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ANTI-INFLAMMATORY AND RELATED PROPERTIES OF 4-(*P*-BIPHENYLYL)-3-HYDROXYBUTYRIC ACID

BY

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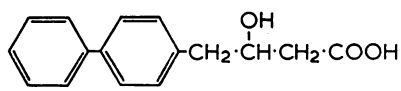
In recent years a variety of non-steroidal anti-inflammatory compounds of differing chemical types has been introduced for the treatment of rheumatic diseases. Such compounds include pyrazolones, anthranilic acids, indoles and substituted phenyl or phenoxyacetic acids. The synthesis and biological testing of a series of substituted phenyl and phenoxybutyric acids as potential anti-inflammatory agents led to the discovery of a high degree of activity in 4-(*p*-biphenylyl)-3-hydroxybutyric acid (I; BDH 7538) (Barron, Bysouth, Clarke, Copley, Stephenson, Vallance & Wild, unpublished results). The anti-inflammatory and related properties of this compound have been compared with those of phenylbutazone, indomethacin and aspirin. The results of these comparisons are reported below.



I

METHODS

General

Specific pathogen-free albino mice (*ca.* 20 g) and rats (*ca.* 150 g) were obtained from our own colonies, which originated from Caesarean-derived barrier-maintained stocks of the Charles River Breeding Laboratories Inc., U.S.A. Albino guinea-pigs (*ca.* 300 g) were bred from animals obtained from the Virus Research Institute, Pirbright. Unless otherwise stated, the compounds, dissolved or suspended in 5% w/v acacia in tap water, were administered by stomach tube at varying dose levels to groups of twenty animals. Control groups received the vehicle only. The use of the oral route minimized counter-irritant effects (Benitz & Hall, 1963). All agents given intravenously or intraperitoneally were, with the exception of bacterial endotoxin, dissolved in physiological saline. Dose volumes were 25 ml./kg body weight (orally) or 10 ml./kg (intravenously and intraperitoneally) for mice and rats and 10 ml./kg for guinea-pigs. Median effective or lethal doses were estimated, where appropriate, by the method of Litchfield & Wilcoxon (1949).

Anti-inflammatory activity

Ultraviolet erythema formation. The compounds were administered to male guinea-pigs from which the hair on one flank had previously been removed with a depilatory mixture containing barium sulphide. Each dose was administered in two equal parts with an interval of 1 hr between. Immediately after receiving the second part of the dose, the depilated skin of each animal was exposed to ultraviolet radiation (Hanovia Kromayer lamp, Model 10) for 45 sec through three circular holes (diameter *ca.* 6 mm) in a plastic mask protecting the remainder of the body. Two hours after exposure, the degree of erythema formation was scored as described by Winder, Wax,

Burr, Been & Rosiere (1958). The median effective dose of each compound was derived from the numbers of animals protected at each dose level.

In further experiments, groups of ten animals received varying doses of the compounds 1, 3, 6 or 24 hr before exposure, the total dose being administered at these times.

Rat paw oedema formation. One hour after administration of the compounds to male rats, the volume of the left hind paw of each animal was measured by the plethysmometric method of Harris & Spencer (1962). This was followed immediately by injection of a 1% w/v suspension of carrageenin (Gelozone ST1, Whiffen and Sons) in sterile physiological saline into the planar surface of the paw (Winter, Risley & Nuss, 1962), the volume injected corresponding to 5% of that of the paw. The volume of the paw was again measured 3 hr later and the increase in volume calculated. The mean percentage inhibition of oedema formation as compared with a control group was calculated for each treated group and the relative potencies of the compounds determined by graphical means.

Additional experiments were carried out in a similar manner using irritants other than carrageenin, each compound being administered at a single dose level to a group of five animals. The irritants, and the time intervals between their injection and measurement of the final volume of the paw, were as follows: 4% w/v formaldehyde, 8% w/v mustard, 1% w/v yeast (3 hr), 0.02% w/v 5-hydroxytryptamine creatinine sulphate, 1% w/v chymotrypsin (1 hr).

Cotton-pellet-induced granuloma formation (Meier, Schuler & Desaulles, 1950). Sterile cotton wool pellets (cut from Johnson's absorbent cotton rolls, No. 2), weighing 50 ± 2 mg, were implanted subcutaneously, one in each axilla of male rats. The compounds were then administered once daily for 7 days, the first dose being given immediately after implantation. Twenty-four hours after the final dose the animals were killed and the pellets, together with the surrounding granulomae, were dissected out, dried at 60° C for 48 hr, and weighed. The extent of granuloma formation was calculated by subtracting the original pellet weight. The adrenals and thymus were also removed from each rat and weighed wet. The mean percentage reduction of granuloma weight as compared with a control group was calculated for each treated group and the relative potencies of the compounds determined graphically.

In a further experiment with BDH 7538 alone, using forty animals at each dose level, half the animals were bilaterally adrenalectomized at the time of pellet implantation and given saline instead of drinking water for the duration of the test. Experiments were also carried out in which BDH 7538 was incorporated in one pellet of each pair before implantation, instead of being given orally. The pellets were removed after 7 days and the difference in granuloma formation between treated and untreated pellets determined.

Adjuvant-induced arthritis (Newbould, 1963). The compounds were administered once daily to groups of five male rats for 15 consecutive days. After the second dose, 0.05 ml. of 0.5% w/v suspension of killed human tubercle bacilli (strains *PN*, *DT* and *C*) in liquid paraffin B.P. was injected into the plantar surface of the left hind paw of each rat. The volume of the paw was then measured daily and the increase in volume from day 2 to day 15 calculated. The mean percentage inhibition of oedema formation as compared with a control group was calculated for each treated group. The severity of the secondary lesions was assessed on day 15 using an arbitrary scoring system.

Increase of capillary permeability and oedema formation induced by xylol (Brown & Robson, 1964). "Pontamine" Sky Blue (60 mg/kg) was administered intravenously to male mice and immediately afterwards two drops of xylol were applied to each surface of the left ear of each animal. Fifteen minutes later those animals in which the treated ear appeared bright blue were randomly divided into groups of ten and the compounds administered. One hour later xylol was applied to the right ear of each animal and after a further 15 min the numbers of animals in which the degree of blueing of the right ear was less than that of the left ear were determined visually ("protection"). The median effective doses were calculated from the numbers of animals protected at each dose level.

In order to assess the effects on oedema formation, the compounds were administered to groups of ten male mice and 1 hr later two drops of xylol were applied to each surface of the left ear of each animal. After a further 30 min the animals were killed and both ears removed and weighed. The difference in weight between the two ears was taken as the degree of oedema formation. The mean percentage inhibition of oedema formation was calculated for each treated group as compared with a control group and the dose of each compound required to produce 50% inhibition determined graphically.

Antipyretic activity

The method employed was similar to that of Winter & Nuss (1963). The oesophageal temperatures of male rats were recorded with an Elektrolaboratoriet TE3 thermometer immediately before the intraperitoneal injection of either *E. coli* endotoxin (lipopolysaccharide B, O26: B6, Difco), 250 μg/kg, or the vehicle (pyrogen-free water). The oesophageal temperatures were again recorded 30 and 60 min later and the compounds (previously warmed at 37° C) then administered. Recording of the oesophageal temperatures was continued at 30 min intervals for a further 4.5 hr. The mean oesophageal temperature for each group of animals at each time interval was calculated and the differences between the value 1.5 hr after administration of endotoxin and each subsequent value determined. The sum of these differences is the " temperature index " for the group and the relative potencies of the compounds were obtained graphically by plotting these temperature indices against the logarithm of the dose.

Analgesic activity

Phenylquinone-induced " writhing " (Hendershot & Forsaith, 1959). The compounds were administered to male mice housed at a constant temperature of 34° C. Each animal was injected intraperitoneally 1 hr later with phenylquinone (2 mg/kg) and the number of times each animal exhibited the characteristic " writhing " response between 5 and 10 min after the injection was recorded. The percentage reduction in the number of " writhes " as compared with a control group was calculated for each treated group and the relative potencies of the compounds determined graphically.

Other analgesic tests. The analgesic activity of BDH 7538 was further examined in groups of five mice by the hot plate and tail pinch methods, the criteria of analgesia being as described by Barron, Hall & Vallance (1966).

Phenol red retention

The method was essentially that of Kreppel (1959). The compounds were administered to groups of six male rats and 45 min later anaesthesia was induced with pentobarbitone sodium (50 mg/kg, intraperitoneally). After a further 15 min phenol red (75 mg/kg) was injected intravenously. Blood samples were taken 10, 30 and 60 min later from the retro-orbital plexus and the phenol red content of the plasma determined in an EEL colorimeter (filter 605). The mean percentage increase in plasma phenol red concentration at 60 min as compared with a control group was calculated for each treated group and the relative potencies of the compounds determined graphically.

Bradykinin-induced bronchoconstriction

Male guinea-pigs were anaesthetized with urethane (1.25 g/kg intraperitoneally). The trachea was cannulated and connected to a Palmer miniature respiration pump. Changes in bronchial resistance produced by the intravenous injection of synthetic bradykinin (0.4–3.2 μg) and histamine acid phosphate (2 μg) were measured by the overflow method of Konzett & Rössler (1940). The sodium salt of each compound was administered intravenously and the minimal effective dose (MED) against bradykinin was determined according to the method of Collier & Shorley (1960). The MED was determined three times for each compound, using a fresh animal for each determination, and the mean value calculated.

Effect on rat blood pressure

BDH 7538 was administered to a group of three unanaesthetized rats bearing intra-aortic cannulae (Weeks & Jones, 1960). The arterial pressure of each rat was recorded by means of a pressure transducer (Statham P23Dc) and pen-recorder (Grass Polygraph, Model 7) before and 0.5, 1, 3, 6 and 24 hr after administration of the compound.

Acute toxicity

The compounds were administered to groups of twenty male mice or rats and to groups of two male guinea-pigs. The median lethal doses were estimated from the mortalities after 7 days.

Inhibition of protein denaturation (Mizushima & Suzuki, 1965).

Samples (1 ml.) of varying concentrations of the sodium or potassium salts of the compounds in aqueous solution were added to 2 ml. of bovine albumin solution (0.75% w/v in M/15 phosphate buffered saline, pH 5.2) and allowed to stand at room temperature for 20 min. The mixtures were then heated in a water bath at 67° C for 10 min and allowed to cool at room temperature for 5 min. The turbidity of each sample was determined using an EEL colorimeter (filter 520) and compared with that of a control sample prepared in a similar manner but omitting the compounds. At least two determinations were carried out at each concentration and the mean percentage inhibition of protein denaturation estimated.

Inhibition of sulphur-35 uptake into cartilage (Whitehouse & Boström, 1962).

Freshly excised rat xiphoid cartilage was scraped free of fatty tissue, sliced (*ca.* 1 mm wide) and stored in ice-cold isotonic Krebs-Ringer phosphate (KRP) solution containing magnesium chloride in place of magnesium sulphate. The tissue was removed, lightly blotted on filter paper and weighed into incubation flasks (50 mg/flask) containing 4 ml. of hypertonic KRP. Samples (1 ml.) of varying concentrations of the compounds in 20% v/v dimethylformamide in distilled water were then added, the KRP thereby being rendered isotonic. The flasks were incubated at 37° C for 2 hr and 1.6 μc of carrier-free sulphur-35 as sodium sulphate was added. After a further 2 hr incubation the tissue was filtered off, washed with 200 ml. of tap water and incubated at 60° C with 0.7 ml. of hyamine hydroxide (1 mM) until dissolved. The incorporated sulphur-35 was counted in a liquid scintillation counter (Nuclear Chicago Model 720) after addition of 4 ml. of toluene-ethanol scintillator and compared with that of a control prepared in a similar manner but omitting the compounds. At least two determinations were carried out on each concentration and the mean percentage inhibition of sulphur-35 uptake estimated.

RESULTS

Anti-inflammatory activity

Ultraviolet erythema formation. BDH 7538 was highly effective in delaying erythema formation, its ED50 and confidence limits ($P=0.95$) being 0.77 (0.51–1.15) mg/kg. The corresponding figures for the reference compounds were phenylbutazone, 5.4 (3.5–8.4) mg/kg, indomethacin, 2.7 (1.2–5.9) mg/kg, and aspirin, 52.0 (32.6–73.8) mg/kg. In contrast to phenylbutazone and indomethacin, the activity of BDH 7538 was not markedly reduced by increasing the time interval between dosing and exposure from 1 hr to 24 hr (Table 1), and thus it possesses a considerably longer duration of action than the reference compounds.

Rat paw oedema formation. The results for the inhibition of carrageenin-induced oedema formation are summarized in Table 2. BDH 7538 was 2.5 times as potent as phenylbutazone, 0.5 times indomethacin and 11.7 times aspirin. None of the compounds

TABLE 1

EFFECT OF PRETREATMENT TIME ON THE ORAL ED50 (mg/kg) OF BDH 7538, PHENYLBUTAZONE AND INDOMETHACIN IN DELAYING ERYTHEMA FORMATION INDUCED BY ULTRAVIOLET RADIATION IN THE GUINEA-PIG

Ten animals were used at each dose level. Confidence limits ($P=0.95$) are given in parentheses.

	Pretreatment time			
	1 hr	3 hr	6 hr	24 hr
Expt. 1 BDH 7538	0·80 (0·54–1·18)	0·52 (0·27–0·99)	0·70 (0·40–1·23)	1·65 (1·23–2·28)
Phenylbutazone	3·8 (2·1–6·8)	2·4 (1·3–4·4)	8·4 (4·7–15·1)	43·0 (23·9–77·4)
Expt. 2 BDH 7538	0·46 (0·28–0·76)	0·34 (0·21–0·55)	0·34 (0·21–0·55)	0·60 (0·32–1·14)
Indomethacin	1·50 (0·79–2·85)	0·72 (0·28–1·87)	0·88 (0·43–1·80)	⩾40·5

TABLE 2

COMPARATIVE INHIBITORY EFFECTS OF ORAL ADMINISTRATION OF BDH 7538, PHENYLBUTAZONE, INDOMETHACIN AND ASPIRIN ON CARRAGEENIN-INDUCED OEDEMA FORMATION IN THE RAT PAW

Twenty animals were used at each dose level.

	Dose (mg/kg)	Mean increase in foot volume ±S.E. (ml.)	Percentage inhibition of oedema formation
BDH 7538	30·0	0·46±0·03	44
	10·0	0·59±0·04	29
	3·3	0·66±0·03	21
Phenylbutazone	90·0	0·46±0·03	45
	30·0	0·56±0·04	33
	10·0	0·66±0·04	20
Indomethacin	9·0	0·46±0·03	44
	3·0	0·58±0·03	30
	1·0	0·75±0·03	10
Aspirin	180·0	0·51±0·03	39
	60·0	0·61±0·03	27
	20·0	0·67±0·04	19
Acacia	—	0·83±0·05	—

showed significant activity in inhibiting oedema formation induced by irritants other than carrageenin when tested at a dose level of 200 mg/kg.

Cotton pellet-induced granuloma formation. Table 3 summarizes the results obtained with BDH 7538 in comparison with the three reference compounds. BDH 7538 was 3.5 times as potent as phenylbutazone but only 0.08 times indomethacin, while aspirin was inactive at a dose level of 300 mg/kg/day. The compound had no consistent effect on adrenal or thymus weight and adrenalectomy did not markedly reduce its anti-inflammatory effect (Table 4). No reduction in granuloma formation was observed when BDH 7538 (0.0125–0.4 mg/pellet) was incorporated in pellets before their implantation.

Adjuvant-induced arthritis. A reliable estimate of potency could not be obtained using this procedure because there was little gradation of response over the fourfold range of doses examined. BDH 7538 seemed to be rather more potent than phenylbutazone, how-

TABLE 3

COMPARATIVE EFFECTS OF ORAL ADMINISTRATION OF BDH 7538, INDOMETHACIN, PHENYLBUTAZONE AND ASPIRIN ON COTTON PELLET-INDUCED GRANULOMA FORMATION AND ON ADRENAL AND THYMUS WEIGHT IN THE RAT

Twenty animals were used at each dose level.

Expt.		Daily dose (mg/kg)	Mean wet weights ±S.E. (mg)		Mean dry granuloma weight ±S.E. (mg)	Percentage reduction in granuloma weight
			Adrenals	Thymus		
1	BDH 7538	48·0	32·9±1·3	414·2±20·1	102·1±3·3	35·2
		24·0	35·0±0·9	441·6±17·5	127·6±5·6	19·0
		12·0	33·2±1·1	429·2±25·2	137·4±6·8	12·8
	Indomethacin	4·0	34·6±1·6	355·8±23·0	104·4±4·9	33·8
		2·0	33·8±1·7	377·7±21·1	130·4±4·9	17·2
		1·0	34·7±1·3	455·6±22·9	133·2±5·7	15·4
	Acacia	—	38·3±1·6	494·5±21·7	157·5±5·4	—
2	BDH 7538	48·0	35·1±1·9	429·7±26·4	108·1±5·5	34·4
		24·0	46·0±1·3	437·9±20·8	123·9±6·3	24·9
		12·0	34·4±1·2	454·2±18·3	143·0±7·7	13·3
	Phenylbutazone	120·0	36·6±1·6	399·2±16·5	115·5±7·7	30·0
		60·0	33·9±1·4	405·7±23·0	138·2±5·2	16·2
		30·0	38·8±0·9	476·0±19·2	145·0±6·0	12·1
	Acacia	—	37·0±0·9	440·0±13·3	164·9±7·5	—
3	Aspirin	300·0	33·3±1·1	312·5±40·7	195·8±20·1	—
	Acacia	—	42·0±1·7	471·5±18·3	192·2±19·7	—

TABLE 4

EFFECT OF ADRENALECTOMY ON THE ANTI-INFLAMMATORY ACTIVITY (INHIBITION OF COTTON-PELLET-INDUCED GRANULOMA FORMATION IN THE RAT) OF BDH 7538 AFTER ORAL ADMINISTRATION

Twenty animals were used at each dose level.

Animals	Daily dose (mg/kg)	Mean wet weights ±S.E. (mg)		Mean dry granuloma weight ±S.E. (mg)	Percentage reduction in granuloma weight
		Adrenals	Thymus		
Intact	48·0	39·0±1·7	395·4±24·9	147·3±7·0	24·0
	24·0	36·9±1·1	425·4±18·4	160·2±6·3	17·3
	12·0	39·6±1·3	440·0±20·5	164·3±6·7	15·3
	Acacia	37·6±1·3	438·5±20·5	193·8—7·1	—
Adrenalectomized	48·0	—	565·7±22·7	155·4±6·2	20·7
	24·0	—	589·5±29·2	186·0±7·0	5·1
	12·0	—	591·3±28·0	187·3±7·3	4·4
	Acacia	—	642·7±27·5	196·0±10·8	—

ever, similar degrees of inhibition (*ca.* 50%) being obtained at dose levels of 48 and 120 mg/kg/day respectively. The reduction in severity of the secondary lesions approximately paralleled the reduction in volume of the injected foot. Indomethacin and aspirin were not examined.

Increase of capillary permeability and oedema formation induced by xylol. A marked difference between the compounds in their inhibitory effects on the two inflammatory responses is indicated by results in Table 5. Thus, BDH 7538, like phenylbutazone and indomethacin, was considerably more effective in inhibiting the increased capillary permeability after application of xylol than in inhibiting oedema formation. Aspirin, on the other hand, was about equally effective in both cases.

TABLE 5

COMPARATIVE EFFECTS OF ORAL ADMINISTRATION OF BDH 7538, PHENYLBUTAZONE, INDOMETHACIN AND ASPIRIN ON THE INCREASED CAPILLARY PERMEABILITY AND OEDEMA FORMATION INDUCED BY XYLOL IN THE MOUSE

Ten animals were used at each dose level. Confidence limits (P=0·95) are given in parentheses.

	Inhibition of increased capillary permeability (ED50, mg/kg)	Inhibition of oedema formation (dose producing 50% inhibition, mg/kg)
BDH 7538	42·0 (22·3–79·0)	324·0
Phenylbutazone	20·5 (9·4–44·7)	407·0
Indomethacin	0·09 (0·06–0·14)	34·0
Aspirin	140·0 (60·9–322·0)	331·0

Antipyretic activity

The effect of graded doses of BDH 7538 on the oesophageal temperatures of rats treated with endotoxin are illustrated in Fig. 1. Similar effects were obtained with the reference compounds, BDH 7538 being 2.5 times as potent as phenylbutazone, 0.5 times indomethacin and 4.0 times aspirin. The highest dose of BDH 7538 administered (20 mg/kg) was without effect on the temperature of rats which received pyrogen-free water in place of endotoxin.

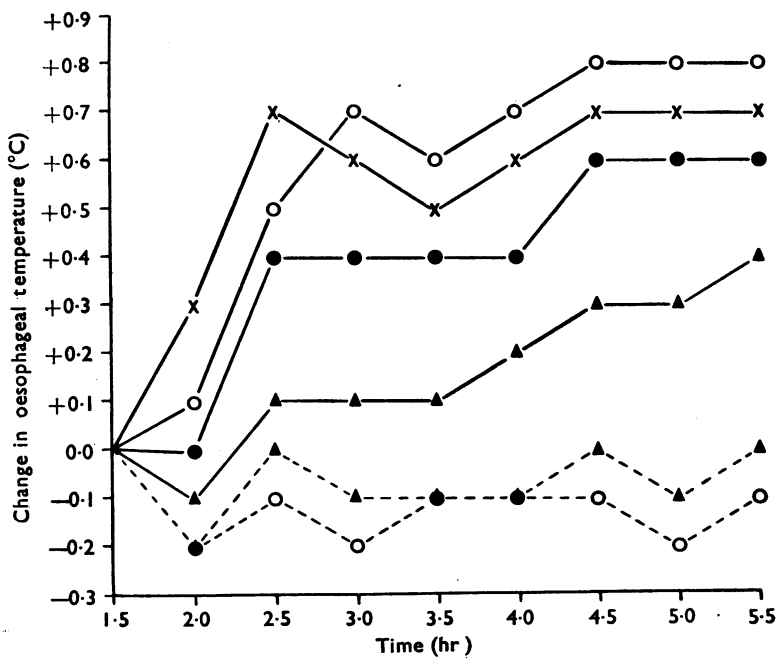


Fig. 1. Effect of oral administration of BDH 7538 or vehicle (5% acacia) on changes in oesophageal temperature induced in rats by intraperitoneal injection of bacterial endotoxin or pyrogen-free water. Endotoxin was administered at 0 hr and BDH 7538 at 1 hr. Each point represents the mean value of a group of twenty animals. Endotoxin + acacia, O——O ; endotoxin + BDH 7538 (5 mg/kg), ×——× ; endotoxin + BDH 7538 (10 mg/kg), ●——● ; endotoxin + BDH 7538 (20 mg/kg), ▲——▲ ; pyrogen-free water + BDH 7538 (20 mg/kg), ▲ - - - ▲ ; pyrogen-free water + acacia, O - - - O.

Analgesic activity

BDH 7538 was effective in reducing the frequency of " writhes " induced by phenylquinone, being 5.6, 0.02 and 1.5 times as potent as phenylbutazone, indomethacin and aspirin, respectively (Table 6). No analgesic effect was obtained in the tail pinch or hot plate procedures at a dose level of 400 mg/kg.

TABLE 6

COMPARATIVE EFFECTS OF ORAL ADMINISTRATION OF BDH 7538, PHENYLBUTAZONE, INDOMETHACIN AND ASPIRIN ON THE FREQUENCY OF "WRITHING" INDUCED BY PHENYLQUINONE IN THE MOUSE

Twenty animals were used at each dose level.

	Dose (mg/kg)	Mean No. of writhes ± S.E.	Percentage reduction
BDH 7538	120·0	1·0±0·1	93
	40·0	2·8±0·9	80
	13·3	8·5±1·4	41
	4·4	10·5±1·2	30
Phenylbutazone	180·0	3·8±0·8	73
	60·0	8·2±1·4	43
	20·0	10·2±1·3	29
	6·7	15·1±1·7	0
Indomethacin	1·8	1·9±0·1	87
	0·6	6·2±1·1	57
	0·2	7·3±1·5	49
	0·07	9·9±1·5	31
Aspirin	120·0	2·9±0·7	80
	40·0	8·0±1·6	44
	13·3	6·3±1·5	56
	4·4	9·0±1·1	37
Acacia	—	14·3±1·5	—

Phenol red retention

The results are summarized in Table 7. All four compounds inhibited the clearance of phenol red from plasma at dose levels in excess of those necessary to produce an anti-inflammatory effect in the rat.

Bradykinin-induced bronchoconstriction

BDH 7538 antagonized the bronchoconstrictor effects of bradykinin but not those of histamine. The MED was 1.7 mg/kg, the corresponding figures for the other compounds being 5.0 mg/kg for phenylbutazone, 0.1 mg/kg for indomethacin and 1.0 mg/kg for aspirin.

Effect on blood pressure in rats

Administration of BDH 7538 (160 mg/kg) caused no change in the mean arterial pressure of a group of three unanaesthetized rats.

Acute toxicity

The acute LD50s, with confidence limits ($P=0.95$), in mice and rats respectively were 1660 (1277–2158) and 385 (335–443) mg/kg for BDH 7538, 1000 (862–1160) and 480 (393–586) mg/kg for phenylbutazone, 24.3 (19.8–29.9) and 25.5 (21.3–30.6) mg/kg for

TABLE 7

COMPARATIVE EFFECTS OF ORAL ADMINISTRATION OF BDH 7538, PHENYLBUTAZONE, INDOMETHACIN AND ASPIRIN ON PLASMA CONCENTRATION OF PHENOL RED IN THE RAT

Six animals were used at each dose level.

Expt.		Dose (mg/kg)	Mean plasma concentration of phenol red at 60 min (μg/ml.)	% increase over control	Relative potency
1	BDH 7538	200·0	41	116	1·0
		100·0	35	84	
		50·0	28	47	
	Phenylbutazone	300·0	38	100	0·7
		150·0	35	85	
		75·0	30	58	
	Acacia	—	19	—	
2	BDH 7538	200·0	52	160	1·0
		100·0	44	120	
		50·0	30	50	
	Indomethacin	40·0	35	80	2·0
		20·0	33	70	
		10·0	24	20	
	Acacia	—	20	—	
3	BDH 7538	200·0	41	116	1·0
		100·0	45	136	
		50·0	32	69	
	Aspirin	400·0	36	100	0·2
		200·0	29	53	
		100·0	32	69	
	Acacia	—	19	—	

indomethacin, and 1500 (1271–1770) and 1550 (1260–1907) mg/kg for aspirin. In the guinea-pig the approximate LD50s for BDH 7538, phenylbutazone and indomethacin were 200, 1200 and >100 mg/kg, respectively; aspirin was not examined in this species.

Inhibition of protein denaturation

The approximate concentrations required to produce 50% inhibition of protein denaturation were BDH 7538 0.15×10^{-3}M, phenylbutazone 0.6×10^{-3}M, indomethacin 0.45×10^{-3}M and aspirin 3.0×10^{-3}M.

Inhibition of sulphur-35 uptake into cartilage

The approximate concentrations required to produce 50% inhibition of sulphur-35 uptake into rat xiphoid cartilage were BDH 7538 1.35×10^{-3}M, phenylbutazone 1.25×10^{-3}M, and indomethacin 0.7×10^{-3}M. Aspirin was ineffective at a concentration of 1.0×10^{-3}M.

DISCUSSION

BDH 7538 has been shown to possess a number of properties in common with the clinically useful anti-rheumatic drugs phenylbutazone, indomethacin and aspirin. As an anti-inflammatory agent it is capable of suppressing inflammatory responses of varying degrees of severity—namely, vasodilatation, increased capillary permeability, oedema formation and deposition of granulation tissue. It is particularly potent in delaying the early inflammatory response of the guinea-pig to ultraviolet radiation even when

administered up to 24 hr before induction of inflammation. This property of BDH 7538 is of particular importance because the potency of non-steroidal anti-inflammatory drugs in this test procedure seems to show good correlation with their clinical anti-rhematic activity (Winder, Wax & Welford, 1965, and references cited). Our results with the three reference compounds seem to provide further confirmation of this correlation, but whether or not it extends to BDH 7538 must await the results of clinical trials now in progress. The inhibitory effect of BDH 7538 on later stages of the inflammatory process is adequately demonstrated by its ability to reduce the increased capillary permeability induced by xylol, oedema formation induced by xylol and carrageenin, and the deposition of granulation tissue around implanted cotton wool pellets. In the mouse, the action of BDH 7538 resembles that of phenylbutazone and indomethacin in that it reduces the leakage of protein-bound dye at dose levels substantially below those required to inhibit oedema formation, but differs from aspirin, which shows little separation of effective dose levels, and from hydrocortisone, which is reported to be effective against oedema formation only (Brown & Robson, 1964). The effect of BDH 7538 on oedema formation in the rat is similar to that of other non-steroidal anti-inflammatory agents inasmuch as it is effective against oedema induced by carrageenin but ineffective, even at high dose levels, against oedema produced by a variety of other irritants. Experiments in which the body temperature or blood pressure of rats was measured after administration of BDH 7538 indicate that the inhibitory effect on oedema formation is unlikely to be due to hypothermia or hypotension. Involvement of the pituitary-adrenal axis in the actions of BDH 7538 also seems unlikely because the experiments concerning inhibition of granuloma formation showed no consistent effect on adrenal or thymus weight on repeated administration and the compound was still active in the absence of the adrenal glands. In these respects it again resembles phenylbutazone (Winder *et al.*, 1965) and indomethacin (Winter, Risley & Nuss, 1963). In the case of indomethacin, adrenal involvement was further excluded by Winter *et al.* (1963) by the demonstration that it was active when incorporated into cotton pellets before implantation. Similar evidence cannot be provided for BDH 7538, for it proved inactive in these conditions although it may be that BDH 7538 is only active after metabolic transformation.

In addition to possessing anti-inflammatory properties, BDH 7538 seems to be an effective antipyretic agent because it reduces the body temperature of febrile rats at dose levels which do not affect normal body temperature. Like phenylbutazone, indomethacin and aspirin, the effect is apparent at effective anti-inflammatory dose levels. The compound is also effective in preventing inflammatory pain in mice as indicated by the antagonism of the characteristic "writhing" syndrome induced by phenylquinone. It does not, however, prevent pain induced by heat or pressure. This selective effect on inflammatory pain seems to be characteristic of non-steroidal anti-inflammatory drugs (Silvestrini, 1965). Like many other non-steroidal anti-inflammatory drugs, BDH 7538 antagonized the bronchoconstrictor action of bradykinin, but not histamine, in the guinea-pig. The significance of this type of activity is not entirely clear, but there seems to be some association with the ability to delay erythema formation after exposure to ultraviolet radiation (Collier & Shorley, 1960, 1963).

The action of BDH 7538 in inhibiting the clearance of phenol red from rat plasma is of some interest because it may be a reflection of competition by the compound for the

secretory " organic acid system " of the renal tubules responsible for the elimination of uric acid, thus indicating the possibility of uricosuric activity (Gutman, 1966). The effect was only apparent at dose levels in excess of those producing anti-inflammatory effects in the rat, however, and it is therefore unlikely to be a prominent feature of the pharmacology of the compound.

No extensive investigations have been carried out on the activity of BDH 7538 *in vitro*. The compound does, however, show activity in two of the many procedures which have been used by others in investigating the biochemical effects of anti-inflammatory drugs—inhibition of the uptake of sulphur-35 by cartilage and inhibition of heat-induced protein denaturation.

BDH 7538 thus seems to be a potent anti-inflammatory agent, having a qualitative resemblance to a number of non-steroidal anti-rheumatic drugs at present in clinical use although there are marked quantitative differences. Thus it is 0.6–7.0, 0.002–3.5 and 0.6–68.0 times as potent as phenylbutazone, indomethacin and aspirin respectively, depending on the test and species of animal used. The closest overall resemblance seems to be to phenylbutazone, although it may have a considerably longer duration of action. Chronic toxicity studies in the rat and dog (unpublished) indicate that the most prominent effect of repeated administration of the compound is gastro-intestinal irritation leading to ulceration at high dose levels. Gastro-intestinal side-effects have been reported to occur with many anti-inflammatory drugs in animals and are probably inseparable from therapeutic effects because both may arise from the same biochemical effect at the subcellular level (Anderson, 1965).

SUMMARY

1. 4-(*p*-biphenylyl)-3-hydroxybutyric acid (BDH 7538) is an effective anti-inflammatory, antipyretic and analgesic agent in animals.

2. Qualitatively, it possesses similar properties to the non-steroidal substances most commonly used for the treatment of rheumatic disorders in man.

3. Quantitatively, it is 0.6–7.0, 0.002–3.5 and 0.6–68.0 times as potent as phenylbutazone, indomethacin and aspirin respectively, depending on the test and species of animal used. A prolonged duration of action has been demonstrated in the guinea-pig.

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