

*The Synthesis of (±)-Apiose and (±)-Cordycepose.**

By R. A. RAPHAEL and C. M. ROXBURGH.

[Reprint Order No. 6403.]

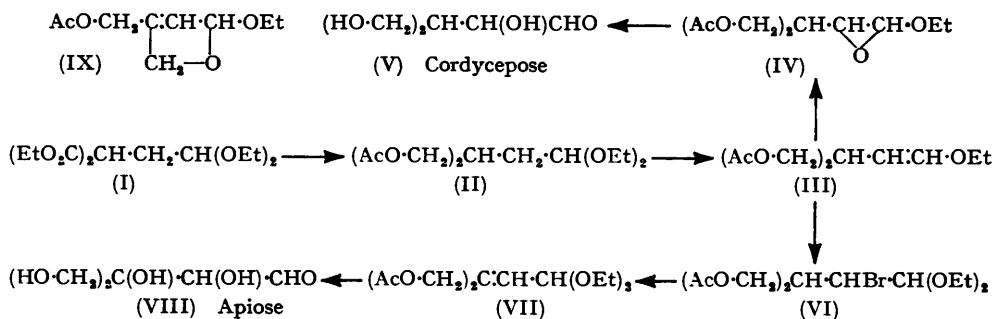
Synthetic confirmation of the structures assigned to the branched chain carbohydrates apiose (VIII) and cordycepose (V) has been obtained. The carbon skeleton is constructed by the condensation of bromoacetal with ethyl sodiomalonate to give ethyl 2:2-diethoxyethylmalonate (I) which, by suitable elaboration, is converted into the racemic forms of the two sugars.

FOR long the only branched-chain carbohydrate known to occur naturally was D(+)-apiose, isolated in 1900 from the glycosides of parsley (*Apium petroselinum*) (see Hudson, *Adv. Carbohydrate Chem.*, 1949, 4, 57; Hemming and Ollis, *Chem. and Ind.*, 1953, 85) and more recently from *Posidonia australis* (Bell, Isherwood, and Hardwick, *J.*, 1954, 3702). The degradative studies of Vongerichten and Schmidt (cf. Hudson, *loc. cit.*) clearly indicated the formulation (VIII) for this sugar. Recently the closely related (–)-cordycepose was found to be the carbohydrate moiety of the glycoside cordycepin, a metabolic product of *Cordyceps militaris* (Linn.) Link (Bentley, Cunningham, and Spring, *J.*, 1951, 2301) and was shown to be 3-deoxyapiose (V) (as the stereochemistry of the cyclic structures of the two sugars is as yet incompletely elucidated, the non-committal *aldehydo*-forms of the structures are used for brevity). These structural assignments have now been confirmed by synthesis of the racemic forms of the two sugars.

Condensation of bromoacetal with ethyl sodiomalonate gave ethyl 2:2-diethoxyethylmalonate (I), reduced by lithium aluminium hydride to 1:1-diethoxy-3:3-di(hydroxymethyl)propane. Acetylation of this diol gave the diacetate (II). On rapid distillation of this ester from a trace of sodium hydrogen sulphate one mol. of ethyl alcohol was smoothly eliminated and 3:3-di(acetoxymethyl)-1-ethoxyprop-1-ene (III) was formed in high yield. This was treated with perbenzoic acid and the resulting epoxy-ether (IV) hydrolysed with aqueous-alcoholic hydrochloric acid. The (±)-cordycepose thus obtained

* A preliminary note on part of this work has already appeared (*Chem. and Ind.*, 1953, 1034).

was characterized as its *p*-nitrophenylosazone which proved to be identical with a specimen obtained from the naturally occurring sugar (we cordially thank Dr. H. R. Bentley for kindly providing a sample of cordycepin). This direct comparison of the (\pm)- and the



(-)-sugar is of course made possible by the elimination of the asymmetry on osazone formation.

The key compound for the synthesis of apiose was 1:1-di(acetoxymethyl)-2-bromo-3:3-diethoxypropane (VI). All attempts to prepare this by bromination of the corresponding acetal (II) were unsuccessful (cf. Simpson, *J. Amer. Chem. Soc.*, 1949, **71**, 754; Marvel and Joncich, *ibid.*, 1951, **73**, 973). It was obtained by addition of the elements of ethyl hypobromite (derived from a solution of *N*-bromosuccinimide in ethanol) to the enol ether (III). Dehydrobromination of the bromo-acetal (VI) with lithamide in liquid ammonia produced the corresponding unsaturated acetal (VII). This product proved to be very labile when distilled, ethyl acetate being continually evolved with the presumed production of the cyclic acetal (IX). Hydroxylation of the double bond of the acetal (VII) followed by acid hydrolysis gave crude (\pm)-apiose, characterised as its *p*-bromophenylosazone, identical with a sample prepared from the (+)-apiose obtained from natural sources (we are indebted to Professor Wilson Baker, F.R.S., for kindly providing this specimen).

EXPERIMENTAL

Ethyl 2:2-Diethoxyethylmalonate (I).—This was prepared in 64% yield as described by Perkin and Pink (*J.*, 1925, **127**, 191). It had b. p. 108—109°/0.7 mm., n_D^{18} 1.4282.

1:1-Diethoxy-3:3-di(hydroxymethyl)propane.—To a solution of lithium aluminium hydride (9 g.) in ether (250 c.c.) was added ethyl 2:2-diethoxyethylmalonate (40 g.) in ether. Ice-cooling was employed to keep the mixture gently boiling. After a further 2 hours' refluxing, ethyl acetate was added, followed by water (100 c.c.). The resulting slurry was extracted with ether for 15 hr. and the extract dried (Na_2SO_4). Evaporation and distillation gave the diol (21.1 g., 75%), b. p. 99—103°/5 $\times 10^{-4}$ mm., 123—125°/0.05 mm., n_D^{17} 1.4511 (Found: C, 56.2; H, 10.3. $\text{C}_9\text{H}_{20}\text{O}_4$ requires C, 56.2; H, 10.5%).

3:3-Di(acetoxymethyl)-1:1-diethoxypropane (II).—The above diol (20 g.), acetic anhydride (33 c.c.), and pyridine (55 c.c.) were mixed and, after the initial exothermic reaction, were heated by steam for 1 hr. and set aside overnight. The mixture was poured on crushed ice (100 g.) and stirred to decompose excess of anhydride. Extraction with ether was followed by careful washing with ice-cold *N*-sulphuric acid and then sodium hydrogen carbonate solution. Drying (Na_2SO_4), evaporation, and distillation gave the acetal (II) (25.4 g., 88%), b. p. 122°/0.6 mm., 142°/2 mm., n_D^{15} 1.4352 (Found: C, 56.4; H, 8.8. $\text{C}_{13}\text{H}_{24}\text{O}_6$ requires C, 56.5; H, 8.75%).

A sample of the acetal was refluxed for 10 min. with a solution of 2:4-dinitrophenylhydrazine in aqueous acetic acid (80%). Cooling and dilution with water gave an oil that soon solidified. Crystallisation from benzene-light petroleum (b. p. 40—60°) gave 3:3-di(acetoxymethyl)propanal 2:4-dinitrophenylhydrazone as yellow plates, m. p. 56—57° (Found N, 14.7. $\text{C}_{15}\text{H}_{18}\text{O}_8\text{N}_4$ requires N, 14.65%).

In an attempt to obtain the free aldehyde, the acetal (1.75 g.) was shaken for 40 min. at 20° with tartaric acid (5 g.) in water (4 c.c.). Isolation with ether, washing with sodium hydrogen carbonate solution, drying (Na_2SO_4), evaporation, and distillation gave a product (0.55 g.), b. p. 145° (bath-temp.)/10⁻⁴ mm., n_D^{21} 1.4417. Analysis showed that this compound was probably 4-acetoxymethyltetrahydro-2-hydroxyfuran formed by hydrolysis of the acetal and

one acetoxy-grouping, followed by hemiacetal formation (Found : C, 52.65; H, 7.55. $C_7H_{12}O_4$ requires C, 52.5; H, 7.55%).

3 : 3-*Di(acetoxymethyl)-1-ethoxyprop-1-ene* (III).—In a 100-c.c. flask fitted with a wide-bore, short stillhead without fractionating column was placed 3 : 3-diacetoxymethyl-1 : 1-diethoxypropane (15.4 g.), sodium hydrogen sulphate (0.05 g.; freshly fused and powdered), and a few porous chips. With the apparatus evacuated to 0.5 mm. the flask and contents were immersed in an oil-bath preheated to 110° and the temperature of the bath then increased to 140° as rapidly as the copious frothing allowed. After 4 minutes' heating distillation began and, carried out as quickly as possible (bath-temp. 140—160°), was substantially complete in 5 min. Fractionation of the distillate (12.2 g.) gave 3 : 3-*di(acetoxymethyl)-1-ethoxyprop-1-ene* (10.6 g., 83%), b. p. 95—97°/0.4 mm., n_D^{20} 1.4446 (Found : C, 57.0; H, 7.3. $C_{11}H_{18}O_4$ requires C, 57.4; H, 7.9%). Treatment with an aqueous-acetic acid solution of 2 : 4-dinitrophenylhydrazine gave the 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 56—57°, described above. This demonstrated that no skeletal rearrangement had occurred during pyrolysis.

(±)-*Cordycepose* (V).—The above enol ether (III) (1 g.) was treated with a solution of perbenzoic acid (0.6 g., 1 mol.) in chloroform (34 c.c.) and set aside at 0° for 24 hr. The solvent was removed under reduced pressure and the residue heated by steam for 1 hr. with ethanol (10 c.c.) and hydrochloric acid (2*N*; 25 c.c.). The cooled mixture was shaken with benzene (3 × 20 c.c.), and the aqueous layer diluted to 70 c.c. with water. Passage through a column of anion-exchange resin (Amberlite IR-4B pre-saturated with carbon dioxide), followed by evaporation to dryness under reduced pressure, gave a brown syrup (0.46 g.) which was dissolved in a little methanol and filtered twice through a charcoal pad. The filtrate was evaporated to dryness and the residue dissolved in water (1 c.c.), washed with ether, evaporated to dryness under reduced pressure, and dried (P_2O_5) for 18 hr. at 0.5 mm. The product, (±)-cordycepose, was a colourless syrup (Found : C, 45.6; H, 8.1. $C_8H_{10}O_4$ requires C, 44.8; H, 7.5%).

A sample of the sugar (42 mg.) in hydrochloric acid (2*N*; 2 c.c.) was treated with *p*-nitrophenylhydrazine (0.3 g.) in hydrochloric acid (2*N*; 5 c.c.). After 4 hr. at room temperature a little sticky solid was filtered off and discarded. Thereafter the solid slowly precipitated and was collected daily for 10 days, washed with water, and dried. Crystallisation of this product (55 mg.) from nitromethane gave cordycepose *p*-nitrophenylosazone as a dark-red micro-crystalline powder, m. p. 259—260° (Kofler block) undepressed on admixture with a specimen derived from acid-hydrolysed cordycep. A mixture of the synthetic and the naturally derived osazone gave a single sharp band when developed in ethyl acetate on alumina.

Attempted preparation of the *p*-bromophenylosazone following Bentley, Cunningham, and Spring's directions (*loc. cit.*) gave cream-coloured clustered blades, m. p. 162—163° (from water), as recorded by them. This compound, however, proved to be, not the expected osazone, but *N*-acetyl-*p*-bromophenylhydrazine, undepressed on admixture with a sample prepared by heating the hydrazine with dilute acetic acid (Found : C, 41.8; H, 4.1; N, 12.3. Calc. for $C_8H_9ON_2Br$: C, 41.95; N, 4.0; N, 12.2%). The calculated analytical figures for the osazone and the *N*-acetyl derivative are very close and thus conducive to misconception.

1 : 1-*Di(acetoxymethyl)-2-bromo-3 : 3-diethoxypropane* (VI).—Finely powdered *N*-bromo-succinimide (4.3 g.; dried over P_2O_5 for 1 hr. at 50°/10⁻⁴ mm.) was added gradually during 20 min. to a vigorously stirred, cooled (−3°) solution of 3 : 3-*di(acetoxymethyl)-1-ethoxyprop-1-ene* (III) (5.3 g.) in dry ethanol. Stirring was continued for 45 min. at −3° and then for 2 hr. at room temperature. The succinimide formed was filtered off, ether (150 c.c.) added, and the solution well shaken with sodium hydrogen carbonate solution (5 × 60 c.c.) and water until the washings were neutral. Drying ($MgSO_4$), evaporation, and fractionation gave 1 : 1-*di(acetoxymethyl)-2-bromo-3 : 3-diethoxypropane* (3.9 g., 48%), b. p. 119—122°/0.35 mm., n_D^{18} 1.4630 (Found : C, 43.7, 43.4; H, 6.0, 6.3. $C_{13}H_{22}O_6Br$ requires C, 43.95; H, 6.5%).

1 : 1-*Di(acetoxymethyl)-3 : 3-diethoxyprop-1-ene* (VII).—A solution of the bromo-acetal (VI) (5.3 g.) in dry ether (20 c.c.) was added rapidly to a well-stirred suspension of lithamide (from lithium, 0.5 g.; formation catalysed by ferric nitrate) in liquid ammonia (250 c.c.) contained in a well-lagged flask. After 30 min. ammonium nitrate (7 g.) was added and the ammonia allowed to evaporate overnight; remaining traces of ammonia were removed under reduced pressure at 30°. The residue was extracted with ether (4 × 40 c.c.); drying, evaporation, and distillation gave a main fraction of ethylenic material (2.4 g.), b. p. 88—100°/0.4 mm., n_D^{18} 1.4544—1.4595. Rapid fractionation gave the substantially pure unsaturated *acetal* (VII) (1.5 g., 37%), b. p. 90—93°/0.5 mm., n_D^{18} 1.4540 (Found : C, 55.3; H, 8.15. $C_{13}H_{22}O_6$ requires C, 56.9; H, 8.1%). If distillation was prolonged, ethyl acetate was evolved with the production of the cyclic *acetal* (IX); this transformation accounts for the unsatisfactory analytical figures.

(±)-*Apiose* (VIII).—The unsaturated acetal (VII) (1.35 g.) was dispersed by vigorous stirring in ice-water (30 c.c.) through which carbon dioxide was rapidly bubbled. Aqueous potassium permanganate (2% ; 30 c.c.) was then added dropwise during 50 min. at 0—3° (cooling). The mixture was then allowed to rise to 7° and a few drops of ethanol added to destroy excess of permanganate. After the precipitate had coagulated, it was filtered off and the filtrate passed through a cation-exchange resin (Amberlite IRC-50) to remove potassium. After ether-extraction the effluent and washings (200 c.c.) were treated with concentrated hydrochloric acid (2 c.c.) and hydrolysis allowed to proceed at room temperature for 24 hr. Passage through an anion-exchange resin (Amberlite IR-4B pre-saturated with carbon dioxide), followed by evaporation to dryness under reduced pressure at room temperature, gave crude (±)-*apiose* (0.53 g., 78%).

A mixture of this product (90 mg.), *p*-bromophenyldiazine (180 mg.), water (2 c.c.), and 50% acetic acid (0.6 c.c.) was heated by steam. A brown oil began to separate almost immediately. After 90 minutes' heating the pale yellow supernatant liquor was decanted while still hot; on being cooled it deposited crystals of *N*-acetyl-*p*-bromophenyldiazine. The remaining brown product was washed with water and dissolved in ethyl acetate to remove tar. This solution was washed with water, then evaporated to dryness, and the residue azeotropically dried by adding benzene and distilling off some of the solvent. The benzene-insoluble residue (*N*-acetyl-*p*-bromohydrazine) was filtered off and the reddish-brown benzene solution reduced in volume to 0.5 c.c. After 24 hr. at room temperature this solution deposited yellowish-brown granules (41 mg.), m. p. 188—196°. Recrystallisation from ethanol gave *apiose p*-bromophenylosazone as tiny bright yellow needles, m. p. 206—208° (Kofler block) undepressed on admixture with a sample of the same m. p. derived from natural sources. The *X*-ray powder photographs of the synthetic and the naturally derived osazones were identical (we cordially thank Professor J. M. Robertson, F.R.S., for arranging these determinations).

One of us (C. M. R.) thanks the Department of Scientific and Industrial Research for a maintenance award).

THE QUEEN'S UNIVERSITY, BELFAST.
THE UNIVERSITY, GLASGOW, W.2.

[Received, May 9th, 1955.]