°. *Anal.* Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.55; H, 6.01; N, 13.92. IR ν_{max}^{KBr} cm^{-1}: 3300, 1650. NMR (DMSO-d_6) δ: 1.60 (6H, s), 5.51 (1H, s), 7.84 (2H, m), 8.23 (1H, m), 8.74 (1H, m).

4,6-Bis(ethoxycarbonyl)-1(2H)-phthalazinone (**41**)——A mixture of 20 g of 2,5-dimethylacetophenone[14] and 200 ml of 5% aq. K_2CO_3 solution was heated to 90°, then 140 g of $KMnO_4$ was added portion-wise with vigorous stirring over a period of 2 hr. After stirring the mixture for an additional 1 hr, the precipitated MnO_2 was filtered off and 17 g of 85% hydrazine hydrate was added to the filtrate. The solution was heated at 90° for 1 hr, then acidified with conc. HCl and allowed to cool to room temperature. The resulting precipitate was washed with H_2O and air-dried to give 13 g of 4,6-bis(carboxy)-1(2H)-phthalazinone, in the form of a white powder. A portion of the powder was recrystallized from MeOH to give fine crystals, which did not melt at 280°.

A suspension of 10 g of 4,6-bis(carboxy)-1(2H)-phthalazinone obtained above in 500 ml of EtOH was treated portion-wise with 20 ml of conc. H_2SO_4, and the mixture was heated for 20 hr, then concentrated under reduced pressure and allowed to stand at room temperature for several hours. The resulting precipitate was filtered, washed with H_2O, and recrystallized from EtOH to give 9.3 g of (**41**), mp 202—203°. *Anal.* Calcd for $C_{14}H_{14}N_2O_5$: C, 57.93; H, 4.86; N, 9.65. Found: C, 57.78; H, 4.78; N, 9.51. IR ν_{max}^{KBr} cm^{-1}: 3240, 1720, 1690. NMR (DMSO-d_6) δ: 1.40 (6H, t, J=7 Hz), 4.38 (4H, q, J=7 Hz), 8.38 (2H, s), 9.17 (1H, s), 13.32 (1H, s).

10) T. Sano, H. Yamazaki, T. Shimamoto, and T. Shimamoto, "Platelets, Thrombosis, and Inhibitors," ed. by P. Disisheim , T. Shimamoto, and H. Yamazaki, Schattauer Verlag, 1974, pp. 425—432.
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13) H. Hidaka, T. Asano, M. Shibuya, and T. Shimamoto, "Platelets, Thrombosis, and Inhibitors," ed. by P. Disisheim, T. Shimamoto, and H. Yamazaki, Schattauer Verlag, 1974, pp. 321—327.
14) Perkin and Stone, *J. Chem. Soc.*, **127**, 2283 (1925).

6-Ethoxycarbonyl-4-hydroxymethyl-1(2H)-phthalazinone (18)——Compound (**41**) (10 g) was added portion-wise to a stirred and ice-cooled solution of 2 g of $NaBH_4$ in 200 ml of EtOH. A solution of 1.5 g of $CaCl_2$ in 30 ml of EtOH was added dropwise to the resulting suspension with external ice-salt cooling. The mixture was stirred at around −5° for 2 hr and then at room temperature for an additional 1 hr. The reaction mixture was concentrated to about 1/4 of its original volume, diluted with 50 ml of H_2O, and acidified with conc. HCl. The resulting precipitate was collected by filtration, washed with H_2O, and then recrystallized from EtOH to give 4.8 g of **18** in needle form, mp 203—204°. *Anal.* Calcd for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87; N, 11.29. Found: C, 58.12; H, 4.69; N, 11.40. IR ν_{max}^{KBr} cm^{-1}: 3500, 1730, 1660, 1290. NMR (DMSO-d_6) δ: 1.40 (3H, t, J=7 Hz), 4.34 (2H, q, J=7 Hz), 5.18 (2H, d, J=5 Hz), 5.47 (1H, t, J=5 Hz), 8.21 (2H, s), 8.52 (1H, s), 12.48 (1H, s).

4,6-Bis(hydroxymethyl)-1(2H)-phthalazinone (16)——A stirred solution of 500 mg of **41** in 100 ml of EtOH was treated portion-wise with 200 mg of $NaBH_4$, and the reaction mixture was stirred overnight at room temperature. The mixture was concentrated under reduced pressure, and the concentrate was then diluted with H_2O, and its pH was adjusted to approximately 5 with acetic acid. The resulting precipitate was filtered off and recrystallized from EtOH to afford 320 mg of **16**, mp 200—201°. *Anal.* Calcd for $C_{10}H_{10}N_2O_3$: C, 58.25; H, 4.89; N, 13.58. Found: C, 58.31; H, 4.85; N, 13.44. IR ν_{max}^{KBr} cm^{-1}: 3500, 3220, 1640. NMR (DMSO-d_6) δ: 4.73 (4H, s), 5.46 (2H, broad), 7.72 (1H, d, J=5 Hz), 8.04 (1H, s), 8.20 (1H, d, J=5 Hz), 12.45 (1H, s).

7-(β-Dimethylaminoethylcarbamoyl)-4-hydroxymethyl-1(2H)-phthalazinone (22)——A mixture of **17** and excess *asym*-dimethylethylenediamine was heated at 90° for 4 hr. After washing out the excess *asym*-dimethylethylenediamine with a small amount of ether, the residue was then allowed to stand in a refrigerator. The resulting crystals were recrystallized from EtOAc–ether to afford **22** in 40—50% yield, mp 198—199°. *Anal.* Calcd for $C_{14}H_{18}N_4O_3$: C, 57.92; H, 6.25; N, 19.30. Found: C, 57.94; H, 6.53; N, 19.21. NMR (DMSO-d_6) δ: 2.24 (6H, s), 2.53 (2H, m), 3.50 (2H, m), 4.73 (2H, s), 4.86 (1H, broad), 8.25 (2H, m), 8.75 (1H, d, J= 1 Hz), 12.63 (1H, s).

The Followeing Compounds were prepared in a Similar Manner——7-(γ-Dimethylaminopropylcarbamoyl)-4-hydroxymethyl-1(2H)-phthalazinone (**23**), mp 179—181° (recryst. from EtOAc). *Anal.* Calcd for $C_{15}H_{20}N_4O_3$: C, 59.19; H, 6.62; N, 18.41. Found: C, 58.96; H, 6.83; N, 18.89. 7-(β-Diethylaminoethylcarbamoyl)-4-hydroxymethyl-1(2H)-phthalazinone (**24**), mp 188—189° (recryst. from EtOAc–*n*-hexane). *Anal.* Calcd for $C_{16}H_{22}N_4O_3$: C, 60.36; H, 6.97; N, 17.60. Found: C, 60.12; H, 7.20; N, 17.44. 7-(γ-Diethylaminopropylcarbamoyl)-4-hydroxymethyl-1(2H)-phthalazinone (**25**), mp 172—174° (recryst. from EtOAc). *Anal.* Calcd for $C_{17}H_{24}N_4O_3$: C, 61.42; H, 7.28; N, 16.86. Found: C, 61.35; H, 7.11; N, 16.72.

7-Ethoxycarbonyl-4-hydroxymethyl-2-methyl-1(2H)-phthalazinone (30)——A mixture consisting of 1.0 g of 7-carboxy-4-hydroxymethyl-1(2H)-phthalazinone, 10 ml of 10% NaOH, 30 ml of MeOH, and 2 ml of CH_3I was heated for 1 hr, and then concentrated to 1/3 of the initial volume. The concentrate was acidified with conc. HCl, and the resulting precipitate was filtered off, washed with H_2O, and recrystallized from MeOH to give 0.7 g of 7-carboxy-4-hydroxymethyl-2-methyl-1(2H)-phthalazinone as colorless leaflets, mp 264—265°. *Anal.* Calcd for $C_{11}H_{10}N_2O_4$: C, 56.41; H, 4.30; N, 11.96. Found: C, 56.38; H, 4.29; N, 12.08. Esterification of 800 mg of 7-carboxy-4-hydroxymethyl-2-methyl-1(2H)-phthalazinone prepared as described above in 20 ml of EtOH and 1 ml of conc. H_2SO_4 gave 550 mg of **30** in the form of colorless needles, on recrystallization from EtOH, mp 164—165°. *Anal.* Calcd for $C_{13}H_{14}N_2O_4$: C, 59.53; H, 5.38; N, 10.68. Found: C, 59.44; H, 5.29; N, 10.48. NMR (DMSO-d_6) δ: 1.40 (3H, t, J=7 Hz), 3.72 (3H, s), 4.41 (2H, q, J=7 Hz), 4.70 (2H, s), 8.31 (2H, broad), 8.75 (1H, s).

4-Hydroxymethyl-7-isopropyloxycarbonyl-1(2H)-phthalazinone (35)——Esterification of 7-carboxy-4-hydroxymethyl-1(2H)-phthalazinone (**19**) with isopropylalcohol and conc. H_2SO_4 gave **35** in the form of colorless needles on recrystallization from acetone–*n*-hexane, mp 212—213° in 80—90% yield. *Anal.* Calcd for $C_{13}H_{14}N_2O_4$: C, 59.53; H, 5.38; N, 10.68. Found: C, 59.42; H, 5.51; N, 10.49. NMR (DMSO-d_6) δ: 1.39 (3H, s), 1.44 (3H, s), 4.74 (2H, s), 5.24 (3H, m), 8.30 (2H, m), 8.75 (1H, s), 12.73 (1H, s).

The Following Esters were prepared by a Similar Procedure——4-Hydroxymethyl-7-methoxycarbonyl-1(2H)-phthalazinone (**33**), mp 224—225° (recryst. from MeOH). *Anal.* Calcd for $C_{11}H_{10}N_2O_4$: C, 56.41; H, 4.30; N, 11.96. Found: C, 56.38; H, 4.41; N, 11.73. 7-*n*-Butoxycarbonyl-4-hydroxymethyl-1(2H)-phthalazinone (**34**), mp 144—146° (recryst. from EtOAc). *Anal.* Calcd for $C_{14}H_{16}N_2O_4$: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.88; H, 5.72; N, 10.23. 4-Hydroxymethyl-7-isobutyloxycarbonyl-1(2H)-phthalazinone (**36**), mp 160—162° (recryst. from EtOAc). *Anal.* Calcd for $C_{14}H_{16}N_2O_4$: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.72; H, 5.84; N, 10.20. 7-(β-Chloroethoxycarbonyl)-4-hydroxymethyl-1(2H)-phthalazinone (**37**), mp 166—168° (recryst. from MeOH). *Anal.* Calcd for $C_{12}H_{11}N_2O_4$: C, 50.97; H, 3.89; N, 9.91. Found: C, 51.12; H, 3.93; N, 9.72.

7-(β-Dimethylaminoethoxycarbonyl)-4-hydroxymethyl-1(2H)-phthalazinone (38)——A mixture consisting of 500 mg of **17**, 20 ml of β-dimethylaminoethanol, and a small amount of KOH powder was refluxed with stirring, at 110—120° for 6 hr. The mixture was then concentrated *in vacuo*, and the concentrate was diluted with H_2O, acidified with acetic acid, and extracted 5 times with $CHCl_3$. The aqueous phase was then made alkaline with aq. $NaHCO_3$ solution, and extracted repeatedly with $CHCl_3$. The latter $CHCl_3$ extracts were combined, dried over anhyd. Na_2SO_4 and concentrated. The resulting residue was recrystal-

lized from acetone to afford 150 mg of **38**, mp 176—178°. *Anal.* Calcd for $C_{14}H_{17}N_3O_4$: C, 57.72; H, 5.88; N, 14.43. Found: C, 57.87; H, 6.04; N, 14.95. NMR (DMSO-d_6) δ: 2.25 (6H, s), 2.68 (2H, t, J=5 Hz), 4.41 (2H, t, J=5 Hz), 4.63 (2H, s), 4.46 (1H, broad), 8.25 (2H, m), 8.69 (1H, d, J=1 Hz), 12.61 (1H, s).

The Following Compounds were prepared by a Similar Procedure——7-(β-Diethylaminoethoxycarbonyl)-4-hydroxymethyl-1(2H)-phthalazinone (**40**), mp 153—154° (recryst. from acetone). *Anal.* Calcd for $C_{16}H_{21}N_3O_4$: C, 60.17; H, 6.63; N, 13.16. Found: C, 60.11; H, 6.54; N, 13.34. NMR ($CDCl_3$) δ: 1.11 (6H, t, J=7 Hz), 2.72 (4H, q, J=7 Hz), 2.94 (2H, t, J=5 Hz), 4.50 (2H, t, J=5 Hz), 4.88 (2H, s), 7.85 (1H, d, J=6 Hz), 8.30 (1H, d, d, J=6 Hz, J=1 Hz), 8.80 (1H, d, J=1 Hz). 7-(γ-Dimethylaminopropyloxycarbonyl)-4-hydroxymethyl-1(2H)-phthalazinone (**39**), mp 141—142° (recryst. from acetone). *Anal.* Calcd for $C_{15}H_{19}N_3O_4$: C, 59.00; H, 6.27; N, 13.76. Found: C, 59.08; H, 6.58; N, 13.71.

Preparation of Rabbit Platelet-rich Plasma (PRP)——Blood was collected from a catheter inserted into the carotid artery of ethyl ether-anesthetized rabbits. The blood was citrated with 3.8% aqueous sodium citrate solution (1 ml of citrate/9 ml of blood) and separated from the red blood cells 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 *B*orn[15] was used to assess the ability of test compounds to inhibit platelet aggregation 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-247E) set at 37° and 1200 rpm, and a solution of the test compound (1×10^{-4} M final concentration) in 2.5 μl of DMSO and a siliconized stirring bar were added. After preincubation for 3 min, 10 μl of an aqueous solution of ADP (10 μM final concentration) or 10 μl of a aqueous solution of AA (137 Mμ 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 μl 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 were administered orally at a dose of 10 mg/kg 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 the intravenous administration of adrenaline at a dose of 10 μg/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 by optical microscopy. Assessment of the inhibitory effect of test compounds was performed by comparing the degree of edematous change with that observed in the case of placebo (potato starch) ingestion. Each compound was tested three times and the inhibitory effect shown in Table I is the mean value obtained from the three experiments.

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