Tetrahedron Letters No.44, pp. 3289-3293, 1964. Pergamon Press Ltd.
Printed in Great Britain.

THE S-MONOXIDES OF CYSTINE, CYSTAMINE AND HOMOCYSTINE

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(Received 11 August 1964; in revised form 15 September 1964)

The S,S-dioxides (thiolsulphonates) of the biologically important disulphides cystine (I) and cystamine, though wrongly assigned the S,S'-dioxide ("disulphoxide") structure by early workers (1, 2, 3, 4, 5), are now well-authenticated compounds (6, 7, 8). However, the existence of the corresponding S-monoxides (thiolsulphinates), while sometimes assumed (9, 10, 11, 12), has not been unequivocally established (cf. 13, 14, 15). We have now synthesized these monoxides and also the monoxide and dioxide of the related disulphide homocystine.

L-Cystine (±)-S-monoxide (II, Cy.SO.S.Cy, Cy = $NH_2CH(COOH)CH_2$-) is obtained as a diastereoisomeric mixture (the sulphoxide group represents a third asymmetric centre) in 80% yield on oxidation of cystine in 2N sulphuric or perchloric acid with 1 to 2 molar proportions of peracetic acid (16 hr) or performic acid (2 hr) at 0°, followed by addition of ethanolic pyridine to precipitate the product at pH 4. It gives a double spot (an unusual pink ninhydrin reaction) on paper electrophoresis (1 N acetic acid, pH 2.3, toluene coolant, 50 V/cm), intermediate in mobility between I and cystine S,S-dioxide (III, $Cy.SO_2.S.Cy$).

Complete separation (as shown by electrophoresis) into "α" (IIa) and "β" (IIb) isomers is effected by repeated fractional precipitation of II from aqueous solution (solubility 1-2% at 20°, pH 4.5) with ethanol. IIa, the slightly preponderant isomer, has a higher solubility and a lower electrophoretic mobility than IIb. Whereas both I and III are laevorotatory, both IIa (α_D^{20} + 62°, c 2.5) and IIb (α_D^{20} + 14°) are dextrarotatory (cf. 9) in 1 N

sulphuric acid. A strong absorption maximum characteristic of the SO stretching vibration (16) occurs in the infra-red spectra (KBr disc) of both IIa (1060 cm^{-1}, 9.43μ) and IIb (1078 cm^{-1}, 9.28μ) (17); this is distinct from the symmetric SO_2 stretching vibration at 1120 cm^{-1} (8.93μ) given by III (17, cf. 6).

Cystamine (±)-S-oxide is obtained as the di-p-toluenesulphonate (needles, m.p. 160° (dec.) from aqueous ethanol) in 55% yield on oxidation of cystamine at pH 0-5 in the absence of halide, either rapidly (1 hr. 0°) with aqueous peracetic acid, or slowly (5 days, 25°) with aqueous hydrogen peroxide. Cystamine monoxide and dioxide are distinguishable by paper chromatography in isobutyric acid/2-methoxyethanol/water (8:3:3).

DL-Homocystine (±)-S-oxide (ν_{SO} 1070 cm^{-1}) and S,S-dioxide (ν_{SO_2} sym. 1120 cm^{-1}) are obtained by peracetic acid oxidation of homocystine in the respective absence or presence of hydrochloric acid.

When previously described preparations of supposed monoxide of cystine (9, 10, cf. 13, 15), cystamine (10, 12) or homocystine (10) via peracid oxidation are followed, the product is found to consist mainly of the monoxide and to contain variable proportions of the disulphide and the disulphide dioxide. Our results confirm an early claim by Toennies (9) that impure II is obtained as the (dextrarotatory) sulphate on oxidation of I with a molar proportion of permonosulphuric acid. We reaffirm (13) that II is not obtained on reduction of III with hydriodic acid (cf. 11). We can find no evidence in support of the claim by Utzinger (14) that the S,S'-dioxide, isomeric with III, is formed in oxidations of I with peracids.

Chloride, bromide and iodide can all promote rapid disproportionation of the above disulphide monoxides into the disulphide and the disulphide dioxide. When several previously described syntheses of the dioxide of cystine (5, 18) or cystamine (3, 4, 7) are followed, yields are dependent more on the effectiveness of the halide present in bringing about disproportionation of the initially formed monoxide than on the oxidant:disulphide ratio.

Disproportionation of II in the presence of hydrochloric acid is preceded by racemization at sulphur (cf. 19). A much slower disproportionation, unaccompanied by racemization, also occurs at pH 0-1 in the absence of halide.

Aqueous solutions of II, like III (2), are generally unstable, and each gives I and alaninesulphinic acid ($Cy.SO_2H$) as eventual hydrolysis products at 20°; complete hydrolysis can occur during paper electrophoresis at pH 2-3 in the absence of adequate external cooling. Maximum stability occurs for III at pH 0-1 (2) and for II at pH 3-4. Although III is more stable than II at pH 0-4, the reverse is true at pH 5-7, where II is found to be an intermediate in the hydrolysis of III:

$$2\ Cy.SO_2.S.Cy + H_2O \longrightarrow Cy.SO.S.Cy + 2\ Cy.SO_2H$$

$$3\ Cy.SO.S.Cy + H_2O \longrightarrow 2\ Cy.S.S.Cy + 2\ Cy.SO_2H$$

The monoxides and dioxides of cystamine and homocystine are generally more stable than the cystine analogues in aqueous solution.

As hydrolysis of both II and III is especially rapid above pH 7, their chemical properties were investigated mainly at pH 4-7, where they (especially II) readily react at 20° with particular thiol-blocking reagents to give the cysteine derivative and alaninesulphinic acid. Thus formaldehyde gives L-thiazolidine-4-carboxylic acid; N-ethylmaleimide gives the cysteine-adduct together with the corresponding sulphoxide and sulphone. The reactions of II with mercurials and with sulphite are implicated in thiol estimations — particularly for proteins which have undergone mild oxidation (20).

As reported for the unpurified compound (10, 15), II reacts rapidly with 2 molar proportions of certain thiols, giving almost exclusively the unsymmetrical disulphide. With hydrogen sulphide, however, II gives dialanyl trisulphide in high yield:

$$Cy.SO.S.Cy + H_2S \longrightarrow Cy.S.S.S.Cy + H_2O.$$

Some of the chemical properties of II and III suggest that, in aqueous solution under near-neutral conditions, initial fission occurs thus:

$$Cy.SO.S.Cy + OH^- \longrightarrow Cy.SO_2H + Cy.S^-$$
$$\rightarrow [Cy.\bar{S}O] + [Cy.SOH]$$

$$Cy.SO_2.S.Cy + OH^- \longrightarrow Cy.SO_2^- + [Cy.SOH]$$

Some fragments could then undergo secondary reactions, e.g.

$$Cy.S^- + [Cy.SOH] \longrightarrow Cy.S.S.Cy + OH^-$$

or combine with an added reagent.

Further details of this work will appear elsewhere. We wish to thank Dr. B. J. Sweetman for helpful suggestions.

All the new compounds reported gave satisfactory elemental analyses.

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