amino acid carbon atoms of the ergotamine side chain.

The development of a relatively high-yielding strain of *Claviceps purpurea* (Fr.) Tul. (Tonolo, 1966) producing ergotamine as a principal alkaloidal component, when grown in submerged culture on a defined medium, has provided a suitable system for study.

Radioactive precursors were added to these cultures at 10 days, provided that the minimal alkaloid content was 300–500 µg/ml. Ergotamine was isolated by preparative t.l.c. after a further 24 h or 48 h fermentation in the presence of L-[U-¹⁴C]-phenylalanine, L-[U-¹⁴C]-proline and L-[U-¹⁴C]-alanine. Further, short-time incubation studies of 6.5 h duration were carried out with L-[U-¹⁴C]-alanine and DL-[1-¹⁴C]alanine. After acid or mild alkaline hydrolysis of the alkaloid, the degradation products were radioassayed as phenylalanine, proline, lysergic acid and the *p*-bromophenylhydrazine derivative of pyruvate, derived under these hydrolytic conditions from α -hydroxy- α -alanine.

L-Phenylalanine and L-proline were efficiently incorporated into the crude peptide alkaloid fraction (2–5%). Phenylalanine was found to specifically label the phenylalanyl moiety (93–96%) and proline the prolyl moiety (73–76%) of the side chain of ergotamine. An examination of the mycelial amino acid pool of *Claviceps purpurea* at 10 days showed that the radioactivity from proline appeared more rapidly in the other amino acids, whereas radioactivity from phenylalanine mainly appeared in tyrosine and phenylalanine.

Both L-[U-¹⁴C]alanine and DL-[1-¹⁴C]alanine were incorporated into the peptide alkaloid fraction with relatively low efficiency (0.07–0.34%). Radioactivity from L-alanine was found to be distributed mainly in the α -hydroxy- α -alanyl (52–54%), prolyl (21–25%) and lysergyl (20–23%) moieties of ergotamine. In contradistinction, DL-[1-¹⁴C]alanine labelled the α -hydroxy- α -alanyl carbon atoms specifically (84–89%). These results show that all three carbon atoms of alanine are incorporated into α -hydroxy- α -alanine of the side chain, whereas C-2 and C-3 are incorporated into proline and lysergic acid, following conventional pathways.

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The Metabolism *in vitro* of Phenothiazine Drugs

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Beckett & Hewick (1967) found that *N*-oxidation was the major metabolic route for the biotransformation of [³⁵S]chlorpromazine *in vitro* by liver microsomal fraction from male rats. We have performed further studies on a series of medicinally used and experimental phenothiazine derivatives, to compare the results with chlorpromazine.

The compounds metabolized were: (1) 10-(2-dimethylaminoethyl)phenothiazine (fenethazine); (2) 10-(3-dimethylaminopropyl)phenothiazine (promazine); (3) 10-(4-dimethylaminobutyl)phenothiazine; (4) 2-chloro-10-(3-dimethylaminopropyl)phenothiazine (chlorpromazine); (5) 10-(3-dimethylaminopropyl)-2-trifluoromethylphenothiazine (trifluorpromazine); (6) 10-(2-dimethylaminopropyl)phenothiazine (promethazine); (7) 10-(2-dimethylamino-1-methylethyl)phenothiazine (isopromethazine); (8) 10-[3-(dimethylamino)-2-methylpropyl]phenothiazine (trimeprazine); (9) 10-(2-diethylaminoethyl)phenothiazine (diethazine); (10) 10-(2-diethylaminopropyl)phenothiazine (ethopropazine); (11) 2-chloro-10-(2-diethylaminopropyl)phenothiazine (chlorproethazine); (12) 10-[2-(1-pyrrolidinyl)ethyl]phenothiazine (pyrathiazine); (13) 10-[3-(1-pyrrolidinyl)propyl]phenothiazine; (14) 2-chloro-10-[3-(1-pyrrolidinyl)propyl]phenothiazine; (15) 10-[3-(1-piperidinyl)propyl]phenothiazine; (16) 2-chloro-10-[3-(4-methylpiperazin-1-yl)propyl]phenothiazine (prochlorperazine); (17) 10-[3-(4-methylpiperazin-1-yl)propyl]trifluoromethylphenothiazine (trifluoperazine).

The basic procedures for metabolizing each compound (0.8 mM) and identifying metabolites were as described by Beckett & Hewick (1967). Where possible *N*-demethylation was measured by the method of Cochin & Axelrod (1959).

All the tested compounds underwent sulfoxidation and aromatic hydroxylation. Compounds containing *N*-methyl or *N*-ethyl groups (namely 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 16 and 17) were *N*-dealkylated. The percentage *N*-demethylation undergone by chlorpromazine was 10%. On normalization, taking the amount of *N*-demethylation occurring with chlorpromazine as unity, the relative amounts of *N*-demethylation undergone by compounds (1), (2), (3), (4), (5), (6), (7), (8), (16) and (17) were 1.3, 1.5, 1.9, 1.0, 1.0, 1.4, 2.3, 2.6, 0.9 and 1.0 respectively.

Demethylation tended to increase with increase in length of side chain (compare compounds 1, 2 and

3) but decreased on introducing electronegative substituents into position 2 of the phenothiazine ring (compare compounds 4 and 5 with compound 2). *N*-Demethylation was almost doubled by the introduction of a branched-chain methyl group β to the side-chain nitrogen atom (compare compounds 1 and 7 and compounds 2 and 8).

Like chlorpromazine, all compounds with *NN*-dimethylaminoalkyl side chains except trimeprazine appeared to be extensively *N*-oxidized. Trimeprazine was negligibly *N*-oxidized. The *NN*-diethyl compounds (9), (10) and (11), unlike their respective methyl analogues compounds (1), (6) and (4), were not detectably *N*-oxidized. Incorporation of the side-chain nitrogen into a pyrrolidine or piperidine ring also virtually abolished *N*-oxidation. However, trifluoperazine and prochlorperazine appeared to be *N*-oxidized as extensively as chlorpromazine, the *N*-oxidation of the perazines probably occurring at the *N*-methyl nitrogen atom.

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The Extraction of a Protein Fraction Rich in Glycine, Serine, Tyrosine and Phenylalanine from Merino Wool

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The elaboration of procedures for the separation of low-sulphur and high-sulphur protein fractions from Merino wool revealed the presence of a third class of protein fraction. This fraction has an amino acid composition characterized by high contents of glycine, serine, tyrosine and phenylalanine, and has been described as the 'glycine-rich' or 'aromatic' protein (Corfield, 1962; Harrap & Gillespie, 1963; Moschetto & Biserte, 1963; O'Donnell & Thompson, 1964; DeDeurwaerder, Dobb & Sweetman, 1964; Zahn & Biela, 1968). Although it is a minor component, comprising between 1 and 3% by weight, its structure and morphological significance are clearly important for a complete understanding of the structure of wool. We report here two procedures for the isolation and purification of the proteins in this fraction.

Merino wool (40 g) was oxidized with peracetic acid by the procedure of Corfield, Robson & Skinner (1958) and shaken with 1 litre of 5M-urea, pH 2.7, for 24 h. A precipitate (1.5 g) separated during dialysis against 25% aqueous ethanol and was

extracted with 200 ml of 0.4M-sodium acetate buffer, pH 4.4, leaving an insoluble residue (0.8 g). The residue was separated into three fractions by chromatography on a 4 cm $\times$ 130 cm column of Sephadex G-100, with 0.2M- Na_2HPO_4 made 5M in urea as eluent. The first and second of these had amino acid compositions like those of α -keratose and γ -keratose respectively. The third fraction consisted of three overlapping bands all of which had amino acid compositions close to that of the protein fraction isolated by Zahn & Biela (1968).

A protein fraction of almost identical composition can be extracted preferentially from unmodified wool. Merino wool (10 g) was extracted with a mixture of 100 ml of formamide and 100 ml of 0.01M-sodium phosphate, pH 7.1, containing sodium dodecyl sulphate (1.0%) and 2-mercaptoethanol (1.0%). After dialysis for 16 h against 0.01M-sodium phosphate, pH 7.1, containing sodium dodecyl sulphate (0.1%) and 2-mercaptoethanol (0.1%) (buffer A) the extracted protein was precipitated by the addition of 9 vol. of acetone. A portion of the protein was chromatographed on a 1.5 cm $\times$ 80 cm column of Sephadex G-75, when more than 90% emerged in a single sharp peak. The protein in this peak moved as a single band during subsequent electrophoresis on polyacrylamide gel. Its estimated molecular weight of 12000 is in good agreement with the keratine fraction of similar amino acid composition isolated from reduced wool by Zahn & Biela (1968).

From electron-microscopic studies DeDeurwaerder *et al.* (1964) have suggested that this protein fraction is of intercellular origin. The ease and manner of its preferential extraction from the wool fibre support this view, but additional electron-microscopic examinations of extracted wool fibres are being undertaken to confirm this.

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