The Stability of the Disulphides of Thiamine Analogues.

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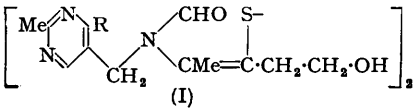
Disulphides have been prepared from a number of thiamine analogues, including *N*-methylthiamine (Nesbitt and Sykes, *J.*, 1954, 3057), and their stability in hot *iso*butanol has been investigated. Confirmation has thereby been obtained of the mechanism previously suggested (Sykes and Todd, *J.*, 1951, 534) for the disproportionation of thiamine disulphide.

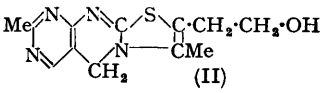
IN a previous paper (Sykes and Todd, *J.*, 1951, 534), a mechanism was suggested for the disproportionation of thiamine disulphide (I; R = NH_2) in hot, high-boiling hydroxylic solvents, thiochrome (II) and thiamine thiazol-2-one (III; R = NH_2) (Sykes, *J.*, 1951, 2507) being produced. It was then suggested that the initiating step of the reaction was the dehydrative cyclisation of the pyrimidine 4-amino-group in (I; R = NH_2) with the nearby *N*-formyl group which is liberated when the thiazole nucleus of the thiamine chloride hydrochloride is opened by alkali and prevented from reclosing by oxidation of the sodium salt of the thiol (VI; R = NH_2, R′ = Na), so formed, to the disulphide. To test this hypothesis, disulphides were required which resembled that from thiamine as closely as possible, but in which this particular dehydrative ring-closure could not take place. 4-Hydroxythiamine chloride hydrochloride was thought to be a suitable parent thiazolium compound and was readily available (Rydon, *Biochem. J.*, 1951, **48**, 383). *N*-Methylthiamine chloride hydrochloride was an apparently even more satisfactory model and this was synthesised for the purpose (Nesbitt and Sykes, *J.*, 1954, 3057).

4-Hydroxythiamine chloride hydrochloride was converted into the disulphide (I;

R = OH) by oxidation in alkaline solution with hydrogen peroxide, and *N*-methylthiamine chloride hydrochloride was oxidised to (I; R = NHMe) with iodine. Both compounds, like thiamine disulphide itself, become solvated with almost any solvent from which they have been recrystallised and retain the solvent of crystallisation most tenaciously.



(I)



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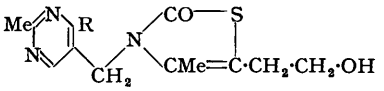


(III)

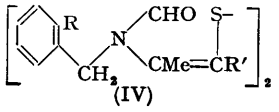
Although neither of these disulphides could, on heating, yield thiochrome or a similar compound (*N*-methylthiamine disulphide could theoretically yield a dihydrothiochrome derivative), there would be no formal structural difficulty in their being converted into the thiazol-2-ones (III; R = NHMe and OH) (Sykes and Todd, *loc. cit.*), provided the disulphide linkage undergoes fission. In fact, however, this does not occur; refluxing of either disulphide in *iso*butanol leads only to recovery of the starting material, and paper chromatography of the *iso*butanol solution failed to reveal any other pyrimidine component. It might still be possible that both these disulphides dissociate into free thiol radicals on heating but that these are only able to reform the disulphides, no alternative reaction being open to them. Refluxing a 1 : 1 mixture of the two disulphides in *iso*butanol, however, failed to yield any trace of a mixed disulphide, even on paper chromatography. The evidence thus seems overwhelming that both disulphides remain undissociated in hot *iso*butanol, in contrast to thiamine disulphide itself, which disproportionates very rapidly, and also that the occurrence of the dehydrative ring closure, mentioned above, is an essential preliminary to the dissociation of disulphides of this type.

Further evidence that the ease of fission of the S–S linkage in thiamine disulphide is exceptional is provided by a study of the stability of the benzyl analogues (IV; R = R′ = H; R = NO_2, R′ = H; and R = NO_2, R′ = CH_2·CH_2·OH). Refluxing each of these in *iso*butanol or xylene does not lead to breakdown, as indicated by recovery of the starting material and paper chromatography of the solutions. Refluxing the compounds in xylene in the presence of diphenyldiazomethane did not give a mixed product derived from a free thiol radical, the diazo-compound being converted into diphenylketazine (V). Similar results were obtained with diphenyldiazomethane and thiamine disulphide but here any free thiol radicals formed have available to them alternative intramolecular reactions (formation of thiochrome or thiamine thiazol-2-one), which would be expected to the exclusion of intermolecular reaction with diphenyldiazomethane. Refluxing of mixtures of pairs of the benzyl disulphide models, or all three together, again failed to reveal any component other than the starting material on paper chromatography.

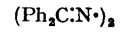
Further evidence was obtained from a mixed disulphide of a thiamine and a simple alkyl or aryl residue. Reaction of the sodium salt (VI; R = NH_2, R′ = Na) of the " open-chain " form of thiamine with phenylsulphenyl chloride, however, yielded only the symmetrical products, thiamine disulphide (I; R = NH_2) and diphenyl disulphide. We have observed this type of reaction previously, when reaction of phenylsulphenyl chloride with a thiol yielded the expected product, while reaction with the ion RS^- yielded only the two symmetrical disulphides. It seems that in the latter case the mixed disulphide RS·SPh is the initial product but undergoes a rapid exchange with RS^- to yield the symmetrical disulphides. Reaction of phenylsulphenyl chloride with a suspension of the covalent silver derivative (VI; R = NH_2, R′ = Ag) in dioxan, however, yielded phenyl thiamine disulphide (VI; R = NH_2, R′ = PhS). Reaction of the sodium salt (VI; R = NH_2, R′ = Na) with sodium toluene-ω-thiolsulphonate (cf. Matsukawa *et al.*, *J. Pharm. Soc. Japan*, 1953, **73**, 497) yielded benzyl thiamine disulphide (VI; R = NH_2, R′ = Ph·CH_2·S). This ionic reaction also yields only symmetrical products in some cases; thus the reaction of the sodium salt (VI; R = OH, R′ = Na) of the " open-chain " form of 4-hydroxy-

thiamine with sodium toluene-ω-thiolsulphonate yields only dibenzyl disulphide and 4-hydroxythiamine disulphide.

Refluxing of the mixed disulphides (VI; R = NH_2, R′ = PhS and $Ph{\cdot}CH_2{\cdot}S$) in *iso*-butanol resulted in almost quantitative conversion into thiochrome (II) and diphenyl and



(IV)



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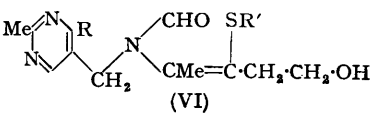
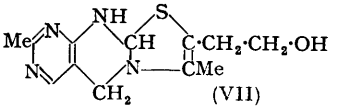


(VI)



(VII)

dibenzyl disulphide, respectively; no other product could be detected by paper chromatography in either case. Repetition of each experiment in an atmosphere of nitrogen yielded a non-fluorescent solution, which developed a brilliant thiochrome fluorescence immediately air was admitted, when either hot or cold. It is thus clear that the decomposition of these mixed disulphides proceeds through an autoxidisable intermediate (dihydrothiochrome; VII), as does thiamine disulphide (Sykes and Todd, *loc. cit.*; Nesbitt and Sykes, following paper). The effect of the presence of a 4-amino-group in the pyrimidine nucleus on the stability of thiamine disulphide and similar compounds is thus clear, and there can now be no doubt that the initiating step in the ready disproportionation of thiamine disulphide is the cyclisation detailed above.

Experimental

Disulphide (I; R = OH) *from* 4-*Hydroxythiamine*.—4-Hydroxythiamine chloride hydrochloride (Rydon, *Biochem. J.*, 1951, **48**, 383) (2 g.) was dissolved in water (20 ml.) and treated with 9·2N-sodium hydroxide (1·93 ml., 3 equiv.). 6·45N-Hydrogen peroxide (0·91 ml., 1 mol.) was added dropwise and the pH of the solution then brought to 7 by means of N-hydrochloric acid. The solution was freeze-dried and the residue extracted with propanol; evaporation under reduced pressure and recrystallisation of the residue first from aqueous methanol–acetone and then from methanol yielded the *di*-[2-N-(4-*hydroxy*-2-*methyl*-5-*pyrimidinyl*)*methylformamido*-1-(2-*hydroxyethyl*)*propenyl*] *disulphide* (0·42 g., 23%) as needles, m. p. 201—202° (Found: C, 50·7; H, 6·3; N, 14·3. $C_{24}H_{32}O_6N_6S_2,CH_3{\cdot}OH$ requires C, 50·4; H, 6·1; N, 14·1%).

Disulphide (I; R = NHMe) *from* N-*Methylthiamine*.—An aqueous solution of *N*-methylthiamine chloride hydrochloride (Nesbitt and Sykes, *J.*, 1954, 3057) (1·032 g., 1 equiv. in 10 ml.) was treated with 1·065N-sodium hydroxide (8·3 ml., 3 equiv.), and 1·15N-iodine in potassium iodide solution was added dropwise until no longer decolorised (2·4 ml., 94%). The solution was freeze-dried and the residue extracted with butanol; evaporation under reduced pressure and recrystallisation of the residue from aqueous acetone yielded the *bis*-5-*methylamino*-analogue (0·33 g., 35%) of the previous product as fine needles, m. p. 155—157° [Found: C, 51·4; H, 6·6; N, 17·8. $C_{26}H_{38}O_4N_8S_2,H_2O,\frac{1}{2}(CH_3)_2CO$ requires C, 51·7; H, 6·7; N, 17·6%. Loss on drying at 120°/10^{-4} mm. (some decomp.), 7·3. Required: 7·4%].

Heating of the Foregoing Disulphides in iso*Butanol*.—A solution of "*N*-methylthiamine disulphide" (I; R = NHMe) (0·265 g.) in dry *iso*butanol (15 ml.) was refluxed under nitrogen for 2 hr.; slight darkening took place. The solvent was removed at room temperature at 10^{-4} mm. and the residue crystallised from methanol–acetone–ether and then from acetone, yielding unchanged disulphide, m. p. and mixed m. p. 155—157° (60%). Paper chromatography of the *iso*butanol solution with saturated aqueous butanol as solvent failed to reveal any compound other than the disulphide (ultra-violet light).

4-Hydroxythiamine disulphide (I; R = OH) gave similar results.

4-*Methyl*-3-2′-*nitrobenzylthiazolium Bromide*.—A solution of 2-nitrobenzyl bromide (10·8 g.) and 4-methylthiazole (5 g.) in dry benzene (20 ml.) was refluxed for 6 hr. The crystals which separated were filtered off and washed with ether. Recrystallisation from ethanol (charcoal) yielded the *thiazolium* compound (13·5 g., 88%) as colourless plates, m. p. 204° (decomp.) (Found: C, 42·1; H, 3·6; N, 9·2. $C_{11}H_{11}O_2N_2BrS$ requires C, 41·9; H, 3·5; N, 8·9%).

Refluxing an ethanolic solution of the thiazolium bromide with an excess (5 equiv.) of

freshly precipitated silver chloride yielded the more stable thiazolium chloride. Recrystallised from methanol–ether (yield, quantitative), this had m. p. 198° (decomp.) (Found: C, 48·5; H, 3·8; N, 10·4. Calc. for $C_{11}H_{11}O_2N_2SCl$: C, 48·8; H, 4·1; N, 10·4%).

Di-[2-(N-2′-nitrobenzylformamido)propenyl] Disulphide (IV; R = NO_2, R′ = H).—4-Methyl-3-2′-nitrobenzylthiazolium bromide (1·5 g.) in water (20 ml.) was treated with 10% aqueous sodium hydroxide (4 ml., 2 equiv.), and hydrogen peroxide (20-vol.; 1·5 ml., 0·5 equiv.) was then added dropwise. The sticky solid that separated was dissolved in benzene, and the solution dried (Na_2SO_4), concentrated and treated with light petroleum (b. p. 60—80°). Colourless plates of the *disulphide* (0·61 g., 43%), m. p. 53°, separated after several days (Found: C, 57·9; H, 4·5; N, 10·0. $C_{22}H_{22}O_6N_4S_2,C_6H_6$ requires C, 57·9; H, 4·8; N, 9·7%). Storage for several days at 10^{-4} mm., followed by recrystallisation from aqueous acetone, yields the unsolvated *disulphide*, m. p. 115° (Found: C, 52·4; H, 4·4; N, 11·0. $C_{22}H_{22}O_6N_4S_2$ requires C, 52·5; H, 4·4; N, 11·2%).

Similar oxidation of 5-2′-hydroxyethyl-4-methyl-3-2′-nitrobenzylthiazolium chloride (Livermore and Sealock, *J. Biol. Chem.*, 1947, **167**, 699) yielded the corresponding analogous *1-2′-hydroxyethylpropenyl disulphide*, m. p. 110° (decomp.) (Found: C, 52·6; H, 5·3; N, 9·3. $C_{26}H_{30}O_8N_4S_2$ requires C, 52·9; H, 5·1; N, 9·5%).

Heating of the Disulphide (IV; R = NO_2, R′ = H) *in Xylene with Diphenyldiazomethane.*—A solution of the disulphide (1·04 g.) and diphenyldiazomethane (1·4 g.) in dry xylene (50 ml.) was refluxed for 4 hr. Removal of the solvent and fractional crystallisation of the residue from aqueous acetone led to almost quantitative recovery of the disulphide and to diphenylketazine, m. p. and mixed m. p. 163° (Found: N, 7·4. Calc. for $C_{26}H_{20}N_2$: N, 7·7%). A similar result was obtained with di-(2-*N*-benzylformamidopropenyl) disulphide (IV; R = R′ = H) (Sykes and Todd, *J.*, 1951, 534) and with the disulphide (IV; R = NO_2, R′ = $CH_2{\cdot}CH_2{\cdot}OH$) derived from 5-2′-hydroxyethyl-4-methyl-3-2′-nitrobenzylthiazolium chloride.

Refluxing a 1 : 1 mixture of any two of the above disulphides in *iso*butanol failed to yield a mixed disulphide: on paper chromatography, only the two initial compounds were detected (disulphides being detected by use of sodium cyanide–nitroprusside).

" Thiamine Phenyl Disulphide " (VI; R = NH_2, R′ = PhS).—Thiamine chloride hydrochloride (3·37 g.) in water (15 ml.) was treated with N-sodium hydroxide (30 ml., 3 equiv.), and the solution immediately freeze-dried. The residue was extracted with boiling dry ethanol, and the ethanolic solution then concentrated under reduced pressure (N_2 leak). The residue was dissolved in the minimum volume of boiling, dry ethanol. A small quantity of insoluble material was filtered off (1·13 g.; 2 equiv. of NaCl = 1·17 g.). The ethanolic solution was evaporated to dryness (N_2 leak) and the residual solid dissolved in water (150 ml.). Aqueous silver nitrate (1·7 g. in 70 ml.) was then added dropwise to the stirred solution. The pale yellow flocculent precipitate was filtered off, washed with water, and dried (KOH) for several days *in vacuo*. (The silver salt is stable in light, unless it contains silver chloride, whereupon it decomposes rapidly.) The dry silver salt (finely powdered) was suspended in stirred, dry dioxan (200 ml.), and phenylsulphenyl chloride (1·5 g.) in dioxan (50 ml.) was added dropwise. After 3 hr. the solution was filtered and evaporated, the residue dissolved in water, and the solution brought to pH 4 with N-hydrochloric acid. The solution was freeze-dried and the residue recrystallised from ethanol–ether, yielding *2-N-(4-amino-2-methyl-5-pyrimidinyl)methylformamido-1-(2-hydroxyethyl)propenyl phenyl disulphide hydrochloride* (1·44 g., 34%) as colourless prisms, m. p. 183° (Found: C, 50·5; H, 5·5; N, 12·9. $C_{18}H_{23}O_2N_4S_2Cl$ requires C, 50·6; H, 5·4; N, 13·1%).

Treatment of an aqueous solution of the hydrochloride with the calculated volume of N-sodium hydroxide, followed by freeze-drying, extraction of the residue with ethanol, and dilution with ether yielded the free *disulphide* as colourless needles, m. p. 141° (Found: C, 55·5; H, 5·5; N, 14·0. $C_{18}H_{22}O_2N_4S_2$ requires C, 55·4; H, 5·6; N, 14·3%).

Heating of " Thiamine Phenyl Disulphide " in iso*Butanol.*—A solution of the disulphide (1 g.) in *iso*butanol (150 ml.) was refluxed for 2 hr.; a pale blue fluorescence slowly developed. The solution was evaporated, adjusted to pH 4 with N-hydrochloric acid, and subjected to continuous ether-extraction. Evaporation of the dried (Na_2SO_4) ethereal layer yielded diphenyl disulphide (0·27 g., 96%), m. p. and mixed m. p. 61°. The aqueous residue was brought to pH 8 with sodium hydrogen carbonate and continuously extracted with chloroform. Concentration of the dried (Na_2SO_4) extract yielded thiochrome (II) (0·44 g., 65%), m. p. and mixed m. p. 228° (decomp.). Dilution of the original *iso*butanol solution, followed by fluorimetric assay, indicated thiochrome in almost quantitative yield. Paper chromatography of the original *iso*butanol solution (saturated aqueous butanol as solvent) yielded only the blue

fluorescent spot of thiochrome, no other ultra-violet-absorbing component (*e.g.*, pyrimidine) being detected. Repetition of the experiment under nitrogen (in solvent boiled out under nitrogen) yielded a non-fluorescent solution that became brilliantly fluorescent as soon as air was admitted to the apparatus (cf. Sykes and Todd, *loc. cit.*).

Heating of "thiamine benzyl disulphide" (VI; R = NH_2, R′ = Ph·CH_2·S), m. p. 156° (decomp.) [prepared by treating the sodium salt of thiamine (VII; R = NH_2) with sodium toluene-ω-thiolsulphonate (cf. Matsukawa *et al.*, *J. Pharm. Soc. Japan*, **1953**, **73**, 497)] in *iso*-butanol led to dibenzyl disulphide (97%) and thiochrome (70%; almost quantitative yield by fluorimetric estimation). Paper chromatography (saturated aqueous butanol as solvent) again failed to reveal any pyrimidine component other than thiochrome.

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