contains equimolar quantities of iron and copper, the iron result will be almost 2% too high, but, as shown by Bastian *et al.* (1956), this interference can be reduced by measurement at 260 m μ instead of 240 m μ , with some sacrifice of sensitivity. The effect of other common cations is given in Table 2.

DISCUSSION

Spectrophotometry of ferric perchlorate has proved to be a quick and simple method for analysis of total iron. The determination can be completed during the course of one day and the total working time is small. It is possible to shorten the method a little by measuring the optical density of the diluted perchloric acid digest (without taking to dryness), but it is essential to remove the last traces of nitric acid, and it is therefore convenient to evaporate the digest completely and redissolve the inorganic material in a fresh portion of perchloric acid. Recovery experiments have shown that no iron is lost even when the dry residue is heated for several hours.

Digestions with perchloric acid have a bad reputation for explosions. In the present method, organic matter receives a thorough treatment with boiling concentrated nitric acid before any perchloric acid is added. We have carried out many

digestions in this way without incident. In the final stages a solution of inorganic salts in perchloric acid is evaporated to dryness; in the absence of organic matter this process is acceptable as safe. However, in view of the consequences of mismanagement, all digestions are conducted behind a plastic screen and precautions are taken to avoid contact of hot liquid or vapour with inflammable materials.

SUMMARY

A rapid and precise method for determining iron in biological samples is described. Iron in the range 0.02–1 mg. is estimated by digestion with nitric acid–perchloric acid and ultraviolet spectrophotometry at 240 m μ . Precautions, and the effect of other cations, are discussed.

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Studies in Detoxication

73. THE METABOLISM OF ALKYL BENZENES: PHENYLACETYLENE AND PHENYLETHYLENE (STYRENE)*

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In the previous papers in this series (Smith, Smithies & Williams, 1954*a-c*; Robinson, Smith & Williams, 1955; Robinson & Williams, 1955; El Masri, Smith & Williams, 1956), the metabolism of alkylbenzenes containing saturated side chains was investigated. These studies have now been extended to include the unsaturated derivatives styrene and phenylacetylene. Of these compounds styrene, a compound of considerable importance in the plastics and other industries, has been previously investigated. In man, rats and rabbits, it appears to be metabolized largely by loss of one of its side-chain carbon atoms to yield benzoic acid, which is excreted as hippuric acid (Spencer, Irish,

Adams & Rowe, 1942; Carpenter, Shaffer, Weil & Smyth, 1944), and in rats and rabbits it is reported that 50–90% of the dose may be excreted as hippuric acid. Recently, however, Danishefsky & Willhite (1954) reported briefly some experiments using [β - ^{14}C]styrene and found that, when styrene was injected subcutaneously into rats (dose about 0.1 ml./100 g. rat), about 12% of the radioactivity appeared in the expired air as carbon dioxide whereas over 70% appeared in the urine. Spencer *et al.* (1942) suggested that a small amount of a mercapturic acid was formed, but, apart from hippuric acid, small amounts of unchanged styrene and respiratory CO_2 , the metabolites of styrene have not been identified. Phenylacetylene has not been investigated hitherto.

* Part 72: Mead, Smith & Williams (1958).

EXPERIMENTAL

Materials and Methods

Animals. Rabbits kept on a constant diet of 50 g./day of rat cubes (Diet 41, Associated Flour Millers) were used.

Reference compounds. Phenylacetylene (b.p. 143°/760 mm.) and styrene (b.p. 58°/20 mm., n_D^{25} 1.545) were redistilled immediately before use. (–)-Mandelic acid [m.p. 133°; $[\alpha]_D^{25}$ –154° in water (c, 1)] was converted into *S*-benzylthiuronium (–)-mandelate which formed plates from water, m.p. 180° and $[\alpha]_D^{25}$ –57° in 20% (v/v) aqueous ethanol (c, 1) (Found: C, 60.6; H, 5.9. $C_{16}H_{18}O_3N_2S$ requires C, 60.4; H, 5.7%) [the (±)-mandelate is a known compound, m.p. 166°; see Wild (1947)]. (±)-Phenylglycol, m.p. 67°, was prepared by hydrolysis of styrene epoxide with water acidified with a few drops of $HClO_4$. Phenacyl alcohol, m.p. 73°, was prepared by boiling phenacyl bromide with barium carbonate for 12 hr. The properties of these compounds agreed with those found in the literature.

Analytical methods. Glucuronic acid in urine was determined according to Paul (1951) (see Mead *et al.* 1958), ethereal sulphates according to Sperber (1948), hippuric acid according to El Masri *et al.* (1956) and mercapturic acid by iodine titration according to Stekol (1936). Styrene and phenylacetylene did not interfere with the mercapturic acid estimations.

Phenylacetylene or styrene in expired air was collected and determined spectrophotometrically by using the apparatus described by Parke & Williams (1950; see also Azouz, Parke & Williams, 1952). The expired air of the animals dosed with phenylacetylene or styrene was dried over anhydrous $MgClO_4$ and the hydrocarbons present were then trapped in ethanol contained in wash bottles cooled in acetone/solid CO_2 . The phenylacetylene or styrene content of the ethanol was estimated at intervals by measuring the light-absorption at 235 $m\mu$ for phenylacetylene (the ϵ_{max} found at this wavelength for pure phenylacetylene was 16 000), and at 245 $m\mu$ for styrene (ϵ_{max} found was 14 100), and identity of the material analysed was checked by determining the absorption curve over the range 220–

270 $m\mu$ with a Unicam SP. 500 spectrophotometer (for published spectra see Magat & Maier, 1943). Quantities of 0.5 g. of phenylacetylene on a watch glass placed in the tank were recovered quantitatively in 12 hr. by aeration into the ethanol absorption bottles. With styrene in similar quantities, the maximum recovery in two experiments was 89.1 and 84.2% after 12 hr. aeration. Recovery was incomplete because styrene tends to polymerize, and in these experiments 2.2 and 2.7% of the original styrene remained on the watch glass as polymeric material.

Paper chromatography. The separation and identification on paper of hippuric, phenaceturic and *p*-hydroxyphenylacetic acid in urine were carried out as described by El Masri *et al.* (1956). Rough estimates of phenaceturic acid, which is the only metabolite of phenylacetylene, were obtained by comparing the spots from the unknown material with spots from known amounts of the acid added to urine. Mandelic acid, phenylglycol and phenylglycol glucuronide (about 50 μg .) were detected chromatographically on paper; the solvent systems, R_F values and colour reactions are quoted in Table 1.

Isolation of metabolites from phenylacetylene

The urine from rabbits receiving oral doses of phenylacetylene (0.4 g./kg.) was non-reducing. It contained no ketones when tested with 2,4-dinitrophenylhydrazine and no glucuronides when tested with naphtharesorcinol. Quantitative measurements showed no significant increase above normal in the glucuronic acid or ethereal sulphate outputs. Paper chromatography of the urine indicated that phenaceturic acid was being excreted in small amounts for at least 4 days after dosing, and by comparison with reference chromatograms it was estimated that 5–15% of the dose of phenylacetylene was being excreted daily as phenaceturic acid for 3 days after dosing. No significant increase in hippuric or *p*-hydroxyphenylacetic acid was found. Mandelic acid was not detected in the urine by

Table 1. R_F values and colour reactions for the detection of the metabolites of styrene

Solvent systems used were: A, benzene–acetic acid–water (1:1:2, by vol.) with Whatman no. 1 paper; B, ethyl methyl ketone–2*N*-ammonia (1:1) with Whatman no. 1 paper; C, butanol–acetic acid–water (4:1:5, by vol.) on Whatman no. 4 paper; and D, propanol–aq. ammonia (sp.gr. 0.88) (7:3, v/v) with a Whatman no. 4. All the descending chromatograms were run for 5 hr. For the detection of mandelic acid and phenylglycol, the paper was soaked on a glass plate with a mixture of concentrated sulphuric acid (20 ml.) and 40% (w/v) formalin (0.2 ml.), which showed up these compounds as brown spots. Hippuric and phenaceturic acid gave no colours with this reagent.

	R_F values in solvent			
	A	B	C	D
Mandelic acid	0.13	0.13	0.90	0.55
Phenylglycol	0.22	0.71	0.93	0.86
Phenylglycol glucuronide	0.00	0.00	0.55	0.26
Hippuric acid	0.07	0.07	0.86	0.55

Fig. 1. Elimination of phenylacetylene in the expired air of a rabbit which had received a 1.5 g. oral dose of the hydrocarbon. Curve A shows the total phenylacetylene eliminated and curve B the amount eliminated every 6 hr.

direct paper chromatography, but traces of it were detected by its R_F values in four solvents (see above) in concentrated extracts of the acidified urine.

Isolation of phenaceturic acid. A rabbit was given 2 g. of phenylacetylene by stomach tube and urine collected daily for 4 days. Each day's urine was adjusted to pH 2 by the addition of conc. HCl and continuously extracted with ether for 18 hr. No ketones were detected in the extracts. On removal of the ether each extract left a tarry residue which crystallized on adding 5 ml. of water. The first and second days' urine each yielded 0.1 g. of phenaceturic acid, m.p. and mixed m.p. 142°, and the combined extracts from the third and fourth days' urine gave another 0.1 g. (total, 7.5% of the dose).

Elimination of unchanged phenylacetylene. Two experiments were made in which the expired air of rabbits which had received 1.5 g. of phenylacetylene orally was collected and analysed. In the first experiment 25% and in the second 37% of the dose was found in the expired air as unchanged phenylacetylene during 3 days (72 hr.) after dosing. At the end of 3 days phenylacetylene was still being eliminated in the expired air. The slow rate of elimination of this hydrocarbon is shown in Fig. 1.

Isolation of metabolites from styrene

The urine of rabbits which had received styrene (0.5 g./kg.) orally gave a weak naphtharesorcinol reaction, but was non-reducing and gave no reaction for ketones. Paper chromatography of the urine showed the presence of large amounts of hippuric acid, but no significant amounts of phenaceturic acid. Mandelic acid was readily detected, together with an unidentified product which gave a pink colour with the sulphuric acid-formalin reagent (see Table 1).

Isolation of (+)-mandelic acid. The 24 hr. urine of eight rabbits which had received collectively 16 g. of styrene was acidified with 10N-HCl to a pH of about 2 and then continuously extracted (24 hr.) with CHCl_3 . The latter on evaporation left a dark-brown mixture of tar and crystals which was stirred with cold water (20 ml.) and filtered. After being kept overnight the crystals were collected and identified as hippuric acid (m.p. and mixed m.p. 189°). The filtrate from the crystals was neutralized and treated with benzylthiuronium chloride (see Wild, 1947) when a precipitate (0.55 g.) of *S*-benzylthiuronium (+)-mandelate separated. On recrystallization from water, this salt formed colourless plates, m.p. 176° and $[\alpha]_D^{25} + 57^\circ$ in 20% (v/v) aqueous ethanol (c, 1) (Found: C, 60.1; H, 5.6; N, 8.6. $\text{C}_{16}\text{H}_{18}\text{O}_3\text{N}_2\text{S}$ requires C, 60.4; H, 5.7; N, 8.8%). This salt was similar to the corresponding salt of (-)-mandelic acid already prepared (see above) but had the opposite sign of rotation.

Isolation of a glucuronide. The urine from twelve rabbits which had been fed with a total of 22 g. of styrene was fractionated with lead acetate (Kamil, Smith & Williams, 1951). The aqueous glucuronide fraction was separated from the basic lead acetate precipitate and concentrated *in vacuo* to small bulk. The concentrated solution which had a positive rotation was extracted with CHCl_3 (3×500 ml.) to remove mandelic acid, which was eventually isolated as *S*-benzylthiuronium (+)-mandelate (1.5 g.), m.p. 176° and $[\alpha]_D^{25} + 56^\circ$ (in 20%, v/v, aqueous ethanol). The aqueous residue, which had now a small negative rotation, was evaporated *in vacuo* to a gum. The latter was dissolved in a

little methanol and methylated with diazomethane, and the product was acetylated with acetic anhydride and pyridine to form the acetylated methyl ester of the glucuronide present. This was obtained as a partly crystalline solid on pouring the acetylation mixture into an excess of water. This solid was purified by solution in CHCl_3 , drying over anhydrous CaCl_2 , filtration and evaporation of the solvent to leave again a partly crystalline solid. It was now heated to 160° at 0.5 mm. pressure to distil off a few drops of an unidentified oil (possibly methyl mandelate). The residue now readily crystallized (0.1 g.) on rubbing with ethanol. Repeated recrystallization with ethanol yielded colourless needles (55 mg.) of methyl (phenylglycol monoacetate tri-*O*-acetyl- β -D-glucosid)uronate, which melted at 140° then solidified and melted again at 155°, and showed $[\alpha]_D^{25} + 1 \pm 2^\circ$ in CHCl_3 (c, 1) (Found: C, 55.9; H, 5.7; OMe, 6.1%. $\text{C}_{23}\text{H}_{28}\text{O}_{12}$ requires C, 55.7; H, 5.7; OMe, 6.3%). This ester gave an intense naphtharesorcinol reaction and was identical with the glucuronide prepared by feeding phenylglycol (see below). The presence of phenylglycol in the glucuronide was proved by hydrolysing the ester with N-NaOH at 60° for 0.5 min., then treating the solution with a preparation of snail-gastric-juice glucuronidase at pH 4.5 (Britton and Robinson buffer mixture; British Drug Houses Ltd.). The enzymic hydrolysate was extracted with ether and the presence of phenylglycol in the extract was shown by paper chromatography with the three solvent systems already described for the identification of this glycol (see Table 1).

Elimination of unchanged styrene. In two experiments carried out as with phenylacetylene, 2.1 and 3% of doses of 1.8 g. of styrene were recovered in the expired air.

Isolation of metabolites from phenylglycol

The metabolism of phenylglycol was investigated because this glycol was a metabolite of styrene. A qualitative examination of the urine of rabbits given orally 0.8 g./kg. of ($\pm$)-phenylglycol showed that its metabolites were hippuric acid, mandelic acid and a glucuronide, the first two being readily detected by paper chromatography.

The basic lead acetate fraction of the 24 hr. urine of ten rabbits dosed with a total of 20 g. of ($\pm$)-phenylglycol was prepared in the usual manner and freed from lead. The lead-free filtrate was concentrated to 20 ml. and the hippuric acid and mandelic acid removed with CHCl_3 (4×500 ml.) as described above. Evaporation of the chloroform extract and solution of the residue in water (10 ml.) yielded a small amount of hippuric acid (m.p. and mixed m.p. 189°) and treatment of the residue with benzylthiuronium chloride yielded 5.1 g. of *S*-benzylthiuronium mandelate, which appeared to be mainly the (+)-isomer ($[\alpha]_D^{25} + 36^\circ$, in 20% aqueous ethanol; c, 1). The aqueous solution after extraction with CHCl_3 was evaporated to dryness, and the residue methylated with diazomethane and acetylated with pyridine and acetic anhydride. The acetylated methyl ester of the glucuronide was purified exactly as described in the section on styrene. Methyl (phenylglycol monoacetate tri-*O*-acetyl glucosid)uronate (120 mg.) was obtained as long needles again showing two melting points, 140° and 155°, ($[\alpha]_D^{25} + 1^\circ$ in CHCl_3) (Found: $\text{CH}_3 \cdot \text{CO}$, 34.2. $\text{C}_{23}\text{H}_{28}\text{O}_{12}$ requires $\text{CH}_3 \cdot \text{CO}$, 34.7%), and it did not depress the melting points of the compound obtained from styrene. That it contained phenylglycol was

again proved by deacetylating and demethylating, hydrolysing the product with snail glucuronidase, and separating the phenylglycol by paper chromatography.

Styrene oxide. Smith *et al.* (1954*b*) had previously shown that this epoxide when fed to rabbits yielded conjugated glucuronic acid corresponding to about 15% of the dose (0.4 g./kg.), but they were unable to isolate and identify the glucuronide. We have now found that hippuric acid (9–10% of the dose, see Table 2) and mandelic acid are also metabolites of this compound.

A sample (0.05 ml.) of the urine of a rabbit which had received 1.6 g. of styrene epoxide was chromatographed in the four solvent systems *A*, *B*, *C* and *D* (see Table 1). Hippuric acid was readily detected in amounts of the order of 10% of the dose; mandelic acid was also found in amounts of the same order. However, neither phenylglycol nor its glucuronide could be detected.

Methylphenylcarbinol. The main metabolites of this compound are already known (Smith *et al.* 1954*b*). The urine of a rabbit which had received 2 g. of this compound orally was chromatographed as above, but neither phenylglycol nor its glucuronide was found, although small amounts of mandelic acid and very large amounts of a glucuronide (probably methylphenylcarbinol glucuronide) were detected.

Phenacyl alcohol ($C_6H_5 \cdot CO \cdot CH_2 \cdot OH$). Quantitative determinations showed that when phenacyl alcohol (0.8 g./kg.) was fed to rabbits the normal ethereal sulphate and hippuric acid outputs were unaltered, but the glucuronides rose slightly to not more than about 5% of the dose. The main object in feeding phenacyl alcohol was to find out whether it gave a glucuronide of (+)- or (-)-phenylglycol, but neither phenylglycol nor its metabolite, mandelic acid, was excreted. A glucuronide, however, was isolated. Two rabbits were each given 1.9 g. of phenacyl alcohol by stomach tube. The 24 hr. urine (100 ml.) gave a positive naphtharesorcinol reaction, a small precipitate with 2,4-dinitrophenylhydrazine in HCl, but a negative test for reducing sugars. This urine was fractionated by the lead acetate procedure. Neither the normal nor basic lead acetate fraction contained any mandelic acid. The basic lead acetate fraction, however, contained a small amount of

a glucuronide. The lead was removed from this fraction and the resulting aqueous solution was evaporated to dryness. The residue was dissolved in water (100 ml.), filtered and treated with an excess of Brady's reagent (2,4-dinitrophenylhydrazine in dilute HCl). A red microcrystalline precipitate (120 mg.) was formed which, on recrystallization from ethanol containing a trace of HCl, yielded fine red needles, m.p. 225° (decomp.) and $[\alpha]_D^{25} - 154^\circ$ in pyridine (*c*, 0.8). This substance analysed as the 2:4-dinitrophenylhydrazone of benzoylmethyl glucosiduronic acid (phenacyl alcohol glucuronide) (Found: C, 48.2; H, 4.4; N, 11.0. $C_{20}H_{20}O_{11}N_4$ requires C, 48.8; H, 4.1; N, 11.4%). Phenylglycol glucuronide was not found and the metabolism of phenacyl alcohol was not further investigated.

RESULTS AND DISCUSSION

A summary of the quantitative results obtained is given in Table 2.

Phenylacetylene. The metabolism of phenylacetylene in the rabbit appears to be a relatively simple process and the formation of its single metabolite can be explained by the simple addition of water to the triple bond. The compound, however, appears to be very slowly metabolized since unchanged phenylacetylene (see Fig. 1) and its urinary metabolite, phenaceturic acid, are eliminated for at least 4 days after dosing, and it appears that approximately half of the dose (0.4 g./kg.) is eliminated unchanged and the other half converted into phenylacetic acid. The addition of water to the triple bond appears to be asymmetric, since the formation of phenylacetic acid requires that a hydroxyl group be added to the β - and a hydrogen atom to the α -carbon atom, thus:

Table 2. *Quantitative aspects of the metabolism of phenylacetylene, styrene and phenylglycol in the rabbit*

The figures are the average for three animals, the ranges being given in parentheses.

	Dose (m-moles/kg.)	Phenylacetylene* 4	Styrene† 5	Styrene† epoxide 4	Phenylglycol‡ 3
Percentage of dose eliminated as	Unchanged in expired air	31 (25, 37) ^a	2 (2, 3) ^a	—	—
	Glucuronide	0 (0–1)	6 (4–7)	15†	34 (20–29)
	Ethereal sulphate	0 (0–0)	0 (0–0)	0†	0 (0–0)
	Hippuric acid	0§	40 (37–44)	9.5 (9–10)	7 (4–13)
	Phenaceturic acid	About 30§	0§	0§	0§
	Mandelic acid	Trace	+ + (2–3% by isolation)	About 10%	+ + + (11% by isolation)
	Mercapturic acid	0 (0–0)¶	5 (2–7)¶	—	—

* These figures are for 3 days after dosing, after which time the excretion of metabolites was still incomplete.

† Here excretion of metabolites was complete in 1–2 days.

‡ Values taken from Smith *et al.* (1954*b*).

§ As shown by paper chromatography.

|| Mandelic acid was a significant metabolite of these compounds, but we did not find a quantitative method which gave satisfactory results.

¶ As estimated by the iodometric method of Stekol (1936).

If the addition occurs in the reverse manner, then acetophenone and its metabolites—methylphenylcarbinol, hippuric acid and mandelic acid—would be expected to be formed (Smith *et al.* 1954*b*) and excreted thus:

Except for traces of mandelic acid, none of these metabolites was found and therefore it can be concluded that the hydration of the triple bond is asymmetrical. The view that the triple bond can be hydrated in biological systems is supported by the observation of Eimhjellen (1956) that cell-free extracts of a strain of bacteria from soil and sewage (Enterobacteriaceae) can convert acetylenedicarboxylic acid by direct hydration into oxaloacetic acid:

Styrene. The metabolism of styrene is more complicated than that of phenylacetylene. The metabolites which we have to account for are hippuric acid, phenylglycol and mandelic acid. The following routes of metabolism might be expected:

Routes (a) and (b) can be eliminated because it is known that phenylethanol (a) and ethylbenzene (b) give rise to phenaceturic acid (Smith *et al.* 1954*b*; El Masri *et al.* 1956) which is not a metabolite of styrene. Routes (c) and (e) are possible since both methylphenylcarbinol (c) and styrene epoxide (e) yield hippuric acid and mandelic acid in the urine, but neither of them gives rise to phenylglycol, although this compound could be an intermediate in their metabolism without appearing in the urine. Route (d) is a 'perhydroxylation', a reaction which has been postulated for the formation of dihydrodiols during the metabolism of aromatic hydrocarbons. If this process is postulated in the metabolism of styrene it would account for the formation of all three of its metabolites.

Two further comments appear to be necessary: the first concerns the monoglucuronide of phenylglycol, of which four isomers are possible. This glucuronide could be a 1- or 2-glucuronide of the (+)- or (-)-glycol [(+)- or (-)-Ph·CH(OG)·CH₂OH or (+)- or (-)-Ph·CH(OH)·CH₂·OG, where G is -C₆H₄O₆]. At present we have no data to decide which isomer we isolated. The second concerns the optical nature of the mandelic acid isolated as metabolite of styrene. The isomer isolated was the (+)-form and this suggests that the double bond of styrene is saturated in such a way as to produce an asymmetric α-carbon atom in the metabolites.

SUMMARY

1. The metabolic fate of phenylethylene (styrene) and phenylacetylene in the rabbit has been investigated.

2. Phenylacetylene is slowly metabolized to phenaceturic acid, which is excreted in the urine for several days after a single oral dose (0.4 g./kg.). Roughly 30–40% of the dose is eliminated in the unchanged state in the expired air over 3 days, and about the same amount is excreted in the urine as phenaceturic acid. It is suggested that phenylacetylene is asymmetrically hydrated *in vivo* to the enol form of phenacetaldehyde.

3. Styrene does not yield phenaceturic acid *in vivo*. Its main metabolite is hippuric acid (30–40% of the dose). It also forms mandelic acid and phenylglycol, the latter being excreted as a monoglucuronide. Only about 2% of the styrene administered is eliminated unchanged in the expired air.

4. (±)-Phenylglycol when fed to rabbits also yields hippuric acid, mandelic acid and phenylglycol glucuronide, and it appears to be an intermediate in the metabolism of styrene. It is suggested that styrene may undergo 'perhydroxylation' *in vivo* to phenylglycol.

5. Styrene epoxide and methylphenylcarbinol, which are possible intermediates in styrene metabolism, do not lead to the excretion of phenylglycol

when administered to rabbits, although they yield hippuric and mandelic acids.

6. Phenacyl alcohol is not reduced to phenylglycol in the rabbit. A small proportion (< 5 %) of it appears to be excreted as phenacyl alcohol glucuronide, which was isolated.

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The Preparation and Properties of Ergothioneine Disulphide

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Ergothioneine occurs both intracellularly in blood (Hunter & Eagles, 1927) and extracellularly in seminal plasma (Mann & Leone, 1953), but it has never been shown to occur in either a combined form or in a higher state of oxidation.

Although ergothioneine is usually written in the thiol form (I) it is not a normal thiol compound as it does not give a nitroprusside reaction; neither, however, does it give the blue colour with the Grote reagent (Grote, 1931) typical of thiourea and substituted thioureas (Kjaer & Rubinstein, 1953) (II).

The chemical reactions of the sulphur in the mercaptoglyoxalines are different from those of

normal thiols, in that the sulphur is completely unaffected by boiling 50 % aqueous potassium hydroxide (Barger & Ewins, 1911), but is rapidly cleaved from the glyoxaline ring in the form of sulphate by even weak oxidants such as ferric sulphate (Ashley & Harington, 1930), to yield the corresponding glyoxaline. As a generalization it can be stated that the majority of the 2-mercaptoglyoxalines do not form stable disulphides, sulphenic or sulphinic acids, but this depends on the substituents in the 4 and 5 positions of the glyoxaline ring and there are a few exceptions to this rule. For a complete review of the chemistry of these compounds see Heath (1951). There is only one report of a higher oxidation product of ergothioneine (Barger & Ewins, 1911). By the action of iodine on ergothioneine they obtained black, steel-grey or blue, mixed crystals of a periodide which they stated was that of ergothioneine disulphide. No evidence or analysis in support of this statement was given; it is probable that the product, besides being a periodide, is also iodinated in the 4(5) position, since this halogen substitution readily occurs as was shown in the synthesis of 4-bromo-5-methyl-2-glyoxalyl disulphide (Heath, Lawson & Rimington, 1951).

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