ated material (as the iodide) shows additional peaks at 1,780, 1,760 and 1,200 cm^{-1} characteristic of phenolic acetates. At least one hydroxyl group is still present in the acetylated derivative.

The molecular formula and spectral evidence suggested that this base might belong to the *bis*benzylisoquinoline group of alkaloids, but none of the degradations usually effective in this group has afforded interpretable results. Almost all chemical reactions give rise to viscous, highly coloured oils. Similar alkaloids have been found in *C. rhamnifolius*, *C. xanthochlorus*, Croiz and *C. speciosus*, M. Arg.

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BIOCHEMISTRY

Fusicoccin: a New Wilting Toxin produced by *Fusicoccum amygdali* Del.

Our attention was directed by Dr. A. Graniti, of the Istituto di Patologia Vegetale of the University of Bari, to the observations of Grosclaude[1] and Graniti[2] that culture filtrates of *Fusicoccum amygdali* Del., the causative agent of a disease of the almond tree (*Prunus amygdalus* St.) common in Italy, exhibited phytotoxic activity. This manifested itself in bringing about the wilting of leaves on small branches of almond trees, such as occurs under natural conditions in the infected plant.

The original strain of *F. amygdali*, given to us by Dr. Graniti, was grown in shake flasks and stirred fermenters. A modified Czapek–Dox medium, containing 3 per cent glucose in place of sucrose, was found suitable for producing the active principle. The fermentations were carried out at 24° C for 3–4 days, when the culture medium showed maximal activity, detectable in a test on young tomato plants after 30–100-fold dilution. The biological assay was run according to the semi-quantitative techniques developed by Gaümann[3] in the course of his detailed studies of wilting phenomena. We are obliged to Dr. Graniti for advice concerning this assay method.

The phytotoxic principle was exhaustively extracted with *n*-butyl acetate from filtered batches of 50–300 l. culture medium, and purified by the following steps: (1) evaporation to dryness; (2) residue taken up in $CHCl_3$ (insoluble fraction discarded); (3) residue from $CHCl_3$ extract taken up in methanol (insoluble fraction discarded); (4) residue from methanol extract washed with hexane to remove lipoid impurities, then taken up in ethyl ether (insoluble fraction discarded); (5) residue from ether extract (usually 20 mg/l. culture fluid) dissolved in about 10 times the volume of chloroform containing 3 per cent acetone, and passed through a 'Florisil' column (1 g for each 3 litres of culture medium) on which the active material was firmly adsorbed. The column was washed with about three column volumes of the same solvent. Fractional elution was effected with chloroform containing 6–10 per cent acetone. The fractionation was followed at first by biological assay and later by thin layer chromatography with chloroform/*iso*-propanol (92:8), using a sulphuric acid spray. During the work it became clear that the phytotoxic activity was due to several substances. The present investigation is concerned with the component present in the largest amount, which proved to be the least polar and migrated faster in the chromatographic test. It was termed fusicoccin *A*.

The fractions containing this active substance were combined and evaporated. The residue consisted of biologically active crystals embedded in an oil which exhibited negligible biological activity. It was filtered through a coarse sintered glass plate to remove the oil and the solid was crystallized from ethyl acetate.

Crystalline fusicoccin *A* showed phytotoxic activity in the tomato plant test in concentration of 0·1–0·2 μg/ml. It is a white solid, m.p. 150°–152° C, soluble in alcohols, ether, chloroform, benzene, insoluble in hexane; it crystallizes readily from esters and *cyclo*hexane. The solubility in water is very low, but the compound can be brought into aqueous solution by first dissolving in a small amount of alcohol.

Elementary analysis indicated the formula of fusicoccin *A* to be $C_{38}H_{58}O_{13}$ (found: C, 63·07, 63·16; H, 8·03, 8·01 per cent; $C_{38}H_{58}O_{13}$ required: C, 63·10, H, 8·03 per cent). It contained one methoxy and two acetoxy groups (direct analysis and nuclear magnetic resonance spectrum), and at least one double bond (one mole of hydrogen taken up on catalytic hydrogenation). It is optically active, $[\alpha]_D^{25}$ + 73 (c = 0·6, in chloroform). Molecular weight determination by X-rays (kindly carried out by Dr. M. Bonamico and Prof. A. Vaciago, C.N.R. Research Centre for Structural Chemistry, Rome) gave a value of 733·5 (± 2 per cent); isothermal distillation (kindly carried out by Dr. W. Simon of the Eidgenossische Technische Hochschule, Zürich) gave a value of 697·3 (± 4 per cent). The ultraviolet spectrum showed only strong end absorption. The infra-red spectrum showed the presence of hydroxy and ester (acetate) groups and of unsaturation, and the absence of aromatic structure. The mass spectrum (kindly carried out by Dr. J. S. Shannon, Sydney, Australia) showed a very complex pattern. A peak corresponding to the molecular ion was absent; the highest mass peak was at *m/e* 704, probably due to an ion formed through loss of water molecule from $C_{38}H_{58}O_{13}$ (mol. wt. 722). A similar behaviour was shown by dihydrofusicoccin *A* (prepared by catalytic hydrogenation of fusicoccin *A*, m.p. 151°–153° C), which exhibited the highest mass peak at *m/e* 706, the value expected for the ion derived by dehydration of a $C_{38}H_{60}O_{13}$ compound (mol. wt. 724). Dihydrofusicoccin *A* had about half the biological activity of fusicoccin *A*. On acid hydrolysis (in N sulphuric acid at 100° C) fusicoccin *A* liberated one mole of glucose (identified by paper chromatography and by reaction with glucose oxidase), demonstrating the glucoside nature of the substance. The structure of the aglycone is under investigation.

We have been informed by Dr. Graniti that crystalline fusicoccin *A* after injection into a wound made near the stem of a branch of an intact almond plant produced wilting of the leaves, as occurs after infection with the fungus under natural conditions.

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