fusicoccin A. The structure of fusicoccin A was also established simultaneously by an independent X-ray structure determination on a heavy-atom derivative by Ballio *et al.* (1968). The basic ring system of the fusicoccins is that of the C_{25} sesterterpenes, the ophiobolins (see Tsuda *et al.* 1967, and references cited therein) (metabolites of *Ophiobolus* spp.) and the insect products the ceroplastols (Iitaka, Watanabe, Harrison & Harrison, 1968).

Preliminary biosynthetic studies have indicated a difference in the incorporation efficiency of [2-^{14}C]mevalonic acid into the C_5 unit on the sugar and the four isoprene units of the aglycone and therefore argue in favour of the fusicoccin aglycone being a diterpene and not a degraded sesterterpene.

Several new fusicoccins having less oxygen substituents on the aglycone have been isolated and are currently being tested for phytotoxicity.

Fusicoccins B, C and D and the aglycone are much less phytotoxic than fusicoccin A. The ophiobolins are also much less phytotoxic than fusicoccin A, and there may be distinct differences between both their effect and their mode of action. The phytotoxicity of fusicoccin A is non-specific, and though highly active when administered via the vascular system this substance is poorly absorbed by intact plants (E. B. Chain, P. G. Mantle & B. V. Milborrow, unpublished work).

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COMMUNICATIONS

Gut Flora and the Metabolism of Prontosils in the Rat

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The antibacterial activity *in vivo* of Prontosil [*p*-(2,4-diaminophenylazo)benzenesulphonamide] and Neoprontosil [sodium 2-(*p*-sulphamyl-phenylazo)-7-acetamido-1-hydroxynaphthalene-3,6-disulphonate] is due to the metabolite, sulphanilamide (Trefouel, Trefouel, Nitti & Bovet, 1935; Fuller, 1937). The site of reduction is believed to be the liver (Bernheim, 1941), but some food azo dyes are reduced in the gut (see Golberg, 1967). It is thus possible that Prontosil, a fat-soluble dye, and Neoprontosil, a water-soluble dye, are converted into an antibacterial agent by gut bacteria.

Prontosil (56mg./kg.) was given orally to rats, and 53% of the dose was excreted in the urine in 3 days as total sulphanilamide. Given intraperitoneally (i.p.) the sulphanilamide output was 32%, and given orally after suppression of gut bacteria with oral antibiotics it was 31%. The biliary excretion of Prontonsil (mol.wt. 291) was low, being 5% of dose in 24hr. after oral administration and 10% after i.p. injection. The biliary material was a coloured azo metabolite of Prontosil. Neoprontosil (100mg./kg.) given orally gave 43% of the dose as sulphanilamide in 2 days and 16% after antibiotics. When given i.p. the sulphanilamide output was 37% with 9% of the dose as unchanged Neoprontosil. The biliary excretion of Neoprontosil (mol.wt. 588) was 60% in 6hr. after i.p. injection and 1% in 24hr. after oral administration.

When Neoprontosil was given i.p. to rats with ligated bile ducts, 76% was excreted in the urine unchanged and 18% as total sulphanilamide, whilst after a sham operation for ligation the outputs were 0 and 38% in 2 days.

Preparations of rat caecal contents readily reduced anaerobically both dyes, but in preparations from rats pretreated with antibiotics the reduction was markedly inhibited. Sulphanilamide and Prontosil were readily taken up by everted gut sacs of rat, but Neoprontosil was poorly taken up. Pretreatment of the rats yielding the gut sacs with antibiotics did not affect the uptake.

These observations and other data suggest that the fat-soluble Prontosil is split to sulphanilamide both by the liver and gut flora, whereas the water-soluble Neoprontosil is split mainly by the gut flora in the rat.

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The work was supported by a grant from Roche Products Ltd.

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The Enzymic Deacetylation of *p*-Acetamidobenzoic Acid

By M. R. Franklin, J. W. Bridges* and R. T. Williams. (*Department of Biochemistry, St Mary's Hospital Medical School, London W.*2)

Earlier work (Bridges & Williams, 1963) suggested that the enzymic deacetylation of N^4-acetylsulphanilamide varied with tissue and species. The deacetylation of *p*-acetamidobenzoic acid (AABA) *in vitro* shows similar variations since it has been found in homogenates of rat, guinea-pig and mouse kidney and guinea-pig and mouse liver, but only slight activity was found in rat and rabbit liver and rabbit kidney. The activity when found was similar in both sexes and occurred in the cell-sap fraction (105000*g* supernatant).

The AABA deacetylase activity in the rat was found only in the kidney. The activity was considerably increased (150%) by Mn^{2+}, but not by other metal ions (Mg^{2+}, Ca^{2+}, Fe^{2+}, Na^+, K^+) or a wide range of common cofactors. The effect of Mn^{2+} on the mouse and guinea-pig kidney enzyme was less marked (50 and 30% respectively). The optimum Mn^{2+} concentration was 7·5mM for the rat kidney enzyme with an optimum pH range of 7·5–8·0. This enzyme preparation did not deacetylate N^4-acetylsulphanilamide, but acetanilide was deacetylated to some extent, although the main acetanilide-deacetylating activity was associated with the microsomes.

The rat kidney enzyme showed an Mn^{2+}-dependent and an Mn^{2+}-independent activity. Storage of the enzyme preparation (10000*g* supernatant) at 4° for 4–5 days (alone or in the presence of nicotinamide, glutathione or added Mn^{2+}) destroyed the Mn^{2+}-dependent activity, but the Mn^{2+}-independent activity remained constant. *p*-Chloromercuribenzoate also destroyed the Mn^{2+}-dependent activity, but did not affect the activity of the preparation that had been stored at 4° for 5 days. CoA, thioglycollate, Hg^{2+}, Cu^{2+} and Zn^{2+} inhibited both the Mn^{2+}-dependent and Mn^{2+}-independent activity.

These and other observations suggest that the rat kidney AABA deacetylase may possess either two active sites, one requiring Mn^{2+} for its activity, or a Mn^{2+}-binding site that causes allosteric activation of the active site. The Mn^{2+} binding or activated site is much more susceptible to inactivation than the original active site.

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This work was supported by a grant from the Ministry of Defence.

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Observations on the Metabolism of Allyl Compounds in the Rat

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1-Menaphthylmercapturic acid has been isolated from the urine of rats after the injection of various 1-menaphthyl compounds including 1-menaphthyl acetate and 1-menaphthyl benzoate (Hyde & Young, 1968), and these appear to be the first examples of mercapturic acid formation arising from the administration of carboxylic acid esters to animals. It is generally accepted that biosynthesis of mercapturic acids takes place by the mechanism proposed by Barnes, James & Wood (1959) and Bray, Franklin & James (1959*a*,*b*), whereby the administered compound or an active derivative reacts with glutathione to give an *S*-substituted glutathione, which is subsequently metabolized to the mercapturic acid. As it is known that the esters of allylic alcohols are able to transfer an allylic group to a strongly nucleophilic anion such as a thiol (Albert, 1965) it became of interest to determine whether an ester such as allyl acetate gives rise to a mercapturic acid *in vivo*.

The urine of rats that had been dosed with allyl acetate by subcutaneous injection was shown by paper chromatography and thin-layer chromatography to contain a hydroxypropylmercapturic acid. This compound was identified as 3-hydroxypropylmercapturic acid, i.e. *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine, by the gas–liquid-chromatographic examination of its methyl ester by the procedure of James, Waring & White (1966). There was no evidence for the presence of allylmercapturic acid, i.e. *N*-acetyl-*S*-allyl-L-cysteine, in the urine of the dosed animals. Similar results were obtained after the administration of allyl alcohol to rats.

Some allyl compounds were found to give rise to allylmercapturic acid in the organism, however, for this compound was isolated from the urine of rats that had been injected subcutaneously with allyl chloride, and by chromatographic examination was