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INHIBITION OF RAT SERUM GLUTAMIC-PYRUVIC TRANSAMINASE *IN VITRO* BY CONGENERS OF SALICYLATE

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Abstract—The relation between chemical structure and inhibitory activity against rat serum glutamic–pyruvic transaminase was studied in congeners of salicylate. The presence of a phenolic hydroxyl in the *ortho*-position to a carboxyl group was found to be a necessary structural requirement for inhibitory activity except that a thiol group may replace the phenolic hydroxyl.

INTRODUCTION

SALICYLATE produces effects on the incorporation of radioactivity from labelled glucose and acetate into the soluble metabolic intermediates of isolated tissues which are explicable in terms of an uncoupling action on oxidative phosphorylation processes.[1] However, using [3-^{14}C]pyruvate as the labelled substrate, salicylate, but not the more powerful uncoupling reagent 2:4-dinitrophenol, produced an increased accumulation of radioactivity in glutamic acid.[2] This result suggested that salicylate exerted an effect on glutamic acid metabolism distinct from its uncoupling action and it was found that salicylate inhibited rat serum glutamic–pyruvic transaminase activity *in vitro*.[2] The present paper is concerned with a study of the relation between chemical structure and the inhibition of the transaminase activity in congeners of salicylate.

EXPERIMENTAL

Materials

The salicylate congeners were obtained commercially and recrystallized from suitable solvents until their melting points remained constant. They were dissolved in 0·1 M KH_2PO_4 solution at pH 7·4 to give solutions which after admixture with the other constituents of the reaction mixture produced a final concentration of 5 mM. Some of the congeners were too insoluble for this final concentration to be attained and were used as saturated solutions at 37 °C.

Measurement of transaminase activity

Glutamic–pyruvic transaminase activity was measured in pooled serum obtained from adult male rats of the Wistar strain by the method of Reitman and Frankel[3]. The determinations were made with a Technicon Autoanalyser (Technicon Instrument Co. Ltd., London) which consists essentially of a flow system in which the constituents of the reaction mixture (Serum, congener solution, substrate and colour reagent) are added successively and the final colour, developed after incubation,

is measured by a recording absorptiometer thus enabling continuous analyses to be performed. A minimum of four determinations was made for each congener and corresponding control mixtures, in which the congener solutions were replaced by equal volumes of water, were also analysed at suitable intervals during the experiments. The possibility that the congener may have interfered with the reaction between

TABLE 1. INHIBITION OF RAT SERUM GLUTAMIC–PYRUVIC TRANSAMINASE ACTIVITY BY SALICYLATE CONGENERS

(The results, which are expressed as mean percentage inhibitions (±s.e.m.), have been analysed by the *t*-test and values of *P* are included. The minimum acceptable level of significance has been taken as $P = 0{\cdot}05$.)

Congener	No. of observations	Concn. (mM)	% Inibition	*P*
Salicylic (2-Hydroxybenzoic)	12	1·25	6·3 ± 0·9	0·5
	12	2·5	14·0 ± 0·7	0·1
	12	5	26·4 ± 1·1	0·01
	12	10	40·0 ± 1·3	0·001
Thiosalicylic	8	sat.	46·0 ± 2·1	0·02
Benzoic	4	5	5·7 ± 1·2	0·8
3-Hydroxybenzoic	4	5	9·5 ± 1·4	0·6
4-Hydroxybenzoic	4	5	8·7 ± 1·3	0·6
2-Methoxybenzoic	4	5	7·2 ± 1·2	0·7
trans-Hexahydrosalicylic	4	5	6·2 ± 1·4	0·7
Phenol	4	5	12·5 ± 3·7	0·6
Salicylamide	4	5	7·0 ± 1·7	0·8
2-Hydroxyphenylacetic	4	5	5·2 ± 1·4	0·7
3-Methylsalicylic	8	sat.	41·7 ± 1·5	0·01
3-Ethylsalicylic	8	5	55·5 ± 0·4	0·001
3-Propylsalicylic	8	5	61·0 ± 0·6	0·001
3-*iso*Propylsalicylic	8	5	61·5 ± 0·6	0·001
3-Allylsalicylic	8	5	63·7 ± 0·9	0·001
3-Phenylsalicylic	6	5	72·0 ± 0·6	0·001
3-*cyclo*Hexylsalicylic	4	5	80·0 ± 0·9	0·001
3-Hydroxysalicylic	6	5	42·6 ± 0·8	0·01
3-Nitrosalicylic	8	sat.	33·6 ± 1·3	0·05
3-Carboxysalicylic	8	5	22·0 ± 0·9	0·01
4-Methylsalicylic	8	sat.	28·2 ± 1·2	0·05
4-Hydroxysalicylic	6	5	32·4 ± 1·0	0·05
4-Aminosalicylic	8	5	15·2 ± 0·6	0·05
5-Hydroxysalicylic	6	5	34·5 ± 1·0	0·05
5-Nitrosalicylic	8	5	51·7 ± 2·2	0·01
5-Aminosalicylic	6	sat.	43·5 ± 1·7	0·02
5-Bromosalicylic	6	5	53·3 ± 2·8	0·02
6-Hydroxysalicylic	8	5	53·6 ± 2·7	0·02
1-Hydroxy-2-naphthoic	8	5	49·1 ± 0·3	0·001
2-Hydroxy-3-naphthoic	8	5	49·9 ± 1·0	0·001
3:5-Di-*iso*propylsalicylic	7	5	76·3 ± 0·5	0·001
2:4-Dinitrophenol	6	0·5	0·6 ± 0·5	0·9

the colour reagent (2:4-dinitrophenylhydrazine) and the α-keto acids (pyruvic and α-ketoglutaric) present in the substrate, was excluded by analysing appropriate mixtures in which the serum was omitted.

RESULTS

The results are given in Table 1 and show that salicylic acid itself produced a significant inhibition of the transaminase activity at concentrations of 5 and 10 mM.

The hydroxyl group could be changed by replacement of the phenolic oxygen by sulphur (thiosalicylic) without loss of activity but its absence (benzoic), alteration of its position relative to the carboxyl group (3- and 4-hydroxybenzoic acids), or methylation (2-methoxybenzoic), produced inactive compounds. The corresponding hydrogenated compound (hexahydrosalicylic) was also inactive showing that the presence of a phenolic, rather than an alcoholic hydroxyl group was important for inhibitory activity. The absence of the carboxyl (phenol), the presence of an amide group (salicylamide) or the introduction of a methylene bridge between the benzene ring and the carboxyl (2-hydroxyphenylacetic) also caused a loss of activity. A general structural requirement for inhibitory activity against the transaminase in salicylate congeners therefore appeared to be a phenolic hydroxyl group in an *ortho*-position to a carboxyl group. This combination was also present in the ring-substituted salicylic acids investigated in the present work. Mono- or di-substitution in the 3-, 4-, 5- or 6-positions with a variety of groups did not remove the activity of the parent salicylic acid. Thus, the introduction of alkyl, aryl, hydroxyl, nitro and carboxyl groups in the 3-position, methyl, hydroxyl and amino groups in the 4-position, hydroxyl, nitro, amino and bromo groups in the 5-position, a hydroxyl in the 6-position, additional benzene rings in the 3:4(1-hydroxy-2-naphthoic)- and 4:5(2-hydroxy-3-naphthoic)-positions and *iso*propyl groups in the 3:5-positions, produced active compounds.

DISCUSSION

All the salicylate congeners showing inhibitory activity against rat serum glutamic–pyruvic transaminase, with the exception of thiosalicylic, contained a phenolic hydroxyl in the *ortho*-position to a carboxyl group. This combination therefore appears to be the general structural requirement for activity in this group of compounds with the reservation that a thiol group may be substituted for the hydroxyl. Substitution of the remaining positions on the benzene ring by a variety of groups did not cause a loss of activity.

Some of the salicylate congeners which inhibit the serum transaminase also uncouple oxidative phosphorylation reactions.[4] However, there is no general correlation between the two effects since the classical uncoupling reagent, 2:4-dinitrophenol had no inhibitory action in the present work. A more striking finding was that the introduction of an extra hydroxyl group in the benzene ring of salicylic acid, as in 2:5- and 2:6-dihydroxybenzoic acids, produced substances which inhibited the serum glutamic–pyruvic transaminase but which are completely devoid of uncoupling activity.[5]

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