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ON THE ANTI-INFLAMMATORY ACTIVITY OF SOME SUBSTITUTED PHENOLIC COMPOUNDS

BY

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Some substituted dihydroxybenzenes have been examined for suppression of the yeast-induced inflammatory reaction in the rat paw. This anti-inflammatory activity is greatest in those compounds derived from resorcinol and in particular the halogenated 5-methylresorcinols. The significance of the internuclear distance between the hydroxyl groups in the *meta*-position to each other in resorcinol and the importance of the enhanced activity due to the halogen atom(s) is discussed. In this series the toxicity and activity could not be divorced.

In a previous paper (Lightowler & Rylance, 1963) we described the anti-inflammatory activity of a group of substituted isomeric dihydroxybenzoic acids. Within this series activity is confined to the 2,6-dihydroxybenzoic acid derivatives. The enhancement of activity by the introduction of a halogen atom was commented on. It has been suggested (Reid, Watson, Cochran & Sproull, 1951) that a carboxyl group and an adjacent hydroxyl group are essential for anti-inflammatory activity in this type of compound. To verify this suggestion we prepared a few substituted dihydroxybenzenes, based on the isomeric dihydroxybenzenes and on phloroglucinol, but found that activity is retained in the absence of the carboxyl group provided that the hydroxyl groups are in the *meta*-position to each other and that certain other molecular requirements are fulfilled (see discussion).

This paper deals with the preparation and testing of these compounds and with an attempt to deduce some structure-activity relationship within the group of anti-inflammatory agents inspired by the salicylates.

METHODS

The assessment of anti-inflammatory activity was carried out by a yeast-induced oedema test on the rat paw (Lightowler & Rylance, 1963).

RESULTS

The results are summarized in Table 1 ; in subsequent references to compounds in the text the compound number in this table is given in parentheses. The figures given are the percentage increase in paw circumference (compared to the size before

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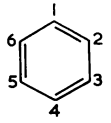
TABLE 1

STRUCTURE AND ANTI-INFLAMMATORY ASSESSMENT OF COMPOUNDS

Doses were injected intraperitoneally. Increases in paw circumference are expressed as percentages of the paw size before injection. LD50's were for intravenous injections and are approximate



Compound number	1	2	3	4	5	6	Dose (mg/kg)	Increase in paw circumference (%) 2 hr	4 hr	No. of rats	Assessment	Mouse LD50 (mg/kg)
1	OH	—	OH	Cl	CH_3	Cl	150	24	26	15	Slightly active	100
							300	22	14	15		
2	OH	—	OH	Cl	CH_3	—	150	19	28	15	Active	160
							300	13	10	15	Active	
								5/15 died	5/15 died			
3	OH	Cl	OH	Cl	CH_3	Cl	37·5	29	29	15	Inactive	125
							75	14	19	15	Active	
4	OH	—	OH	Br	CH_3	—	75	24	30	15	Inactive	215
							150	11	16	15	Active	
5	OH	Br	OH	Br	CH_3	Br	150	18	16	15	Active	600
							300	14	14	15	Active	
6	OH	I	OH	I	CH_3	I	37·5	30	30	15	Inactive	
							75	25	27	15	Inactive	
							150	16	18	15	Active	720
							300	18	14	15	Active	
7	OH	CH_3	Br	OH	Br	Br	75	33	33	15	Inactive	220
							150	29	38	10	Inactive	
8	OH	OH	Br	CH_3	Br	Br	75	27	37	15	Inactive	85
							150	All died	—	10	Inactive	
9	OH	Br	OH	Br	—	Br	150	29	39	15	Inactive	395
							300	21	31	15	Inactive	
10	OH	Br	CH_3	Br	—	Br	75	32	41	15	Inactive	170
							150	36	46	15	Inactive	

11	OH	Br	OH	CH_3	—	Br	75 150	40 35	41 40	10 15	Inactive Inactive	—
12	OH	CH_3	OH	Br	—	Br	75 150	29 34	35 37	15 10	Inactive Inactive	705
13	OH	Br	OH	Br	OH	Br	37·5 75	13 8	15 7	15 15	Active Active	100
14	OH	—	OH	$CH_2.C_6H_5$	—	—	150 300	14 8	12 All died	15 15	Active Active	73
15	OH	—	OH	—	$CH_2.C_6H_5$	—	150 300	15 10 2/15 died	13 6 9/15 died	15 15	Active Active	—
16	OH	Br	OH	$CH_2.C_6H_5$	—	Br	75 150	27 23	30 28	15 15	Inactive Inactive	160
17	OH	CO_2H	OH	NO	CH_3	NO	37·5 75 150	32 22 13	34 20 13/15 died	15 15 15	Inactive Inactive Active	375
18	OH	—	OH	—	$CO.C_6H_5$	—	75 150	42 35	43 38	15 15	Inactive Inactive	78
19	OH	—	OH	$CO.C_6H_5$	—	—	37·5 75	41 44	41 47	15 15	Inactive Inactive	85
20	OH	$CO.CH_3$	OH	Br	OH	Br	150 300	22 8 1/12 died	27 7 2/12 died	15 12	Inactive Active	290
21	OH	—	OH	—	CH_2OH	—	300 600	40 33	38 37	15 15	Inactive Inactive	750
22	OH	C_6H_5	—	OH	—	—	11·25 22·5	31 37	34 43	15 15	Inactive Inactive	22
23	$O.CO.CH_3$	Br	$O.CO.CH_3$	Br	CH_3	Br	150 300	31 44	32 27	10 10	Inactive Inactive	900
24	$O.CO.CH_3$	CO_2H	$O.CO.CH_3$	Cl	CH_3	Cl	150	31	40	10	Inactive	390

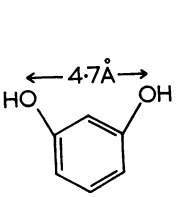
injection of the yeast). The swelling in control animals (135 in all, during the period in which this work was carried out; means and standard deviations) was $35 \pm 3\%$ at 2 hr and $40 \pm 4\%$ at 4 hr. A value of 20% swelling or less was taken as indicating activity by the compound being examined.

DISCUSSION

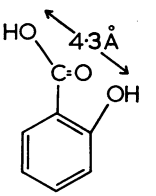
In a previous paper (Lightowler & Rylance, 1963) we noted the high anti-inflammatory activity of 3,5-dichloro-2,6-dihydroxy-4-methylbenzoic acid. The starting compound of the present series was therefore the corresponding compound without the carboxyl group, 4,6-dichloro-5-methylresorcinol (1); this shows anti-inflammatory activity. Other halogenated 5-methylresorcinols (2,3,4,5 and 6) are also active. The possibility that the resorcinol nucleus might be the best structure for activity (within the isomeric dihydroxybenzenes) was confirmed when bromo-compounds from other isomers were examined. Thus the bromo-derivatives from 2-methylhydroquinone (7) and from 4-methylcatechol (8) show no activity.

The importance of the two hydroxyl groups is underlined by the fact that 2,4,6-tribromo-3-methylphenol (10) is inactive. This is the corresponding monohydroxy compound to 2,4,6-tribromo-5-methylresorcinol (5).

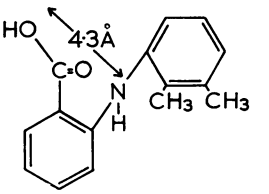
The need for two hydroxyl groups in the *meta*-position to one another suggested that the distance between these two groups is important, although other factors (steric and/or electronic) will influence whether a particular compound has anti-inflammatory activity. The internuclear distance between the oxygen atoms was found to be 4.7Å, as measured on Dreiding molecular models.



Resorcinol



Salicylic acid



Mefenamic acid

The corresponding distance between the oxygen atom of the phenolic group and the oxygen atom of the carboxylic hydroxyl group in one possible configuration of salicylic acid was similarly found to be 4.3Å.

The agreement of these results suggested that the activity of the resorcinol nucleus was due to its ability to attach itself to the same receptor site as did salicylic acid, the distances between the important centres being roughly the same and both compounds containing the aromatic nucleus which is probably also essential. The same internuclear distance applies also to acetylsalicylic acid. With mefenamic acid (*N*-2,3-xylyl anthranilic acid), a new anti-inflammatory agent (Winder, Wax, Scotti, Scherrer, Jones & Short, 1962), we found that the distance between the oxygen atom of the carboxylic hydroxyl group and the nitrogen atom was also 4.3Å. The nitrogen atom of mefenamic acid and the phenolic oxygen atom of salicylic acid possibly fulfil the same function at the receptor site.

Although a carboxyl group as such is not essential the possibility that an acidic group is necessary to secure attachment to some receptor site is not ruled out, since most of the active compounds listed have a highly acidic (phenolic) hydroxyl group in the molecule. The acidity in these compounds is enhanced by the presence of halogen atoms in the molecule. An acidic point of attachment is therefore still retained. One of the hydroxyl groups in the resorcinol molecule therefore functions in a similar manner to the carboxyl group of the salicylic acid group of anti-inflammatory compounds, whereas the other hydroxyl group (which must be in the *meta*-position to the first) represents the hydroxyl group of the salicylic acid molecule (or the corresponding oxygen of acetylsalicylic acid or the nitrogen atom or mefenamic acid).

Within our present series the presence and position of the methyl group is of great importance. 2,4,6-Tribromoresorcinol (9) is inactive ; this compound lacks only the 5-methyl group compared with the active compound 2,4,6-tribromo-5-methylresorcinol (5). The dibromo-derivatives of 4-methylresorcinol (11) and 2-methylresorcinol (12) also have no activity. The symmetrical 5-methylresorcinol seemed therefore to be the best nucleus for anti-inflammatory activity.

The methyl group can be replaced by a hydroxyl or benzyl group without loss of activity. With a benzyl group the effect is such that 5-benzylresorcinol (15) is active without the presence of halogen atoms in the molecule. 4-Benzyl-resorcinol (14) also shows anti-inflammatory activity. From models it can be shown that in one possible configuration the benzene rings of these two compounds can be superimposed one upon the other leaving the hydroxyl groups also in super-imposition. This fact probably explains the similar activities of the compounds, and the same explanation would account for the anti-inflammatory activity of both 3- and 4-benzyl-2,6-dihydroxybenzoic acid (Lightowler & Rylance, 1963).

The presence of an area of high electron density in the position occupied by the benzyl group appears to be advantageous. One puzzling result was the lack of activity shown by 4-benzyl-2,6-dibromo-resorcinol (16) ; this we cannot explain.

No activity was found in the corresponding benzophenones ; this may be due entirely to the change in electronic character.

The increased anti-inflammatory activity with the introducton of halogen atoms into the molecule is presumably due to increased acidity. We had previously noted this effect in a series of substituted γ-resorcylic acids (Lightowler & Rylance, 1963). With these the enhanced acidity of the hydroxyl groups may allow the possibility of several spatial correspondences of atoms on the receptor site. One of the most active compounds is 3,5-dichloro-2,6-dihydroxy-4-methylbenzoic acid ; the replacement of the halogen atoms in this compound by nitroso-groups (17) did not affect the anti-inflammatory activity. The halogen atoms and nitroso-groups can both be expected to enhance acidity in the hydroxyl groups by the process of electron withdrawal. The comparable anti-inflammatory action of these compounds was therefore expected.

The size of the group at position 2 (between the two hydroxyl groups) in this present series (see compounds 3, 5, 6, 9, 11, 12, 13, 16, 17 and 20) does not appear

to be important, since compounds with iodine, bromine or chlorine atoms or an acetyl group in this position still retain activity. This statement may need modification if bulkier groups are introduced.

Neither of the acetoxy compounds we prepared showed any anti-inflammatory activity. The lack of activity by the diacetate of 2,4,6-tribromo-5-methylresorcinol (23) (3,5-diacetoxy-2,4,6-tribromotoluene) was to be expected since with both hydroxyl groups esterified there is no acidic group available to bind with the receptor site. The result obtained with the diacetate of 3,5,dichloro-2,6-dihydroxy-4-methylbenzoic acid (24) was somewhat unexpected. The oxygen atom linking the aromatic nucleus to the acetyl group in this compound has a lower electron density than the normal phenolic oxygen atom due to the electron withdrawal by the acetyl group itself and the substituents in the benzene ring, for example halogen atoms. The corresponding oxygen atom in acetylsalicylic acid similarly has a lower than normal electron density. Since it is commonly accepted that acetylsalicylic acid is a better anti-inflammatory agent than salicylic acid we expected the above diacetate to be active.

The alternative hypothesis, that in this series the compounds were active by virtue of the free hydroxyl group and that the acetyl derivatives were not hydrolysed under the conditions of the test, merited consideration. Thus the hydroxyl group could link to the receptor site through the formation (using the hydrogen atom of the hydroxyl group) of a hydrogen bond. This could also apply to the imino-group of mefenamic acid. The oxygen and nitrogen atoms respectively are thus one end of a hydrogen bond binding the drug to a $\delta-$ site on the receptor. Further points of attachment are provided by the carboxyl group and perhaps by the benzene ring.

Further study is needed for a complete explanation of the results obtained with the acetyl compounds.

APPENDIX

Chemistry of the compounds studied

All melting points are uncorrected. Microanalyses were by Messrs Weiler and Strauss.

Brominated phenols. These were prepared by the action of bromine on the phenol in ether or acetic acid.

The phenol (0.05 moles) was dissolved in acetic acid (50 ml.) and bromine (0.1 moles) was added slowly in the cold. After several hours the reaction mixture was poured into water, the solid separated and recrystallized from aqueous ethanol. Compounds made in this way are shown in Table 2.

The unhalogenated starting materials were obtained as follows: 2-methylresorcinol and 5-methylresorcinol (orcinol) were available commercially, 2,4,6-trihydroxyacetophenone was prepared by the method of Gulati, Seth & Venkataraman (1935) and 4-methylresorcinol (cresorcinol) by the method of Nevile & Winther (1882).

3,5-Diacetoxy-2,4,6-tribromotoluene (23). 2,4,6-Tribromo-5-methylresorcinol (7.2 g), acetic anhydride (8 ml.) and acetic acid (100 ml.) were refluxed for 5 hr.

TABLE 2
CHEMISTRY OF HALOGENATED PHENOLS

Compound	Melting point (°C)	Analysis					
		Found			Theory		
		C	H	Br	C	H	Br
2,6-Dibromo-4-benzylresorcinol (16)	94–95	43·8	2·8	44·4	43·6	2·8	44·7
4,6-Dibromo-2-methylresorcinol (12)	104–104·5	29·8	2·1	56·9	29·8	2·1	56·7
2,6-Dibromo-4-methylresorcinol (11)	86–87	29·8	2·1	57·3	29·8	2·1	56·7
3,5-Dibromo-2,4,6-trihydroxy-acetophenone (20)	199–201	29·0	1·7	49·3	29·45	1·8	49·1

On pouring into water an oil separated which was crystallized from alcohol to give material with melting point 137 to 139° C (3 g). Analysis showed: found, C 29.9, H 2.2 and Br 54.1 ; $C_{11}H_9Br_3O_4$ requires, C 29.7, H 2.0 and Br 53.9.

2,6-Diacetoxy-3,5-dichloro-4-methylbenzoic acid (24). 3,5-Dichloro-2,6-dihydroxy-4-methylbenzoic acid (10 g), prepared as described by Lightowler & Rylance (1963), was mixed with acetic anhydride (15 ml.) and concentrated sulphuric acid (5 drops) and allowed to stand at room temperature for 4 hr. The mixture was poured into water and a solid separated which was crystallized from aqueous ethanol to give material with melting point 153 to 154° C (11 g). Analysis showed: found, C 44.9, H 3.0 and Cl 22.0 ; $C_{12}H_{10}Cl_2O_6$ requires C 44.9, H 3.1 and Cl 22.1.

2,6-Dihydroxy-4-methyl-3,5-dinitrosobenzoic acid (17). 2,6-Dihydroxy-4-methylbenzoic acid (12.3 g), prepared by the method of Robertson & Robinson (1927), was dissolved in ethanol (100 ml.), concentrated hydrochloric acid (50 ml.) was added and the solution cooled to 0° C in a bath containing an ice-salt mixture. Sodium nitrite (14.4 g) was then added slowly with stirring, stirring continued for a further 15 min after the addition had been completed and the solution was poured into water (800 ml.). The solution was allowed to stand for several hours and the compound (14 g) was filtered off. Its melting point was 133° C (d). Analysis showed: found, C 39.9, H 3.7 and N 11.3 ; $C_8H_6N_2O_6.H_2O$ requires C 39.3, H 3.3 and N 11.5. Other compounds used were prepared by the methods described in the literature.

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