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Studies on Benzhydryl Derivatives. I. Synthesis and Anti-Inflammatory Activity[1]

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A series of N-diphenylmethyl derivatives of glycine was synthesized and their anti-inflammatory and some other pharmacological properties were studied. The inhibition of carrageenin edema-induction and the protection of albumin from heat denaturation by these derivatives were studied in relation to the substituents in one of the phenyl groups.

The most potent compounds, 3,4-dichloro- (Compd. **22**) and 4-fluoro- (Compd. **16**) diphenylmethylglycine derivatives, showed the higher activity in carrageenin-edema and weaker toxicity tested by gastric irritation and acute toxicity study than 2-(4-isobutylphenyl)propionic acid (ibuprofen). Compd. **16** showed the significant and interesting synergic effect by co-administration with cortisone acetate in the various tests of anti-inflammatory activity.

Keywords——N-diphenylmethylglycine derivatives; anti-inflammatory activity; carrageenin edema inhibition; analgesic activity; acute toxicity test; synergic effect

There have been a number of reports showing the involvement of benzhydryl group in the wide varieties of biological activities. Negwer[3] surveyed 3326 compounds having 13 or more carbon atoms and cited 302 compounds including benzhydryl derivatives as biologically active substances. Harms *et al.*[4] suggested that even much greater number of diarylmethyl derivatives may be found to be biologically active if the structural features for the investigation are expanded so as to include those compounds having alicyclic or heterocyclic rings as substituents for one or both of the phenyl groups.

Anti-inflammatory activity is known to require the planar part such as an aromatic or heterocyclic ring and a free carboxylic acid group in the molecule.[5] Benzhydryl group should satisfy one of the requirements for this activity and also can be expected to lead the better absorption of the molecule through the intestinal membrane of the animal due to its hydro-

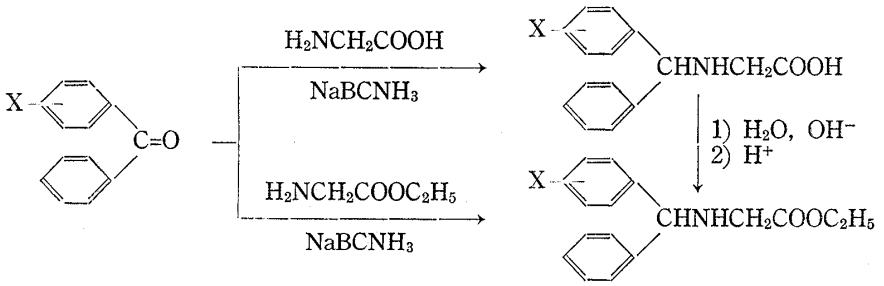


Chart 1. Preparation of N-Diphenylmethylglycine Derivatives

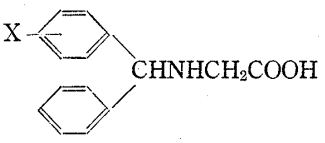
1) A part of this work has been reported in the 28th Meeting of the Kinki Branch of the Pharmaceutical Society of Japan, Nishinomiya (1978).
2) Location: *a) 3-11 Shodai-Tajika, Hirakata, Osaka, Japan*; *b) Kowakae, Higashi-Osaka.*
3) M. Negwer, "Organic-Chemical Drugs and Their Synonymes," Akademie-Verlag, Berlin, 1971.
4) A.F. Harms, W. Hespe, W. Th. Nauta, R. Rekker, H. Timmerman, and J. de Vries, "Drug Design," Vol. VI, E.J. Ariens, Ed., Academic Press, New York, San Francisco, London, 1975, p. 2.
5) T.Y. Shen, *Topics Med. Chem.*, 1, 29 (1967).

phobic or lipophilic property. Therefore N-diphenylmethyl derivatives of glycine, alanine and β-alanine were synthesized and their anti-inflammatory activity was studied.

Vazquez *et al.*,[6] Fox and Wenner[7] reported the synthesis of N-diphenyl methyl glycine and its ethyl ester by condensing benzhydryl amine with the amide and ester of chloroacetic acid, respectively. We synthesized N-diphenylmethyl derivatives by one step reaction, the reductive condensation of benzophenones with amino acids or their esters in the presence of sodium cyanoborohydride (Chart 1). The yield of the product from condensation reaction is shown in Table I, together with some analytical data.

As N-diphenylmethylglycine (Compd. **1**) showed the higher activity than other diphenylmethyl and diphenylacetyl compounds tested (Table II; will be discussed in Pharmacology Section) we applied the Topliss's manual method,[8] which was a practical application of Hansch's theory,[9] to obtain the highly active compound and synthesized additional four derivatives, 3,4-dichloro-, 4-chloro-, 4-methyl-, and 4-methoxy-derivatives, (Compd. **22**, **13**, **20**, and **21**), substituted in the aromatic ring of Compd. **1**. As the anti-edematous assay of these five compounds suggested the positive dependency on the electron withdrawing property (σ-dependency), 2,4-dichloro- (σ: 0.46) and 3,5-dichloro- (σ: 0.75) derivatives were synthesized according to Topliss.[8] No positive relationship between this activity and the σ-value, however, was concluded from the detailed assay. Therefore we synthesized several other compounds substituted with halogens in the aromatic ring, since 4-chloro-derivative and three dichloro-derivatives were highly active in the system.

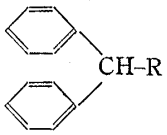
Table I. N-Diphenylmethyl Amino Acids synthesized

Compd.	X	Yield (%)	mp (°)[a]	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
Glycine derivatives										
1	H	63, 54[b]	205—208	$C_{15}H_{15}NO_2$	74.66	6.26	5.81	74.51	6.16	5.99
13	4-Cl	58	181	$C_{15}H_{14}ClNO_2$	65.34	5.12	5.08	65.31	5.15	5.21
14	3-Cl	40	185—186	$C_{15}H_{14}ClNO_2$	65.34	5.12	5.08	65.44	5.23	5.20
15	2-Cl	51	198—199	$C_{15}H_{14}ClNO_2$	65.34	5.12	5.08	65.42	5.18	5.22
16	4-F	58	211	$C_{15}H_{14}FNO_2$	69.49	5.44	5.40	69.35	5.40	5.52
17	3-F	45	199—200	$C_{15}H_{14}FNO_2$	69.49	5.44	5.40	69.38	5.33	5.56
18	2-F	41	205—206	$C_{15}H_{14}FNO_2$	69.49	5.44	5.40	69.43	5.42	5.50
19	4-Br	38	187—188	$C_{15}H_{14}BrNO_2$	56.27	4.41	4.38	56.25	4.39	4.55
20	4-CH_3	90	184—185	$C_{16}H_{17}NO_2$	75.27	6.71	5.49	75.03	6.70	5.54
21	4-OCH_3	60	182—183	$C_{16}H_{17}NO_3$	70.83	6.32	5.16	71.01	6.36	5.30
22	3,4-Cl_2	65	180	$C_{15}H_{13}Cl_2NO_2$	58.08	4.22	4.52	57.96	4.51	4.66
23	2,4-Cl_2	51	154	$C_{15}H_{13}Cl_2NO_2$	58.08	4.22	4.52	58.15	4.45	4.63
24	3,5-Cl_2	49	185	$C_{15}H_{13}Cl_2NO_2$	58.08	4.22	4.52	58.10	4.25	4.55
β-Alanine derivative				$(C_6H_5)_2CHNHCH_2CH_2COOH$						
2	H	28	137—139	$C_{16}H_{17}NO_2$	75.27	6.71	5.49	75.20	6.66	5.32

a) Not corrected.
b) Prepared by the alternative method (see Experimental Section).

6) A.G. de Vazquez, J.D. Bonafede, and M.J. Vernengo, *Anales Asoc. Quim. Argentina*, **60**, 501 (1972).
7) H.H. Fox and W. Wenner, *J. Org. Chem.*, **16**, 225 (1951).
8) J.G. Topliss, *J. Med. Chem.*, **15**, 1006 (1972); *ibid.*, **20**, 463 (1977).
9) C. Hansch, A. Leo, S.H. Unger, K.H. Kim, D. Nikaitani, and E.J. Lien, *J. Med. Chem.*, **16**, 1207 (1973).

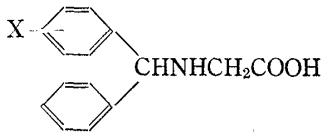
Table II. Anti-Edematous Activity of Diphenylmethyl and Diphenylacetyl Derivatives



Compound	R	Carrageenin edema inhibition (%)[a]
1	$NHCH_2COOH$	31.6**[b]
2	$NHCH_2CH_2COOH$	14.1*[b]
3	$2\text{-}NH\text{-}C_6H_4COOH$[d]	−0.3
4	$2\text{-}O\text{-}C_6H_4COOH$[d]	7.8
5	$2\text{-}O\text{-}C_6H_4CONH_2$[d]	13.0
6	$CONHCH_2COOH$[c]	3.3
7	$CONHCH(CH_3)COOH$[c]	2.9
8	$CONHCH_2CONH(CH_3)_2$[c]	−0.3
9	$CONHCH(COOH)CH_2CH_2CONH_2$[c]	1.3
10	$CONHCH(COOH)CH_2CONH_2$[c]	5.3
11	$CONHCH_2CH_2COOH$[c]	5.0
12	Ibuprofen	34.1**

a) Each test compound or ibuprofen was administered orally in a dose of 50 mg/kg 1 hr before the carrageenin treatment. Percent inhibition of 3 hr after the injection of carrageenin was calculated in comparison with the value for the control group which received only vehicle.
b) The figure with a star (*) designates the significant difference from the control ($p<0.05$), and the double stars (**): $p<0.01$.
c) The syntheses of them were reported.[e]
d) The synthesis will be reported somewhere else.
e) S. Takemura, H. Terauchi, and Y. Inamori, *et al.*, *Yakugaku Zasshi*, **98**, 869 (1978).

Table III. Anti-Edematous Activity of N-Diphenylmethylglycine Derivatives



Compound	X	σ-Value	Carrageenin edema inhibition (%)[a]
1	H	0	31.6**
13	4-Cl	0.23	33.2**
14	3-Cl	0.37	31.0**
15	2-Cl	0.23	32.3**
16	4-F	0.06	41.8**
17	3-F	0.06	33.6**
18	2-F	0.06	20.4*
19	4-Br	0.23	13.0
20	$4\text{-}CH_4$	−0.17	17.9*
21	$4\text{-}OCH_3$	−0.27	14.3
22	$3,4\text{-}Cl_2$	0.52	43.3**
23	$2,4\text{-}Cl_2$	0.46	22.6*
24	$3,5\text{-}Cl_2$	0.75	31.5**
12	Ibuprofen	—	35.9**

a) As for the experimental conditions and the notations of stars (* and **), see the footnote for Table II.

Pharmacology and Discussion

Anti-Edematous Activity of Diphenylmethyl and Diphenylacetyl Derivatives

Table II shows the inhibition rates against carrageenin-induced inflammation on rat's hind paw by diphenylmethyl and diphenylacetyl derivatives, among which N-diphenylmethylglycine (Compd. **1**) showed the highest anti-edematous activity, comparable to 2-(4-isobutylphenyl)propionic acid (ibuprofen). Much lower activity was demonstrated by β-alanine derivative (Compd. **2**) which differed only slightly in its structure from Compd. **1**. No anti-edematous activity was observed in anthranilic acid (Compd. **3**), salicylic acid (Compd. **4**) and salicylic amide (Compd. **5**) derivatives. The diphenylacetyl derivatives (Compd. **6**—Compd. **11**) were found to be inactive in this system.

Anti-Edematous Activity of N-Diphenylmethylglycine Derivatives

All the diphenylmethylglycine derivatives showed appreciable anti-edematous activity (Table III). The activities of Compd. **19** (4-bromo-derivative), Compd. **21** (4-methoxy derivative) and of Compd. **20** (4-methyl derivative) were much lower than those of other active derivatives. All the chloro- (Compds. **13**, **14**, and **15**), fluoro-(Compds. **16**, **17**, and **18**) and dichloro- (Compds. **22**, **23**, and **24**) derivatives had the significant activity depending on the substitutes and the positions of the substitution in the aromatic ring. Compd. **16** (4-fluoro-derivative) and Compd. **22** (3,4-dichloro-derivative) had the highest activity among the compounds tested and their anti-edematous activity was higher than that of ibuprofen.

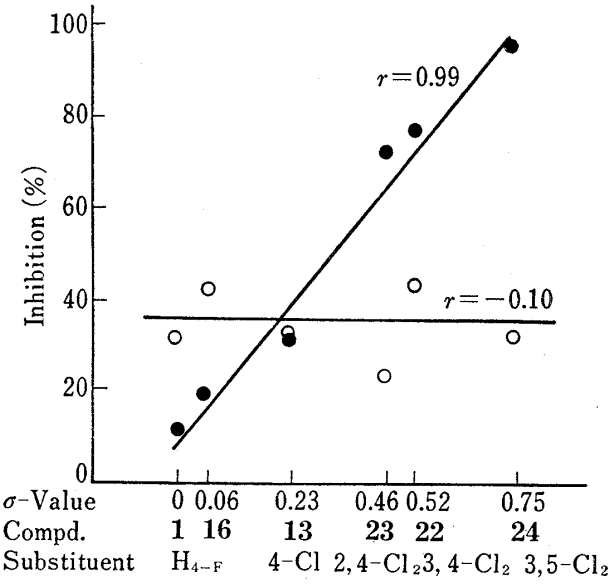


Fig. 1. Correlation between σ-Value and the Anti-Inflammatory Activities

Inhibition percentages of carrageenin edema at the dose of 50 mg/kg (○) and of heat denaturation of albumin at 0.3 mM (●) were plotted against σ-values of compounds. Experimental details for carragenin-edema were in the footnote for Table II and in Experimental Section, and for heat denaturation, in Experimental Section. The "r" values in figure are the correlation-coefficients.

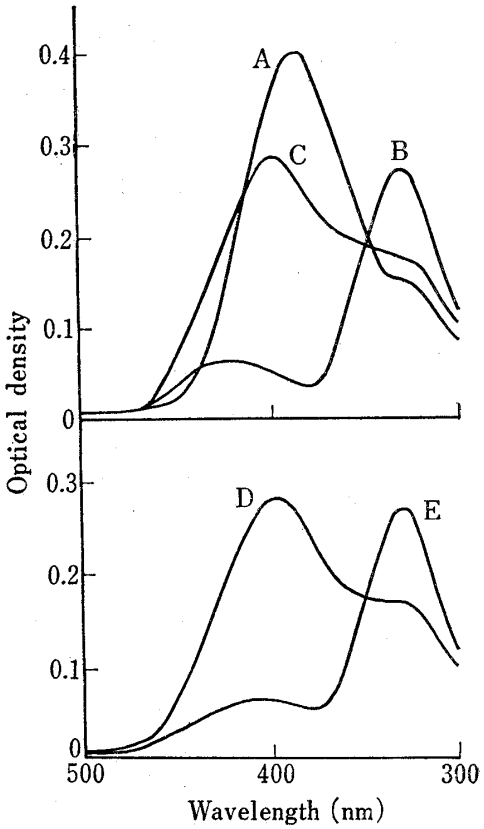


Fig. 2. Absorption Spectra of Free Pyridoxal-5-phosphate and Its Complex with Bovine Serum Albumin

Absorption of 0.1 mM pyridoxal phosphate in 0.1 M phosphate buffer, pH 7.5, were taken with and without BSA or other test compounds. A: Free pyridoxal phosphate; B: 0.1 mM BSA present; C: 0.1 mM BSA and 1 mM Compd. **22** present; and E: 0.1 mM BSA and 1 mM Compd. **16** present.

The relationships between the electronic property of the substituents in the aromatic ring of the compounds and their biological and biochemical activities are illustrated in Fig. 1. Electron-withdrawing property of the substituents, σ-value, had neither positive nor negative effect on the apparent anti-edematous activity of the compounds. The strong σ-value dependency, however, was observed in the protective activity for albumin from the heat denaturation, which had been proposed as one of the anti-inflammatory tests *in vitro*.[10] All the diphenylmethyl drivatives protected the protein from the heat denaturation to some extent depending on the their potencies and concentrations in the reaction mixture. Compounds which had the substituents with the higher σ-values such as Compd. **23**, **22** and **24** showed the stronger protection against the heat denaturation at various concentrations than those with the low σ-values (Compd. **1**, **16** and **13**). The correlation-profile between σ-value and the protective activity at 0.3 mM is in Fig. 1.

Dempsey and Christensen[11] reported the binding of pyridoxal phosphate with serum albumin *via* ε-amino groups of lysine residues resembling with the enzyme-coenzyme interaction in histidine decarboxylase, transaminase, and so on. Various anti-inflammatory drugs are known to inhibit this binding detected by the inhibition of the spectral shift from 387 nm to 332 nm due to the complex formation.[12] Compd. **23**, **22**, and **24** which showed the strong protection against heat denaturation inhibited complex formation of albumin with pyridoxal phosphate at 1 mM similarly to ibuprofen, while Compd. **16** which had much weaker protective activity did not interfere this interaction at 1 mM (Fig. 2). Therefore the protection of albumin from heat denaturation may be mainly due to the interaction or binding of the diphenylmethyl derivatives with the protein.

Table IV. Pharmacological Activities of N-Diphenylmethylglycine Derivatives

Compound[b]	Anti-inflammatory activities inhibition (%)[a]				Analgesic activity Writhing syndrome inhibition(%)[a]	Ulcergenic activity Lesion area (mm^2)	Approx. LD_{50} (mg/kg)[g]
	Vascular permeability[c]	Leucocyte emigration[d]	Kaolin edema[e]	Granuloma formation[f]			
1	10.6	15.2	12.5	3.3	15.9	0.10	>1000
16	28.1**	31.5**	22.6**	4.0	37.5**	1.16	>1000
22	22.2**	24.7**	25.7**	9.0	29.5**	0.63	>1000
Ibuprofen	19.7*	25.5*	26.1**	10.2	30.7*	1.95	800
Cortisone acetate	−1.1	55.5**	NT[h]	20.1**	−1.1	NT[h]	NT[h]

a) The inhibition rates were calculated in comparison with the values for the control groups. Percentage with a star (*) designates the significant difference from the control ($p<0.05$), and the double stars (**): ($p<0.01$).
b) Test compounds and ibuprofen were administered orally in the dose of 50 mg/kg and cortisone acetate, 25 mg/kg excepting the acute toxicity study.
c) Vascular permeability induced by acetic acid was estimated by the amount of protein exuded in peritoneal fluid.
d) Leucocyte-emigration was estimated by counting the numbers of leucocytes in CMC-pouch.
e) Inhibition for kaolin edema was the result from 8 hr after the induction.
f) The effect on granuloma formation was obtained by the successive administrations for 7 days.
g) LD_{50} was obtained graphically.
h) Not tested.

Pharmacological Properties of N-Diphenylmethylglycine Derivatives

Some other pharmacological properties of Compd. **1** (the parent compound), Compd. **16** and **22** were studied further. The results are summarized in Table IV. Compd. **16** and **22** and ibuprofen showed the significant anti-inflammatory activity tested by vascular permeability of proteins and leucocyte emigration into CMC-pouch. Cortisone acetate

10) Y. Mizushima and H. Suzuki, *Arch. Int. Pharmacodyn. Ther.*, **157**, 115 (1965).
11) W.B. Dempsy and H.N. Christensen, *J. Biol. Chem.*, **237**, 1113 (1962).
12) I.F. Skidmore and M.W. Whitehouse, *Biochem. Pharmacol.*, **15**, 1965 (1966).

TABLE V. Synergistic Effect of 4-Fluorodiphenylmethylglycine (Compd. **16**) with Cortisone Acetate[a]

Dosage of Compd. **16** or ibuprofen (mg/kg/day)	Carrageenin edema swelling (%)	CMC-Pouch method		Wet weight of carrageenin granuloma (mg/g body wt.)	Ulcerated area[b] (mm^2)
		Leucocyte Emigration ($10^2/mm^3$)	Protein exudation (mg/ml)		
Control	65.0±1.8 (100)	48.9±3.6 (100)	5.87±0.18 (100)		
Compd. **16**, 25	52.6±2.2** (80.9)	32.2±3.1** (65.8)	4.65±0.16** (79.2)		
Ibuprofen, 25	49.4±2.9** (76.0)	36.7±3.9* (75.1)	5.18±0.13** (88.2)		
Control				98.8±4.6 (100)	0
Compd. **16**, 50				97.4±7.1 (98.6)	0.33±0.15
Ibuprofen, 50				89.6±7.0 (90.7)	1.53±0.39
Cortisone Acetate (12.5 mg/kg)[c]					
Control	55.7±3.2 (100)	42.6±3.8 (100)	5.94±0.16 (100)		
Compd. **16**, 25	43.3±2.8** (77.7)	26.7±2.6** (62.7)	4.19±0.20** (70.5)		
Ibuprofen, 25	45.5±3.0* (81.7)	34.3±3.0 (80.5)	5.23±0.15** (88.0)		
Cortisone Acetate (25 mg/kg)[c]					
Control	47.8±2.5 (100)	25.9±2.6 (100)	5.58±0.20 (100)		0.05±0.04
Compd. **16**, 25	41.1±1.6* (86.0)	19.8±2.3 (76.4)	4.33±0.16** (77.6)	89.9±6.7 (100)	
Ibuprofen, 25	40.9±2.3 (85.6)	22.8±2.5 (88.0)	5.07±0.25 (90.9)		
Compd. **16**, 50				68.5±6.9* (76.2)	0.57±0.22
Ibuprofen, 50				71.5±5.7 (79.5)	1.21±0.42
Cortisone Aectate (50 mg/kg)[c]					
Control				80.1±5.4 (100)	0.55±0.24
Compd. **16**, 50				57.6±3.4** (71.9)	0.36±0.21
Ibupro en, 50				64.0±5.0* (79.9)	0.98±0.83

a) Each value is shown as the mean and standard error of 10 rats for the swelling percentage by carrageenin, the number of leucocyte emigrated, the amount of proteins exuded (mg/ml) into CMC-pouch, wet weight of carrageenin granuloma (mg/g body weight) and ulcerated area (mm^2). The value in parentheses is the inhibition rate by Compd. **16** or ibuprofen in comparison with the value of the corresponding control. Inhibition rate for the group treated with cortisone and Compd. **16** or ibuprofen was calculated on the base of the corresponding value of the control group treated with cortisone alone. Values with stars, * and **, designate the statistically significant difference from the corresponding control, ($p<0.05$) and ($p<0.01$) respectively.

b) Ulcerogenic reaction shown here was from the same experiment for granuloma formation. Rat's a stomach was examined for ulceration by the same way as described in Experimental Section after the treatment with compounds for 7 days.

c) Cortisone acetate at the desired amounts was administered orally 30 min before the induction of carrageenin edema or the injection of CMC-solution. The effect on granuloma was from the treatment for 7 days similar to other test compounds.

appeared to be greatly different in these properties. It inhibited the leucocyte emigration at much greater rate compared with ibuprofen and three diphenylmethyl compounds, but did not inhibit the vascular permeability to any extent. The edema-induction by kaolin was inhibited significantly and to the comparable extent by Compd. **16**, **22** and ibuprofen. Granuloma formation was weakly inhibited by both Compd. **22** and ibuprofen, but not by Compd. **1** and **16**. Cortisone acetate was also different in this property and showed the higher and significant inhibition on granuloma formation. Compd. **1** showed the similar, but much lower activities than Compd. **16** and **22** in all of these anti-inflammatory tests.

Compd. **16**, **22** and ibuprofen were found to have the analgesic activity judged from the effective reduction of writhing syndrome induced by acetic acid but cortisone acetate did not show this activity. This analgesic activity of these compounds suggested the possibility that the apparent anti-inflammatory activity might have been due to their inhibitory function on the central nervous system. In fact, one of the inhibitory drugs for the central nervous system, chlorpromazine, inhibited carrageenin edema significantly. The treatment with chlorpromazine, however, gave only slight effect on the anti-inflammatory function of Compd. **16** and **22**, which showed the strong activity in addition to the probable inhibition by chlorpromazine due to the action on the central nervous system. Namely, Compd. **16** and **22** at 50 mg/kg inhibited edema formation for additional 32% and 26%, respectively, by co-administration of 5 mg/kg chlorpromazine against the control group which received the same amount of chlorpromazine 30 min before edema induction. Therefore the anti-inflammatory activity of these compounds, Compd. **16** and **22**, should be mainly due to some other biological mechanism but not due to the inhibition on the central nervous system.

These three diphenylmethyl derivatives were less toxic than ibuprofen tested by the gastric irritation and acute toxicity studies in rats. Ulcer formation caused by the oral administration was almost negligible in Compd. **1** and **22**, but Compd. **16** gave the higher ulcer formation rate which was closer to that by ibuprofen. Almost no toxic phenomena were observed by oral administration of Compd. **1** up to the dose of 1000 mg/kg in the observation of body weight and the general behavior of animals for 7 days. Compd. **16** suppressed the increase in the body weight depending on the dosages and the administration of 1000 mg/kg of compound **16** gave only 36g-gain after 7 days compared with 60 g in the control group. However the observable effect on the general behavior of the animals died within 7 days. Compd. **22** was more toxic in this criterion compared with Compd. **1** and **16**. Significant reduction in the body weight was observed at the dosages more than 450 mg/kg depending on the amount administered, but no animals died whithin 7 days and animals showed the recovery and increase in the body weight 3 to 5 days after the administration. Ibuprofen inhibited the increase in the body weight at 300 mg/kg and the significant decrease, more than 450 mg/kg. LD_{50} of ibuprofen was calculated as 800 mg/kg.

Synergistic Effect of 4-Fluorodiphenylmethylglycine (Compd. 16) with Cortisone Acetate

Mechanism of anti-inflammatory reaction of cortisone acetate may entirely different from that of the diphenylmethyl derivatives. In order to see whether they could show their individual or synergistic activity, cortisone acetate was co-administered with Compd. **16**, 4-fluoro-derivative. The swelling by carragenin was inhibited for some 20% by 25 mg/kg (half of the amount used in the former experiments) of Compd. **16** and ibuprofen (Table V). Treatment with cortisone gave the significant inhibitions depending on the amounts administered, 14% by 12.5 mg/kg and 26% by 25 mg/kg, and the co-administration of Compd. **16** or ibuprofen with cortisone led to the additional inhibition of the swelling. The additional inhibition-rate in comparison with the swelling for the group treated with cortisone alone was around 20% for both Compd. **16** and ibuprofen.

The effect of co-administration on leucocyte emigration was also similar to that on carrageenin edema. The inhibition of protein exudation into the CMC-pouch by Compd. **16** and ibuprofen was not affected by cortisone. Ulcer-inducing activity did not change by

co-administration of cortisone and with the higher amount of cortisone this toxicity was not additional although cortisone itself also had some ulcer inducing activity.

Significant synergic effect was observed in carrageenin granuloma. Compd. **16** itself did not show the inhibitory effect on granuloma, but gave the apparent inhibition by the co-administration with cortisone. Cortisone acetate alone inhibited the granuloma formation for 9% and 19% at 25 and 50 mg/kg/day, respectively. Compd. **16** at 50 mg/kg/day induced the additional some 25% inhibition by the co-administration with cortisone (25 and 50 mg/kg) calculated on the base of corresponding value of sole administration of cortisone. This activity detected only by the co-administration with cortisone may be explained as the strong stimulation of the activity of cortisone by Compd. **16**, the indirect effect of Compd. **16** to prepare the physiological conditions in animals for the action of cortisone or/and the development of the activity in Compd. **16** by some special mechanism due to the interaction or co-existence with cortisone. Similar but much weaker synergic effect was observed in ibuprofen.

These pharmacological results obtained suggest the possibility of the useful clinical application of these diphenylmethyl derivatives in the consideration of the strong anti-inflammatory activity tested in various methods, the significant synergic effect with cortisone acetate and relatively low toxicity including gastric irritation.

Experimental

General Method for the Synthesis——A mixture of the diarylketone (10 mmol), glycine (10 mmol), methanol (20 ml), H_2O (2 ml) and $NaBCNH_3$ (15 mmol) was refluxed on a water bath for 26 hr. The solvent was removed by distillation under reduced pressure and a mixture of 10% aqueous NaOH (2 ml) and H_2O (20 ml) was added to the residue to give a suspension. The pH of H_2O-layer obtained from repeated ether extraction was brought to pH 5.0 with 10% HCl and the solution was allowed to stand overnight. The crystals were collected by filtration and washed with H_2O.

Compd. 1 was recrystallized from MeOH and Compd. 2 and Compd. **13**—**24** were purified by repetition of dissolution, precipitation and washing procedures described above.

Alternative Synthetic Method for N-Diphenylmethylglycine (Compd. 1)——Compound 1 was synthesized also by the following alternative method. Benzophenone (10 mmol) and glycine Et-ester hydrochloride (10 mmol) in MeOH (15 ml) were refluxed with $NaBCNH_3$ (15 mmol) on a water bath for 5 hr and 10 ml of H_2O were added to the residue obtained from evaporation under reduced pressure. The ether-layer obtained from repeated extractions (twice) from this suspension was condensed to give an oil. To this oil was added a mixture of 10% NaOH (4 ml) and MeOH (6 ml) and the resulted mixture was refluxed for 2 hr. The MeOH was removed by distillation and the residue was dissolved in H_2O (10 ml). Further procedures involving ether-extraction, pH-adjustment and so on were exactly the same as those described above in the General Method. The recrystallized sample was identical with Compd. 1 synthesized by the former method in the mixed melting point determination and the comparison of IR spectra.

Assay for Anti-Inflammatory Activities——Animals were fasted for 16 hr prior to the treatment with the inflammation-inducing reagents and test compounds at appropriate doses suspended in 0.2% carboxymethyl cellulose (CMC) were administered orally in a volume of 10 ml/kg body weight. The percent inhibition of inflammatory reactions was calculated for each test compound in comparison with the value for the corresponding control group. Each group consisted of 10 animals. The statistical significance was evaluated by Student's t-test.[13]

[A] Carrageenin-Induced Paw Edema: The modified Winter's method[14] was applied for the assay of the carrageenin-induced edema on rat hind paw. Edema was provoked by the subcutaneous injection of 0.2 ml of 1.5% carrageenin on the sole of the right hind paw of young male rats, weighing 180—200 g (Sprague-Dawley strain). The swelling percentage was calculated from the difference of the paw-volumes before and 3 hr after the carrageenin treatment estimated from the volume of the displacement of mercury. Test compounds except cortisone acetate were administered orally 1 hr before the carrageenin treatment. Cortisone acetate was administered to animals 30 min before the carrageenin-injection.

[B] Vascular Permeability induced by Acetic Acid: Inflammatory reaction was induced by intraperitoneal injection of 0.25 ml of 1.2% acetic acid[15] in mice weighing 30—35 g (ddy strain). Animals were sacrificed 2 hr after the injection of acetic acid. The total amount of protein exuded in peritoneum was

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determined by biuret reaction according to the method by Gornall *et al.*[16] as an index of vascular permeability. Test compound was administered 1 hr before the treatment with acetic acid.

[C] Carboxymethyl Cellulose (CMC) Pouch Method: CMC-pouch was made on the dorsum of the rat by subcutaneous injection of 5 ml of 2% CMC solution[17] sterilized previously at 120° for 30 min. After 3 hr was taken 0.1 ml of the pouch fluid in a Tuberculin syringe, mixed in 5 ml of physiological saline and the amount of proteins exuded was measured by Lowry method.[18] As for the measurement of the numbers of leucocytes, pouch fluid (0.1 ml) taken 6 hr after the injection of acetic acid was mixed with 0.9 ml Türk solution to stain leucocytes. The number of leucocytes was counted with Fucks-Rosenthal haemocytometer. Test compounds were administered orally 1 hr before the CMC-injection.

[D] Carrageenin-Induced Granuloma: Granuloma pouch was induced by the modified Selye's method.[19] Carrageenin solution (2% in 0.9% NaCl) was sterilized at 110° for 30 min and 4 ml of the solution containing 0.1 mg/ml of dihydrostreptomycin sulfate and penicillin G (200000 units) were injected subcutaneously on the dorsum of each rat. Test compounds were administered orally once a day for successive 7 days and during this period animals received food and water *ad libitum*. Animals were fested for 24 hr after the last administration of the test compounds and the capsules of the granulomatous tissue were taken carefully from the sacrificed animals. The pouch fluid was removed and the weight of the capsule was measured.

[E] Kaolin-Induced Edema: According to the method of Vinegar[20] 0.2 ml/body of 10% kaolin was injected subcutaneously in the sole of the hind paw of the rat fasted for 16 hr. Rats having edema at about the same degree were chosen after 3 hr induction period and test compounds were administered orally 4 hr after the kaolin-treatment. Paw-volumes were measured at 2 hr intervals in the same way described for carrageenin induced edema.

[F] Heat Denaturation of Albumin:[10] The mixture of 3.6 ml of 0.75% bovine serum albumin in 0.2 M phosphate buffer, pH 5.3, and 0.4 ml of test compounds at the desired concentrations in 10% dimethylsulfoxide was incubated at room temperature for 15 min and then treated at 63° for 10 min. The reaction mixture was cooled down immediately and the turbidity of the solution was measured at 660 nm. The protection rate from denaturation was calculated from the difference between the control and the sample.

[G] Binding of Pyridoxal-5-Phosphate with Bovine Serum Albumin:[12] One and half ml of 0.3 mM bovine albumin (BSA) in 0.1 M phosphate buffer, pH 7.5, was mixed with 1.5 ml of 3 mM test compound in the same buffer and the mixture was incubated at room temperature for 15 min. To this mixture was added 1.5 ml of 0.3 mM pyridoxal-5-phosphate and this reaction mixture was kept at room temperature for 90 min. Absorption spectrum was recorded against BSA solution and the solution of test compound as the reference.

Other Pharmacological and Toxicological Studies——[A] Analgesic Activity: The effect on writhing syndrome induced by acetic acid[21] was studied as one of the indices of the analgesic activity. The mouse weighing 30 to 35 g (ddy strain) was placed in an individual observation chamber immediately after the intraperitoneal injection of 0.1 ml/10 g body weight of 1.2% acetic acid. After 15 min were recorded the numbers of writhing syndrome in 5 min. Test compounds were administered orally 1 hr before the injection of acetic acid. Each group consisted of 10 mice.

[B] Ulcergenic Activity: Test on gastric irritation was by the method of Jahn.[22] Test compounds were administered orally to male rats weighing 180—200 g which had been fasted for 24 hr previously. After 4.5 hr duration, animals were sacrificed and the whole area with mucosal erosion and ulcer in stomachs was measured under the magnification by 10. Each group consisted of 10 rats.

[C] Acute Toxicity Test: A group consisting of 5 male rats weighing 180—200 g was fasted for 24 hr and test compounds were administered orally at the doses of 200, 300, 450, 675 and 1000 mg/kg. Animals were kept under observation including the body weight-measurement for 7 days and during this period were available food and water for rats *ad libitum*.

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