inhibits the same step in the last-named two extracts, but is ineffective in *E. macrocarpa* endosperm. Tributyl-2,4-dichlorobenzylphosphonium chloride (Phosfon), on the other hand, was less selective in that it inhibited both steps of the cyclization of geranylgeranyl pyrophosphate to (-)-kaurene in the *G. fujikuroi* extracts. Phosfon was also shown to block the formation of other cyclic diterpene hydrocarbons from geranylgeranyl pyrophosphate in *R. communis* seedling extracts.

- Birch, A. J., Richards, R. W. & Smith, H. (1958). *Proc. chem. Soc.* p. 192.
 Cross, B. E., Galt, R. H. B. & Hanson, J. R. (1964). *J. chem. Soc.* p. 295.
 Cross, B. E., Norton, K. & Stewart, J. C. (1968). *J. chem. Soc. C*, p. 1054.
 Dennis, D. T., Upper, C. D. & West, C. A. (1965). *Plant Physiol.* **40**, 948.
 Dennis, D. T. & West, C. A. (1967). *J. biol. Chem.* **242**, 3293.
 Kende, H., Ninnemann, H. & Lang, A. (1963). *Naturwissenschaften*, **50**, 599.
 Ninnemann, H., Zeveaart, J. A. D., Kende, H. & Lang, A. (1964). *Planta*, **61**, 229.
 Oster, M. O. & West, C. A. (1968). *Arch. Biochem. Biophys.* **127**, 112.
 Upper, C. D. & West, C. A. (1967). *J. biol. Chem.* **242**, 3285.
 Verbisar, A. J., Cragg, G., Geissman, T. A. & Phinney B. O. (1967). *Phytochemistry*, **6**, 807.

The Wilting Toxins of *Fusicoccum amygdali* Del.

By K. D. BARROW and E. B. CHAIN. (*Department of Biochemistry, Imperial College of Science and Technology, London S.W.7*)

Cultures of *Fusicoccum amygdali* Del., a wilting pathogen of peach and almond in Southern Europe, have been shown to produce a number of phytotoxic metabolites termed the fusicoccins (Ballio *et al.* 1964; Barrow *et al.* 1968a). Strain selection and modifications of earlier media have raised the total fusicoccin yields to 250–350 µg./ml. in both shake flasks and stirred fermenters. The increased yield has led to a simplified isolation and separation procedure (G. T. Banks, E. B. Chain & R. Thomas, unpublished work).

Wilting is induced in plant tissues by the major metabolite fusicoccin A at concentrations as low as 0.1 µg./ml.

The structure of fusicoccin A is shown (I); it was determined by chemical degradation and extensive use of physical methods (Barrow *et al.* 1968a,b), and by an X-ray structure determination of a suitable heavy-atom derivative of the fusicoccin aglycone (Hough, Hursthouse, Neidle & Rogers, 1968).

(I) Me, methyl; Ac, acetyl

The empirical formula of fusicoccin A is $C_{36}H_{56}O_{12}$. It contains one methoxyl and two acetoxy groups, forms a dihydro derivative, $C_{36}H_{58}O_{12}$, on catalytic hydrogenation and a penta-acetate, $C_{42}H_{62}O_{15}$, on acetylation. Treatment of fusicoccin A with acid liberates (per mole) 1 mole of glucose and 1,1-dimethylallyl alcohol, which dehydrates to isoprene. At alkaline pH fusicoccin A is transformed into the metabolites fusicoccin B ($C_{36}H_{56}O_{12}$) and fusicoccin C ($C_{34}H_{54}O_{11}$) and finally into fusicoccin D (deacetyl fusicoccin, $C_{32}H_{52}O_{10}$). The distribution of acetoxy groups between the aglycone and sugar residues was readily deduced from mass-spectral measurements. Fusicoccin A and B have one acetate group on the sugar and one on the aglycone, fusicoccin C has one acetate group on the aglycone, and the penta-acetate has three acetate groups on the sugar and two on the aglycone. The glucose must therefore be disubstituted and the consumption of 2 moles of periodate by fusicoccin D establishes the 1,6-disubstitution. Two of the products from the reaction of fusicoccin A with acid, fusicoccin E ($C_{29}H_{46}O_{11}$) and fusicoccin F ($C_{27}H_{44}O_{10}$), are glucosides, and the group attached to position 6 of the glucose is therefore 1,1-dimethylallyl alcohol. The aglycone ($C_{21}H_{34}O_5$) can be prepared in low yield by acid hydrolysis, but more efficient methods are the Smith degradation (Abdel-Akher, Hamilton, Montgomery & Smith, 1952) (yield 65–75%) or reaction of the product from the periodate oxidation of fusicoccin D with strong base (Dugan & de Mayo, 1965) (yield 30–40%). Extensive n.m.r. and mass-spectral studies on the aglycone and its derivatives, including a number of selectively oxidized products, were used to elucidate the structure. The reaction of the aglycone with mercuric acetate followed by treatment with bromide ions gave a suitable derivative for the X-ray structure (Hough *et al.* 1968). A similar derivative was also prepared from

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 C₂₅ 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 [2-¹⁴C]mevalonic acid into the C₅ 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).

- Abdel-Akher, M., Hamilton, J. K., Montgomery, R. & Smith, F. (1952). *J. Amer. chem. Soc.* **74**, 4970.
 Ballio, A., Brufani, M., Casinovi, C. G., Cerrini, S., Fedeli, W., Pellicciari, R., Santurbano, B. & Vaciago, A. (1968). *Experientia*, **24**, 631.
 Ballio, A., Chain, E. B., De Leo, P., Erlanger, B. F., Mauri, M. & Tonolo, A. (1964). *Nature, Lond.*, **203**, 297.
 Barrow, K. D., Barton, D. H. R., Chain, E. B., Conlay, C., Smale, T. C., Thomas, R. & Waight, E. S. (1968a). *Chem. Commun.* p. 1195.
 Barrow, K. D., Barton, D. H. R., Chain, E. B., Ohnsorge, U. F. W. & Thomas, R. (1968b). *Chem. Commun.* p. 1198.
 Dugan, J. J. & de Mayo, P. (1965). *Canad. J. Chem.* **43**, 2033.
 Hough, E., Hursthouse, M. B., Neidle, S. & Rogers, D. (1968). *Chem. Commun.* p. 1197.
 Iitaka, Y., Watanabe, I., Harrison, I. T. & Harrison, S. (1968). *J. Amer. chem. Soc.*, **90**, 1092.
 Tsuda, K., Nozoe, S., Morisaki, M., Hirai, K., Itai, A., Okuda, S., Canonica, L., Fieocchi, A., Galli Kienle, M. & Scala, A. (1967). *Tetrahedron Lett.* p. 3369.

COMMUNICATIONS

Gut Flora and the Metabolism of Prontosils in the Rat

By R. GINGELL, J. W. BRIDGES* and R. T. WILLIAMS. (*Department of Biochemistry, St. Mary's Hospital Medical School, London W.2*)

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 (56 mg./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 Prontosil (mol.wt. 291) was

low, being 5% of dose in 24 hr. after oral administration and 10% after i.p. injection. The biliary material was a coloured azo metabolite of Prontosil. Neoprontosil (100 mg./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 6 hr. after i.p. injection and 1% in 24 hr. 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.

* Present address: Department of Biochemistry, University of Surrey, London S.W.11.