Anti-Inflammatory and CNS Depressant Properties of 1-(4-Chlorophenyl)-4-{2-[3-(2-pyridyl)acrylyloxy]-ethyl}-piperazine (DA 1529)

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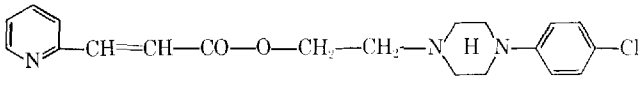
1-(4-Chlorophenyl)-4-{2-[3-(2-pyridyl)acrylyloxy]ethyl}-piperazine (DA 1529) has been found the most active among 29 alkylpiperazine esters studied. Following oral administration, DA 1529 prevents the edema formation provoked in rats by subplantar injection of carrageenin, formalin, and dextran; it has antinociceptive effect on inflamed (not on normal) foot of rats; it has antipyretic effect in yeast-treated pyrexial rats. DA 1529 is as active or only a little less active than phenylbutazone as anti-inflammatory; it is more active than phenylbutazone as analgesic and antipyretic. The acute toxicity in mice of DA 1529 is similar to that of phenylbutazone. DA 1529 has been found to possess mild CNS depressant properties as evidenced in mice by the results of the barbiturate potentiation test, of the rotarod test, by the reduced spontaneous motility, by analgesia in the hot-plate test, by the hypothermic effect.

AMONG 29 alkylpiperazine esters synthesized (1) and tested for anti-inflammatory, analgesic, and antipyretic activity, the 1-(4-chlorophenyl) - 4 - {2 - [3 - (2 - pyridyl)acrylyloxy]-ethyl}-piperazine (DA 1529) proved to be the more interesting one. Since the completion of the above-mentioned researches, the pharmacological properties of the compound have been explored more fully, and this report is a complete account of the results obtained.

The prevention in rats of the carrageenin-induced edema has been regarded as a reliable method for testing the anti-inflammatory properties of nonsteroid anti-inflammatory drugs (2). Since it has been proved that many compounds unrelated to the anti-inflammatory drugs are very active on this test (3), it was thought advisable to expand the investigation on our drug and look for many other activities in order to draw a clear and correct picture of its pharmacological profile. From this research DA 1529 turned out to be an anti-inflammatory drug with some mild CNS depressant properties.

The compound is a colorless, crystalline material, soluble in water as hydrochloride, and has a molecular weight of 371.86 and the following formula:



METHODS

Male albino mice of 18–22 Gm. and male Sprague-Dawley albino rats of 140–180 Gm. were used in these experiments.

In all the tests, DA 1529 was administered as hydrochloride in aqueous solution at the constant volume of 10 ml./Kg. in rats and 20 ml./Kg. in mice. The control animals received corresponding volumes of saline. The median effective doses (LD_{50} or ED_{50}) were calculated according to Litchfield and Wilcoxon (4) for the quantal responses, and according to Burn (5) for the graded responses. The significance of the differences was estimated using the Student t test as test of significance.

Acute Toxicity.—The acute toxicity tests were performed on mice and rats. The animals in groups of 10 to 20 for each dose tested were treated intraperitoneally or orally and observed for 96 hr. The effects appearing within 3 hr. after treatment were studied according to the scheme suggested by Irwin (6). The doses used ranged from 25 to 200 mg./Kg. for the intraperitoneal route and from 50 to 300 mg./Kg. for the oral route.

Anti-Inflammatory Activity.—The anti-inflammatory activity was estimated in rats following subplantar injection in the left hind paw of formalin (0.1 ml./rat of a 0.75% v/v solution), dextran (0.05 ml./rat of a 6% w/v solution), and carrageenin (0.1 ml. of a 1% w/v suspension in sterile normal saline) (7).

The degree of the edema was expressed as percentage increase of the thickness of the paw after the injection of the edema-provoking agent (measured with a dial thickness gauge) over the thickness of the same paw before injection of the edema-provoking agent. The anti-inflammatory effect was estimated at different time intervals, as specified in the tables, following oral administration of 50 100, and 200 mg./Kg. Phenylbutazone, 200 mg./Kg. orally, was used as reference compound.

Antipyretic Activity.—The antipyretic activity was studied according to the method of Smith and Hambourger (8) in groups of 5 rats for each dose. The animals were housed in a thermostatically controlled room at 23°. The rectal temperatures were measured using a thermocouple applicator before ad-

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ministration of DA 1529 and at hourly intervals for 5 hr., following intraperitoneal administration of 25, 50, and 100 mg./Kg., and oral administration of 50, 100, and 200 mg./Kg. Phenylbutazone, 100 mg./Kg. intraperitoneally and orally, was used as reference compound.

Analgesic Activity on Inflamed Tissue.—The analgesic activity was tested in rats, according to the technique of Randall and Selitto (9). DA 1529 was administered 90 min. after subplantar injection of brewer's yeast (0.1 ml./rat of a 20% suspension in normal saline); 10 to 15 animals were used for each dose. In evaluating the results, the regression line and the corresponding ED_{50} were calculated correlating the logarithm of the dose with the percentage increase of the pain threshold, 90 min. after treatment, of the inflamed paw and of normal paw in treated animals over the corresponding thresholds in control animals. DA 1529 was administered intraperitoneally at the dose of 25, 50, and 100 mg./Kg. and orally at the dose of 50, 100, and 200 mg./Kg. Phenylbutazone administered intraperitoneally, 100 mg./Kg., and orally, 200 mg./Kg., was used as reference compound.

Analgesic Activity—Hot Plate Method.—The analgesic activity was studied in mice, according to the method previously described in detail in another paper (10). DA 1529 was administered intraperitoneally at the dose of 50, 100, and 200 mg./Kg. and orally at the dose of 50, 100, 150, and 200 mg./Kg. to groups of 29 to 40 mice for each dose. Phenylbutazone administered intraperitoneally, 50 mg./Kg., and orally, 200 mg./Kg., was used as reference compound.

Hypothermic Activity.—The activity on the body temperature was evaluated in mice housed in a thermostatically controlled room at 23°. The rectal temperatures were recorded by means of a thermocouple applicator before treatment and 1, 2, 4, and 6 hr. after intraperitoneal administration of 25, 50, and 100 mg./Kg. and oral administration of 12.5, 25, 50, and 100 mg./Kg. of DA 1529. Twenty animals were used for each dose.

Activity on Spontaneous Motility.—The spontaneous motility was studied in mice, using Dews technique (11). DA 1529 was administered intraperitoneally at the dose of 12.5, 25, 50, and 100 mg./Kg. and orally at the dose of 50, 100, and 200 mg./Kg. 15 min. before the test. Six groups of five animals each were used for each dose and route of administration. Corresponding groups of normal mice were used as controls. The results were evaluated by determining the percentage variations of the motility of the treated groups compared with the controls and calculating the corresponding ED_{50} from the activity-log dose line.

Activity of *Hexobarbital* and *Pentobarbital* Sleeping Time.—Hexobarbital and pentobarbital were injected intraperitoneally in mice, at the respective doses of 100 mg./Kg. and 60 mg./Kg., 15 min. after intraperitoneal or oral administration of DA 1529. When hexobarbital was used DA 1529 was adminstered intraperitoneally at 12.5, 25, and 50 mg./Kg. and orally at 12.5, 50, and 100 mg./Kg. When pentobarbital was used DA 1529 was administered intraperitoneally at 25, 50, and 100 mg./Kg. and orally at 12.5, 25, 50, and 100 mg./Kg. The sleeping time was defined as the interval between the loss and the spontaneous return of the righting reflex. Ten to 20 animals were used for each dose and a group of control animals was always prepared in parallel. In evaluating the results the regression line and the corresponding ED_{50} were calculated correlating the logarithm of the dose with the percentage increase of the sleeping time of the treated group over that of the control group.

Anticonvulsant Activity.—The anticonvulsant activity was tested by means of: (*a*) protection from the tonic phase of convulsions due to maximal electroshock (10 ma., alternating current, 0.2 sec.); (*b*) protection from tonic-clonic type convulsions and from death due to lethal doses of pentamethylenetetrazole (125 mg./Kg. i.p.); (*c*) protection from the convulsive and lethal effects of lethal doses of strychnine (3 mg./Kg. i.p.).

The experiments were carried out on unfasted mice. DA 1529 was administered by the intraperitoneal route, 15 min. before the convulsant, to groups of 10 animals each at the dose of 100 mg./Kg.

Activity on the Movement Coordination.—The muscular activity and coordination were studied in mice, using the following methods: (*a*) Boissier's rotarod test (12). The number of mice which lost the capacity to cling to a rotating rod (2.5 cm. diameter, rough surface, 12 r.p.m.) was determined. Before the experiment the mice were selected so as to reject animals falling within 5 min. (*b*) Traction test described by Boissier (12). The mice were suspended by their forepaws from a horizontally stretched wire. The number of animals which lost the capacity of clinging to the wire with at least one hind paw within 5 sec. was determined. (*c*) Inclined screen test according to Randall *et al.* (13). The number of animals incapable of clinging to a fine mesh wire net inclined at approximately 30° to the horizontal was recorded. (*d*) Paralyzing action test according to Berger (14). The loss of the righting reflex for at least 1 min. was taken as test of paralysis.

The tests were performed 2, 5, and 15 min. after intraperitoneal administration and 5, 15, and 30 min. after oral administration of DA 1529. For the rotarod test the doses used were 25, 50, and 100 mg./Kg. intraperitoneally and orally. For the traction test the doses used were 50, 100, and 200 mg./Kg. intraperitoneally and orally. For the inclined screen and righting reflex tests the doses used were 50, 100, and 200 intraperitoneally and 100, 200, and 300 orally.

Action on the Pinna and Corneal Reflexes.—The changes in the pinna and corneal reflexes were determined in mice, according to the method of Witkin *et al.* (15). The tests were performed 2, 5, and 15 min. after intraperitoneal administration and 5, 15, and 30 min. after oral administration of DA 1529. The doses used were 25, 50, and 100 mg./Kg. intraperitoneally and 200, 300, and 350 mg./Kg. orally, 10–15 animals/dose being used.

Activity on the Cardiovascular System and Respiration.—The effect of DA 1529 on the arterial pressure and respiration was studied in male rabbits weighing 2.3–2.5 Kg. in ethyl-urethan narcosis (750 mg./Kg. i.p.). The blood pressure was recorded at the carotid artery using a mercury manometer, and the respiration by means of a Marey tambour connected to a pneumograph fixed to the chest. DA 1529 was administered to the animal

Table I.—Acute Toxicity in Mice and Rats

Animal	Route of Administration	Animals, No.	Doses, No.	LD_{50}, mg./Kg.	Confidence Limits $P = 95\%$
Mouse	i.p.	80	4	323.0	303.3–349.9
Mouse	oral	40	4	529.0	496.7–563.4
Rat	i.p.	50	5	311.0	275.2–351.4
Rat	oral	50	5	478.0	437.4–524.5

Table II.—Activity on Carrageenin-Induced Edema in Rats

Compd.	Dose, mg./Kg. Orally	Animals, No.	Edema, 6 hr. After Administration of the Compd. Degree[a]	Inhibition Compared with Controls, %
Saline	30 ml.	19	27.2 ± 1.40	...
DA 1529	50	20	23.8 ± 1.24	13
DA 1529	100	20	23.0 ± 1.43	16[b]
DA 1529	200	20	18.6 ± 1.29	32[b]
Saline	30 ml.	20	25.1 ± 0.85	...
Phenylbutazone	200	20	12.9 ± 1.32	49[b]

[a] Expressed as percentage increase of the thickness of the inflamed paw compared with the thickness before injection of carrageenin ± S.E. [b] Change significant at 0.02 level.

Table III.—Activity on Formalin-Induced Edema in Rats

Compd.	Dose, mg./Kg. Orally	Animals, No.	Edema, at Intervals After Administration of the Compd.—2 hr., 15 min.— Degree[a]	Inhibition Compared with Controls, %	—4 hr., 15 min.— Degree[a]	Inhibition Compared with Controls, %
Saline	10 ml.	10	69.0 ± 2.36	...	66.9 ± 2.59	...
DA 1529	50	10	62.4 ± 3.24	10	68.6 ± 2.64	0
DA 1529	100	10	55.4 ± 2.94	20[b]	57.0 ± 2.33	15[b]
DA 1529	200	10	43.8 ± 2.07	37[b]	46.8 ± 2.16	30[b]
Saline	10 ml.	20	75.4 ± 2.47	...	78.5 ± 2.51	...
Phenylbutazone	200	20	61.7 ± 2.55	18[b]	63.5 ± 2.55	19[b]

[a] Expressed as percentage increase of the thickness of the inflamed paw compared with the thickness before injection of formalin ± S.E. [b] Change si gnificant at 0.02 level.

Table IV.—Activity on Dextran-Induced Edema in Rats

Compd.	Dose, mg./Kg. Orally	Animals, No.	Edema, 90 min. After Administration of the Compd. Degree[a]	Inhibition Compared with Controls, %
Saline	10 ml.	10	91.1 ± 2.52	...
DA 1529	50	10	94.5 ± 2.11	0
DA 1529	100	10	91.2 ± 3.06	0
DA 1529	200	10	80.0 ± 2.97	12[b]
Saline	10 ml.	10	97.5 ± 2.23	...
Phenylbutazone	200	20	82.0 ± 4.05	16[b]

[a] Expressed as percentage increase of the thickness of the inflamed paw compared with the thickness before injection of dextran ± S.E. [b] Change significant at 0.02 level.

through the marginal ear vein at various doses, maintaining constant the volume and rate of administration (1 ml. in 30 sec.). The coronary vasodilator activity of the compound was determined on isolated rabbit heart prepared according to Langendorff and perfused at constant pressure according to the technique described by Setnikar (16); the amplitude and rate of the heart beats were recorded simultaneously.

Activity on Smooth Muscle.—The action on smooth muscle was studied on segments of guinea-pig ileum suspended in oxygenated Tyrode's solution, in a thermostatically controlled bath kept at 34°. The intestine was stimulated with standard doses of acetylcholine (10^{-7} Gm./ml. of chloride), histamine (10^{-6} Gm./ml. of hydrochloride), nicotine (2×10^{-6} Gm./ml. of bitartrate), and 5 HT (10^{-6} Gm./ml. of creatinine sulfate monohydrate).

RESULTS

Acute Toxicity.—The results of the toxicity tests on DA 1529 are given in Table I. The compound showed slight toxicity when administered by intra-

peritoneal and oral route in either mice or rats. The oral LD_{50}/i.p. LD_{50} ratio was found to be 1.64 for mice and 1.54 for rats. These values are almost identical for the two species and indicate good absorption of the substance under test through the digestive tract. The direct observation in mice performed according to the indications of Irwin (4) revealed that DA 1529 at nonlethal doses (100–200 mg./Kg. i.p. and 100, 200, 300 mg./Kg. orally) caused reduction of the spontaneous motility, curiosity, and pain sensitivity as well as muscular hypotonia, a very slight motor deficit, and reduction of the ipsilateral flexor reflex.

Reduced pain sensitivity was still evident at 50 mg./Kg., for both routes of administration.

Anti-Inflammatory Activity.—Tables II, III, and IV report the data relative to DA 1529's oral inhibiting effect of carrageenin, formalin, and dextran-induced edemas. The results of the experiments performed, reported in Table II, indicate the inhibiting effect of DA 1529 on carrageenin-induced edema; this effect is slightly less intense than that exerted by phenylbutazone. As shown by Table III, DA 1529 demonstrated to be capable of significantly reducing formalin-induced edema, exerting an anti-inflammatory action quantitatively superior to that of phenylbutazone. As for dextran-induced edema, DA 1529 exerted a slight anti-inflammatory action only at the 200 mg./Kg. dose; at this dose the action of DA 1529 was slightly less than that of phenylbutazone.

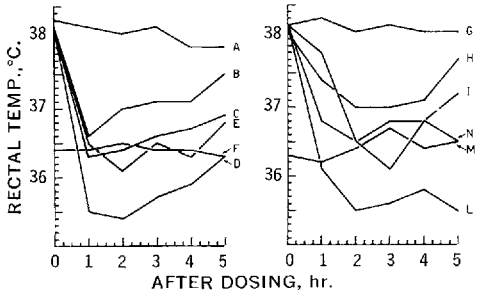

Fig. 1.—Antipyretic activity in rats. Key: A, fevered controls; B, DA 1529, 25 mg./Kg.; C, DA 1529, 50 mg./Kg.; D, DA 1529, 100 mg./Kg.; E, phenylbutazone, 100 mg./Kg.; F, nonfevered controls; G, fevered controls; H, DA 1529, 50 mg./Kg.; I, DA 1529, 100 mg./Kg.; L, DA 1529, 200 mg./Kg.; M, nonfevered controls; N, phenylbutazone, 100 mg./Kg.

Antipyretic Activity.—Figure 1 gives the variations of the rectal temperature. As the graphs show, DA 1529, administered by either the intraperitoneal or oral route, exerted a distinct inhibiting action on experimentally induced pyrexia in rats, the effect still evident, although to a slighter extent, 5 hr. after treatment. The antipyretic effect observed was found to be proportional to the dose administered.

Analgesic Activity on Inflamed Tissue.—Table V summarizes the results of the experiments performed with the gradual pressure method according to Randall and Selitto (19) on the inflamed and controlateral normal paw. The data reported show that DA 1529 exerted a marked effect on the inflamed paw but did not increase the pain threshold in the normal paw.

Under the same experimental conditions phenylbutazone is active only on the inflamed paw. The median effective doses calculated by plotting the percentage increase of the pain threshold in the inflamed paw in function of the logarithm of the dose were found to be 9.5 mg./Kg. for intraperitoneal administration and 40.1 mg./Kg. for oral administration.

Analgesic Activity–Hot-Plate Method.—Table VI summarizes the data relative to the hot-plate analgesic action, and gives the percentage increase of the reaction time (RT) to the heat nociceptive stimulus 1 and 2 hr. after treatment (i.p. and oral) with DA 1529, the total number of mice insensitive to pain during the 2 hr. of the experiment, and the ED_{50} for the two routes of administration. In this test, DA 1529 significantly increased the reaction time to the heat stimulus and provoked an analgesia proportional to the dose administered. The effective dose, producing analgesia in 50% of the animals, was found to be 105 mg./Kg. i.p. and 109 mg./Kg. orally.

Hypothermic Activity.—The results reported in Table VII indicate that after intraperitoneal or oral administration of 100 mg./Kg. of DA 1529, the rectal temperature of mice fell approximately 2°,

Table V.—Analgesic Activity (Randall-Selitto Method) in Rats

Compd.	Dose, mg./Kg.	Animals, No.	Inflamed Paw mm. Hg ± S.E.[a] Pain Threshold	Increase of Pain Threshold, %	ED_{50},[b] mg./Kg.	Normal Paw mm. Hg ± S.E.[a] Pain Threshold	Increase of Pain Threshold, %
Saline	10 ml. i.p.	10	59.2 ± 3.00	...	...	193.6 ± 10.12	...
DA 1529	25 i.p.	10	117.4 ± 8.27	98	9.50	217.2 ± 6.39	12[c]
DA 1529	50 i.p.	10	145.4 ± 16.29	146	...	237.0 ± 6.26	22[c]
DA 1529	100 i.p.	10	161.2 ± 13.15	172	...	199.0 ± 8.33	3[c]
Saline	10 ml. oral	15	53.3 ± 2.67	...	...	168.0 ± 7.59	...
DA 1529	50 oral	15	96.3 ± 9.16	81	40.15	170.5 ± 8.59	1[c]
DA 1529	100 oral	15	129.3 ± 13.95	142	...	203.9 ± 7.92	21[c]
DA 1529	200 oral	15	180.7 ± 14.92	239	...	195.3 ± 9.62	16[c]
Saline	10 ml. i.p.	15	76.0 ± 2.93	...	...	189.7 ± 9.50	...
Phenylbutazone	100 i.p.	15	146.8 ± 9.53	93	...	186.9 ± 5.99	0
Saline	10 ml. oral	15	61.6 ± 2.15	...	...	164.5 ± 5.51	...
Phenylbutazone	200 oral	15	129.2 ± 8.19	109	...	177.1 ± 5.58	8[c]

[a] 90 min. after administration of the compound. [b] Dose provoking a 50% increase in the pain threshold. [c] Difference nonsignificant at 0.05 level.

TABLE VI.—ANALGESIC ACTIVITY (HOT-PLATE METHOD) IN MICE

Compd.	Dose, mg./Kg.	Increase of Reaction Time at Intervals After Administration, % 1 hr.	% 2 hr.	Total No. of Mice Insensitive to Pain No.	%	ED_{50},[a] mg./Kg.	Confidence Limits $P = 95\%$
DA 1529	50 i.p.	124	114	8/40	20	105.0	82.03–134.40
DA 1529	100 i.p.	181	143	22/39	56	...	...
DA 1529	200 i.p.	227	161	28/40	70	...	...
DA 1529	50 oral	96	100	10/39	26	109.0	88.62–134.07
DA 1529	100 oral	131	122	16/39	41	...	...
DA 1529	150 oral	196	165	18/30	60	...	...
DA 1529	200 oral	187	180	21/29	72	...	...
Phenylbutazone	50 i.p.	12	37	0/19	0	...	...
Phenylbutazone	200 oral	47	36	0/10	0	...	...

[a] Dose inducing analgesia in 50% of the animals.

TABLE VII.—ACTIVITY ON BODY TEMPERATURE IN MICE

Compd.	Dose, mg./Kg.	Animals, No.	Body Temp., °C. ± S.E. Before Treatment	After Treatment 1 hr.	2 hr.	4 hr.	6 hr.
Saline	2 ml. i.p.	20	38.0 ± 0.14	37.5 ± 0.15	37.7 ± 0.12	37.2 ± 0.23	37.2 ± 0.19
DA 1529	25.0 i.p.	20	38.7 ± 0.09	38.0 ± 0.15	38.0 ± 0.14	37.5 ± 0.18	37.7 ± 0.13
DA 1529	50.0 i.p.	20	38.1 ± 0.18	37.1 ± 0.25	37.5 ± 0.18	37.5 ± 0.14	37.3 ± 0.14
DA 1529	100.0 i.p.	20	38.4 ± 0.12	34.9 ± 0.39	36.6 ± 0.22	37.6 ± 0.15	37.4 ± 0.17
Saline	2 ml. oral	20	37.9 ± 0.15	38.1 ± 0.13	37.9 ± 0.19	37.5 ± 0.16	37.7 ± 0.13
DA 1529	12.5 oral	20	37.9 ± 0.15	37.7 ± 0.13	37.9 ± 0.11	37.6 ± 0.13	37.3 ± 0.15
DA 1529	25.0 oral	20	37.8 ± 0.17	37.6 ± 0.15	37.6 ± 0.15	37.5 ± 0.15	37.5 ± 0.15
DA 1529	50.0 oral	20	38.3 ± 0.10	37.3 ± 0.27	37.6 ± 0.20	37.5 ± 0.20	37.5 ± 0.15
DA 1529	100.0 oral	20	38.1 ± 0.10	36.8 ± 0.21	37.4 ± 0.19	37.6 ± 0.12	37.3 ± 0.10

TABLE VIII.—ACTIVITY ON SPONTANEOUS MOTILITY, BARBITURATE DEPRESSION, MOVEMENT COORDINATION, AND ON PINNA AND CORNEAL REFLEXES IN MICE

Test	Time After Dosing, min.	Route of Administration	Animals, No.	Doses, No.	ED_{50}, mg./Kg.
Spontaneous motility	15	i.p.	120	4	27.5[a]
	15	os	90	3	94.3[a]
Hexobarbital sleeping time	15	i.p.	30	3	13.39[b]
	15	os	40	4	24.41[b]
Pentobarbital sleeping time	15	i.p.	60	3	35.35[b]
	15	os	70	4	26.74[b]
Rotarod	5	i.p.	30	3	56.0[c]
	15	os	30	3	55.0[c]
Traction	5	i.p.	30	3	88.0[c]
	15	os	30	3	117.0[c]
Inclined screen	5–15	i.p.	30	3	>200[c]
	5–15–30	os	30	3	>300[c]
Righting reflex	5–15	i.p.	30	3	>200[c]
	5–15–30	os	30	3	>300[c]
Pinna reflex	5	i.p.	60	4	98.0[c]
	15	os	30	3	298.0[c]
Corneal reflex	5–15	i.p.	60	4	>200[c]
	5–15–30	os	30	3	>300[c]

[a] Dose provoking a 50% reduction in the spontaneous motility compared with controls. [b] Dose provoking a 50% increase in the sleeping time compared with controls. [c] Dose inducing the effect considered in 50% of the animals.

compared with the controls. The compound exerted only a slight hypothermic effect at 50 mg./Kg. by the oral route. No significant change in the temperature was observed for the other doses tested (25–50 mg./Kg. i.p. and 12.5–25 mg./Kg. orally).

Activity on Spontaneous Motility.—As may be seen from Table VIII, DA 1529 administered intraperitoneally or orally, induced a significant reduction of the spontaneous motility.

Action on Hexobarbital and Pentobarbital Sleeping Time.—On both intraperitoneal and oral administration, DA 1529 markedly potentiated the hypnotic effect of hexobarbital and pentobarbital (Table VIII).

Anticonvulsant Activity.—DA 1529 injected intraperitoneally at the dose of 100 mg./Kg. was found to be inactive on convulsions induced by electroshock, pentamethylenetetrazole, or strychnine.

Action on Movement Coordination and Pinna and Corneal Reflexes.—Table VIII gives the results of the test on muscular activity and coordination and on the pinna and corneal reflexes. Table VIII reveals that the compound administered

intraperitoneally or orally exerted a marked effect on the rotarod and traction tests, at doses far from the toxic levels and from those causing behavioral changes. The effect was well marked 5 min. after intraperitoneal administration and 15 min. after oral administration. Fifteen and 30 min. after dosing the mice were normal. DA 1529 had no action on the inclined screen test and righting reflex. As for the alterations of the superficial reflexes, the pinna reflex was markedly influenced when the product was administered intraperitoneally, and only slightly so on oral administration.

By contrast the corneal reflex was not altered at any of the doses tested.

Activity on the Cardiovascular System and Respiration.—DA 1529, administered intravenously to rabbits at doses of 2, 4, and 8 mg./Kg., lowered blood pressure and increased respiration. These phenomena were only temporary and were all the greater the higher the dose. On isolated rabbit heart, DA 1529 perfused at the concentration of 10 mcg./ml., caused a slight increase in the coronary flow, and a slight reduction in the amplitude of the pulse rate and heart beat.

Action on Smooth Muscle.—On guinea pig ileum *in vitro*, DA 1529 exerted a very slight action against acetylcholine, 5-HT, and nicotine, while the antihistamine activity was still apparent, although slight (17% inhibition of histamine-induced contraction), at the concentration of 10^{-8} Gm./ml.

DISCUSSION

DA 1529 prevents the formation of edemas provoked in rats by subplantar injection of formalin, carrageenin, and dextran. More than as an anti-inflammatory, DA 1529 is active as an analgesic and antipyretic. The antinociceptive effect is very marked on the inflamed foot, insignificant on the normal foot. The antipyretic effect is well marked. Relative to phenylbutazone, DA 1529 is more active as an analgesic and antipyretic, as active or only a little less active as an anti-inflammatory. The acute toxicity of DA 1529 is similar to that of phenylbutazone. The data in Table I relative to DA 1529 have to be compared with data relative to phenylbutazone obtained in this laboratory in comparable experimental conditions (LD_{50} in mg./Kg.): 327 and 680 in mice; 215 and 637 in rats. The first value is relative to the intraperitoneal administration, the second one to the oral administration.

The therapeutic indexes (LD_{50}/ED_{50}) are as favorable for DA 1529 as for phenylbutazone. When the analgesic effect is considered the therapeutic index in rats (Randall-Selitto method) is 33 and 11 after intraperitoneal and, respectively, oral administration; in mice (hot-plate method) it is 3.0 intraperitoneally and 4.8 orally.

The anti-inflammatory, analgesic, and antipyretic effects of DA 1529 are apparent at doses provoking in rats only a mild sedation and not overt neural deficit or derangement of the autonomic nervous system or of the neuromuscular system.

DA 1529 has, however, some CNS depressant properties as the results obtained in mice seem to prove. It is active in the barbiturate potentiation test; in the rotarod test, it reduces the spontaneous motility and the sensitivity to painful stimulation. In addition, it has hypothermic properties. As it is well proved (17) that hypothermia, potentiation of the barbiturate depression, and depression of spontaneous motility are strictly correlated, it may be that the depressant effects observed in these experiments performed at 22–23° have been caused or exaggerated by the hypothermic effect. It may be the same as far as the analgesic effect observed in the hot-plate method is concerned since the thermal sensitivity of the feet of the mice is involved. Drugs modifying rectal or superficial temperature are known to have an effect on the response of mice in the hot-plate method (18–23). The analgesic effect, the superficial temperature of the paw, and the rectal temperature may, therefore, be causally related.

In the authors' opinion, however, the mild hypothermic effect is not fully responsible for the effects observed in their experiments since calming effects were still evident following administration of doses not causing hypothermia. Furthermore, the hypothermic effect of the compound was minimal compared with that caused by reserpine or chlorpromazine since, following oral administration of 50 or 100 mg./Kg., the rectal temperature, while lower than normal, was still in the range of normal values of healthy animals.

From what has been said, it is legitimate to think of DA 1529 as a mild CNS depressant agent. This mild depressant effect does not, however, detract from the potential value of the compound as an anti-inflammatory–analgesic–antipyretic agent since the depressant effect is of very short duration, while the duration of the main effect is considerably longer.

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