obtained by preparative paper chromatography on Whatman 3MM paper.

The selective acylation of the thymidine-5'-hydroxyl is also possible with organic acid chlorides provided these have bulky substituents. Thus *5'-trichloroacetyl thymidine'* was obtained as follows: A solution of thymidine (250 mg, 1.033 m*M*) in acetonitrile (40 ml) containing pyridine (0.2 ml) was cooled to 10° and stirred while a solution of trichloroacetyl chloride (188 mg, 1.03 m*M*) in acetonitrile (10 ml) was added over 45 min, stirring was continued for a further 2 h after which the solution was filtered from a small amount of thymidine, evaporated to dryness and taken up in methylene chloride (20 ml). On standing overnight, 40 mg of thymidine crystallized out. The solution after filtration of thymidine was evaporated to dryness and the residue chromatographed over silicic acid (20 g). 5'-Trichloroacetyl thymidine was eluated with $CHCl_3$-EtOH (9:1) and crystallized from chloroform (300 mg), m.p. 167–168°, λ_{max} 5.65 μ (CH_2Cl_2), 264.5 mμ (EtOH) (ε 12,220). Found: C 36.68, H 3.18, Cl 27.72. $C_{12}H_{13}O_6N_2Cl_3$ requires: C 37.16, H 3.50, Cl 27.48%.

In a similar manner, using the respective acid chlorides, *5'-D,L-α-chloro phenylacetyl thymidine* (m.p. 135-145°), *5'-bromoacetyl thymidine* (m.p. 159–160°), and *5'-chloroacetyl thymidine* (m.p. 160°) have been obtained[9].

Zusammenfassung. Ein einfacher Weg zur selektiven Esterbildung mit der 5'-Hydroxylgruppe von Thymidin wird beschrieben. Beispiele: Thymidin-5'-phosphat, Thymidin-5'-sulfat und Thymidin-5'-trichloracetat.

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[9] Gifts of chemicals obtained through the Wellcome Trust are gratefully acknowledged.

In vitro Production of Indole Acetic Acid by *Fusarium vasinfectum* Atk.

The importance of indole acetic acid (IAA) and other growth regulators in plant diseases has been recently reviewed[1,2]. The cotton wilt pathogen *Fusarium vasinfectum* Atk. has been reported to produce growth-promoting substances in various culture media[3–5]. The presence of indole compounds was not explored by HIRATA[3] or by KALYANASUNDARAM and LAKSHMINARAYANAN[4], while VENKATARAM[5] discounted the production of IAA in culture filtrates.

Erlenmeyer flasks of 250 ml capacity with 50 ml Czapek's medium as well as flasks with 50 ml Czapek's medium plus 0.1% DL-tryptophan were sterilized and inoculated with 8 mm culture discs of the actively growing fungus, grown on Czapek's agar medium and incubated at laboratory temperature (26–29°C) in the dark. After incubating for 6 and 9 weeks, the fungal mats were separated and the filtrates adjusted to pH 4.0, with 2*N* HCl. The filtrates were shaken with equal volumes of peroxide-free ether and extracted in a refrigerator at 4° ± 1°C for a period of 24 h with solvent changes at 8 and 16 h. At the end of 24 h, all the ether phases were pooled and evaporated to dryness under reduced pressure between 35° and 40°C. The residue was taken in a small volume of distilled methanol, spotted on Whatman No. 1 paper and developed ascendingly for 17 h in isopropanol water (80:20), isopropanol-ammonia-water (10:1:1) and in *n*-butanol-ammonia-water (10:1:1)[6]. The dried chromatograms thus developed were sprayed either with Salkowski or Ehrlich reagents[7]. The Rf values of the fungal IAA and of the known IAA samples were identical in all systems tested. There was no secretion of IAA in the extracts from the fungal cultures grown in Czapek's medium. The IAA-like spots were observed only with tryptophan cultures.

The IAA-like compound was further purified chromatographically, employing water as the solvent, and eluted in distilled methanol. The concentration of the compound was estimated by GORDON and PALEG's method[8], employing 4 ml Salper reagent (50 ml of 35% perchloric acid and 1 ml of 0.5*M* ferric chloride) and 2 ml of the eluate and determining the absorbancy at 535 mμ in a Hilgar Spectrophotometer, after allowing 1 h for colour stability. There were indications to show that the fungus synthesized 6.02 mg IAA during the 6th week of incubation, while the compound increased by 25% by the end of the 9th week.

The results show that *F. vasinfectum* is capable of synthesizing indole acetic acid from tryptophan and the concentration of the growth promoter increased with the age of the cultures. Further studies are required to investigate the IAA metabolism in *Fusarium* infected cotton plants[9].

Résumé. Fusarium vasinfectum Atk., le champignon qui flétrit le coton synthétise l'acide indol-3-acétique (IAA) de tryptophan et la concentration augmente avec l'âge de la culture.

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[1] L. SEQUIRA, Ann. Rev. Phytopath. *1*, 5 (1963).
[2] H. CRUEN, Ann. Rev. Pl. Phys. *10*, 405 (1959).
[3] S. HIRATA, Rept. of Faculty of Agri. Miyazaki Univ. *3*, 46 (1958).
[4] R. KALYANASUNDARAM and K. LAKSHMINARAYANAN, Nature *171*, 1120 (1953).
[5] C. S. VENKATARAM, Proc. Indian Acad. Sci. *49* (B), 167 (1959).
[6] JOYCE BENTLEY, Meth. Biochem. Anal. *9*, 75 (1962).
[7] C. DAWSON et al., *Data for Biochemical Research* (Oxford Univ. Press, 1959).
[8] S. A. GORDON and L. G. PALEG, Physiol. Plant *10*, 39 (1957).
[9] I thank Prof. G. RANGASWAMI for critical reading of the manuscript.