tuberosum dürfte im Hinblick auf die Biogenese dieser Verbindungen von Interesse sein.

Summary. The 'sterol fraction' from leaves of the potato plant, *Solanum tuberosum*, was found to contain a minor constituent, the structure of which has been established as 4α-methyl-5α-stigmasta-7,24(28)-diene-3β-ol (I). This sterol is most probably identical with α_1-sitosterol and has the same constitution as citrostadienol, differing only in the configuration of the $\Delta^{24(28)}$-double bond.

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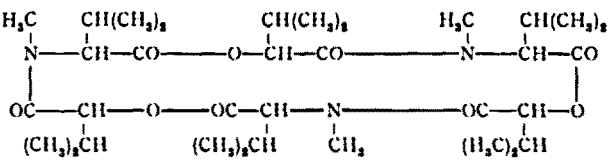
Synthesis of Enniatin B

In 1947 Plattner et al.[1] isolated from a number of fusarium strains the antibacterial metabolite Enniatin B for which they proposed the structure of a cyclic tetrapeptolide[2]. Recently Shemyakin et al.[3] were able to demonstrate by synthesis that Enniatin B did not possess this structure. In the meantime one of us (W. K.-Sch.) repeated the molecular weight determination for this natural product according to a novel method developed by Simon[4]. To our surprise we found a value of 655 (ethyl acetate) according to this thermo-electric method instead of 426 as calculated for a cyclotetrapeptolide. These measurements suggested the possibility that Enniatin B could be a cyclohexapeptolide (molecular weight = 639) rather than a cyclotetrapeptolide since all the degradation results presented by Plattner et al.[2] would still be in agreement with such a formula.

The cyclohexapeptolide structure has now been confirmed by the synthesis of an active substance with all the properties of naturally occurring Enniatin B.

The open chain hexapeptolide IX was built up through the steps outlined in the accompanying reaction scheme.

Our synthesis starts with N-methyl-*L*-valine recently prepared in our laboratory by a new procedure[5]. It was thereby possible to use benzyloxycarbonyl as the N-protecting group. This represents a considerable advance over the use of *p*-nitro-benzyloxycarbonyl-N-methyl-*L*-valine applied in the preparation of the cyclotetrapeptolide[3]. Tert. butyl-*D*-α-hydroxy-isovalerate was obtained via the corresponding O-acetyl derivative. The acid chloride coupling method, first applied in the peptolide series by Shemyakin[6] was used throughout. For the ester bonds benzene sulfochloride proved to be very suitable, whereas for the methylated peptide bonds PCl_5 was preferred. Deblocking of the protecting groups was carried out in the usual way.



X, Natural Enniatin B

Full details will be given in Helvetica Chimica Acta.

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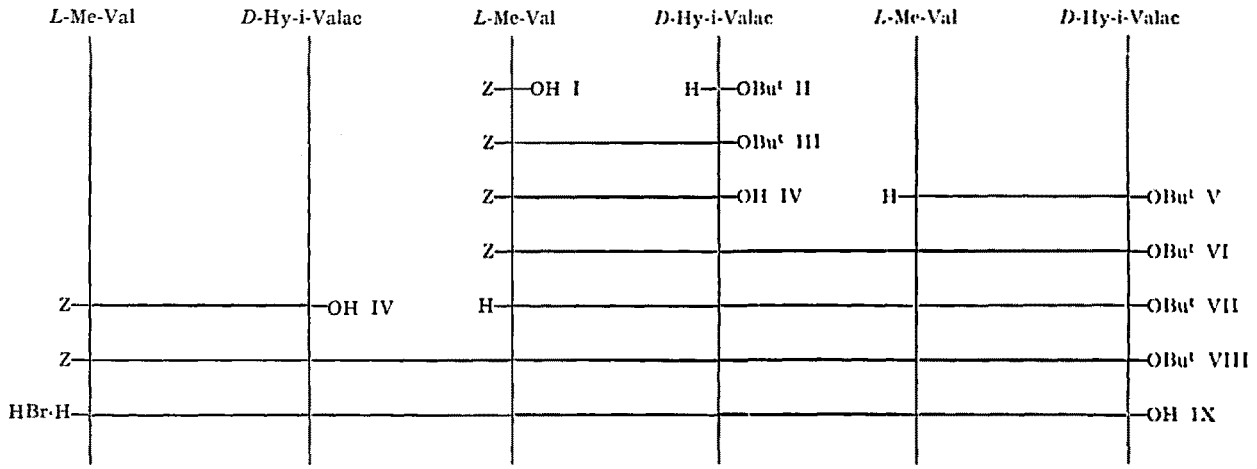

Reaction scheme

L-Me-Val = N-methyl-*L*-valine; *D*-Hy-i-Valac = α-hydroxy-*D*-iso-valeric acid; OBu^t = tert. butyl ester; Z = benzyloxycarbonyl.

The acid chloride of IX was cyclized in high dilution (benzene) in the presence of triethylamine.

X has been shown to be identical in melting point, IR-spectrum, optical rotation, chromatographic behaviour and microbiological activity[7] (Staph. aureus ATCC 6538-P, *in vitro*) with naturally occurring Enniatin B.

[7] We are very much indebted to Prof. B. FUST and Dr. ERIKA BÖHNI from our Microbiological Department for this bioassay.

Zusammenfassung. Die Struktur von Enniatin B, eines antimikrobiellen Wirkstoffes aus Fusarienstämmen (ETH, Zürich), wird durch Synthese als die eines Cyclohexapeptolids bewiesen (vgl. X).

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The Stability of Purified Preparations of Substance P

Crude preparations of the pharmacologically active polypeptide known as Substance P are reasonably stable in solution, but various workers have found that preparations containing 600 U/mg or more, lose activity when the solutions are diluted for testing (STERN[1]).

Experiments were undertaken to discover the cause of this instability and means of overcoming it. 1 mg of a highly purified preparation of Substance P was supplied by Dr. W. Haefely of Hoffmann-La Roche & Co. Ltd., Basle. Solutions of this preparation were compared with a standard preparation of Substance P (75 U/mg) by their action on isolated guinea-pig ileum. When the purified preparation was dissolved in Tyrodes solution and added to the bath in less than 1 min, the activity appeared to be about 800 U/mg and this fell to 350 U/mg 30 min later.

The presence of proteins in the solution had two effects on the responses to Substance P.

(1) The effect of the standard preparation was potentiated. In one experiment, for example, the presence of human plasma albumin (10^{-5} g/ml in the solution added in the bath) increased the potency of the standard 3.5 times. Beef γ-globulin (10^{-5} g/ml) or gelatin (5%) had a smaller effect (1.6 times). This effect is like that described by GRANT, HOOD, and RAMWELL[2] who found that certain proteins increased the action of acetylcholine on frog rectus abdominis. In order to avoid complications in later experiments, the standard was dissolved and diluted in the same solutions as the preparations compared with it.

(2) The stability of the purified preparation was increased. In one series of experiments 120 μg of the purified preparation was weighed out, dissolved in 1 ml of Tyrode solution containing beef γ-globulin (10^{-3} g/ml), and diluted 10 times for the test. The activity of the original solution was estimated as 4200 U/mg of the purified preparation, and it appeared to be stable for a week at 4°. On dilution with 10^{-3} globulin the activity fell 30–60% in 1 h. The loss was not abolished by using polythene vessels, and was increased when the glass vessel was treated with silicone. When the diluting fluid contained 5% glycerol no loss was detected in 1 h.

In three experiments in which 10^{-3} γ-globulin was used as the diluting fluid, the glass surface was increased by adding small glass particles (ballotini) and the loss was 84, 86 and 94% in less than 40 min. This increase in the rate of disappearance of Substance P suggested that it was being adsorbed on the surface of the glass, and it was found that over 50% of the activity could be recovered by washing the ballotini with $N/50$ HCl.

In another series of experiments 120 μg of the preparation was dissolved in 1 ml of 0.9% saline containing 5% gelatin. This solution was diluted with 5% gelatin 100 times for testing, and estimated to contain 6700 U/mg of the preparation. The activity fell to 5000 in 90 min and to 4700 in 24 h. These strong solutions of gelatin thus gave better protection than γ-globulin.

A similar phenomenon has been studied by HILL[3,4] who found that insulin was adsorbed on glass, and that 5% gelatin prevented this adsorption and could be used to elute insulin from glass. Others have made similar observations[5]. Presumably the gelatin competes with the active substances for receptors in the glass, though it is possible that the active substances form a stable but still active compound with gelatin. STOUFFER and LIPSCOMBE[6] have studied a similar phenomenon in experiments which showed that highly active A.C.T.H. preparations were adsorbed on glass and could be eluted with acid.

Other workers have also described the adsorption of small quantities of active substances on glass. For example, MARSHALL[7] found that organic bases were adsorbed on glass unless the glass was cleaned by soaking in concentrated nitric acid.

VEALL and VETTER[8] state that albumin labelled with radioiodine is lost on glass, but that this can be prevented if the total concentration of albumin is kept over 1%. HJORTH[9] found that pollen extracts lost their potency as antigens in glass vials, and that this loss was prevented by adding 0.01% of the nonionic detergent known as Tween 80; in this case the loss depended on the presence of air in the vial.

It is possible that the apparent increase of activity, when proteins were added to the standard preparation, as described above, was due to the fact that in the absence of added protein there was some loss on the walls of the bath in which the assay was made. However, the fact that the standard preparation seemed to be stable casts doubt on this explanation.

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