RESEARCH PAPERS

SUBSTITUTED DIHYDROXYBENZOIC ACIDS AS POSSIBLE ANTI-INFLAMMATORY AGENTS

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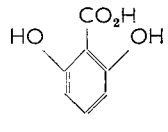
A series of substituted dihydroxybenzoic acids was prepared and examined for anti-inflammatory activity. The compounds which showed activity were derived from γ-resorcylic acid. The introduction of a halogen atom at positions 3 and 5 and a benzyl or methyl group at position 4 gave marked activity but also increased toxicity.

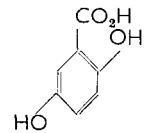
As a result of the reported anti-inflammatory activity of γ-resorcylic acid (I) (Reid and others, 1951) a series of related compounds was prepared and examined for anti-inflammatory activity. These compounds were based on the six isomeric dihydroxybenzoic acids (I–VI). The enhancement of the activity by the introduction of a halogen atom was noted early in this investigation and this paper therefore deals mainly with the halogenated, methyl and benzyl derivatives of these acids, of which a number have previously been reported (see Experimental).



2,6-dihydroxybenzoic acid

(γ-resorcylic acid)

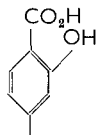
(I)



2,5-dihydroxybenzoic acid

(Gentisic acid)

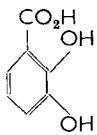
(II)



2,4-dihydroxybenzoic acid

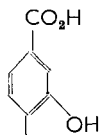
(β-resorcylic acid)

(III)



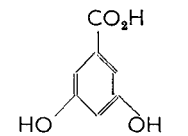
2,3-dihydroxybenzoic acid

(IV)



3,4-dihydroxybenzoic acid

(V)



3,5-dihydroxybenzoic acid

(VI)

EXPERIMENTAL

Chemical

All m.ps. are uncorrected. Micro analyses were by Messrs. Weiler and Strauss.

2,6-Dihydroxy-4-methylbenzoic acid (Robertson and Robinson, 1927), 3,5-dibromo-2,6-dihydroxybenzoic acid (Beilstein, 10, I, 186), 3,5-dibromo-, 3,5-dichloro- and 3-chloro-2,6-dihydroxy-4-methylbenzoic acid

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(British Patent 877,355), 2,5-dihydroxy-3-methylbenzoic acid (Beilstein, 10, 419), 2,5-dihydroxy-4-methylbenzoic acid (Beilstein, 10, 421), 2,4-dihydroxy-5-methylbenzoic acid (Beilstein, 10, II, 275), 5-chloro-2,4-dihydroxybenzoic acid (Sandin and McKee, 1935), 3,5-dichloro-2,4-dihydroxybenzoic acid (Pectyrin and Kirchine, 1947), 4,5-dibromo-2,3-dihydroxybenzoic acid (Beilstein, 10, I, 175), 5-bromo-3,4-dihydroxybenzoic acid (Beilstein, 10, 400) and 3,5-dihydroxybenzoic acid (Birkinshaw and Bracken, 1942) were known compounds and were prepared by standard methods.

The unhalogenated acids in general were prepared by carboxylation of the corresponding *m*-dihydroxybenzene using $KHCO_3/CO_2$. The general preparation was as follows.

1 part by weight of the dihydroxybenzene and 2 parts by weight of $KHCO_3$ were mixed with 2 parts by volume of glycerol. The mixture was then heated, preferably with stirring to 140° (bath or jacket temperature) with CO_2 passing through during the reaction. After 6 hr. the mixture was taken up in water, strongly acidified with sulphuric acid and filtered. The solid obtained was then recrystallised from water or ethanol/water.

The carboxylation of 4-benzylresorcinol gave a mixture of the isomers 3-benzyl-2,6-dihydroxybenzoic acid and 5-benzyl-2,4-dihydroxybenzoic acid. These were separated chromatographically on an alumina column (Table I).

TABLE I

ACIDS OBTAINED BY THE CARBOXYLATION OF *m*-DIHYDROXYBENZENES

Name	m.p. °C	Found		Theory	
		C	H	C	H
2,6-Dihydroxy-4-(hydroxymethyl)benzoic acid ..	190–193	52·2	4·3	52·2	4·35
4-Benzyl-2,6-dihydroxybenzoic acid	172–173	68·8	5·1	68·85	4·9
3-Benzyl-2,6-dihydroxybenzoic acid	174–176	67·7	4·6	68·85	4·9
2,6-Dihydroxy-4-(*p*-tolylmethyl)benzoic acid	168–170	69·7	5·5	69·8	5·4
2,4-Dihydroxy-3-methylbenzoic acid	204–206	57·0	5·0	57·1	4·8
5-Benzyl-2,4-dihydroxybenzoic acid	211–214	68·2	4·8	68·85	4·9

Halogenated acids generally were prepared by the addition of sulphuryl chloride or bromine to a solution of the dihydroxybenzoic acid in ether or acetic acid.

The phenolic acid (0·05M) was dissolved in ether (50 ml.) and sulphuryl chloride (0·05 or 0·1M depending on whether mono- or di-chlorination was required) added slowly in the cold. After standing overnight the reaction mixture was poured into water, extracted with ether and the ethereal layer extracted with aqueous sodium bicarbonate; after acidification of the alkaline solution, the acid was precipitated and recrystallised from water or ethanol/water (using charcoal for purification if necessary).

A similar procedure substituting bromine for sulphuryl chloride gave the bromo-compounds (see Table II).

The structure 4-chloro-3,5-dihydroxybenzoic acid was assigned to one compound; it could, however, be the 2-chloro-isomer, the exact structure not having been proved so far.

The preparation of intermediates is listed below; where no reference is made to the preparation of an intermediate it can be assumed that the material was available commercially.

3,5-*Dihydroxybenzophenone* was prepared by the method of Fischer and Fischer (1913).

TABLE II

HALOGENATED ACIDS

Name	m.p. °C	Analyses							
		Found				Theory			
		C	H	Cl	Br	C	H	Cl	Br
4-Benzyl-3,5-dibromo-2,6-dihydroxybenzoic acid ..	192–193	42·1	2·7	—	39·3	41·8	2·5	—	39·8
3,5-Dichloro-2,6-dihydroxybenzoic acid ..	210–212	38·5	1·6	32·7	—	37·7	1·8	31·8	—
3-Chloro-2,6-dihydroxybenzoic acid	190–192	44·3	2·7	19·1	—	44·5	2·7	18·8	—
3-Chloro-2,5-dihydroxybenzoic acid	220–222	44·4	2·8	19·1	—	44·5	2·7	18·8	—
5-Chloro-2,3-dihydroxybenzoic acid	223–228	44·5	3·0	18·8	—	44·5	2·7	18·8	—
4-Chloro-3,5-dihydroxybenzoic acid	250–251	44·6	2·8	19·4	—	44·5	2·7	18·8	—

5-*Benzylresorcinol* (for preparation of 4-benzyl-2,6-dihydroxybenzoic acid). 3,5-Dihydroxybenzophenone (34·9 g.), diethylene glycol (447 ml.), hydrazine hydrate (39·2 ml.) and potassium hydroxide pellets (51·1 g.) were mixed, heated on the steam-bath to dissolve the potassium hydroxide, refluxed for 1 hr. and the reaction mixture then distilled until the temperature of the solution was between 190–200°. The solution was then refluxed for a further 5 hr., cooled, diluted with water, acidified with sulphuric acid and the potassium sulphate filtered off. The filtrate was thoroughly extracted with ether, the extract dried over magnesium sulphate and the solvent distilled off. The residue was distilled to give a yellow viscous oil (17·8 g.), b.p. 194–198°/0·5 mm. This material was used as such for the carboxylation stage. Similar procedures give 5-(*p*-tolylmethyl)-resorcinol, which decomposed on attempted distillation, the crude product was therefore carboxylated as such.

4-*Benzylresorcinol* was prepared by the method of Dohme (1928), m.p. 78–79° (lit. m.p. 78–79°).

Ethyl 3,5-*dihydroxybenzoate* was prepared from the acid and ethanol in the presence of H_2SO_4.

5-(*Hydroxymethyl*)*resorcinol* was prepared by lithium aluminium hydride reduction of ethyl 3,5-dihydroxybenzoate. It has been previously prepared by Boehm and Parlasca (1932) by a less direct method. Ethyl 3,5-dihydroxybenzoate (28·3 g.) was dissolved in dry ether (200 ml.) and added slowly dropwise to a stirred suspension of lithium aluminium hydride (23·6 g.) in dry ether (550 ml.). After all the ester had been added the mixture was refluxed for a further 2 hr. Water (approximately 160 ml.)

was then added slowly with vigorous stirring to the cooled mixture to decompose excess lithium aluminium hydride and the reaction mixture poured into ice-cold diluted sulphuric acid. The ether layer was separated, the aqueous solution saturated with sodium sulphate and continuously extracted with ether for 2 days. The ether extracts were dried over $MgSO_4$, the ether removed by distillation to give 5-(hydroxymethyl)-resorcinol (15·1 g.), m.p. 170–178°. This material is satisfactory for carboxylation. A sample was recrystallised from ether/60–80° light petroleum to give material m.p. 175–179°. Found: C, 59·1; H, 5·8. $C_7H_8O_3$ requires C, 60·0; H, 5·7.

Methyl 3,5-dichloro-2,6-dihydroxy-4-methylbenzoate. 3,5-Dichloro-2,6-dihydroxy-4-methylbenzoic acid (52 g.) was dissolved in acetone (1200 ml.) and sodium bicarbonate (17·8 g.) and dimethyl sulphate (22·2 ml.) were added. The mixture was refluxed for 2 hr., cooled and water (500 ml.) added, whereupon the excess of sodium bicarbonate dissolved and the methyl ester was precipitated. After standing for several hours the mixture was filtered to give the ester m.p. 166–169°.

3,5-Dichloro-2,6-dihydroxy-4-methylbenzamide. The methyl 3,5-dichloro-2,6-dihydroxy-4-methylbenzoate (3 g.) was suspended in concentrated ammonia solution (20 ml.) and shaken mechanically overnight. The amide was filtered off and recrystallised from water to give material (2 g.) m.p. 210–212°. Found: C, 40·5; H, 3·0; Cl, 30·4; N, 5·9. $C_8H_7Cl_2NO_3$ requires C, 40·7; H, 3·0; Cl, 30·1; N, 5·9.

3-Chloro-4-methylbenzoic acid. This compound has been prepared before (Beilstein, 9, 498, and 9, II, 331) by various methods.

Sulphuryl chloride (36 ml.) was added to *p*-toluic acid (20 g.) in glacial acetic acid (100 ml.) and the solution refluxed for 5 hr. After standing overnight the product was filtered off and recrystallised from ethanol to give material (7 g.) m.p. 201–203° (lit. m.p. 200–202°). Mol. wt. found (by titration) 169·2; mol. wt. theory 170·5.

Methods

Anti-inflammatory assessment was made using either or both the granuloma pellet test and reduction of yeast-induced oedema.

Granuloma Pellet Test

This was modified from the method of Meyer, Stucki and Auslebrook (1953) and Meier, Schuler and Desaulles (1950). Groups of 10 albino rats of either sex, within the weight range 50–60 g. were anaesthetized with ether. Dental cotton wool pellets of 6–10 mg. were implanted via a trochar and cannula under the skin of the groin and axilla. Subsequently the animals received five daily subcutaneous injections of the solution (or vehicle) into the scapular region and were killed on the sixth day for examination. Tissue infiltration was measured by determining the increase in weight of pellets after drying 24 hr. at 110° and by micro-Kjeldahl estimation of the nitrogen content of the dried pellet. The two values obtained gave good agreement. Values obtained with treated animals were compared with those from controls by Students "*t*" test.

Reduction of Yeast-induced Oedema

This was modified from the method of Selitto and Randall (1954), and Eckhart, Thomas and Garner (1958). Groups of 15 albino rats within the weight range 55–60 g. were used for each dose level. The paw circumference was measured immediately before the injection of 0·1 ml. of a 20 per cent suspension of brewer's yeast into the plantar surface of the right hind foot. One min. after the yeast injection the compound was given intraperitoneally and the paw circumference then measured 2 hr. and 4 hr. later. Pooled values obtained with a total of 135 control animals, over the period of the tests, showed that the percentage increase (with standard deviation) in the circumference of yeast injected paws was 35 ± 3 at 2 hr. and 40 ± 4 at 4 hr. Compounds were considered to be effective if the swelling did not exceed 20 per cent.

In the rat-paw test two dose levels only (changed by a factor of two) were used. Table III shows either the lowest active dose or the highest tolerated dose which failed to confer protection.

TABLE III

ANTI-INFLAMMATORY ACTIVITY IN A SERIES OF SUBSTITUTED DIHYDROXYBENZOIC ACIDS

Name	(mg./kg.)	Activity: Granuloma pellet test	Activity: Rat paw oedema test	Approximate LD50 i.v. mouse (mg./kg.)
2,6-Dihydroxy-4-methylbenzoic acid	150	0	+	380
3,5-Dibromo-2,6-dihydroxybenzoic acid	300		+	350
3,5-Dibromo-2,6-dihydroxy-4-methylbenzoic acid	150	0	+	138
3,5-Dichloro-2,6-dihydroxy-4-methylbenzoic acid	150	+ (P=0·001)	+	125
2,6-Dihydroxy-4-(hydroxymethyl)benzoic acid	600		0	1200
4-Benzyl-2,6-dihydroxybenzoic acid	150		+	100
3-Benzyl-2,6-dihydroxybenzoic acid	75		+	
2,6-Dihydroxy-4-(*p*-tolylmethyl)benzoic acid	37·5		+	45
4-Benzyl-3,5-dibromo-2,6-dihydroxybenzoic acid	75		+	105
3,5-Dichloro-2,6-dihydroxy-4-methylbenzamide	75		+	110
2,4-Dihydroxy-5-methylbenzoic acid	150	0	0	545
5-Chloro-2,4-dihydroxybenzoic acid	150	0	0	1075
3,5-Dichloro-2,4-dihydroxybenzoic acid	150	0	0	1000
2,4-Dihydroxy-3-methylbenzoic acid	300		0	780
5-Benzyl-2,4-dihydroxybenzoic acid	150		0	
4,5-Dibromo-2,3-dihydroxybenzoic acid	75		0	75
*5-Chloro-2,3-dihydroxybenzoic acid	150		0	
5-Bromo-3,4-dihydroxybenzoic acid	150		0	180
3,5-Dihydroxybenzoic acid	150		0	2000
4,Chloro-3,5-dihydroxybenzoic acid	150		0	2000
3-Chloro-4-methylbenzoic acid	160	+ (P=0·01)	+	208

0 Ineffective at the highest tolerated dose.
+ Effective, swelling less than 20 per cent.
* Miss M. E. Farquharson (personal communication).

Toxicity. Albino mice within the weight range 18–22 g. were used. LD50 values were determined graphically by plotting log dose against probability using not less than 5 groups of 5 animals.

RESULTS

The results are given in Table III.

The undermentioned compounds were tested only by the granuloma pellet test and found to be inactive. $P = >0{\cdot}05$ ("*t*" test): 3-Chloro-2,6-dihydroxy-4-methylbenzoic acid, 3,5-dichloro-2,6-dihydroxybenzoic acid,

3-chloro-2,6-dihydroxybenzoic acid, 2,5-dihydroxy-3-methylbenzoic acid, 2,5-dihydroxy-4-methylbenzoic acid and 3-chloro-2,5-dihydroxybenzoic acid.

Discussion

Anti-inflammatory activity, in the compounds tested in this series, was apparently restricted to the 2,6-dihydroxybenzoic acids (Table III). Activity was enhanced by the presence of halogen atoms at positions 3 and 5. This was further increased when a methyl or benzyl group was also present at position 4 in the ring. 4-Benzyl-2,6-dihydroxybenzoic acid, with a benzyl group at position 4 but without halogens in the ring, was also active.

Clarke and Mosher (1953) have claimed that both the hydroxyl and carboxyl groups are essential for anti-inflammatory activity; 3-chloro-4-methylbenzoic acid, which possesses no hydroxyl groups has been shown to be active and is thus an exception to the general statement. The most active compound was 3,5-dichloro-2,6-dihydroxy-4-methylbenzoic acid. Unfortunately whenever anti-inflammatory activity was found, the doses given were very near to the toxic levels (Table III) and attempts to divorce the two (i.e. give a permissible therapeutic index) met with failure.

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References

Birkinshaw, J. H. and Bracken, A. (1942). *J. chem. Soc.*, 368–370.
Boehm, T. and Parlasca, H. (1932). *Arch. Pharm.*, **270**, 168–182.
Clarke, N. E., and Mosher, R. E. (1953). *Circulation*, **7**, 337–344.
Dohme, A. R. L. (1928). U.S. Patent 1,658,229.
Eckhart, E. T., Thomas, G. P. and Garner, W. M. (1958). *Proc. Soc. exp. Biol., N.Y.*, **98**, 211–212.
Fischer, E. and Fischer, H. O. L. (1913). *Ber.*, **46**, 1138–1148.
Meier, R., Schuler, W. and Desaulles, P. (1950). *Experientia*, **6**, 469–471.
Meyer, R. K., Stucki, J. C. and Auslebrook, K. A. (1953). *Proc. Soc. exp. Biol., N.Y.*, **84**, 624–628.
Pectyrin, P. A. and Kirchine, A. S. (1947). *J. gen. Chem.*, **17**, 278–282.
Reid, J., Watson, R. D., Cochran, J. B. and Sproull, D. H. (1951). *Brit. med. J.*, **2**, 321–325.
Robertson, A. and Robinson, R. (1927). *J. chem. Soc.*, 2196–2206.
Sandin, R. B. and McKee, R. A. (1935). *J. Amer. chem. Soc.*, **57**, 1077–1078.
Selitto, J. J. and Randall, L. O. (1954). *Fed. Proc.*, **13**, 403–404.