508. *Cordycepin, a Metabolic Product from Cultures of* Cordyceps militaris *(Linn.) Link. Part I. Isolation and Characterisation.*

By K. G. Cunningham, S. A. Hutchinson, William Manson, and F. S. Spring.

Cordyceps militaris (Linn.) Link has been promoted on various nitrogenous media, yielding solutions which inhibit the growth of many strains of *Bacillus subtilis*. From the culture solutions a crystalline metabolic product, cordycepin, $C_{10}H_{13}O_3N_5$, has been isolated; it appears to be responsible for the total antibacterial activity of the culture solutions.

Previous work on *Cordyceps militaris* (Linn.) Link has been confined to morphology, life history and relationships (Massee, *Ann. Bot.*, 1895, **9**, 1; Varitchak, *Compt. rend.*, 1927, **184**, 622; Petch, *Trans. Brit. Mycol. Soc.*, 1936, **20**, 216), and physiology (Atkinson, *Bot. Gaz.*, 1894, **19**, 129; Pettit, *Bull. Cornell Univ. Agric. Exp. Station*, 1895, **97**, 339). That the mould might produce an antibiotic was deduced from the observation that the residue of host tissue incorporated in the pseudosclerotum is resistant to decay.

A monoascospore culture from a sporophore collected at Tollymore Park, Co. Down, in the autumn of 1948, was promoted on a peptone (0·5%)–Lemco (0·3%)–glucose (1·0%)–agar (1·5%) medium. The cultures grew vigorously with the development of typical *Cladosporium* conidial stages (Petch, *loc. cit.*) after 5—7 days. The development of antibacterial activity in the liquid medium from a surface culture of the mould was measured by using *B. subtilis*, strain 6752 N.C.T.C., as test organism.

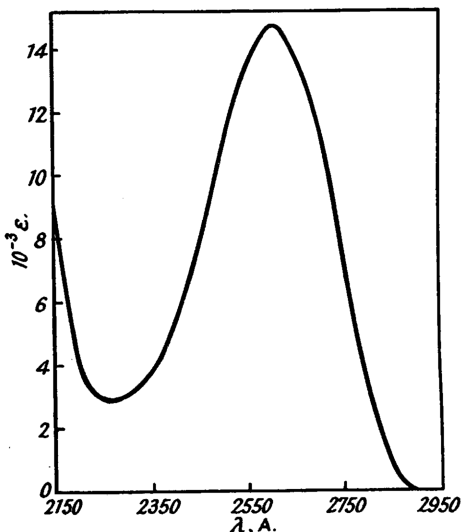
When a peptone (0·5%)–Lemco (0·3%)–glucose (1·0%) medium was used with and without a phosphate buffer, the mould grew vigorously with the production of maximum antibacterial activity after 20—24 days in each case. Similar results were obtained by using a peptone (0·5%)–glucose (1·0%) medium, with and without a phosphate buffer. On the other hand, growth and development of antibacterial activity were very poor when a peptone (0·5%)–Lemco (0·3%) medium was used with a phosphate buffer. Established mycelia maintain their capacity to produce antibacterial activity over a prolonged period on being repeatedly flooded with fresh medium.

The simplest medium which has been found satisfactory for the development of the mould and the production of antibacterial solutions is one containing glucose and a source of amino-acid. In the large-scale production of active culture solutions we employed a medium of the casein hydrolysate "Pronutrin" (0·5%) and glucose (1%). The medium was inoculated from a bulk suspension of conidia; submerged growth was observed on the second day, and was quickly followed by vigorous surface growth and the development of acidity. The medium inhibited the growth of the test strain of *B. subtilis* after 12 days; the first well-defined inhibition, however, was obtained after 20 days; harvesting was commenced on the 28th day. After treatment with charcoal the exhausted medium showed no inhibitory action towards the control strain of *B. subtilis* and was discarded. From the charcoal we isolated a crystalline compound, which we name cordycepin, in yields varying between 25 mg. and 100 mg. per litre of medium.

In aqueous solution, at a concentration between 10 μg. and 100 μg. per ml., cordycepin was found to inhibit the growth of *B. subtilis*, strain 6752 N.C.T.C., in Bouillon broth. Of 45

strains of *B. subtilis* tested, 43 were inhibited in Bouillon broth to varying degrees by cordycepin at a concentration of 1·0 mg. per ml. Cordycepin does not inhibit the development of *Staph. aureus* Oxf. H, *Sar. lutea*, *E. coli*, *B. welchii*, *B. proteus*, *Strep. hæmolyticus*, *S. flexneri*, *Strep. fæcalis*, and *Pasteurella septica*; it inhibits avian-tubercle bacillus at a concentration of 0·1 mg. per ml., Youman's medium being used at 37°, and with Dubos–Tween–albumin medium it inhibits a bovine-type tubercle bacillus at a dilution of 1 : 60,000. The toxicity of cordycepin is of a low order.

Cordycepin crystallises from ethanol and *n*-propanol as transparent needles, from *n*-butanol as lustrous needles or plates, and from water it separates as dull matted needles; in all cases it separates with solvent of crystallisation. A solution of cordycepin in water is nearly neutral (pH 7·1). Analyses of the metabolic product and of its picrate and picrolonate establish the molecular formula, $C_{10}H_{13}O_3N_5$, for cordycepin. A molecular-weight determination by Mrs. Dorothy Hodgkin and Dr. G. J. Pitt, using the *X*-ray crystallographic method, gave the value 247 ± 10, for cordycepin, which is in close agreement with that required for $C_{10}H_{13}O_3N_5$ (251). Cordycepin exhibits an ultra-violet absorption maximum at 2600 A. ($\varepsilon = 14,600$) in ethanol (see figure), and is optically active, $[\alpha]_D^{20}$ $-47°$ (in water). The molecule contains three active hydrogen atoms (Zerewitinoff), but contains neither a *C*-methyl group (Kuhn–Roth) nor a methoxyl group (Zeisel); a Herzig–Meyer estimation showed the absence of a *N*-methyl group.



Experimental.

Isolation of Cordycepin.—To a solution of glucose (440 g.) in water (44 l.) was added one of "Pronutrin" (220 g.) in warm water (500 ml.). This medium was dispensed in culture flasks (400 ml. per flask) and sterilised by being autoclaved at 15 lbs./sq. in. for 20 minutes. The cooled flasks were inoculated from a bulk aqueous suspension of conidia of *Cordyceps militaris* (Linn.) Link, which was prepared from a sub-culture of the mould on peptone (0·5%), glucose (1%), and agar (2%). After incubation at 24° for 28 days, the mixture was filtered, and the filtrate stirred with activated charcoal (500 g.) for 4 days After 24 hours. the supernatant liquor was removed by a siphon. The charcoal was filtered off under suction, air-dried at 24° for 7 days, and extracted (Soxhlet) with acetone for 7 days. The acetone liquor (2 l.) was evaporated, and the residual brown gum washed with cold acetone (20 ml.) and dissolved in hot *n*-butanol (50 ml.). On cooling, the filtered solution deposited a crop of needles (1·1 g.), m. p. 210—220°. Recrystallisation from ethanol gave *cordycepin* as needles, m. p. 225—226°. For analysis a specimen was dried for 6 hours over phosphoric oxide at 78°/0·1 mm. (Found : C, 47·9, 47·8; H, 5·2, 5·2; N, 27·6, 28·3. $C_{10}H_{13}O_3N_5$ requires C, 47·8; H, 5·2; N, 27·9%). Cordycepin crystallises from *n*-propanol as needles, m. p. 225—226°, containing solvent of crystallisation which is difficult to remove. For analysis a specimen was dried for 8 hours over phosphoric oxide at 100°/0·5 mm. (Found : C, 48·1, 48·2; H, 4·9, 5·4%). From water it separates as *hydrated* needles, which were dried for 2 hours at 78°/0·5 mm. (Found : C, 45·3; H, 5·9. $C_{10}H_{13}O_3N_5,H_2O$ requires C, 44·6; H, 5·6%).

Cordycepin Picrate.—To a warm solution of cordycepin (15 mg.) in water (1·0 ml.) was added an excess of saturated aqueous picric acid. The yellow flocculent precipitate was crystallised from water giving *cordycepin picrate* (12 mg.) as needles, m. p. 195° (decomp.) (Found : C, 39·7; H, 3·2; N, 23·4. $C_{10}H_{13}O_3N_5,C_6H_3O_7N_3$ requires C, 40·0; H, 3·4; N, 23·3%).

Cordycepin Picrolonate.—Excess of a saturated aqueous-ethanolic solution of picrolonic acid was added to a warm solution of cordycepin (15 mg.) in water (1·0 ml.). The precipitate of yellow prismatic needles which separated on cooling was collected (10 mg.) and recrystallised from water, yielding *cordycepin picrolonate* as needles, m. p. 240° (decomp.) (Found : C, 46·7; H, 4·2; N, 24·2. $C_{10}H_{13}O_3N_5,C_{10}H_8O_5N_4$ requires C, 46·6; H, 4·1; N, 24·5%).

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The Royal Technical College, Glasgow.
The University of Glasgow.

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