509. *Cordycepin, A Metabolic Product from Cultures of* Cordyceps militaris *(Linn.) Link. Part II.* The Structure of Cordycepin.*

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Acid hydrolysis of cordycepin yields adenine and cordycepose. Cordycepose (VI) is shown to be a 3-deoxypentose with a branched carbon chain, and cordycepin is shown to be 9-cordyceposidoadenine (IX).

Part I * described the isolation of a crystalline metabolic product, cordycepin, from culture fluids of *Cordyceps militaris* (Linn.) Link. Analyses of cordycepin and its derivatives indicated a molecular formula $C_{10}H_{13}O_3N_5$ for the former, and preliminary examination revealed that the molecule contains three active hydrogen atoms but no methoxyl, *C*-methyl or *N*-methyl groupings.

The first indication of the nature of cordycepin was obtained from a consideration of its ultra-violet absorption spectrum which exhibits a well-defined maximum at 2600 A. ($\varepsilon \simeq 15{,}000$) in neutral, acid, or alkaline solution. This absorption spectrum is very similar to those of adenine, 9-methyladenine, and adenosine (9-D-ribofuranosidoadenine) (Gulland and Holiday, *J.*, 1936, 768; Table I) but substantially different from that of 7-methyladenine.

Table I.

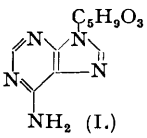
	λ, A.		$\varepsilon_{max.}$	
	N/20-NaOH.	N/20-HCl.	N/20-NaOH.	N/20-HCl.
Cordycepin	2600	2600	14,600	14,400
Adenosine [1]	2600	2600	14,300	14,200
Adenine [1, 2]	2580	2600	13,600	13,200
9-Methyladenine [1]	2600	2600	14,700	14,200
7-Methyladenine [1]	2690	2690	11,400	14,600

[1] Gulland and Holiday (*loc. cit.*). [2] Cf. Loofbourow and Stimson, *J.*, 1940, 844.

This relationship has been confirmed by degradation experiments. Cordycepin dissolves readily in warm, dilute mineral acids to give a solution from which adenine picrate separates on the addition of aqueous picric acid. Furthermore adenine hydrochloride separates rapidly from solutions of cordycepin in concentrated hydrochloric acid, and was identified by analysis and by direct comparison with an authentic specimen in respect of melting point and ultra-violet (Fig. 1) and infra-red absorption spectra (Fig. 2).

Solutions of acid-hydrolysed cordycepin react slowly with 2 : 4-dinitrophenylhydrazine in hydrochloric acid to give a dinitrophenylosazone, $C_{17}H_{16}O_{10}N_8$, which gives the blue-violet coloration with sodium hydroxide in aqueous ethanol characteristic of nitrophenylosazones. By using similar conditions a *p*-nitrophenylosazone, $C_{17}H_{18}O_6N_6$, was obtained which gives a bright blue coloration with the alkali reagent.

Deamination of cordycepin with nitrous acid followed by acid hydrolysis gives hypoxanthine. The isolation of adenine and the nitrophenylosazones shows that cordycepin is an adenine-glycoside, and the deamination of cordycepin to a product from which hypoxanthine was obtained by acid hydrolysis limits the structure of the metabolic product to either a 9- or a 7-glycosidoadenine. The ultra-violet absorption characteristics of cordycepin show that the former is the more likely.



(I.)

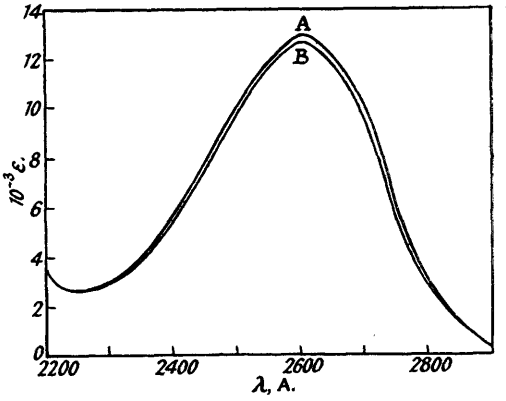
The isolation of a 2 : 4-dinitrophenylosazone, $C_{17}H_{16}O_{10}N_8$, and a *p*-nitrophenylosazone, $C_{17}H_{18}O_6N_6$, from acid-hydrolysed cordycepin corresponds to the formation of a deoxypentose, $C_5H_{10}O_4$. Thus cordycepin is a 9-deoxypentosidoadenine (I). The formation of the osazones, moreover, shows that cordycepin is not a 2-deoxypentoside. Cordycepin is not oxidised by periodate, the conditions described by Lythgoe and Todd (*J.*, 1944, 592) being used; under the same conditions oxidation of adenosine was complete in 4 hours. It follows that cordycepin is a 3-deoxypentoside.

Hydrolysis of cordycepin in dilute hydrochloric acid, followed by the quantitative removal of adenine by means of a cation-exchange resin and then of hydrochloric acid by means of silver carbonate, gave a solution from which an analytically pure sugar, $C_5H_{10}O_4$, has been

* Part I, preceding paper.

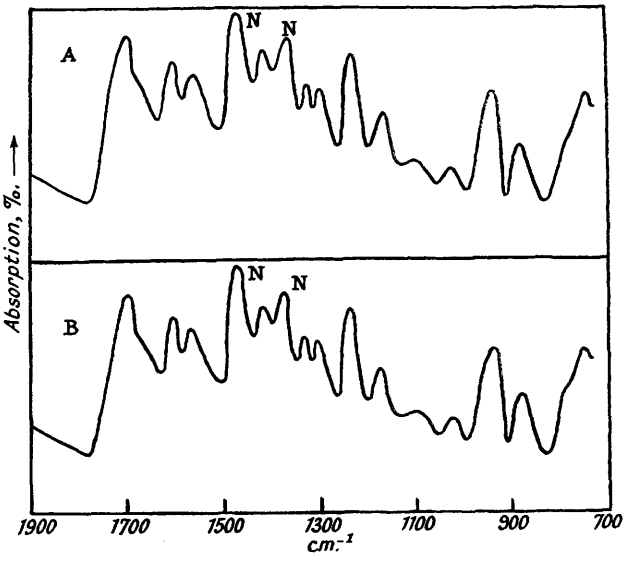
isolated as a pale straw-coloured syrup. The sugar readily reduces Fehling's solution and is oxidised by bromine water to yield a lactone, $C_5H_8O_4$. The latter reacts with phenylhydrazine to yield a crystalline phenylhydrazide, $C_{11}H_{16}O_4N_2$, m. p. 151°, $[\alpha]_D$ +26°. The sugar $C_5H_{10}O_4$ is therefore a 3-deoxyaldopentose.

FIG. 1.



A, *Adenine hydrochloride in ethanol.*
B, *Adenine hydrochloride from cordycepin in ethanol.*

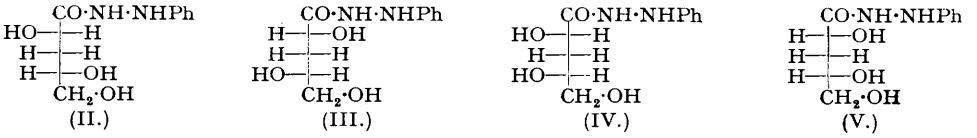
FIG. 2.



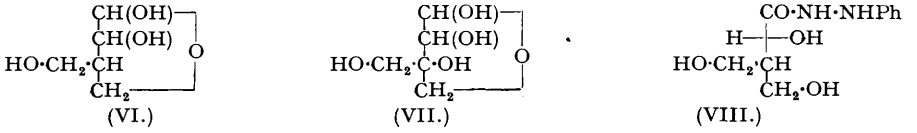
A, *Adenine hydrochloride.* } *in Nujol suspension.*
B, *Adenine hydrochloride from cordycepin* }
N, *Nujol absorption bands.*

Of the 3-deoxyaldopentoses, 3-deoxy-L-xylose (3-deoxy-L-ribose) has been prepared by Mukherjee and Todd (*J.*, 1947, 969), and 3-deoxy-D-xylose (3-deoxy-D-ribose) by Kent, Stacey, and Wiggins (*J.*, 1949, 1232). The four phenylhydrazides [(II)—(V)] of the corresponding αγδ-trihydroxyvaleric acids have been described by Nef (*Annalen*, 1910, **376**, 1). These comprise two pairs of enantiomorphs, the D- and the L-*threo*-phenylhydrazide {(II) and (III), m. p. 110°, $[\alpha]_D$ ±26°} and the D- and the L-*erythro*-phenylhydrazide {(IV) and (V), m. p. 150°, $[\alpha]_D$ ±9°}. L-*erythro*-αγδ-Trihydroxyvaleric acid phenylhydrazide (IV) has also been prepared from 3-deoxy-L-xylose by Mukherjee and Todd (*loc. cit.*). The phenylhydrazide,

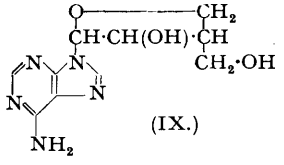
$C_{11}H_{16}O_4N_2$, obtained from the glycosidic fragment of cordycepin is markedly different in melting point from the D- and the L-*threo*-phenylhydrazide (II) and (III), and a comparison of the phenylhydrazide from cordycepin with a specimen of L-*erythro*-$\alpha\gamma\delta$-trihydroxyvaleric acid phenylhydrazide, m. p. 149° $[\alpha]_D$ +4·5°, kindly supplied by Professor A. R. Todd, F.R.S., showed the two isomers to be different. Thus the phenylhydrazide from cordycepin is not identical with any one of the phenylhydrazides (II)—(V).

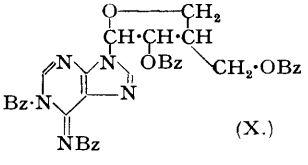


The sugar obtained by hydrolysis of cordycepin, which we name cordycepose, is not therefore a straight-chain 3-deoxyaldopentose, and the only structure which agrees with its properties is that of a 3-deoxypentose with a branched carbon chain (VI). This represents cordycepose as a relative of apiose (VII), a sugar isolated from parsley (Vongerichten, *Annalen*, 1901, **318**, 121, and subsequent papers; Schmidt, *Annalen*, 1930, **483**, 115).



Accordingly, cordyceponic acid phenylhydrazide is formulated as (VIII); it contains only one asymmetric centre and the phenylhydrazide rule indicates that its dextrorotation requires the configuration shown. In support of the structure (VI) for cordycepose it was found that the *p*-bromophenylosazone of cordycepose, like that of apiose, shows no detectable optical rotation in alcohol. Cordycepin is represented by the structure (IX) in which the configuration of the 2′-centre is known.





Although a crystalline acetate of cordycepin could not be obtained, benzoylation of cordycepin readily gave a crystalline benzoyl derivative, analysis of which indicated that it is a tetrabenzoate and not the expected tribenzoate. The tetrabenzoate is formulated as (X). This behaviour appears to be characteristic of 9-glycosidoadenines since similar benzoylation of adenosine gives a pentabenzoate and not a tetrabenzoate.

Experimental.

Acid Hydrolysis of Cordycepin.—A solution of cordycepin (150 mg.) in hydrochloric acid (2N.; 3·0 ml.) was heated on the steam-bath for 30 minutes. The solution was concentrated under reduced pressure to 1 ml., and the crystalline mass which separated from the cooled solution was collected, washed with ethanol (0·5 ml.), and dried (60 mg., 59%). Recrystallisation from ethanol gave adenine hydrochloride as fine needles, m. p. 285—286° (decomp.) alone or mixed with an authentic specimen (Found, in a sample dried for 3 hours at 78°/0·1 mm.: C, 33·2, 33·4; H, 3·7, 4·1; N, 39·0; Cl, 19·5. Calc. for $C_5H_5N_5,HCl,\frac{1}{2}H_2O$: C, 33·2; H, 3·9; N, 38·8; Cl, 19·6%). A warm solution of the hydrochloride (15 mg.) in water (1·0 ml.) was mixed with a cold saturated aqueous solution of picric acid (2·0 ml.) and the yellow precipitate (25 mg.) was collected, washed with water, and recrystallised from water, giving adenine picrate as long yellow needles, m. p. 290° alone or mixed with an authentic specimen (Found, in a sample dried for 3 hours at 100°/0·1 mm.: C, 36·3, 36·4; H, 2·3, 2·2; N, 30·9. Calc. for $C_5H_5N_5,C_6H_3O_7N_3$: C, 36·3; H, 2·2; N, 30·8%).

Cordycepose 2 : 4-*Dinitrophenylosazone.*—A solution of cordycepin (400 mg.) in hydrochloric acid (2N.; 5·0 ml.) was heated on the steam-bath for 30 minutes, and the solution cooled and filtered from separated adenine hydrochloride. The filtrate was mixed with a saturated solution of 2 : 4-dinitrophenylhydrazine in hydrochloric acid (2N.; 40 ml.) and set aside at room temperature. Amorphous material which separated during 24 hours was collected and discarded, and precipitates were then collected from the filtrate every day for 10 days. The combined red solid (50 mg.) was washed with

water, dried, and crystallised twice from a small volume of glacial acetic acid whereupon *cordycepose* 2 : 4-*dinitrophenylosazone* was obtained as dark red, microcrystals, m. p. 254—256° (decomp.) (Found : C, 41·6; H, 3·4; N, 22·6. $C_{17}H_{16}O_{10}N_8$ requires C, 41·5; H, 3·3; N, 22·8%). Cordycepose 2 : 4-dinitrophenylosazone gives a blue-violet coloration with sodium hydroxide in ethanol.

Cordycepose p-*Nitrophenylosazone*.—Under similar conditions a solution of cordycepin (100 mg.) in hydrochloric acid (2N.; 2·0 ml.), mixed with a saturated solution of *p*-nitrophenylhydrazine in hydrochloric acid (2N.; 20 ml.), gave *cordycepose* p-*nitrophenylosazone* (50 mg.) which separated from ethanol as a dark-red powder, m. p. 260° (decomp.) (Found : C, 49·8; H, 4·6; N, 20·5. $C_{17}H_{18}O_6N_6$ requires C, 50·7; H, 4·5; N, 20·9%). The *p*-nitrophenylosazone gives a bright blue coloration with sodium hydroxide in ethanol.

Deamination of Cordycepin.—Sodium nitrite (300 mg.) and glacial acetic acid (0·5 ml.) were added to a solution of cordycepin (100 mg.) in hot water (8·0 ml.), and the mixture kept at 70° for 30 minutes. The solution was then made alkaline (brilliant yellow) with aqueous sodium hydroxide. Evaporation of the solution under reduced pressure gave a white residue which was extracted with boiling ethanol (30 ml.). The extract was cooled, filtered from sodium acetate, concentrated, again cooled, and filtered, and finally evaporated to dryness under reduced pressure. The residue was dissolved in hydrochloric acid (*d* 1·18; 2·0 ml.) and heated on the steam-bath for 10 minutes. Evaporation of the solution under reduced pressure gave a dark residue which was extracted with boiling ethanol (25 ml.) (charcoal). The filtered extract was concentrated (10 ml.), and on cooling deposited needles (20 mg.), m. p. 360° (decomp.). A solution of the solid (10 mg.) in hot water (1 ml.) was mixed with a saturated aqueous solution of picric acid and the yellow precipitate was collected, washed with water, and recrystallised from water, giving orange-yellow blades, m. p. 240—250° (decomp., after sintering at 200—220°) alone or mixed with an authentic specimen of hypoxanthine picrate.

Attempted Periodate Oxidation of Cordycepin.—Under the conditions described by Lythgoe and Todd (*loc. cit.*) cordycepin was not oxidised in 3 days; under identical conditions of oxidation adenosine was complete in 4 hours.

Cordycepin Tetrabenzoate.—Cordycepin (50 mg.) was dried over phosphoric oxide for 3 hours at 78°/0·1 mm. and suspended in dry pyridine (3·0 ml.). Benzoyl chloride (1·0 ml.) was added and the mixture shaken to complete solution, set aside for 18 hours at room temperature, and heated on the steam-bath for 5 minutes. The solution was then mixed with crushed ice (20 g.), and the supernatant liquor decanted from a dark red oil which was washed with water (10 ml.) and dissolved in warm ethanol (3·0 ml.). On cooling, the solution deposited a crystalline solid (100 mg.), m. p. 170—175°, which, after recrystallisation from ethanol, gave *cordycepin tetrabenzoate* as lustrous needles, m. p. 179—180° (Found : C, 68·2, 68·5; H, 4·2, 4·2; N, 10·6, 10·5. $C_{38}H_{29}O_7N_5$ requires C, 68·4; H, 4·4; N, 10·5%).

Adenosine Pentabenzoate.—Under similar conditions adenosine (300 mg.) and benzoyl chloride (4·0 ml.) in dry pyridine (5·0 ml.) gave *adenosine pentabenzoate*, which crystallised from aqueous ethanol as needles, m. p. 183—184° (Found : C, 68·6; H, 4·3; N, 8·6. $C_{45}H_{33}O_9N_5$ requires C, 68·6; H, 4·2; N, 8·9%).

Cordycepose.—A solution of cordycepin (3·0 g.) in warm hydrochloric acid (N/10; 100 ml.) was boiled under reflux until the optical rotation became constant. The specific rotation, initial value $[\alpha]_D^{25}$ −38°, became constant at a value of $[\alpha]_D^{25}$ −12° after 270 minutes, and a sample of the solution treated at this stage with saturated aqueous picric acid gave adenine picrate as yellow needles, m. p. 285—290° alone or mixed with an authentic specimen. The solution was neutral and a further addition of hydrochloric acid (N/10; 10 ml.) was made and the mixture was heated for a further 30 minutes to ensure complete hydrolysis. The cooled solution was freed from adenine by passage through a column (30 cm. × 0·5 cm.) of Zeo Carb 215 (Permutit Co., Ltd.) previously washed by decantation and conditioned by treatment alternately with hydrochloric acid (2N.) and ammonia solution (N.) and used in the acid form. The column was washed with water until free from acid, and the total effluent was shown by means of ultra-violet light absorption measurement to contain less than 1 mg. of adenine. The effluent was neutralised with freshly precipitated silver carbonate, filtered, saturated with hydrogen sulphide, and filtered again through charcoal. The clear filtrate was evaporated to dryness under reduced pressure, the residual syrup was dissolved in ethanol (30 ml.), and the solution filtered from a small quantity of inorganic material and evaporated to dryness, finally under high vacuum, giving *cordycepose* as a pale straw-coloured syrup (1·06 g. 66%), $[\alpha]_D^{20}$ −26° (*c*, 0·640 in ethanol) (Found, in a sample dried for 6 hours at 20°/0·1 mm. : C, 44·5; H, 7·9. $C_5H_{10}O_4$ requires C, 44·8; H, 7·5%).

Cordyceponolactone.—Bromine (0·3 ml.) was added to a solution of cordycepose (610 mg.) in water (7·0 ml.), and the mixture was shaken in a closed flask for 60 hours in the dark. After removal of excess of bromine under reduced pressure by means of a stream of air, the solution was diluted (20 ml.) and freed from hydrobromic acid by means of silver carbonate and hydrogen sulphide, as previously described. The solution obtained by filtration through charcoal was evaporated to dryness under reduced pressure and the residual pale brown gum dissolved in ethanol (10 ml.). The solution was filtered from a trace of inorganic material and evaporated to dryness, and the residue heated at 80°/0·1 mm. for 30 minutes, giving *cordyceponolactone* as a pale brown syrup (350 mg., 58%) soluble in aqueous sodium hydrogen carbonate with effervescence, and having $[\alpha]_D^{20}$ +32° (*c*, 0·433 in ethanol) (Found : C, 45·8; H, 6·4. $C_5H_8O_4$ requires C, 45·5; H, 6·1%).

Cordyceponic Acid Phenylhydrazide.—A mixture of cordyceponolactone (55 mg.) and freshly distilled phenylhydrazine (0·1 ml.) in ethanol (0·2 ml.) was heated for 30 minutes under reflux on the steam-bath and then cooled for several hours in the refrigerator. The crystalline product (60 mg., 60%) was collected, a small volume of ethanol being used, and recrystallised from ethyl acetate giving *cordyceponic acid phenylhydrazide* as clustered needles, m. p. 151°, $[\alpha]_D^{22}$ +26° ±3° (*c*, 0·3 in ethanol) (Found : C, 54·8; H, 6·6; N, 11·6. $C_{11}H_{16}O_4N_2$ requires C, 55·0; H, 6·7; N, 11·7%).

Cordycepose p-*Bromophenylosazone.*—A mixture of cordycepose (200 mg.), *p*-bromophenylhydrazine hydrochloride (0·5 g.), and sodium acetate (600 mg.) in acetic acid (15% *v/v*; 5·0 ml.) was heated on the steam-bath for 90 minutes. The discoloured semi-solid product which separated from the cooled mixture was collected and washed free from oily material with the minimum quantity of ice-cold ethanol. The residue (113 mg., 16%) was recrystallised from water (charcoal) giving *cordycepose* p-*bromophenylosazone* as clustered blades, m. p. 163—164°, $[\alpha]_D^{20}$ 0° ±1° (*c*, 0·800 in ethanol) (Found: C, 43·1; H, 3·8; N, 12·0; Br, 35·2. $C_{17}H_{18}O_2N_4Br_2$ requires C, 43·4; H, 3·9; N, 11·9; Br, 34·0%).

The infra-red absorption spectra were determined by Dr. I. A. Brownlie to whom we express our thanks.

Grateful acknowledgment is made to the Governors of the Royal Technical College for the award of the Nobel Scholarship (to K. G. C.).

THE ROYAL TECHNICAL COLLEGE, GLASGOW. [*Received, April 27th,* 1951.]