Alanine-3-sulphinic Acid in Partial Hydrolysates of Oxidized Proteins

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Oxidation of proteins can lead to substantial conversion of the disulphide groups of the cystyl residues into disulphide monoxide ($-SO-S-$) and other partial oxidation states intermediate between disulphide and sulphinic acid (Harris & Smith, 1937; Alexander, Fox & Hudson, 1951; Stein & Guarnaccio, 1959; Maclaren, Leach & Swan, 1960; Strasheim & Buijs, 1961; Maclaren, Savige & Sweetman, 1965; Sweetman, Eager, Maclaren & Savige, 1966; Savige & Maclaren, 1966).

The presence of these groups has been established by indirect methods only. None of the partial oxidation products of cystine has, until now, been isolated from oxidized proteins. This has been attributed to the instability of these amino acids under conditions commonly used for acid hydrolysis of proteins. Cystine *SS*-dioxide, and more particularly cystine ($\pm$)-*S*-monoxide, are unstable in aqueous HCl (Lavine, 1936; Savige, Eager, Maclaren & Roxburgh, 1964). Alanine-3-sulphinic acid, another oxidation product of cystine, was also reported to be unstable under these conditions (Maclaren *et al.* 1960), particularly when hydrolysed in the presence of wool (Schirlé & Meybeck, 1952). Nevertheless, Conden & Gordon (1950), using paper chromatography, detected a product in an hydrolysate of oxidized wool, which they believed to be either alanine-3-sulphinic acid or cystine dioxide.

It is now found that alanine-3-sulphinic acid is moderately stable to hydrolysis in 6*N*-HCl at 105° under N_2 or vacuum, and can readily be detected when some partially oxidized samples of wool, bovine serum albumin or insulin are hydrolysed for 1–20 hr. After this time the amount of alanine-3-sulphinic acid becomes small and it cannot be detected at all after 30 hr.

Substantial amounts of alanine-3-sulphinic acid were isolated from oxidized wool thus. Solvent-degreased Merino 64s top (10 g.) was oxidized in 98% (v/v) formic acid (300 ml.) at 0° for 1 hr. with performic acid [prepared by keeping a mixture of 30% (w/w) H_2O_2 (1.0 ml.) and 98% formic acid (9 ml.) at room temperature for 1 hr.]. It was then washed and dried at room temperature. The oxidized wool was then partially hydrolysed by refluxing in distilled 6*N*-HCl (500 ml.) under N_2 for 6 hr. After evaporation and freeze-drying, the

residue was dissolved in water (10 ml.) and applied to a column (40 cm. $\times$ 2 cm.) of Dowex 50 (X4, H^+ form). Elution with water gave first cysteic acid, closely followed by alanine-3-sulphinic acid. Fractions of eluate were examined by paper electrophoresis in *N*-acetic acid, with ninhydrin as location reagent. The fractions containing alanine-3-sulphinic acid were combined and concentrated. Addition of acetone gave alanine-3-sulphinic acid as the optically inactive monohydrate (95 mg.), which was recrystallized from aqueous acetone and dried *in vacuo* (20°, P_2O_5 , 2 hr.) [Found: C, 21.4; H, 5.3; N, 8.0; O, 46.3; S, 18.5. $C_3H_7NO_4S \cdot H_2O$ requires C, 21.1; H, 5.3; N, 8.2; O, 46.7; S, 18.7%; weight loss on drying *in vacuo* (80°, P_2O_5 , 4 hr.), 10.0%; theory for $1H_2O$, 10.1%]. Equivalent weight as determined by reduction with HI (Lavine, 1936) was 174 (theory 171). Identification of the product as DL-alanine-3-sulphinic acid was confirmed by paper chromatography with both butan-2-ol–2-methoxyethanol–formic acid–water (8:2:1:3, by vol.) and phenol–water (3:1, w/v) as solvents and by paper electrophoresis. L-Alanine-3-sulphinic acid ('L-cysteine sulfinic acid'; Calbiochem, Los Angeles, Calif., U.S.A.) was used as reference compound.

Alanine-3-sulphinic acid was also detected after hydrolysis of bovine serum albumin and insulin that had been oxidized under similar conditions.

Both cystine monoxide and cystine dioxide are known to decompose on acid hydrolysis, giving cystine and alanine-3-sulphinic acid (Savage *et al.* 1964), so that any monoxycystyl and dioxycystyl residues in oxidized proteins should also yield alanine-3-sulphinic acid on short-term hydrolysis in 6*N*-HCl. Thus alanine-3-sulphinic acid found in protein hydrolysates may not necessarily have been present in the protein as such.

Either of two factors could explain the failure of previous workers to detect alanine-3-sulphinic acid in hydrolysates of oxidized wool: (1) under some conditions of oxidation and with certain oxidants, cysteic acid is the only detectable product derived from cystine, no partial oxidation products being present (B. J. Sweetman, unpublished work); (2) when times of hydrolysis are prolonged, substantial destruction of alanine-3-sulphinic acid occurs so that detection becomes difficult.

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The Synthesis of Labelled 24-Methylenelanosterol and its Conversion into Ergosterol

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Continuing studies on the mechanism of biosynthesis in the steroid field (Wilton, Akhtar & Munday, 1966), we now report observations pertinent to the biosynthesis of ergosterol. Recent work from several Laboratories has shown (Alexander, Gold & Schwenk, 1957; Parks, 1958; Badar, Guglielmetti & Arigoni, 1964; Castle, Blondin & Nes, 1963) that biological systems elaborate the normal steroid side chain of the type (I) into branched-chain structures of the types (II) and (III) through the participation of *S*-adenosyl-methionine (see Scheme 1).

Lederer (1964) reported that ergosterol (IV) when biosynthesized in the presence of [*Me*-D₃]-methionine, incorporated only two of the three deuterium atoms. This observation has given rise to considerable speculation, and a number of compounds of the type shown in Scheme 2 have been postulated as precursors of ergosterol (Lederer, 1964). The intermediary role, however, of these compounds (a, b, c and d; Scheme 2) in ergosterol biosynthesis has hitherto not been subjected to experimental verification.

We now report the first synthesis of 24-methylene-

Scheme 1.

Scheme 2.