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SOME BIOCHEMICAL PROPERTIES OF THIO ANALOGUES OF SALICYLIC ACID

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Abstract—The relationship between chemical structure and ability to uncouple oxidative phosphorylation in rat liver mitochondria was investigated with aromatic and aliphatic thio compounds related to salicylic acid. Of these, only 2-mercaptobenzoic acid, thionthiolsalicylic acid and phenol-2-sulphinic acid were significantly active as uncoupling agents. Thiophenol, thiobenzoic acid, 2-mercaptotropone, 2- and 4-methylmercaptophenols and certain aromatic zinc-complexing agents (toluene dithiol, 1,10-phenanthroline, 'Zincon') also uncoupled oxidative phosphorylation. There was no simple relationship between uncoupling potency and either (1) the acid dissociation constants of these compounds, which range in pK from less than 2 up to 10, or (2) their ability to complex zinc ions.

4-Mercaptobenzoic acid was a potent inhibitor of yeast alcohol dehydrogenase and pig heart malic dehydrogenase: 2-mercaptobenzoic acid only inhibited the malic dehydrogenase.

2-Mercaptocyclohexane-carboxylic acid, 2-methylsulphoxyphenol and 4-N-picrylamino-2-hydroxy-benzenesulphonamide were prepared and characterised.

2-MERCAPTOBENZOIC acid ("thiosalicylic" acid, Fig. 1, X = OH) is more potent

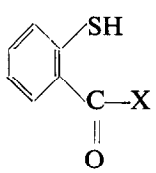

FIG. 1. Pharmacophore for uncoupling activity among thio analogues of salicylic acid.

than salicylic acid in uncoupling oxidative phosphorylation.[1, 2] Certain thiolphilic reagents also uncouple oxidative phosphorylation.[3, 4] It is believed they selectively block thiol groups participating in the energy-linked mitochondrial phosphorylation of ADP* by which ATP is synthesised.

Mercaptobenzoic acid could perhaps owe its uncoupling activity to the formation of disulphide linkage(s) with these thiol groups. Alternatively, mercaptobenzoic acid

* Abbreviations used:
ADP, ATP = adenosine-5′-di (tri) phosphate.
NAD = Nicotinamide-adenine-dinucleotide (formerly designated DPN).
NADH = reduced NAD.
EDTA = Sodium ethylenediaminetetra-acetate (Versene).
BAL = 2,3-Dimercaptopropanol.
Zincon = 5-*o*. Carboxyphenyl-1-(2-hydroxy-5-sulphophenyl)-3-phenylformazan.

might inhibit mitochondrial phosphorylation by the same mechanisms as salicylic acid and other non-thiol uncoupling drugs; the presence of the thio(phenol) group potentiating the intrinsic drug activity of the salicylate pharmacophore. We have tried to resolve this particular question and throw some light on the mechanism of action of uncoupling agents in general, by examining the effects of several aliphatic thiols, thiophenols and other thio-analogues of salicylic acid, upon oxidative phosphorylation in rat liver mitochondria.

Salts of salicylic acid inhibit many NAD-linked dehydrogenase systems[5] for which zinc is an essential cofactor[6]. We found that mercaptobenzoate had no consistent salicylate-like effect on two salicylate-sensitive dehydrogenases but other thio-analogues proved to be rather potent inhibitors of these enzyme systems.

MATERIALS

Pig heart malic dehydrogenase and yeast alcohol dehydrogenase were purchased from Boehringer & Soehne, G.m.b.H., Manheim, Germany.

Chemicals were obtained from the following sources: toluene 3,4-dithiol and zincon; Hopkin & Williams, Chadwell Heath, Essex; 1,10-phenanthroline, thiophenol, ammonium sulphide and 2-mercaptobenzoic acid, British Drug Houses, Poole, Dorset; β-mercaptopropionic acid and β-mercaptobutyric acid, Lights and Co., Colnbrook, Bucks.; thiolacetic and thiobenzoic acids, 2- and 4-methylmercaptophenols, Aldrich Chemical Co., Milwaukee, Wisc., U.S.A.; tropolone, Koch Laboratories, Haverhill, Suffolk; hexahydrosalicylic acid, Fluka A.G., Buchs, Switzerland; 2-Hydroxybenzene-sulphonamide and its 4-amino derivatives were donated by Dr. S. S. Adams, Boots Pure Drug Co., Nottingham.

Phenol-2- and 4- sulphinic acids were prepared from 2- and 4-aminophenol respectively.[7] 2-Mercaptotropone was prepared from tropolone via 2-chlorotropone[8] and (a) methylated with diazomethane in ether to give 2-methylmercaptotropone and (b) oxidised with iodine to give 2,2′-dithiotropone (2,2′-ditropinyl disulphide). Other compounds were prepared by procedures described in Beilstein's Handbuch with the exception of the following:

2-Methylsulphoxyphenol

2-Methylmercaptophenol (3 gms) was dissolved in aqueous potassium hydroxide (1·8 gm) at 0° and acetylated with 2 ml acetic anhydride. The precipitated acetate (1·5 gm) was dissolved in glacial acetic acid (5 ml), cooled to 0° and treated with 1 ml hydrogen peroxide (100 vol.). After standing for 0° for 2 hr, the mixture was kept at 20° for 3 days. The acetic acid was then removed by distillation under reduced pressure at 60°. The acetylated sulphoxide was saponified with 1 N sodium hydroxide at 100° for 2 hr. After cooling, the solution was acidified with dilute hydrochloric acid and extracted with chloroform. Evaporation of the chloroform yielded the product which was crystallised from benzene–pet. ether (b.p. 40/60°), m.p. 125·5–127·5° (uncorr.); yield 0·8 gm. ($C_7H_8O_2S$ requires C, 53·8 per cent; H, 5·2 per cent; S, 20·5 per cent; found C, 54·0 per cent; H, 5·3 per cent; S, 20·4 per cent).

This compound gives a red-violet colour with ferric chloride in ethanol: methylmercaptophenol gives a green colouration. Thin layer chromatography on Kieselgel 'H' plates in methanol gave one spot R_F, 0·69 (starting material had R_F 0·75).

2-Mercaptocyclohexanecarboxylic acid (hexahydro-2-mercaptobenzoic acid)

This process is based on the preparations of DL-β-mercaptobutyric acid from DL-β-hydroxybutyric acid.[9]

The sodium salt of *cis* hexahydrosalicylic acid (7 gm) was heated with thiourea (3 gm) and 60% (w/v) hydrobromic acid (30 ml) under reflux for 7 hr. The mixture was made alkaline with sodium hydroxide and refluxed for another 2 hr. After cooling and acidification with hydrochloric acid, the products were extracted into ether. The ethereal extract was dried over sodium sulphate and the ether removed by distillation. The residue was fractionally distilled under reduced pressure (12 mm Hg). The fraction collected over the boiling range 140–150° gave a positive test for an aliphatic thiol with sodium nitroprusside. Thin layer chromatography of this fraction on Kieselgel 'H' plates in methanol and paper chromatography with isopropanol-ammonia-water (8:1:1 v/v) indicated two components which stained with iodine vapour; one of which (R_F 0·56 on paper) corresponded to the starting material and gave no reaction with nitroprusside; the other (R_F 0·75 on paper) gave a thiol-positive reaction with nitroprusside. The mixture had a sulphur content of 9·0 per cent; hexahydromercaptobenzoic acid requires 20·0 per cent.

This mixture was tested for uncoupling activity after reduction of any disulphide present with sodium sulphite.[10] Excess sulphite was removed as sulphur dioxide by aspiration with nitrogen after adjusting to pH 4 with dilute hydrochloric acid.

N-Picryl-4-amino-2-hydroxybenzenesulphonamide (5-Picrylamino-phenol-2-sulphonomide).

4-Amino-2-hydroxybenzenesulphonamide (0·2 g) and sodium bicarbonate (0·4 g) were dissolved in 5 ml water and treated with 2,4,6-trinitrobenzene sulphonic acid (0·3 g). After standing for 2 hr in the dark, the product was filtered and crystallised from aqueous ethanol, m.p. 272 (d) ($C_{12}H_9N_5O_9S$ requires N, 17·5 per cent; found N, 17·4 per cent).

When 4-aminosalicylic acid was treated similarly with trinitrobenzenesulphonic acid, N-picryl-4-aminosalicylic acid was obtained, m.p. 251° (d) (found N, 15·4 per cent).

BIOCHEMICAL PROCEDURES

Oxidative phosphorylation by rat liver mitochondria respiring on succinate at 30° was measured as described hitherto.[11] 2-Mercaptobenzoic acid and other compounds to be tested were added to the incubation medium, either as aqueous solutions pH 7 or as solutions in N,N-dimethylformamide (not exceeding 50 μl). Yeast hexokinase activity and mitochondrial ATP-ase activity in the presence of test compounds, were measured at pH 8–9 and pH 7·4 respectively.[12, 13] Malic dehydrogenase and alcohol dehydrogenase activities in the presence of test compounds were assayed by standard procedures[14, 15] with the modification that NADH formation was measured by the increase in extinction at 366 mμ.

RESULTS

Uncoupling of oxidative phosphorylation by thiophenols and thiotropone

Table 1 shows the relative uncoupling activities of some thiophenol analogues of salicylic acid. 2-Mercaptobenzoic acid, 2,2′-dithiobenzoic acid and 2-mercaptotropone

were the only compounds in this group which uncoupled oxidative phosphorylation. Thiophenol itself (in saturated solution) uncoupled oxidative phosphorylation but was less potent than 2-mercaptobenzoic acid.

The weak uncoupling activity of 2,2′-dithiobenzoic acid (*o.* carboxyphenyl disulphide) was not apparently due to contamination by 2-mercaptobenzoic acid,

TABLE 1. EFFECT OF SOME THIOPHENOLS AND OTHER THIOANALOGUES OF SALICYLIC ACID UPON OXIDATIVE PHOSPHORYLATION

P/O ratio (succinate oxidation) in presence of drugs expressed as a percentage of P/O ratio in drug-free controls.

Compound	Conc. (mM)	P/O	Compound	Conc. (mM)	P/O (%)
None	—	100			
Salicylic acid	0·5	60	Thio(n)thiolsalicylic acid	0·25	50
				0·5	0
2-Mercaptobenzoic acid	0·25	20	Thiobenzoic acid	1·0	30
	0·5	0	Benzenesulphinic acid	1·0	95
S-Methyl-2-mercaptobenzoic acid	0·25	90	Phenol-2-sulphinic acid	0·5	55
				1·0	10
2,2′-Dithiobenzoic acid	1·0	70	Phenol-4-sulphinic acid	1·0	85
4-Mercaptobenzoic acid	1·0	80	2-Methylsulphoxyphenol	5·0	100
Hexahydro-2-mercaptobenzoic acid	1·5	100	Phenol-2-sulphonic acid	5·0	95
β-Mercaptopropionic acid	1·0	95	Phenol-2-sulphonamide	2·5	90
Thiophenol	0·6	50			
	1·5*	20			
			4-Picrylamino-2-hydroxy benzenesulphonamide	1·0	100
Tropolone	0·2	50	4-Picrylamino-salicylic acid	0·5	0
2-Mercaptotropone	0·5	85			
	1·0	25			
S-Methyl-2-mercaptotropone	1·0	97	S-Methylmercaptophenol	2·5	55
2,2′-Dithiotropone	0·5	95	S-Methyl-4-mercaptophenol	2·5	40
	1·0*	60			
			Guaiacol	5·0	70
			1,10-Phenanthroline	2·5	60
			Zincon	0·3	55

* Saturated solution in phosphate buffer (pH, 6·8).

since the disulphide gave no reducing reaction with a Fehling's reagent[15] and on paper chromatography in isopropanol-ammonia-water (8:1:1 v/v) it migrated as a single spot, R_F 0·24 (cf. mercaptobenzoic acid, R_f 0·75). The corresponding disulphide formed from mercaptotropone, 2,2′-dithiotropone only uncoupled oxidative phosphorylation when added in saturated solution at pH 6·8 (>0·5 mM). It is possible that the feeble uncoupling activity of these two disulphides was due to their partial reduction within mitochondria to the corresponding thiols. Methylating the thiol group abolished the uncoupling activity of both 2-mercaptobenzoic acid and 2-mercaptotropone.

It was not possible to compare S-acetylmercaptobenzoic acid with O-acetylsalicylic acid because this acetylated thiol was too insoluble in aqueous solutions and in water-miscible solvents (such as dimethylformamide and ethanol) to be tested adequately. *Trans* hexahydromercaptobenzoic acid failed to uncouple oxidative phosphorylation when tested in an (approximately equimolar) admixture with *cis* hexahydrosalicylic acid, which is known to be devoid of uncoupling activity.[1, 2] Other β-mercapto aliphatic acids were likewise inactive.

These findings indicate that a carbonyl group *ortho* to an aromatic thiol group considerably potentiates the ability of simple aromatic thiols to uncouple oxidative phosphorylation.

4,4'-Dithiobenzoic acid almost completely inhibited respiration at 0·5 mM. None of the other compounds examined here depressed respiration at uncoupling concentrations.

Uncoupling activity of phenolic thioanalogues of salicylic acid

Thio(n)thiolsalicylic acid (Fig. 2, X = SH) and phenol-2-sulphinic acid (Fig. 3, X = OH) uncoupled oxidative phosphorylation. Other aromatic sulphinic acids tested were inactive.

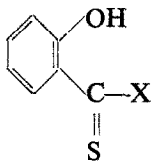

FIG. 2. Pharmacophore for uncoupling activity among thio analogues of salicylic acid.

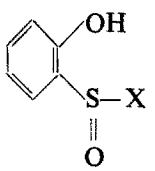

FIG. 3. Pharmacophore for uncoupling activity among thio analogues of salicylic acid.

The uncoupling activity of thio(n)thiol-salicylic acid, which approximated to that of 2-mercaptobenzoic acid, is probably associated with both the *ortho* phenolic group and the acylthiol group. Thio(l)benzoic acid, an acylthiol, also uncoupled oxidative phosphorylation but was less potent than thiothiolsalicylic acid. If this uncoupling activity of thiobenzoic acid was due to liberation of hydrosulphide ions, it must occur in the mitochondrion; since less lipophilic molecules which might hydrolyse similarly (thiolacetic acid, thioacetamide and salts of ethyldithiocarbamic acid, all at 3 mM), solutions of 'ammonium sulphide' (3 mM with respect to H_2S) and benzoic acid (5 mM), all had no effect on oxidative phosphorylation.

2-Methylsulphoxyphenol (Fig. 3, X = CH_3) had no effect as oxidative phosphorylation (contrast phenol-2-sulphinic acid). However, the related desoxy compound, 2-methylmercaptophenol, did uncouple oxidative phosphorylation and so did its isomer (4-methylmercaptophenol) and its oxyanalogue, guaiacol (2-methoxyphenol); though the guaiacol was much less potent in this respect.

Several *ortho* phenolic sulphonic acids were examined ranging in hydrophilic/lipophilic character from 2-phenolsulphonic acid itself to its 4-trinitrophenyl(picryl)-amino derivative, including 1-hydroxy-2-naphthalene sulphonic acid. Although these acids firmly complexed transition metal ions, none of them (at 5 mM) affected oxidative phosphorylation. The corresponding amides were also tested, where these were available, and found not to inhibit mitochondrial phosphorylation.

None of the compounds listed in Table 1 which uncoupled oxidative phosphorylation inhibited the yeast hexokinase used to trap newly synthesised ATP. Phenolsulphinic acid did not stimulate the mitochondrial ATP-ase activity (in contrast to other compounds which uncouple oxidative phosphorylation, see Table 2).

TABLE 2. EFFECT OF SOME PHENOLS ON MITOCHONDRIAL ATP-ASE ACTIVITY

Enzyme activity at 20°, pH 7·4 without added compounds designated 1·0.

Phenol	Conc. (mM)	Relative activity
None	—	1·0
Salicylic acid	1·0	2·8
2-Mercaptobenzoic acid	1·0	4·2
Phenol-2-sulphinic acid	1·0	1·0
4-Picrylamino-salicylic acid	0·5	1·6
2,4-Dinitrophenol	0·05	3·1

Effect of 2-mercaptobenzoic acid and related compounds on yeast alcohol dehydrogenase

Measurements of dehydrogenase activity were made by following the change in extinction at 366 mμ (i.e. NADH formation) over the period 15 sec–45 sec after adding the enzyme to a mixture of the substrate, the compound to be tested and the buffer.

There was a surprising difference between the effect of hydroxybenzoates and mercaptobenzoates on this enzyme system. 2-Mercaptobenzoic acid apparently stimulated the enzyme: control experiments showed that no NADH was formed when alcohol dehydrogenase was incubated with NAD and 2-mercaptobenzoic acid in the absence of alcohol. 4-Hydroxybenzoic acid had no effect, but 4-mercaptobenzoic acid was a much stronger inhibitor of this enzyme than was salicylic acid. At relatively high concentrations, S-methyl-2-mercaptobenzoic acid did inhibit the enzyme, but 2-methoxybenzoate had no significant effect.

In curious contrast to the 2-mercaptobenzoate, thio(n)thiolsalicylic acid was a potent inhibitor.

EDTA did not affect the enzyme, which suggested that a strong zinc-chelator is required to inhibit yeast alcohol dehydrogenase.

Effect of 2-mercaptobenzoic acid and related compounds on malate dehydrogeuase

Pig heart malate dehydrogenase has been shown to be rather more sensitive than alcohol dehydrogenase to millimolar concentrations of salicylate salts.[5, 17]

2-Mercaptobenzoic acid was approximately equipotent with salicylic acid in inhibiting malate dehydrogenase (although it did not inhibit the alcohol dehydrogenase). 4-Mercaptobenzoic acid and S-methyl-2-mercaptobenzoic acid inhibited malate dehydrogenase (as well as alcohol dehydrogenase), while the corresponding oxy-compounds, 4-hydroxybenzoic acid and 2-methoxybenzoic acid had no effect upon either the malate or the alcohol dehydrogenase.

The inhibition of pig-heart malate dehydrogenase by 2- and 4-mercaptobenzoic acids was not simply due to the presence of a thiol group, as simple aliphatic thiols e.g. β-mercaptopropionic acid, had no effect upon the enzyme activity.

DISCUSSION

Inhibition of dehydrogenases

The effect of salicylate analogues on the zinc-containing enzymes, yeast alcohol dehydrogenase and pig heart malic dehydrogenase, was studied in order to find out whether or not there was any correlation between ability to chelate zinc ions (bound

TABLE 3. INHIBITION OF YEAST ALCOHOL DEHYDROGENASE BY SOME AROMATIC ACIDS

Enzyme activity in the presence of acids is expressed as a percentage of the activity of drug-free controls.

Acid	Conc. (mM)	Activity 15–45 sec. (%)	Activity over first 2 mins. (%)
None	—	100	100
Salicylic	10	48	52
	5	71	78
4-Hydroxybenzoic	10	91	103
2-Methoxybenzoic	10	97	81
1-Hydroxy-2-napthoic	4	5	0
	2	31	25
2-Mercaptobenzoic	10	112	95
	5	142	125
	2·5	124	130
4-Mercaptobenzoic	5	—	0
2,2′-Dithiobenzoic	5	—	0
	2·5	37	30
4,4′-Dithiobenzoic	5	—	25
	2·5	55	55
Thio(n)thiolsalicylic	5	—	0
	2·5	—	44
S-Methyl-2-mercaptobenzoic	10	75	70
EDTA	10	97	100

TABLE 4. INHIBITION OF MALATE DEHYDROGENASE BY SOME AROMATIC ACIDS

Enzyme activity in the presence of acids expressed as a percentage of the activity in drug-free controls.

Acid	Conc. (mM)	Activity 15–45 sec. (%)	Activity over first 2 mins. (%)
None	—	100	100
Salicylic	10	41	24
4-Hydroxybenzoic	10	94	96
2-Methoxybenzoic	10	96	92
	5	89	94
Hexahydrosalicylic	10	87	89
2-Mercaptobenzoic	10	40	30
	5	51	30
4-Mercaptobenzoic	10	18	9
	5	46	33
S-Methyl-2-mercaptobenzoic	10	70	72
	5	77	83
β-Mercaptopropionic	10	112	94

to protein) and ability to uncouple oxidative phosphorylation. Cadmium ions, the arsenite-BAL complex, 2,4-dinitrophenol and dicoumarol, which all uncouple oxidative phosphorylation, also inhibit the zinc-containing β-hydroxybutyric dehydrogenase of beef heart mitochondria and yeast alcohol dehydrogenase.[18] Smith

and his colleagues found that salicylic acid inhibited many NAD-linked dehydrogenase.[5, 17] In view of the affinity of zinc for thiol groups, we tested mercaptobenzoic acid and other thiols for their effects upon two dehydrogenases which have been fairly intensively studied as regards their inhibition by uncoupling agents and other drugs. The fact that 2-mercaptobenzoic acid only inhibited one of these enzymes (malate dehydrogenase) whilst 4-mercaptobenzoic acid inhibited them both but fails to uncouple oxidative phosphorylation, suggests that it is rather fortuitous that several uncoupling agents should happen to inhibit these zinc-containing dehydrogenases. It must be concluded that these drugs uncouple oxidative phosphorylation by some other general mechanism than simply binding zinc ions within the mitochondrial lipoprotein complex, even though potent zinc-complexing agents, such as toluene-dithiol, zincon and 1,10-phenanthroline, apparently uncouple oxidative phosphorylation (see Table 1).

Some of our observations extend and confirm those of Smith and Bryant upon the relationship between chemical structure and inhibition of malic dehydrogenase.[19] The *ortho* hydroxybenzoyl group is apparently essential for this type of activity amongst sulphur-free derivatives of benzoic acid. Aromatic thiolacids, but not simple aliphatic thiolacids, are also inhibitors of this enzyme.

TABLE 5. SUMMARY OF RELATIONSHIP BETWEEN STRUCTURE AND UNCOUPLING ACTIVITY FOR SOME ANALOGUES OF SALICYLIC (2-HYDROXYBENZOIC) ACID AND TROPOLONE (2-HYDROXYTROPONE)

Compound	Activity	Compound	Activity
Benzoic acids:		*Benzoic acids:*	
2-Hydroxy	+	2-Mercapto	+
4-Hydroxy	−	4-Mercapto	−
2-Methoxy	−	2-Methylmercapto	−
Hexahydro-2-hydroxy	−	Hexahydro-2-mercapto	−
Tropones:		*Tropones:*	
2-Hydroxy	+	2-Mercapto	+
2-Methoxy	−	2-Methylmercapto	−

Relationship between structure and uncoupling activity

Table 5 summarises the considerable similarity between the structure-action relationships for salicylic (2-hydroxybenzoic)[1, 2] and 2-mercaptobenzoic acids, and for tropolone (2-hydroxytropone)[11] and 2-mercaptotropone. The requirement for an aromatic thiol group is further indicated by (a) the loss of uncoupling activity upon oxidation to the disulphide and (b) the failure of many aliphatic thiols to significantly affect mitochondrial phosphorylation.

The greater uncoupling activity of mercaptobenzoic acid, compared with salicylic acid, is explicable not only by its greater lipophilic character (which largely governs the uncoupling activity of substituted salicylic acids[2]) but also by its higher pK_a—which corresponds more nearly to the pK's of other potent uncoupling agents with considerable lipophilic character, such as phenylbutazone (pK 4·5), dicoumarol (pK 7·2), 2,4 dinitrophenol (pK 4·0) and 2-phenyl-indan-1,3-dione (pK 4·2). The latter drugs are all enols, whereas it is the carboxyl group of 2-mercaptobenzoic acid

which ionises with a pK in water of ~4 (Table 6). This suggests that the *ortho* thio group may not be functionally involved in the uncoupling action, although it may impress uncoupling activity by conferring lipophilic character through intramolecular hydrogen bonding[28] (contrast 4-mercaptobenzoic acid), if indeed the pK primarily determines the uncoupling activity of aromatic compounds.

TABLE 6. ACID DISSOCIATION CONSTANTS (AS pK_a's) OF SOME AROMATIC COMPOUNDS

Values mainly taken from the literature.

Compound	pK_a (H_2O)	pK_a (50% ethanol)	Ref.
2-Hydroxybenzoic acid	3·0	4·0	(20)
2-Mercaptobenzoic acid		5·0	(20)
4-Hydroxybenzoic acid	4·5	6·1	(20)
4-Mercaptobenzoic acid		5·6	(20)
Tropolone	6·9		(21)
2-Mercaptotropone	5·9		(21)
Phenol	10·0	11·3	(20, 22)
Thiophenol	6·5 (7·8)	7·8	(20, 23, 24)
Phenol-2-sulphinic acid	2, 8·2		(25)
2-Methylsulphoxyphenol	ca. 7·8*		
2-Hydroxyacetophenone	10·8		(26)
2-Methylmercaptophenol	ca. 9·3*		
4-Methylmercaptophenol	9·5		(27)
Guaiacol	10·0		(22)
4-Methoxyphenol	10·2		(22)

* determined spectrophotometrically[28]

The fact that 2-mercaptotropone is a weaker uncoupling agent than tropolone, is rather puzzling. None of the following factors would appear to explain this anomaly: (i) acidity, (ii) structure, (iii) lability.

(i) Mercaptotropone is more acidic than tropolone (Table 6). With other drugs the uncoupling activity appears to increase as the pK decreases over the pK range from 7-plus to about 4 (cf. thiophenol and phenol).

(ii) Nozoe and Matsui[29] concluded that 2-mercaptotropone probably normally exists as its isomer, 2-hydroxytropothione, although it behaves chemically as the thiol rather than the thione. This should not, however, affect the relative activities of tropolone and mercaptotropone (hydroxytropothione) if it is only the acidic character of the molecule which confers uncoupling activity.

(iii) Mercaptotropone was not significantly oxidised to the (inactive) disulphide on incubation with phosphorylating liver mitochondria. Over 90 per cent of the added thiol was recovered by extraction with chloroform after the incubation period (as assayed by the extinction of these chloroform solutions at 420 mμ).

Both tropolone and 2-mercaptotropone are highly lipophilic, indicated by their very high partition coefficients between chloroform, butanol, n.octanol, or tributyl phosphate and aqueous salt solutions, pH 6·8.

The uncoupling activity of salicylic acid is abolished on replacing the carboxylic hydroxyl group with a methyl group (giving 2-hydroxyacetophenone, I).[2, 28] Similarly replacing the sulphinic hydroxyl group of phenol-2-sulphinic acid with a methyl

group (giving 2-methylsulphoxyphenol, II) also abolishes the uncoupling activity. These inactive phenols (I and II) each have a high pK (Table 6) but this is not wholly incompatible with uncoupling activity, *vide* guaiacol and the methylmercaptophenols.

Phenol-2-sulphinic acid is a less active drug than salicylic acid probably because of its lower pK_1. Aromatic sulphinic acids as a class are stronger than carboxylic acids[24] and therefore less likely to partition from an aqueous phase into a lipid-rich medium.

Addendum—Contrary to previous reports, malic dehydrogenase does not contain zinc.[30] Therefore its inhibition by 2-mercaptobenzoic acid has nothing to do with zinc-binding.

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