Articles

1*H*-2-Benzopyran-1-one Derivatives, Microbial Products with Pharmacological Activity. Conversion into Orally Active Derivatives with Antiinflammatory and Antiulcer Activities

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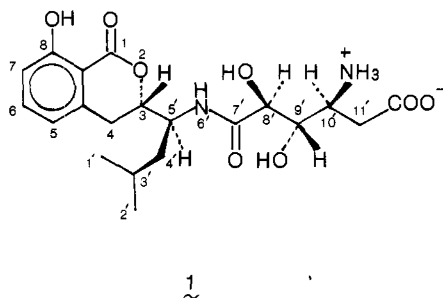
A novel gastroprotective substance, 6-[[1(*S*)-[3(*S*),4-dihydro-8-hydroxy-1-oxo-1*H*-2-benzopyran-3-yl]-3-methylbutyl]amino]-4(*S*),5(*S*)-dihydroxy-6-oxo-3(*S*)-ammoniohexanoate (AI-77-B, 1), isolated from a culture broth of *Bacillus pumilus* AI-77, was chemically modified to prodrugs that are active by oral dosing. Compound 1 was lactonized and then monoalkylated at the primary amine position. Six N-alkylated γ-lactone derivatives of 1 (with alkyl chains being methyl **5a**, ethyl **5b**, *n*-propyl **5c**, *n*-butyl **5d**, *n*-pentyl **5e**, or *n*-hexyl **5f**) were synthesized and eight compounds including 1 and γ-lactone derivative **2** were compared for their gastroprotective activities and blood levels after oral administration in rats. Further, chloroform-water partition coefficients of **5a-f** were also compared as a measure of lipid solubility. The protective effects of these compounds on stress ulcers were mutually related to blood levels of dealkylated compounds (1 and 2). Parent compound 1 was detected in blood at 1 h after each of **5a-d** was administered. When **5b** or **5c** was administered, high activity and high blood levels of 1 were observed in comparison with those levels obtained with **5a** or **5d**. Neither **5e** nor **5f** were detected in any amount in blood by oral administration without special formulation due to extremely low solubilities and agglutinative properties in intestinal fluid. Interestingly, **5b** and **5c** were found to have antiinflammatory activities in addition to potent antiulcerogenicity action.

A novel gastroprotective substance (AI-77-B, 1) has been isolated from a culture broth of *Bacillus pumilus AI-77* as the major component of seven related structures.[1] The structure of 1 has been established as 6-[[1(*S*)-[3(*S*),4-dihydro-8-hydroxy-1-oxo-1*H*-2-benzopyran-3-yl]-3-methylbutyl]amino]-4(*S*),5(*S*)-dihydroxy-6-oxo-3(*S*)-ammoniohexanoate.[2] Compound 1 appears to be part of a



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unique drug class[9] exhibiting noncentral suppressive, nonanticholinergic and nonantihistaminergic properties despite its potent antiulcerogenicity acting against stress ulcers induced in rats by restraint and water immersion. However, 1 shows no significant activity by oral administration apparently due to little gastrointestinal absorption. We have reported on the structural requirements for gastroprotective activity of 1 and derivatives, and it has become apparent that the presence of both the amino acid moiety of the side chain and the 1*H*-2-benzopyran-1-one skelton are important for activity.[3] For the purpose of obtaining drugs that are more effective on oral dosing, we have prepared prodrugs of 1 by introducing various kinds of lipophilic groups onto the primary amine moiety. As a result of these studies a series of N-monoalkylated γ-lactone derivatives of 1 was found to be suitable. Interestingly, some of those compounds exhibited antiinflammatory activity in addition to gastroprotective activity. In this paper, the preparation of N-monoalkyl-substituted γ-lactone derivatives of 1 (with alkyl chains = methyl **5a**, ethyl **5b**, *n*-propyl **5c**, *n*-butyl **5d**, *n*-pentyl **5e**, or *n*-hexyl **5f**) is described. Their gastroprotective activities, concentration in blood following oral administration, and chloroform-water partition coefficients as a measured of lipid solubility are compared. Furthermore, antiinflammatory activity of **5b** and **5c** is evaluated. These are selected for their high absorption from the gastrointestinal tract.

Chemistry. The N-monoalkylated derivatives **5a-f** with alkyl chains of methyl, ethyl, *n*-propyl, *n*-butyl, *n*-pentyl, or *n*-hexyl were prepared by two different methods as shown in methods 1 and 2. Treatment of an amide with 1 equiv of triethyloxonium tetrafluoroborate (Meerwein's reagent)[4] in methylene chloride is known to give the imino ether tetrafluoroborate in excellent yield.[5] In the case of imino ethers from secondary and tertiary amides, replacement of the methylene chloride by absolute ethanol, followed by treatment with excess sodium borohydride at 0 or 25 °C, gives the corresponding amine in essentially

(1) Shimojima, Y.; Hayashi, H.; Ooka, T.; Shibukawa, M. *Agric. Biol. Chim.* **1982**, *46*, 1823.
(2) (a) Shimojima, Y.; Hayashi, H.; Ooka, T.; Shibukawa, M.; Iitaka, Y. *Tetrahedron Lett.* **1982**, *23*, 5435. (b) Shimojima, Y.; Hayashi, H.; Ooka, T.; Shibukawa, M.; Iitaka, Y. *Tetrahedron* **1984**, *40*, 2519.
(3) Shimojima, Y.; Hayashi, H. *J. Med. Chem.* **1983**, *26*, 1370.
(4) Meerwein, H. *Org. Synth.* **1966**, *46*, 113.
(5) (a) Karplus, M. *J. Chem. Phys.* **1959**, *30*, 11. (b) Hanessian, S. *Tetrahedron Lett.* *1967*, 1549. (c) Meerwein, H.; Borner, P.; Fuchs, O.; Sasse, H. J.; Schrodt. H.; Spille, J. *Ber. Dtsch. Chem. Ges.* **1956**, *89*, 2060.

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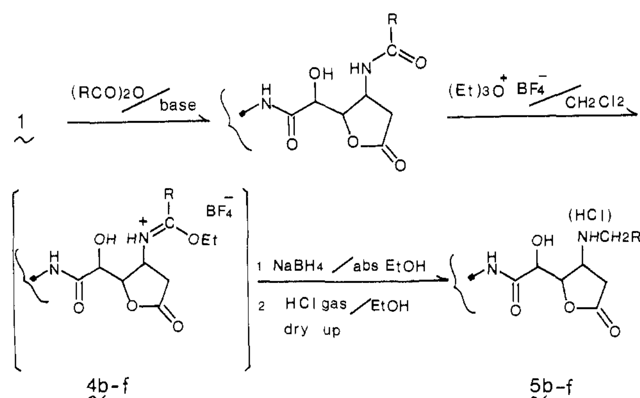
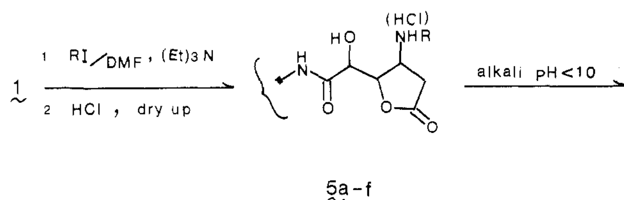
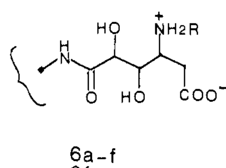
Method 1



b, R = CH_3
c, R = C_2H_5
d, R = n-C_3H_7
e, R = n-C_4H_9
f, R = n-C_5H_{11}

Method 2





a, R = CH_3
b, R = C_2H_5
c, R = n-C_3H_7
d, R = n-C_4H_9
e, R = n-C_5H_{11}
f, R = n-C_6H_{13}

quantitative yield.[6] For preparing **5b**, method 1 was superior to method 2 in yield (Table I). It is very fortunate that triethyloxonium tetrafluoroborate reacts with the amide on C-10′ with high selectivity at room temperature. Even in the treatment of *N*-acetyl derivative **3b** with 3 equiv of triethyloxonium tetrafluoroborate, neither the amide at the center of the molecule nor the phenol on C-8 were attacked. This difference in reactivity between the two amide moieties may be due to steric hindrance around the center amide. If alkylation of the phenol on C-8 had occurred, the resulting derivative would lack the characteristic fluorescence of 3,4-dihydro-8-hydroxy-1*H*-2-benzopyran-1-one;[3] however, no products without fluorescence could be detected from the above reaction. Regarding the possibility of alkylation of the secondary alcohol on C-8′ by the reagent, no clear evidence was obtained. The imino ethers 4 easily suffered hydrolysis to the corresponding amine; therefore, they had to be reduced directly without isolation. Triethylamine is superior to pyridine as a base for acylation of 1, because residual pyridine consumes Meerwein's reagent in the next reaction. Attempted alkylation via method 1 of the formamide resulting from treatment of 1 with dicyclohexylcarbodiimide and formic acid, resulted in recovering only the unchanged formamide. A possible reason for this unsuccessful result may reside in an acceleration of hydrolysis of an intermediate imino ether due to a reduction in bulk of the amide substitutent. Therefore N-methylated derivative **5a** was synthesized with excess methyl iodide by method 2.

In preparing compounds **5c–f** with alkyl chains longer than ethyl, method 2 gave higher yields (reaction yield 54–75%) than method 1 (42–58%). The reason for low yields of the imino ethers **4c–f** is not clear. In order to identify metabolites of **5a–f** by high-performance liquid chromatography (HPLC) analyses, compounds **6a–f** were prepared from corresponding compounds **5a–f** by hydrolysis with aqueous NaOH at pH 7–9.

Table I. N-Alkylated γ-Lactone Derivatives of 1

5a–f

no.	R	% yield method 1	% yield method 2	mp, °C dec	formula[a]
5a	CH_3	*b*	10	149–151	$C_{21}H_{28}N_2O_7$
5b	C_2H_5	69	58	162–164	$C_{22}H_{30}N_2O_7$
5c	n-C_3H_7	58	75	137–139	$C_{23}H_{32}N_2O_7$
5d	n-C_4H_9	42	62	157–158	$C_{24}H_{34}N_2O_7$
5e	n-C_5H_{11}	45	54	159–161	$C_{25}H_{36}N_2O_7$
5f	n-C_6H_{13}	42	54	166–168	$C_{26}H_{38}N_2O_7$

[a] Elemental analyses obtained for C, H, and N were in agreement (0.4%) with the theoretical values. [b] An attempted alkylation by method 1 was unsuccessful.

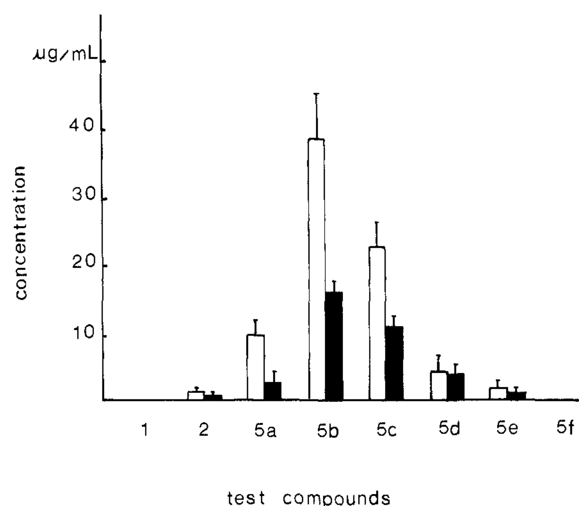


Figure 1. Blood levels of 1, 2, and N-alkylated γ-lactone derivatives (200 mg/kg, po in rats). Test compounds were administered as formulations composed of 5**x**·HCl/glycerin/HCO-60 = 1/12/0.05 (in weight). (□) The cumulative amount of two or three compounds detected in blood samples collected 1 h after administration of test compound; (■) the cumulative amount at 3 h after treatments. Compositions are shown in Table IV. Each column shows the mean drug level in blood samples taken from six to seven rats; each vertical bar indicates the standard error.

Biological Results and Discussion

Compound 1, γ-lactone derivative **2**,[2,3] and six *N*-alkyl derivatives **5a–f** were compared for relative potency of gastroprotective activity against stress ulcers and their concentration in blood following oral administration in rats

(6) (a) Borch, R. F. *Tetrahedron Lett. 1968*, 61. (b) Monteiro, H. J. *Synthesis, 1974*, 137.

Table II. Protective Effects on Stress-Induced Ulcers in Rats

	protection, %,[a,b] at the following doses:[e]						
	dose, mg/kg, po				dose, mg/kg, ip		
no.	100	50	25	12.5	50	25	12.5
1	17	0	0	NT[d]	94	100	78
2[c]	78	66	44	NT	83	83	66
5a[c]	66	56	11	17	56	33	NT
5b[c]	89	78	56	33	100	76	66
5c[c]	100	94	78	17	89	66	44
5d[c]	89	66	33	17	89	44	NT
5e[c]	56	33	44	NT	44	56	NT
5f[c]	44	44	11	NT	11	17	NT

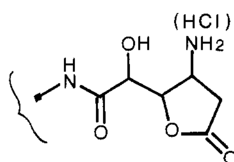
[a] Five rats were treated as a group for each dosage. [b] Treatments in which protective values were more than 60% were evaluated as significantly effective (Student's *t* test, $P < 0.01$). [c] These compounds were administered as HCl salts. [d] NT = not tested. [e] Test compounds were administered as formulation composed of drug/glycerin/HCO-60 = 1/12/0.05 (in weight).

Table III. Solubility and Distribution Coefficient

	solubility, μg/mL		distribution coeff
no.[a]	phosphate buffer[b]	H_2O	$CHCl_3$/phosphate buffer[b]
5a	9.9×10^2	1.5×10^3	3750
5b	1.1×10^4	$>1.0 \times 10^5$	43
5c	1.6×10^3	$>1.0 \times 10^5$	260
5d	3.7×10^2	1.0×10^5	1700
5e	3.3×10^2	7.3×10^3	1900
5f	5.0×10^1	1.6×10^3	3000

[a] Compounds were used as HCl salts. [b] pH 7.5 (10 mM).

(Tables II and IV and Figure 1). Furthermore, as a measure of lipid solubility, chloroform–water partition coefficients of **5a–f** were determined (Table III).



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Ring-opened compounds of the γ-lactone as **1** exhibited no significant activity by oral administration. On the other hand γ-lactone derivative **2** exhibited potent gastroprotective activity by oral administration. However, the protective value of **2** often showed significant differences between individual rats, which was probably attributed to variable absorption of **2** from the gastrointestinal tract. Introduction of an alkyl function at the primary amine of **2** resulted in more reproducible gastroprotective action and higher blood levels (Table II and Figure 1). The highest blood level of each compound was found in blood samples collected 1 h after treatment (data are not shown). *N*-Ethyl derivative **5b** showed the highest blood level of the six *N*-alkyl derivatives **5a–f**. In each compound **5a–c**, an approximate linear relationship existed between the concentration detected in blood and the dosage up to an oral dosage of 200 mg/kg. However, at high dosages of **5d** (over 100 mg/kg), saturation of absorption was observed. In the cases of **5e** and **5f** administered without formulation, no compound related to 1*H*-2-benzopyran-1-one was detected in blood by high-performance liquid chromatography (HPLC) analysis. By means of in vivo tests in rats such as perfusion of small intestines or stomachs, the absorption site of these compounds was proved to be the small intestine. However, **5d–f** showed extremely low solubilities and were found to easily agglutinate in intestinal fluid. Therefore, we studied methods to suitably disperse **5d–f** in intestinal fluid. A formulation consisting of drug, glycerin, and HCO-60 in the ratio of 1:12:0.05 (weight) was selected. Figure 1 shows the levels of **5a–f** in blood at 1 and 3 h after oral administration. Each value plotted is the cumulative amount of three kinds of compounds including the drug, its corresponding ring-opened compound **6**, and dealkylated metabolite **1**, which is the parent. When **5a**, **5c**, and **5d** were administered, **1** was detected as shown in Table IV. In HPLC analysis, complete separation between the peak of **6b** and that of **1** was unsuccessful under the condition in which both peaks were free from interference derived from serum components. Judging from the above results, dealkylation of **5b** could also occur in vivo. On the other hand, no significant amount of **1** was detected in the blood samples collected after administration of the **5e** formulation. Even trace amounts of **5f** were not detected in blood in the administration of the **5f** formulation.

It is generally accepted that N-alkylated drugs undergo dealkylation in liver microsomes by a mechanism that is NADPH dependent and requires oxygen. The system

Table IV. Compositions of Compounds Detected in Blood

200 mg/kg po, no.	compounds detected in blood,[a] μg/mL: 1 h after administration: 2	5x[b]	6x	1	total mean ± SE	3 h after administration: 2	5x	6x	1	total mean ± SE
1				ND[d]	ND				ND	ND
2	0.8			0.7	1.5 ± (0.6)	0.6			0.5	1.1 ± (0.9)
5a		3.8	3.1	3.0	9.9 ± (2.5)		1.2	1.1	0.4	2.7 ± (1.4)
5b		17.5	21.0[c]		38.5 ± (6.5)		8.3	7.9[c]		16.2 ± (1.5)
5c		14.2	6.4	2.0	22.2 ± (3.6)		3.4	3.4	4.2	11.0 ± (1.5)
5d		2.3	2.0	trace	4.3 ± (2.0)		2.3	1.8	ND	4.1 ± (1.4)
5e		1.2	0.8	ND	2.0 ± (1.0)		0.8	0.6	ND	1.4 ± (0.7)
5f		ND	ND	ND	ND ±		ND	ND	ND	ND

[a] Each value shows the mean of blood samples taken from six to seven rats. [b] x represents the corresponding one of a–f. [c] The cumulative amount of **6b** and **1**. [d] ND = not detected.

Table V. Evaluation of the Antiinflammatory Activity and Acute Toxicity of **5b** and **5c**

	ED_{50}, mg/kg, po					
no.	CPE[c] (rats)	BPE[d] (rats)	DPE[e] (rats)	UV erythema (guinea pigs)	ED_{min},[a] mg/kg, po adju arth[f] (rats)	LD_{50}, mg/kg, po (rats)
5b[b]	>200	182 (107–309)[g]	47 (33–66)	13 (7–23)	20	1250 (893–1750)
5c[b]	124 (84–182)	69 (54–88)	43 (32–58)	12 (9–7)	15	1075 (893–1398)
IDM[h]	1.3 (0.7–2.3)	>25	>25	<6	3	12–25

[a] Minimum effect dose. [b] Compounds were administered as HCl salts. [c] Carrageenin paw edema. [d] Bradykinin paw edema. [e] Dextran paw edema. [f] Adjuvant arthritis. [g] 95% confidence limits. [h] Indomethacin.

removes methyl, ethyl, and butyl groups.[7] Therefore, penetrating a drug into microsomes seems essential for dealkylation to occur. Brodie et al.[7] showed the relationship between lipid solubility and metabolic rate in various drugs with an *N*-methyl side chain. They showed that water-soluble drugs such as theobromine, sarcosine, and (dimethylamino)ethanol, whose chloroform–water (pH 7.4) partition coefficients are 0.1, <0.03, and <0.03, respectively, do not undergo demethylation, whereas lipophilic drugs, such as ephedrine, *N*-methylaniline, aminopyrine, and caffeine, whose partition coefficients are higher than 19, were easily demethylated.

Levy et al. and Schanker et al.[8] pointed out that substances with very small (<0.001) chloroform–water partition coefficients were not absorbed or only very poorly absorbed by passive diffusion from the gastrointestinal tract. In the light of results obtained by Brodie et al. or Schanker et al., chloroform–water partition coefficients of **5a–f** are sufficiently high to permit both absorption from the gastrointestinal tract and dealkylation in the liver microsomes. Both **5b** and **5c** of **5a–f** are adequate for our purpose of obtaining drugs that are sufficiently effective by oral dosing; the protective values by oral administration of **5c** are almost equal or somewhat higher than those of intraperitoneal administration. A possible explanation for the weak activity of **5a** compared with **2** may reside in an inability to maintain the desired blood levels of **1** during experimental ulceration. Further studies are required to clarify this.

5b and **5c** were selected to examine in further detail pharmacological actions other than the gastroprotective action. Both compounds were found to have an antiinflammatory action (Table V). They showed inhibitory effects on bradykinin- or dextran-induced paw edema, while indomethacin (an acidic antiinflammatory drug) showed no significant effect even at the high dosage corresponding to the LD_{50} value (12.5–25 mg/kg, po, in rats). Contrary to the case of aspirin, when **5c** was given together with an acidic antiinflammatory drug as indomethacin or diclofenac, significantly synergistic reduction of edema fromation by carrageenin was observed (Figure 2). These findings suggest that **5c** and indomethacin act on one another complementarily to reduce carrageenin-induced paw edema. Interestingly, series **5** compounds have been found to have potent prophylactic action on gastric ulcers caused by indomethacin or aspirin in rats (private information from Prof. Y. Kasuya[9]). It is generally well known that most nonsteroidal antiinflammatory drugs have the side effect of ulceration based on inhibition of prostaglandin biosynthesis. Both **5b** and **5c** exhibit an antiinflammatory action in addition to an antiulcerlogenic activity. It is worthwhile to study the mode of action of these compounds.

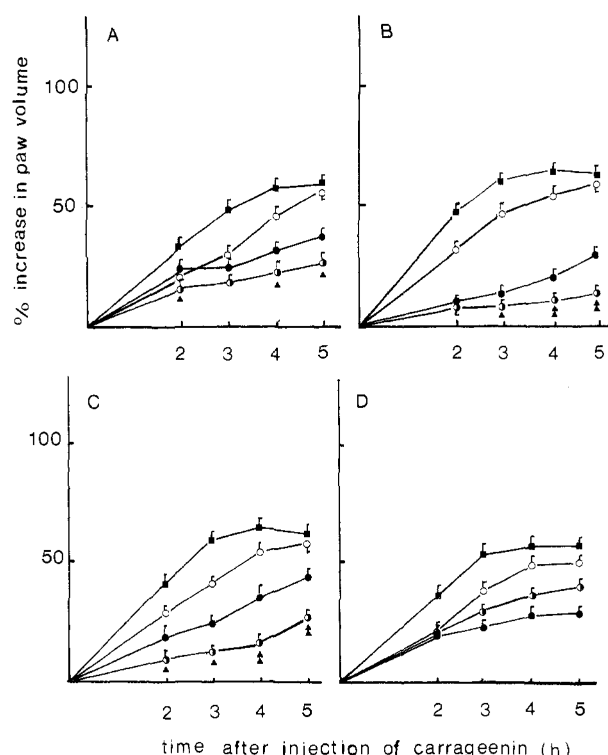

Figure 2. Synergistic effect of compound **5c** with indomethacin or with diclofenac on carrageenin edema. A: (■) vehicle; (○) **5c**, 150 mg/kg; (●) indomethacin, 1.25 mg/kg; (◑) **5c**, 150 mg/kg, was given 30 min before the administration of indomethacin, 1.25 mg/kg. B: (■) vehicle; (○) **5c**, 150 mg/kg; (●) indomethacin, 6 mg/kg; (◑) **5c**, 150 mg/kg, was given 30 min before the administration of indomethacin, 6 mg/kg. C: (■) vehicle; (○) **5c**, 150 mg/kg; (●) diclofena, 5 mg/kg; (◑) **5c**, 150 mg/kg, was given 30 min before the administration of diclofenac sodium. D: (■) vehicle; (○) aspirin, 50 mg/kg; (●) indomethacin, 5 mg/kg; (◑) aspirin, 50 mg/kg, was given 30 min before the administration of indomethacin, 5 mg/kg. (▲) $P < 0.05$, (▲▲) $P < 0.01$; statistically significant difference compared with the mean value of the indomethacin-only treated group or diclofenac sodium-only treated group. Each point represents the mean value of 8–10 rats; each vertical bar indicates the standard error. All of the test compounds were given orally.

Experimental Section

Melting points were determined in capillary tubes by using a silicon oil bath (Yamato) MP-21 and are uncorrected. UV spectra were measured with a Shimadzu UV-210A instrument; IR spectra were measured with a Hitachi Model 285 spectrometer. ^{1}H NMR spectra were recorded on a JEOL JHM-MH 100 spectrometer with Me_4Si as the internal standard. Each analytical sample had spectral data compatible with its assigned structure and was found

(7) Brodie, B. B.; Gaudette L. E. *Biochem. Pharmacol.* **1959**, *2*, 89.
(8) (a) Levy, G.; Jusko, W. J. *J. Pharm. Sci.* **1966**, *55*, 285. (b) Schanker, L. S. *J. Pharmacol. Exp. Ther.* **1959**, *126*, 283.
(9) Urushidani, T.; Kasuya, Y.; Yano, S. *Exp. Ulcer.*, in press: when **5c** (25–100 mg/kg, po) were given to rats 10 min before indomethacin treatments (30 mg/kg, sc), values of gastric ulcer inhibition were 86–99% ($p < 0.01$).

to be a single spot on TLC or a single peak in high-performance liquid chromatography (HPLC). TLC was carried out on plates coated with 0.25-mm layer of silica gel $60F_{254}$ (Merck). For preparative separation, plates with a 2-mm-layer thickness were used. The location of spots was detected by illumination with a UV lamp or by spraying with ninhydrin reagent. HPLC was performed on Waters 200 series chromatography system incorporating a Model 6000A pump operating at a flow rate of 1 mL/min, a Model 440 absorbance detector at 254 mm, a Model U6K injector, and a Waters μBondapak C_{18} column (particle size 10 μm).

Method 2. 4-[1-Hydroxy-2-[[1-(3,4-dihydro-8-hydroxy-1-oxo-1*H*-2-benzopyran-3-yl)-3-methylbutyl]amino]-2-oxoethyl]-3-(methylamino)butan-4-olide (5a). Thoroughly dried 1 (4.24 g, 10 mmol) was placed in a 200-mL glass pressure container and dissolved in *N,N*-dimethylformamide (20 mL). After adding methyl iodide (6 mL, 96 mmol) and triethylamine (4.5 mL, 30 mmol), the container was closed and shaken vigorously at room temperature. Furthermore, methyl iodide (6 mL, 96 mmol) was added to the reaction solution twice at 2-h intervals and then the container was shaken for 20 h. The solvent and unreacted methyl iodide were distilled off in vacuo. To the residue dissolved in tetrahydrofuran was added 4 mL of HCl (44 mmol) with stirring while cooling with ice. The solution then evaporated, forming the γ-lactone ring completely. The residue was dissolved in 0.01 N HCl and extracted first with Et_2O and then with AcOEt to remove the side products. The aqueous layer was adjusted to pH 7.5 with 0.5 M $NaHCO_3$ and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The residue was dissolved in MeOH and purified by preparative TLC, with use of MeOH/$CHCl_3$ (1/10) to give 410 mg (9.8%) of **5a** in the form of a white powder. An analytical sample was obtained by crystallization from hexane/AcOEt: UV max (MeOH) 247 and 315 nm; IR (KBr) 1790 (γ-lactone), 1675 (δ-lactone carbonyl bonded with hydrogen) cm^{-1}; ^1H NMR (CD_3OD) δ 7.40 (dd, 1, J = 7 and 8 Hz, aromatic), 6.79 and 6.74 (each d, each 1, J = 7 and 8 Hz, aromatic), 4.70 (m, 1, $C_{9'}$ H), 4.64 (m, 1, C_3 H), 4.38 (d, 1, J = 2 Hz, $C_{8'}$ H), 4.32 (m, 1, $C_{5'}$ H), 3.30 (m, 1, $C_{10'}$ H), 3.10–2.80 (m, 3, $C_{11'a}$ and C_4 H), 2.35–2.20 (overlapping with $NHCH_3$, 1, $C_{11'b}$ H), 2.28 (s, 3, $NHCH_3$), 2.05–1.10 (m, 3, $C_{3'}$ H and $C_{4'}$ H), 0.98 and 0.94 (2 d, each 3, each J = 6 Hz, $C_{1'}$ H and $C_{2'}$ H). Anal. ($C_{21}H_{28}N_2O_7$) C, H, N. This sample was dissolved in THF containing 1.2 equiv of HCl and then evaporated. The resulting hydrochloride salt of **5a** was used for biological tests.

Method 1. 4-[1-Hydroxy-2-[[1-(3,4-dihydro-8-hydroxy-1-oxo-1*H*-2-benzopyran-3-yl)-3-methylbutyl]amino]-2-oxoethyl]-3-(ethylamino)butan-4-olide (5b). Thoroughly dried N-acetylated derivative **3b**[2,3] (1.79 g, 4 mmol) was suspended in methylene chloride (30 mL) which was freshly distilled. To the suspension, triethyloxonium tetrafluoroborate (1.14 g, 6 mmol) in methylene chloride was added in drops in an argon atmosphere with stirring and the mixture was stirred at room temperature for 4 h. After removal of the methylene chloride in vacuo, dried EtOH (40 mL) was added to the residue with stirring at 0 °C and then sodium borohydride (298 mg, 8 mmol) was added followed by stirring for 30 min; excess borohydride was decomposed with EtOH saturated with HCl gas. After evaporation in vacuo, the resulting solid was dissolved in H_2O/MeOH (1/4) and purified with an Amberite XAD-2 column (280 mL in water). After the column was washed with 560 mL of MeOH/H_2O/1 N HCl (30/63/7), the column was eluted with MeOH/H_2O/1 N HCl (50/45/5). The fractions containing **5b** were collected and evaporated to give 1.41 g (76%) of crude **5b**. This was dissolved in water, adjusted to pH 7.5 with 0.5 M $NaHCO_3$ and then extracted with AcOEt. After the organic phase was dried over Na_2SO_4 and filtered, the AcOEt was evaporated to give 1.27 g (68.8%) of **5b**. An analytical sample was obtained by crystallization from hexane/AcOEt: mp 162–164 °C dec; UV (MeOH) max 247 nm (ε 6119), 315 (4042); ^1H NMR (Me_2SO-d_6) δ 2.44 (q, 2, J = 4 Hz, $NHCH_2CH_3$), 1.90–1.10 (m, 3, $C_{3'}$ H and $C_{4'}$ H), 1.20 (t, 3, J = 4 Hz, $NHCH_2CH_3$), 0.84 and 0.90 (2 d, each 3, each J = 7 Hz, 2 CH_3). Anal. ($C_{22}H_{30}N_2O_7$) C, H, N. The sample was prepared as the hydrochloride salt by the same method used for **5a**.

Method 2. 4-[1-Hydroxy-2-[[1-(3,4-dihydro-8-hydroxy-1-oxo-1*H*-2-benzopyran-3-yl)-3-methylbutyl]amino]-2-oxoethyl]-3-(propylamino)butan-4-olide (5c). Thoroughly dried 1 (5 g, 11.8 mmol) was dissolved in *N,N*-dimethylformamide (20 mL) at 50 °C. To the solution were added propyl iodide (11.4 mL, 118 mmol) and triethylamine (5 mL, 35.4 mmol) and then the mixture was stirred (4 h, 50 °C). Tetrahydrofuran (150 mL) was added to the reaction solution. The resulting precipitate was filtered off. To the filtrate was added HCl (4.0 mL, 44 mmol) with stirring at 0 °C and then evaporated in vacuo. The oily residue was dissolved in AcOEt (200 mL) and extracted with 0.5 M $NaHCO_3$ (150 mL). To the organic layer was added 0.5 M $Na_2S_2O_3$ (70 mL) to decompose excess propyl iodide. The resulting organic layer was washed with water and saturated NaCl, dried over Na_2SO_4, filtered, and then evaporated to give 3.94 g of crude **5c** (75%, purity 80% by HPLC). This sample dissolved in MeOH was charged on a silica gel column (250 g, Wacogel C-100, 40–100 mesh) packed with $CHCl_3$, washed with $CHCl_3$ (300 mL), and then eluted with $CHCl_3$/MeOH (15/1). Fractions containing **5c** only were collected and evaporated. The residue was precipitated from hexane/AcOEt to give 1.05 g (overall yield 25%) of an analytical sample: mp 136.5–138.5 °C: ^1H NMR δ 2.44–2.08 (m, 3, $C_{11a'}$ H and $NHCH_2CH_2CH_3$), 1.16 (m, 2, $NCH_2CH_2CH_3$), 0.94 center (m, 9, 2 CH_3 and $NHCH_2CH_2CH_3$); TLC, R_f 0.70 ($CHCl_3$/MeOH, 10/1); HPLC retention time 10.5 min (MeOH/H_2O/AcOH, 50/47.5/2.5). The sample was prepared as the hydrochloride salt by the same method used for **5a**.

Method 2. 4-[1-Hydroxy-2-[[1-(3,4-dihydro-8-hydroxy-1-oxo-1*H*-2-benzopyran-3-yl)-3-methylbutyl]amino]-2-oxoethyl]-3-(butylamino)butan-4-olide (5d). Thoroughly dried 1 (5 g, 11.8 mmol) was dissolved in *N,N*-dimethylformamide (20 mL) at 50 °C, and then butyl iodide (13.4 mL, 118 mmol) and triethylamine (5 mL, 35.4 mmol) were added. The mixture was stirred for 4 h at 50 °C. The reaction was worked up as described for **5c** to give 5.9 g of crude **5d** (purity 57% by HPLC analysis, yield 62%). The crude sample was purified by column chromatography (Amberite XAD-2, 500 mL). After the crude sample was charged on the column, the column was washed with MeOH/H_2O/1 N HCl (40/55/5) and then eluted with MeOH/H_2O/1 N HCl (60/36/4). Fractions containing **5d** only were combined and evaporated. The residue was dissolved in AcOEt (200 mL) and extracted with 0.05 M $NaHCO_3$ (150 mL) to make the free base form. The organic layer was washed with water, dried over Na_2SO_4, filtered, and evaporated. The residue was crystallized from hexane/AcOEt to give 1.25 g of pure **5d** (overall yield 23%): mp 157–157.5 °C dec; ^1H NMR (Me_2SO-d_6) δ 2.44–2.08 (m, 3, $C_{11a'}$ H and $NHCH_2(CH_2)_2CH_3$), 1.8–1.1 (m, 4, $NHCH_2(CH_2)_2CH_3$), 0.89 (m, 9, 2 CH_3 and $NHCH_2(CH_2)_2CH_3$); HPLC, retention time 7.9 min (MeOH/H_2O/AcOH, 65/33.2/1.8). Anal. ($C_{24}H_{34}N_2O_7$) C, H, N. The sample was converted to the hydrochloride salt for biological experiments as described for **5a**.

Method 2. 4-[1-Hydroxy-2-[[1-(3,4-dihydro-8-hydroxy-1-oxo-1*H*-2-benzopyran-3-yl)-3-methylbutyl]amino]-2-oxoethyl]-3-(pentylamino)butan-4-olide (5e). Thoroughly dried 1 (5 g, 11.8 mmol) was dissolved in *N,N*-dimethylformamide (20 mL) at 50 °C, and then pentyl iodide (23 g, 118 mmol) and triethylamine (5 mL, 35.4 mmol) were added. The mixture was stirred for 4 h at 50 °C. The reaction was worked up as for **5c** to give 6.8 g of crude **5e** (purity 52% by HPLC analysis, yield 53.8%). The crude sample was purified by column chromatography with Amberite XAD-2 (500 mL in water). After a solution of crude sample dissolved in MeOH/H_2O/1 N HCl (30/63/7) was charged on the column, the column was washed with MeOH/H_2O/1 N HCl (40/55/5) and then eluted with MeOH/H_2O/1 N HCl (75/22.5/2.5). Fractions containing **5e** only were combined and evaporated. The residue was dissolved in AcOEt (200 mL) and then extracted with 0.5 M $NaHCO_3$ (150 mL). The organic phase was washed with water, dried over Na_2SO_4, filtered, and evaporated. The residue was crystallized from hexane/AcOEt to give 2.06 g of pure **5e** (overall yield 36.9%): mp 159–161 °C dec; ^1H NMR Me_2SO-d_6) δ 2.44–2.08 (m, 3, $C_{11a'}$ H and $NHCH_2(CH_2)_3CH_3$), 1.9–1.1 (m, 6, $NHCH_2(CH_2)_3CH_3$), 0.89 (m, 9, 2 CH_3 and $NHCH_2(CH_2)_3CH_3$); HPLC retention time 13.7 min (MeOH/H_2O/1 N HCl, 65/33.2/1.8). Anal. ($C_{25}H_{36}N_2O_7$) C, H, N. The sample was prepared as the hydrochloride salt for biological examinations by the same method used for **5a**.

Method 2. 4-[1-Hydroxy-2-[[1-(3,4-dihydro-8-hydroxy-1-oxo-1*H*-2-benzopyran-3-yl)-3-methylbutyl]amino]-2-oxo-

Table VI. Retention Times in HPLC Analyses and Recoveries from Whole Blood

no.	composition of solvent[a] THF[b]:	H$_2$O	retention time, min 1	2	5x[c]	6x	recovery form whole blood, % 1 + 5x + 6x
5a	28	72	18.0		20.4	14.0	50
5b	28	72	18.0		22.8	18.6	20
5c	28	72	18.0		25.6	20.7	40
5d	32	68	14.2		18.4	15.5	30
5e	34	66	12.9		21.3	18.9	50
5f	36	64	7.2		20.3	18.7	80
2	28	72	18.0	18.8			50 (1 + 2)
1	28	72	18.0				50 (1 alone detected)

[a] PIC-B7 (1-heptanesulfonic acid, Waters Associates, Inc.) was added to the solvent in all cases (final concentration = 0.005 M). [b] Tetrahydrofuran. [c] x represents the corresponding one of a–f.

ethyl]-3-(hexylamino)butan-4-olide (5f). Compound **5f** was synthesized from 1 (5 g, 11.8 mmol) and hexyl iodide (23 g, 118 mmol) by the same methods used for **5e**, affording crude **5f**: 5.94 g (purity 52% by HPLC analysis, yield 54%). The crude sample was dissolved in MeOH/H_2O/1 N HCl (35/58.5/6.5) and charged on an XAD-2 column (500 mL in water). The column was washed with MeOH/H_2O/1 N HCl (50/45/5) and eluted with MeOH/H_2O/1 N HCl (80/18/2). Fractions containing **5f** only were combined and then evaporated. The residue was dissolved in AcOEt, extracted with 0.5 M $NaHCO_3$, washed with water, dried over Na_2SO_4, filtered, and then evaporated. The resulting **5f** was crystallized from hexane/AcOEt to give 1.48 g of pure **5f** (overall yield 26%): mp 166–168 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 2.44–2.08 (m, 3, $C_{11a'}$ H and $NHCH_2(CH_2)_4CH_3$), 1.6–1.2 (m, 8, $NHCH_2(CH_2)_4CH_3$), 0.89 center (m, 9, 2 CH_3 and $NHCH_2(CH_2)_4CH_3$); HPLC retention time 13.7 (45% aqueous THF containing PIC-B7). Anal. ($C_{26}H_{38}N_2O_7$) C, H, N.

6-[[1-(3,4-dihydro-8-hydroxy-1-oxo-1*H*-2-benzopyran-3-yl)-3-methylbutyl]amino]-4,5-dihydroxy-6-oxo-3-(methylamino)hexanoic Acid (6a). To open the γ-lactone ring, 0.1 N aqueous NaOH was dripped into a EtOH solution (10 mL) of **5a** (100 mg, 0.24 mmol) with stirring to a pH of 9.0. The pH was held at 9.0–10.0 by the addition of alkali until the spot of **5a** could no longer be detected on TLC (R_f 0.4, $CHCl_3$/MeOH, 10/1), and then the pH was adjusted to 6.5 with 0.1 N HCl. Compound **6a** was purified from the hydrolysate by preparative TLC (R_f 0.48, $CHCl_3$/MeOH, 1/1) to give 52 mg (50%) of **6a**: IR (KBr, carbonyl region) 1690 (sh), 1675 (sh), 1662, 1625; ^{1}H NMR (CD_3OD) δ 7.42 (dd, 1, J = 8 Hz, aromatic), 6.81 and 6.75 (2 d, 2, each J = 8 Hz, aromatic), 4.6 (m, 1, C_3 H), 4.30 (m, 1, $C_{5'}$ H), 4.12 (d, 1, J = 7 Hz, $C_{8'}$ H), 3.92 (dd, 1, J = 7 and 4 Hz, $C_{9'}$ H), 3.62 (m, 1, $C_{10'}$ H), 3.0 center (m, 2, C_4 H), 2.56 center (m, 2, $C_{11'}$ H), 2.28 (s, 3, $NHCH_3$), 2.0–1.1 (m, 3, $C_{3'}$ H and $C_{4'}$ H), 0.95 and 0.92 (2 d, each 3, each J = 6 Hz, 2 CH_3). Anal. ($C_{21}H_{30}N_2O_8$) C, H, N.

6b–f were prepared by the same method used for **6a** from corresponding **5b–f** and each hydrolysate was analyzed by HPLC for the identification of γ-lactone opened compounds of corresponding **5** in vivo without further purification by preparative TLC.

Determination of the Blood Level. To determine the concentration of each compound in blood, the plasma was treated and prepared as follows. Five to seven male Wistar rats (120–160 g) were treated as a group. The animals were deprived of food for 24 h and then test compounds or their formulations in aqueous solutions were administered orally. One or three hours after dosing, 1.8 mL of blood was collected from the jugular vein of each rat (anesthetized with ether) and then mixed with 0.2 mL of 0.9% aqueous citric acid followed by centrifuging (3000 rpm, 15 min, 0 °C). To 1 mL of the resulting plasma was added 2 mL of ethanol (in the case of **5d**, acetone was used instead of ethanol in order to eliminate the interfering factor derived from serum). The solution was mixed, allowed to stand in ice for 30 min, and then centrifuged (3000 rpm, 15 min). Two milliliters of supernatant was dried in vacuo at 25 °C and redissolved in 400 μL of ethanol followed by centrifuging (1 × 10^4 rpm, 2 min). The resulting supernatant was submitted for the HPLC analysis. The blood levels of the test compounds were considered as the sum of concentrations of three kinds of compounds: the N-alkylated compounds **5**, its corresponding ring-opened compound **6**, and their metabolite 1. The recovery of each test compound from whole blood was determined in vitro by the same procedure as above with use of blood of nontreated rats to which the test compound was added at a concentration of 50 μg/mL. All values for blood levels of test compounds were corrected by using these recoveries. The solvent systems, retention times in HPLC, and recoveries are shown in Table VI.

Determination of Partition Coefficients and Solubilities. Chloroform and 10 mM phosphate buffer (pH 7.5) were used as the organic and aqueous phases, respectively. The organic–aqueous phase volume ratio was 1:1; the initial concentration of drug in the aqueous phase was 1 mg/mL. The phases were shaken at 20 °C for 20 min followed by centrifugation (3000 rpm, 5 min) and then both aqueous and organic phases were submitted to HPLC analysis.

Solubilities of the test compounds in water or phosphate buffer (10 mM, pH 7.5) were determined as follows. Enough of each compound was suspended in water or phosphate buffer to give a saturated solution, which was achieved by sonication for 10 min at 20 °C and then centrifugation (3000 rpm, 10 min). The resulting supernatant was submitted to HPLC analysis.

Stress Ulcer. The gastroprotective activity of the test compound was measured by Takagi's method.[10] Male Wistar rats (160–180 g) were deprived of food for 24 h and then administered an aqueous solution of formulated test compound which was composed of test compound/glycerin/HCO-60 (1/12/0.05 in weight) intraperitoneally or orally (HCO-60; polyoxyethylene hydrogenated castor oil derivative, Nikko Chemical Ltd.). Rats treated with vehicle solution only were used as controls. One hour after administration, each rat was restrained in a net cage and immersed in a water bath (21 °C) for 6 h. The percent of ulcer inhibition was calculated as the method described in our previous paper.[3]

Carrageenin-Induced Paw Edema. The method described by Winter[11] was employed. Eight to ten male rats of the Wistar strain, weighing 120–150 g, served as a group for each dosage. The test compounds were administered orally in a suspension or a solution of 1% (hydroxypropyl)cellulose 1 h before the injection of 0.1 mL of 1% carrageenin aqueous solution into the right hind paw. ED_{50} was determined 3 h after carrageenin injection.

Bradykinin-Induced Paw Edema. A physiological saline solution of bradykinin was injected (50 μg/0.1 mL per paw) to the right hind paw instead of the carrageenin solution in the above test. ED_{50} was determined 2 h after bradykinin injection.

Dextran-Induced Paw Edema. A physiological saline solution of dextran (M_r 6 × 10^4–9 × 10^4) was injected (850 μg/0.1 mL per paw) into the right hind paw instead of the carrageenin solution in the above test. ED_{50} was determined 3 h after dextran injection.

Ultraviolet-Induced Erythema.[12] Erythema was induced on the depilated skin of the ventral trunk of male guinea pigs of the Hartlet strain (200–300 g). ED_{50} was determined 1 h after irradiation of ultraviolet light.

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Adjuvant Arthritis. The method described by Peason[13] was employed. Eight to ten male Sprague-Dawly rats (180-200 g) were used as a group. A suspension of the lyophilized powder of *Mycobacterium butyricum* (Difco) in corn oil was injected into the right hind paw (0.6 mg/0.1 mL per paw) to cause adjuvant arthritis. Test compounds were administered orally 1 h after the adjuvant injection and then followed by daily administration for a period of 18 days. Observation was continued for a period of 28 days.

Acute Toxicity. LD_{50} was determined from the 7-day mortality in male Wistar rats (120-140 g). ED_{50} and LD_{50} values were calculated according to the method of Litchfield and Wilcoxon.[14]

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Registry No. 1, 77674-99-8; **2**, 77682-31-6; **2**·HCl, 86594-32-3; **3b**, 77675-00-4; **5a**, 77685-58-6; **5a**·HCl, 92760-76-4; **5b**, 77700-96-0; 5b·HCl, 92760-77-5; **5c**, 77683-10-4; **5c**·HCl, 92760-78-6; **5d**, 77683-11-5; **5d**·HCl, 92760-79-7; **5e**, 77683-14-8; **5e**·HCl, 92760-80-0; **5f**, 77683-15-9; **5f**·HCl, 92760-81-1; **6a**, 92669-85-7; **6b**, 77689-68-0; **6c**, 92669-86-8; **6d**, 92669-87-9; **6e**, 92669-88-0; **6f**, 92669-89-1.

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Comparison of the Hypolipidemic Activity of Cyclic vs. Acyclic Imides

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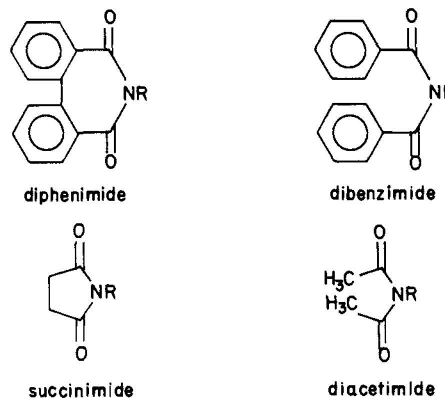
Two series of nitrogen-substituted cyclic and acyclic imides were examined for hypolipidemic activity in mice after dosing for 16 days at a dose of 20 mg/kg per day. The hypolipidemic activity of the unsubstituted, *N*-butyl, *N*-3-oxobutyl, and *N*-2-carboxyethyl derivatives of diacetimide and succinimide were compared as well as the unsubstituted and N-substituted dibenzimide and diphenimide. It was shown that an imide functionality incorporated into a ring was not necessary for hypocholesterolemic activity. Good hypocholesterolemic activity was observed in both series of acyclic and cyclic imides. However, a cyclic imido structure was a necessary requirement for good hypotriglyceridemic activity. A decrease in hypotriglyceridemic activity was noted when comparing the cyclic imides to their respective acyclic congeners.

Chapman and co-workers[1] initially reported the hypolipidemic activity of phthalimide and N-substituted phthalimide in rodents at low doses. Phthalimide, the parent compound, decreased plasma cholesterol and triglyceride levels by 43% and 56%, respectively, in mice after 16 days of dosing at a dose of 20 mg/kg per day.[2] A number of N-substituted phthalimide derivatives including alkyl, methyl ketone, carboxylic acids, and acetate esters of varying chain length were synthesized and tested for hypolipidemic activity in mice. The most significant reduction in serum cholesterol and triglyceride levels were afforded by the administration of *N*-*n*-butylphthalimide, the most active of the alkyl series, *N*-(2-carboxyethyl)phthalimide, the most active of the acid series, and *N*-(3-oxobutyl)phthalimide, the most active of the methyl ketone derivatives.[1] Further structure-activity relationship studies on the hypolipidemic activity of phthalimide were performed by Chapman[3] and were limited to three areas: (i) substitution on the imide nitrogen of phthalimide, (ii) changes in the structure of phthalimide involving the imide ring system, and (iii) changes in the structure of phthalimide involving the aromatic ring. All compounds structurally related to phthalimide that have been synthesized and examined for hypolipidemic activity to date have possessed an intact imide or lactam ring system. Therefore, a study was conducted in which the importance of the rigid imide ring system for hypolipidemic activity was determined. Two series of acyclic and cyclic imides were synthesized and their hypolipidemic activity in mice compared. The hypolipidemic activities of diphenimide and dibenzimide derivatives were compared as well as those of the succinimide and diacetimide derivatives.



R = H, C_4H_9, $CH_2CH_2C(=O)CH_3$, CH_2CH_2COOH, $CH_2CH_2CO_2C_2H_5$

Experimental Section

Chemistry. Melting points are uncorrected and were determined by using a Mel-Temp capillary melting point apparatus. Thin-layer chromatography was performed with silica gel 60 F-254 TLC plates. Column chromatography was performed with silica gel G-60 (60-200 mesh). ^{1}H NMR spectra were obtained on either a JEOL FX60 60-MHz nuclear magnetic resonance spectrometer or a Bruker 250 250-MHz nuclear magnetic resonance spectrometer. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and are correct within ±0.4% of theory. Diacetimide (1) and succinimide (7) were purchased from a commercial source and used as received.

***N*-Butyldiacetimide (2).** By the procedure of Mariella et al.[4] *n*-butylamine (10.0 mL, 0.10 mol) was added dropwise over a period of 15 min to a solution of 0.5 g of anhydrous sodium acetate in acetic anhydride (125 mL, 1.32 mol). The resulting solution was stirred under reflux for 20 h, after which the unreacted acetic anhydride was removed in vacuo. The residue was dissolved in 50 mL of water and the solution was stirred for 45 min and then

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