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SHORT COMMUNICATION

The metabolism of thioamides by the supernatant fraction of rat liver homogenate

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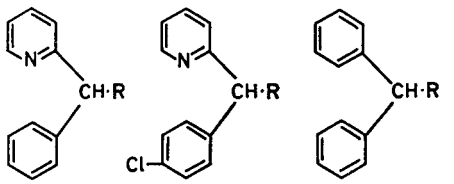
α-Phenyl-α-(2-pyridyl) thioacetamide (SC 15396; antigastrin) is metabolized by the supernatant fraction of rat liver homogenate to give two metabolites. The major metabolite has been identified as α-phenyl-α-(2-pyridyl)acetonitrile. Three other thioamides, analogues of SC 15396 also give the corresponding nitriles in these conditions.

α-Phenyl-α-(2-pyridyl)thioacetamide (SC 15396, antigastrin, G. D. Searle & Co.) (Ia) has been shown to inhibit gastric secretion when administered by the intravenous or oral routes (Gillespie, McCusker & others, 1968; Connell, Sircus & others, 1967). Since little is known about the metabolic fate of thioamides we have examined the effect of incubating SC 15396 and some closely-related thioamides with the supernatant fraction of rat liver homogenate, as a preliminary to examining the fate of the drug *in vivo*.

EXPERIMENTAL



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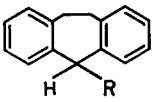

IV

a R= −CS·NH$_2$
b R= −CN

Materials and methods

The thioamides examined, α-phenyl-α-(2-pyridyl)thioacetamide (Ia), α-(4-chlorophenyl)-α-(2-pyridyl)thioacetamide (IIa), diphenylthioacetamide (IIIa) and 10,11-dihydro-5*H*-dibenzo-(*a*,*d*)-cycloheptene-5-thioamide (IVa) were obtained by courtesy of G. D. Searle & Co. α-Phenyl-α-(2-pyridyl)acetonitrile (Ib) and diphenylacetonitrile (IIIb) were obtained from Ralph N. Emanuel Ltd.

The two other nitriles (IIb) and (IVb) were most conveniently prepared by addition of sodium plumbite solution (10% w/v) to alcoholic solutions of the corresponding thioamides. Lead sulphide was precipitated and the nitriles extracted with ether.

Crystallization from ether/light-petroleum gave the pure nitriles (IIb) m.p. 63–65° (Brocades-Stheeman, 1962) and (IVb) m.p. 153° (Sperber, Papa & others, 1951).

In vitro *metabolism*

Male Wistar rats, weighing about 300 g were used in all experiments. They were killed, the livers quickly removed, blotted, weighed and homogenized with 2 volumes of isotonic potassium chloride solution at 0–5° in an Ultra-Turax homogenizer (3 × 12 s). The homogenates were centrifuged 30 min at 10,000 *g* to remove cell debris and the supernatant fraction decanted.

To the supernatant (2·0 ml), in a 25 ml incubation flask there was added NADP (1mg), glucose-6-phosphate (5 mg), magnesium chloride (20 μmol), nicotinamide (60 μmol) and SC 15396 (1a) (10 μmol) as a fine suspension in Sørensen phosphate buffer (pH 7·4) to make a total volume of 7 ml. Control experiments were conducted from which the substrate was omitted.

To eliminate any possibility of purely chemical degradation of the drugs, some control experiments used liver supernatant, denatured by being heated at 65–75° for 45 min followed by further homogenization for 1 min.

The flasks were incubated 1·5 h at 37° in a shaking incubator, cooled and extracted three times with peroxide-free ether (3 × 8·0 ml). The ether extracts were evaporated to dryness at 37° and the residue dissolved in benzene (0·2 ml). The benzene extracts were chromatographed on 20 × 5 cm silica gel plates (Merck G, 250μm) by ascending chromatography with the three solvent systems ether, ether–formic acid (15:1 v/v) and methylene chloride. Extracts from blank control flasks and from flasks containing denatured supernatant fraction, co-factors and substrate were chromatographed at the same time. Spots due to metabolites and, where appropriate, the original materials were located by their fluorescence under ultraviolet light and by exposing the plates to iodine vapour. Results are shown in Table 1.

The large scale separation of the metabolites of SC 15396 (Ia) was conducted with the supernatant fraction from six livers (102 g) and the appropriate quantities of co-factors and substrate. The extracts were chromatographed on large (50 × 20 cm) thin-layer plates using ether as solvent.

The metabolite was eluted from the appropriate portion of the plate with methylene chloride, the solution evaporated *in vacuo*, and the residue dissolved in spectroscopic chloroform (0·2 ml.).

The infrared spectrum of this solution was determined on a Unicam SP 100 spectrophotometer in a 0·5 mm cell and compared with that of an authentic sample of (Ib) (200 mg) chromatographed and extracted in an identical manner.

RESULTS

The chromatographic separation of the metabolites is shown in Table 1. Spots due to substrate and to materials extracted from the supernatant fraction can be identified by comparison of the control extracts with those containing substrate and active supernatant fraction. Apart from a small amount of material on the base-line in most cases, the main components extracted after incubation are the thioamides and the derived nitriles.

For SC 15396 (Ia) the infrared spectrum of the metabolite was identical with that of an authentic sample of the nitrile (Ib). No spot corresponding to this nitrile was present either in blank experiments or in those using denatured liver fractions.

Table 1. *Rf values of the thioamides, their metabolites and corresponding nitriles*

Experiment	Solvent systems I	II	III
Metabolism of Ia	0·40†, 0·56, 0·9	0·34, 0·74	—
Control*	0·38	0·34	—
Ia	0·40	0·28	—
Ib	0·55	0·74	—
Metabolism of IIa	0·39, 0·59†	0·40, 0·78	—
Control*	0·39	0·42	—
IIa	0·38	0·36	—
IIb	0·57	0·79	—
Metabolism of IIIa	0·60, 0·72	—	0·28, 0·61†
Control*	0·57	—	—
IIIa	0·62	—	0·26
IIIb	0·74	—	0·60
Metabolism of IVa	0·60, 0·75†	—	0·32, 0·68
Control*	0·59	—	—
IVa	0·62	—	0·31
IVb	0·77	—	0·67

All results represent values from 6 repeat experiments.
* Denatured liver fraction, co-factors and drugs.
† A very small additional spot at Rf $< 0{\cdot}05$.
Solvent systems: I peroxide-free ether; II ether–formic acid 15:1 v/v; III methylene chloride.

DISCUSSION

There are few previous reports of the metabolic fate of thioamides. Ethionamide (2-ethylisonicotinic acid thioamide) and its sulphoxide are excreted by man as 2-ethylisonicotinamide and 2-ethylisonicotinic acid (Bieder & Mazeau, 1964; Bieder, Brunel & Mazeau, 1966). The same metabolites are reported by Johnston, Kane & Kibby (1967) after administration of ethionamide to rats and mice.

2,6-Dichlorothiobenzamide (Prefix) however, in rats and dogs, is metabolized to the extent of about 40%, to 2,6-dichloro-3-hydroxybenzonitrile (Griffiths, Moss & others 1966), and the pattern of metabolic products is the same from the thioamide or the nitrile whichever is administered.

All four compounds examined in the present investigation are metabolized in the same way *in vitro*; simple hydrolysis of the thioamide or replacement of the sulphur by oxygen can represent only a minor pathway in these conditions. These metabolites can be detected as base-line spots on TLC plates but only traces of base-line materials were produced from extracts of the thioamide metabolic products.

These observations are interesting because of the unexpected course of the metabolic process. The only previous report of this nature, that of Griffiths & others (1966), concerned 2,6-dichlorothiobenzamide, a compound for which the more obvious hydrolytic pathway, would be hindered by the presence of substituents *ortho* to the thioamide group. It is conceivable therefore that in this case, oxidative removal of H_2S is favoured. In the series of compounds we have used however, this explanation is untenable since all the thioamides may readily be hydrolysed.

It seems likely therefore that the formation of nitrile is an oxidative process, mediated by the oxidising enzyme system in the microsomal fraction of rat liver homogenate and further work is in hand to verify this. Preliminary work reported here suggests this may be true since inactivation of the microsomal enzyme system led to recovery of the thioamides unchanged.

The other significant feature is the possibility that a chemically reactive nitrile can be produced, *in vivo*, by the operation of a detoxication process.

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