LETTERS TO THE EDITORS

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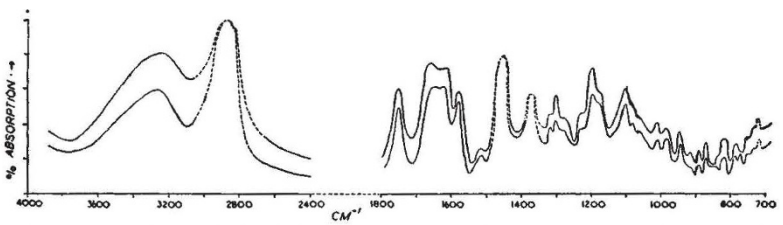
Actinomycin

THE name 'actinomycin' was given by Waksman and Woodruff[1] to a bright red crystalline antibiotic which they isolated from *Actinomyces antibioticus*. A preliminary examination of its chemical nature was made by Waksman and Tishler[2], who reported that it decomposed at 250°, had $[\alpha]_D^{25} = -320° \pm 5°$, and gave analytical values approximating to a formula $C_{41}H\ O_{11}N_8$; molecular weight determinations gave values varying from 768 to 1,000. Actinomycin appeared to contain a quinonoid system, since it underwent reversible reduction with hydrosulphite and it could be both acetylated and reductively acetylated. More recently, red antibiotic substances have been isolated from other Actinomycetes, and all have been considered to be identical with actinomycin[3]. It would also seem that the yellow xanthomycins[4] are related chemically to actinomycin.



Infra-red spectra of actinomycin (upper curve) and 'Antibiotic *X*.45' (lower curve). Samples in nujol suspension

We are at present investigating a bright red crystalline antibiotic isolated from an unidentified strain of Actinomycete by Lehr and Berger[5], and kindly supplied to us under the name 'Antibiotic *X*.45' through the courtesy of Dr. J. A. Aeschlimann of Hoffmann-La Roche, Inc., Nutley, New Jersey. This material is optically active ($[\alpha]_D^{25} = -332°$; *c*, 0·25 in ethanol) and decomposes at 252°. In ultra-violet absorption, toxicity and bacteriological spectrum, it is indistinguishable from actinomycin, and comparison of its infra-red absorption spectrum with that of a small sample of actinomycin generously provided by Dr. Waksman reveals only minor differences (we are indebted to Dr. G. B. B. M. Sutherland and Mr. T. S. Robinson for the determination of these spectra); the chemical properties of the two materials are indistinguishable. Our material, however, gives analytical values (C, 58·0, 57·8, 58·1; H, 6·5, 6·4, 6·4; N, 13·1, 12·9, 13·1 per cent) corresponding to an approximate formula $C_{41}H_{51}O_{12}N_8$ containing four active hydrogen atoms. Although the discrepancy between the analytical values is disturbing, the remarkable degree of coincidence in chemical and physical properties, and in particular the close similarity of the infra-red spectra, make it appear probable that actinomycin and 'Antibiotic *X*.45' are identical, though final confirmation of this point must await further work.

On mild acid hydrolysis a guanidino group is set free (positive Sakaguchi reaction) and a strong fatty acid odour becomes evident. On complete acid hydrolysis followed by paper chromatography, the presence is revealed in the hydrolysate of five substances which react with ninhydrin. Three of these, *A*, *B* and *C*, were quickly identified as the amino-acids threonine, proline and valine. The fourth substance, *D*, travelled somewhat faster than valine on paper strips developed with butanol–acetic acid or phenol; it did not appear as a coloured spot on such strips when ninhydrin development was carried out at 70° but came up strongly above 100°. Substance *D* was finally identified as N-methylvaline, an amino-acid which has also been found in *Fusarium* antibiotics[6]. The fifth substance, *E*, although probably an amino-acid, differs from all normally occurring members of this group and has not yet been identified. It has an R_f value in butanol–acetic acid closely similar to that of threonine, and in phenol to that of valine. On changing from an ammonia to an acid atmosphere in phenol chromatograms, substance *E* is held back slightly more than the other four amino-acids, but it does not appear to be a basic amino-acid and is not precipitated by phosphotungstic acid. Hydrolysis of Dr. Waksman's actinomycin under the same conditions yielded the same five ninhydrin-reacting substances.

Alkaline decomposition of the precipitate obtained by treating the crude acid hydrolysate of '*X*.45' with phosphotungstic acid yielded ammonia (from the guanido group) and suggested that a further base may be present. This is not identical with ethanolamine, which it resembles in smell.

Experiments with D-amino-acid oxidase result in complete removal of valine (we are indebted to Mr. K. Burton for assistance in the enzymatic experiments, and for providing the enzyme). The valine must therefore be D-valine, and the proline must be L-proline. Threonine is too slowly attacked to give significant results by this method, and experiments showed that the same applies to N-methylvaline. Preliminary experiments on partial hydrolysates have revealed the sequence threonyl–proline.

The four amino-acids already identified, assuming they are all present in peptide combination, account for $C_{20}H_{34}O_5N_4$. The association of a peptide with a quinone system is, so far as we are aware, unique among natural products. Further work on this material is continuing, and will be published in full elsewhere.

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Cambridge. July 30.

[1] *Proc. Soc. Exp. Biol. Med.*, **45**, 609 (1940); *J. Bact.*, **42**, 231 (1941).
[2] *J. Biol. Chem.*, **142**, 519 (1942).
[3] Welsch, M., *Bull. Soc. Chim. Biol.*, **28**, 557 (1946). Umezawa, H., *et al.*, *J. Penicillin (Japan)*, **1**, 129 (1947). Trussell, P. C., and Richardson, E. M., *Canad. J. Res.*, **26** C, 27 (1948). Kochalaty, W., *et al.*, *Arch. Biochem.*, **17**, 191 (1948).
[4] Thorne, C. B., and Peterson, W. H., *J. Biol. Chem.*, **176**, 413 (1948).
[5] Lehr, H., and Berger, J., *Arch. Biochem.* (in the press).
[6] Cook, A. H., Cox, S. F., and Farmer, T. H., *J. Chem. Soc.*, 1022 (1949). Plattner, Pl. A., and Nager, A., *Helv. Chim. Acta*, **31**, 665 (1948).

Inflammation of Explosive Vapours and the Influence of Inert Diluents

STUDIES of the initiation of explosion in liquids detonated by light impact[1,2,3] and experiments with greatly diluted vapours[4] led to an investigation of the inflammation of some alkyl nitrates in the gas phase and of the influence of different diluents. The