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STUDIES OF 2-AMINO-2-DEOXY-DL-GLYCERALDEHYDE. IDENTIFICATION OF AN *N*-ACETYL DERIVATIVE AMONGST THE DEGRADATION PRODUCTS OF THE OVALBUMIN GLYCOPEPTIDE

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ABSTRACT

Reduction of ethyl 2-amino-3,3-diethoxypropionate with lithium aluminium hydride yields the acetal, $CH_2OH \cdot CHNH_2 \cdot CH(OC_2H_5)_2$ (**1**). The product obtained on hydrolysis of **1** with concentrated hydrochloric acid is stable only in acid solution and behaves as a hydrate or a polymer of $CH_2OH \cdot CH\overset{+}{N}H_3 \cdot CHO\ Cl^-$. It affords a crystalline semicarbazone hydrochloride (**2**), 2,4-dinitrophenylhydrazone hydrochloride (**3**), and dithioacetal oxalate (**4**). Other acyl and alkyl derivatives have been prepared directly from compound **1**. The aldehyde, $CH_2OH.CH(NHCOCH_3).CHO$, which has only been identified in solution, should be formed after the Smith degradation of certain polysaccharides that contain amino sugars. The diethyl acetal **12** and its *O*-methyl derivative **15**, the 2,4-dinitrophenylhydrazone **13**, and the ethylene dithioacetal **14** of this aldehyde have been prepared. The derivative **14** was also isolated from the Smith-degradation products of the ovalbumin glycopeptide after mercaptolysis.

INTRODUCTION

There are three possible monoaminotrioses, 1-amino-3-hydroxypropan-2-one[1], 3-amino-2-hydroxypropionaldehyde[2], and 2-amino-3-hydroxypropionaldehyde, and the last of these is the subject of this publication. This compound may exist transiently in solution during certain reactions[3,4], and its 2,4-dinitrophenylhydrazone sulphate was characterized amongst the degradation products of cycloserine[5]. The L-isomer of the *N*-benzoyl derivative is formed by oxidation of *N*-benzoyl-L-serine[6]. An attempt to isolate the *N*-acetyl derivative from an aqueous solution[7,8] gave a product that was probably polymeric. These preparations and an earlier one[9] all used periodate oxidation of amino sugar derivatives. Klenk and Faillard[10] isolated a compound from the degradation of acetylsphingosine, which was oxidised to *N*-acetylserine.

The formation of 2-amino-3-hydroxypropionaldehyde or its *N*-acetyl derivative from polysaccharides that contain a 2-acetamido-2-deoxyhexopyranoside residue having free hydroxyl groups in positions 3 and 4 can be expected after periodate oxidation, borohydride reduction, and acid hydrolysis. This reaction sequence will occur when the amino sugar is in a nonreducing, terminal position such as that of 2-acetamido-2-deoxy-D-glucose in ovalbumin[11], or 2-acetamido-2-deoxy-D-galactose

in the characteristic structure responsible for blood-group A specificity[12]. The amino sugar can become terminal after various degradations, as in orosomucoid[13]. The same fragment should occur after the degradation of mucin of the ovine submaxillary gland, where the 2-acetamido-2-deoxy-D-galactoside residue is attached by the 6-hydroxyl group to *N*-acetylneuraminic acid[14].

The C_3 and C_4 compounds having carbonyl, amino, and hydroxyl groups already described are only known in their ionic salt form (Table I). In this publication*, the preparation is described of derivatives of 2-amino-3-hydroxypropionaldehyde, together with the isolation of a derivative from the ovalbumin glycopeptide after Smith degradation.

TABLE I

HYDROCHLORIDES OF AMINO HYDROXY KETONES AND ALDEHYDES HAVING THREE AND FOUR CARBON ATOMS

Compound	*Properties*	*References*
$CH_2NH_2 \cdot CHOH \cdot CHO$, HCl	M.p. 143–145° (dec.) (a chloroplatinate is known)	2 and 16
$CH_2NH_2 \cdot CO \cdot CH_2OH$, HCl	M.p. 136–137°	1
2-Amino-2-deoxy-D-threose, HCl	Amorphous, approximately pure, furanose	17
2-Amino-2-deoxy-D-erythrose, HCl	M.p. 128°, furanose	17
2-Amino-3,3-dimethyl-4-hydroxybutyraldehyde, HCl	Crystalline, hygroscopic, furanose	18

RESULTS AND DISCUSSION

Reduction of ethyl 2-amino-3,3-diethoxypropionate[19] with lithium aluminium hydride gave the diethyl acetal **1** (95%) as a distillable syrup that was purified by recrystallization of its neutral oxalate and characterised as the bis(3,5-dinitrobenzoyl) derivative. Hydrolysis of compound **1** with acid is hindered by the quaternary ammonium group and requires prolonged treatment with concentrated hydrochloric acid.

In acid solution, an aldehyde such as $CH_2OH \cdot CH(\overset{+}{N}H_3) \cdot CHO$ is probably completely hydrated or polymerized. The n.m.r. spectrum in deuterium oxide does not show a signal corresponding to an aldehyde proton. The expected structures have four non-exchangeable protons, and two complex groups are distinguished in the spectrum, centred at 5.3 (one proton), and 4.0 p.p.m. (three protons). A very weak, residual signal in the methyl region enabled the extent of hydrolysis to be determined as 98%. The hydrolysis is confirmed by the reducing properties of the aqueous solution and by the preparation of various derivatives of the aldehyde function (see later). The unfavourable physical properties of the solid hydrochloride do not allow elemental analysis or determination of the infrared spectrum.

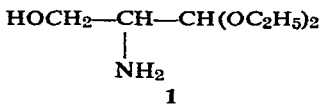
The preparation of several functional derivatives indicates that, at least under certain conditions, the compound reacts as the structure, $CH_2OH \cdot \overset{+}{C}HNH_3 \cdot CHO\ Cl^-$.

*Part of this work has already been communicated in preliminary notes[29–31].

The semicarbazone hydrochloride was obtained by the usual method and has an analytical composition and ultraviolet and infrared spectra corresponding to the chelated structure **2**, which was confirmed by the n.m.r. spectrum[20]. The 2,4-dinitrophenylhydrazone hydrochloride (**3**) crystallizes with a molecule of methanol. The ethylene dithioacetal (**4**) was prepared by treatment with 1,2-ethanedithiol in concentrated hydrochloric acid and purified *via* its neutral oxalate. The structure of the dithioacetal was confirmed by reductive desulphuration to 2-aminopropan-1-ol[21]. The same product was also prepared from the diethyl acetal **1**.

TABLE II

DERIVATIVES OF 2-AMINO-3-HYDROXYPROPIONALDEHYDE



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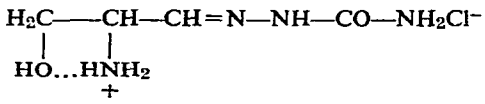

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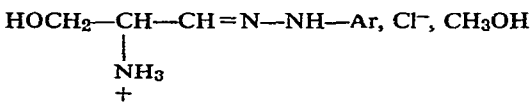

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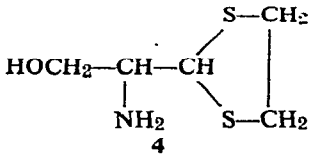

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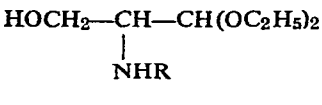
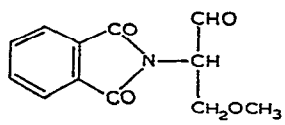


5 R = $C_6H_5CH_2$
6 R = 2,4-dinitrophenyl



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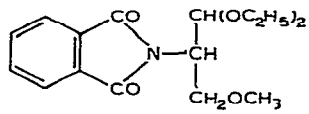

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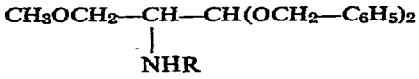
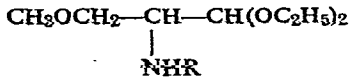
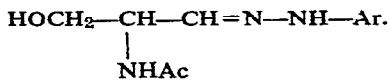


9 R = 2,4-dinitrophenyl



10 R = H
11 R = 2,4-dinitrophenyl
12 R = Ac



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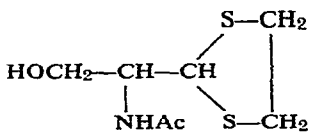

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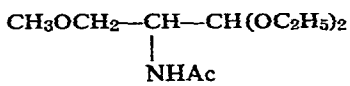

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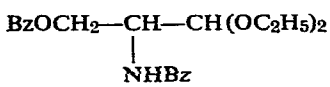

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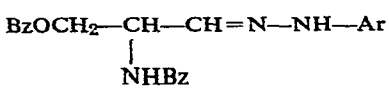

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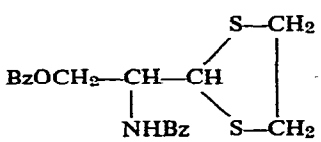

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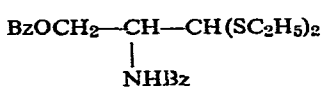

19

With phenylhydrazine in dilute acetic acid, a low yield of hydroxypyruvaldehyde 1,2-bis(phenylhydrazone) was obtained (the yield in the case of glyceraldehyde is also low; 2-amino-2-deoxy-D-threose only gives 10% of D-*glycero*-tetrulose phenylosazone). The main product was an amorphous phenylhydrazone which on hydrogenation over Raney nickel yields 2,3-diaminopropan-1-ol, isolated as the picrate (yield, 20% from "2-amino-3-hydroxypropionaldehyde hydrochloride"). Traces of DL-serine were observed (paper chromatography) after oxidation with mercuric or silver oxide, and no glyceraldehyde was formed after treatment with nitrous acid.

Methyl and benzyl derivatives. — Ethyl 2-benzylamino-3,3-diethoxypropionate is also reduced by lithium aluminium hydride to yield the diethyl acetal **5**. Vigorous hydrolysis of **5** yielded an amorphous powder which is probably a hydrate or a polymer of $CH_2OH \cdot CH(\overset{+}{N}H_2 \cdot CH_2 \cdot C_6H_5) \cdot CHO\ Cl^-$. Treatment with phenylhydrazine, followed by reduction, gave 3-amino-2-benzylaminopropan-1-ol, isolated as the dipicrate.

Attempts to hydrolyse other acetal derivatives did not yield any α-aminoaldehyde products. The *N*-2,4-dinitrophenyl compound **6**, quantitatively obtained from acetal **1**, undergoes C–N rupture on acid hydrolysis to yield 2,4-dinitroaniline.

In order to gain access to the *O*-methyl series, 3-methoxy-2-phthalimidopropionaldehyde[23] (**7**) was converted into the dibenzyl acetal and treated with alcoholic hydrazine to yield 2-amino-1,1-dibenzyloxy-3-methoxypropane (characterized as the *N*-2,4-dinitrophenyl derivative **9**). Similarly, 1,1-diethoxy-3-methoxy-2-phthalimidopropane (**8**), with alcoholic hydrazine, yielded the unstable diethyl acetal **10** which gave no identified product on acid hydrolysis. Concentrated hydrochloric acid at room temperature degrades the corresponding *N*-2,4-dinitrophenyl acetal **11** to 2,4-dinitroaniline. At pH 2.5 and 90°, no hydrolysis was observed after one hour.

Acyl derivatives. — 2-Acetamido-2-deoxy-L-glyceraldehyde has already been prepared in aqueous solution[8], and in a powder form[7] (m.p. 92°), the analytical composition of which has not been determined. This powder was oxidised to serine after acid hydrolysis (yield not indicated), and it also yielded the 2,4-dinitrophenylosazone of pyruvaldehyde[7].

The acetamido acetal **12** is quantitatively obtained when a methanolic solution of the amino acetal **1** is treated by acetic anhydride. Compound **12** was hydrolysed by 0.1N sulphuric acid for five minutes at 100°, but the resulting aldehyde, $CH_2OH \cdot CH(NHCOCH_3) \cdot CHO$, could not be isolated. Paper chromatography indicated that the racemic compound in this solution has the same R_F value as the L-isomer[8]. Sodium borohydride reduction of the aldehyde in solution gives 2-acetamidopropane-1,3-diol[8] (overall yield of 75%). This is an indication of the high yield of the hydrolysis under the conditions chosen. Acetal **12** yielded a 2,4-dinitrophenylhydrazone **13** and an ethylene dithioacetal **14**.

The direct acetylation of the presumed compound, $CH_2OH \cdot \overset{+}{C}HNH_3 \cdot CHO\ Cl^-$, in methanol–acetic anhydride–triethylamine, yielded a white, amorphous solid, which did not give 2-acetamidopropane-1,3-diol with sodium borohydride, but afforded the 2,4-dinitrophenylosazone of pyruvaldehyde with the appropriate reagent[7].

The acetylation of acetal **10** gives the crystalline acetamido acetal **15** which can also be isolated, but in a lower yield, by treatment of the acetamido acetal **12** with methyl iodide and barium oxide in *N,N*-dimethylformamide. The aldehyde corresponding to the dibenzoyl derivative **16** cannot be obtained when the latter is hydrolysed. The 2,4-dinitrophenylhydrazone **17** is precipitated when the acetal **16** is left for a long time in the presence of the reagent dissolved in 2N hydrochloric acid. Attempts to hydrolyse the benzoyl dithioacetals **18** and **19** by mercuric chloride were unsuccessful.

1-Amino-3-hydroxypropan-2-one. — The preparation of 1-amino-3-hydroxypropan-2-one hydrochloride was repeated and confirmed[1]. The n.m.r. spectrum in deuterium oxide has two equal signals, at δ 4.18 and 4.49 p.p.m., which correspond to the two methylene groups. This spectrum is thus entirely different from that of "2-amino-3-hydroxypropionaldehyde hydrochloride". Moreover, the ketone gives a different semicarbazone, the structure of which is confirmed by its n.m.r. spectrum.

Characterization of a derivative of 2-acetamido-2-deoxy-D-glyceraldehyde after degradation of the ovalbumin glycopeptide. — The above results indicate that 2-acetamido-2-deoxy-D-glyceraldehyde is best characterised as the ethylene dithioacetal **14**. This derivative may be characterised chromatographically (see Experimental section) and by its mass spectrum. The parent peak is very weak (0.2% of the base peak), as observed in similar compounds[24]. The fragmentation in Fig. 1 is proposed by analogy with published results[25]. The protonated acetamide, $CH_3\text{-}C(^{+}OH)\text{-}NH_2$ (*m/e* 60), and the acetyl ion, $CH_3\text{-}CO^+$ (*m/e* 43), are also observed.

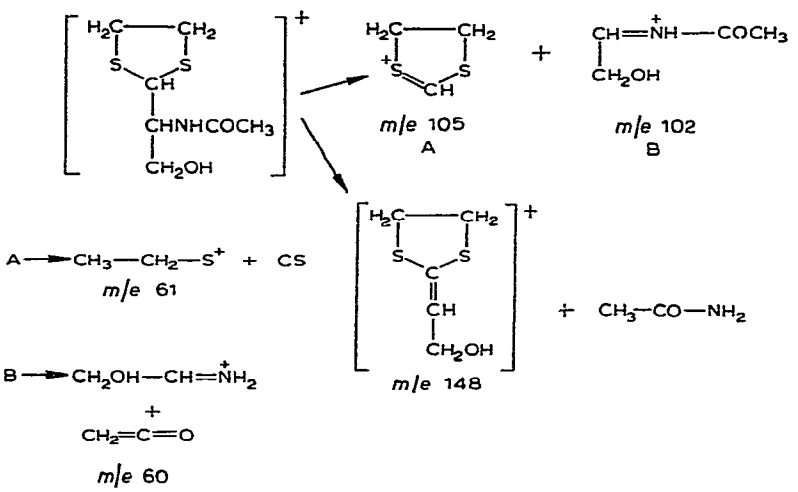

Fig. 1

The glycopeptide was prepared by pronase hydrolysis of ovalbumin, but without the final purification by successive treatments with benzyl chloroformate and carboxypeptidase[26]. It probably still contained traces of serine, threonine, and

especially leucine residues. Of the three 2-acetamido-2-deoxy-D-glucose residues present in this glycopeptide, only one is terminal and non-reducing, and thus open to attack by periodate[27]. The periodate oxidation and the borohydride reduction were carried out under the described conditions[27], but the mild, acid hydrolysis (final stage of the Smith degradation[15]) was replaced by a mercaptolysis with 1,2-ethanedithiol in concentrated hydrochloric acid at room temperature. This treatment does not break the *N*-acetyl bonds[25]. The reaction sequence is as follows:

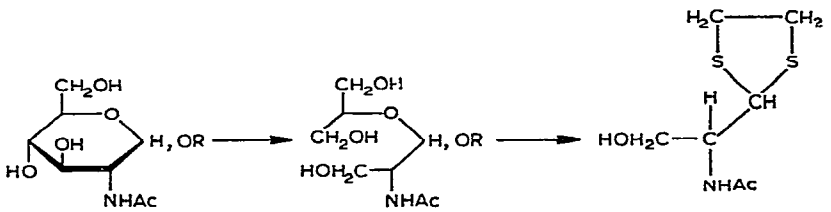

Fig. 2

These reactions were carried out on 410 mg of glycopeptide, from which 17 mg of compound **14** were obtained. This corresponds to a yield of *ca.* 30% if the molecular weight of the glycopeptide is taken as 1551.

EXPERIMENTAL

All solvents were removed under diminished pressure in a rotary evaporator at 45° unless otherwise stated. Melting points were determined on a Kofler hot-stage apparatus and are corrected. N.m.r. spectra were determined for solutions in D_2O at 60 MHz on a Varian A-60 spectrometer at 35°. Mass spectra were determined on an MS 9 instrument with a direct-insertion probe and operation at 70 ev. I.r. spectra were recorded with a Perkin–Elmer spectrophotometer (Model 237), either as thin films or Nujol mulls. U.v. spectra were measured with a Jobin and Yvon spectrophotometer (Model Maroc IV) at concentrations of about 10^{-4}M. Microanalyses were performed by the C.N.R.S. Laboratories, Thiais, France.

2-Amino-3-hydroxypropionaldehyde diethyl acetal (**1**). — A solution of ethyl 2-amino-3,3-diethoxypropionate (49 g) in dry ether (400 ml) was added with gentle stirring and mild reflux for 3 h to a suspension of lithium aluminium hydride (19.7 g) in dry ether (600 ml). Water (65 ml) was added to the reaction mixture cooled in an ice–salt bath. The ethereal layer was decanted off, and the inorganic residue was washed with boiling ethanol (5 × 200 ml). The ethanolic solution was evaporated to dryness at 35°, and the residue was taken up in a mixture of ethanol (350 ml) and dry ether (350 ml). This solution was stored for one night in a refrigerator and filtered. This filtrate, together with the former ethereal layer, was evaporated to dryness, and the brown, residual syrup was dried (P_2O_5, *in vacuo*) and distilled to give a colourless liquid (33.4 g, 85%) which solidified at 0°; b.p. 72°/0.2 mm, n_D^{25} 1.443.

Anal. Calc. for $C_7H_{17}NO_3$: C, 51.51; H, 10.50; N, 8.58. Found: C, 51.62; H, 10.51; N, 8.30.

The crude syrup (489 mg, 3 mmoles) was dissolved in ethanol (10 ml) and treated with oxalic acid dihydrate (189 mg, 1.5 mmoles) in ethanol (10 ml). The slight precipitate of lithium oxalate was filtered off, and anhydrous ether was added to the filtrate to give the neutral oxalate, m.p. 150–151° (from ethanol–ether).

Anal. Calc. for $C_{16}H_{36}N_2O_{10}$: C, 46.14; H, 8.71; N, 6.73. Found: C, 46.01; H, 8.65; N, 6.76.

An aqueous solution of the oxalate (5 g) was treated with saturated, aqueous barium hydroxide (75 ml). The filtered solution was evaporated, the residue was dissolved in ethanol, and the filtered solution was evaporated to give acetal **1** as a light-yellow syrup (3.74 g, 100%), b.p. 80–82°/0.5 mm.

The bis-*O,N*-(3,5-dinitrobenzoyl) derivative had m.p. 171–173° (dec.) (from ethanol–benzene).

Anal. Calc. for $C_{21}H_{21}N_5O_{13}$: C, 45.74; H, 3.84; N, 12.70. Found: C, 45.74; H, 3.77; N, 12.70.

With benzoyl chloride (4 equiv.) in 10% sodium hydroxide, acetal **1** gave a di-*O,N*-benzoyl derivative, m.p. 110° (from ethanol).

Anal. Calc. for $C_{21}H_{25}NO_5$: C, 67.90; H, 6.79; N, 3.77. Found: C, 67.76; H, 6.82; N, 3.66.

2-Amino-3-hydroxypropionaldehyde hydrochloride. — The acetal **1** (10 g) was added dropwise to conc. hydrochloric acid (20 ml) at −15°. The reaction mixture was kept for 15 h at room temperature, and then the hydrochloric acid was rapidly evaporated at ~15°/0.1 mm. The residual solid was treated with conc. hydrochloric acid (20 ml) at room temperature overnight, after which the hydrochloric acid was evaporated as above. The solid was washed several times with dry ether, finely ground, and dried *in vacuo* over KOH and P_2O_5 at 0°. The white, amorphous solid (quantitative yield) obtained was very hygroscopic, unstable in the atmosphere, soluble in water, slightly soluble in methanol, and insoluble in both ethanol and ether.

Semicarbazone hydrochloride **2**. — The above product (250 mg, 2 mmoles) was dissolved in water (5 ml) and treated with semicarbazide hydrochloride (223 mg, 2 mmoles) and sodium acetate trihydrate (272 mg, 2 mmoles). A small amount of biuret was filtered off, and the filtrate was heated for 1 h at 100°. The water was removed, and the residue was crystallised from methanol–ether; yield 75%; m.p. 155–156° (dec.); λ_{max}^{water} 228 nm (ε 8200); n.m.r.: ref. 20.

Anal. Calc. for $C_4H_{11}ClN_4O_2$: C, 26.31; H, 6.07; N, 30.68; HCl, 20.0. Found: C, 26.33; H, 6.32; N, 30.76; HCl, 20.1.

2,4-Dinitrophenylhydrazone hydrochloride **3**. — The amorphous aldehyde was dissolved in the minimal amount of methanol and treated with the reagent (1 equiv.) dissolved in methanol in the presence of a few drops of conc. hydrochloric acid; a light-red precipitate was discarded, and the filtrate was evaporated to dryness. The residue was dissolved in a little water, and the solution was filtered, and concentrated until yellow crystals appeared. These crystals were recrystallized with difficulty from methanol; yield, 47%; m.p. 187–189° (dec.).

Anal. Calc. for $C_9H_{12}ClN_5O_5 \cdot CH_3OH$: C, 35.53; H, 4.73; N, 20.73. Found: C, 34.75; H, 4.73; N, 20.91.

Phenylhydrazone and its reduction. — A solution of 2-amino-3-hydroxypropionaldehyde hydrochloride (900 mg) in water (9 ml) was treated with phenylhydrazine (775 mg) in 50% acetic acid (1 ml). A precipitate of hydroxypyruvaldehyde 1,2-bis-(phenylhydrazone) (m.p. 133–134°) was removed, and the filtrate was reduced by catalytic hydrogenation in the presence of Raney nickel at room temperature and atmospheric pressure. One mol of hydrogen was absorbed after 18 h, and the catalyst was then filtered off and washed with water. The aniline was extracted with benzene, the aqueous phase was evaporated, and the residue was treated with an aqueous solution of picric acid to yield 2,3-diaminopropan-1-ol dipicrate (20%), m.p. 216–217° (dec.); lit.[27], m.p. 215° (dec.).

Anal. Calc. for $C_{15}H_{16}N_8O_{15}$: C, 32.85; H, 2.94; N, 20.44. Found: C, 32.85; H, 3.37; N, 20.23.

Ethylene dithioacetal derivative **4**. — The amorphous aldehyde (1.2 g) dissolved in concentrated hydrochloric acid (10 ml) was treated at 0° with 1,2-ethanedithiol (1 g) and then stirred for 24 h at room temperature. The reaction mixture was poured on to a mixture of 40% aqueous sodium hydroxide (25 ml) and crushed ice (60 g). The product was extracted with chloroform, the extract was dried (Na_2SO_4) and evaporated, and distillation of the residue gave dithioacetal **4**; yield, 80%; b.p. 170–180°/0.2 mm; m.p. 60–63°.

An ethanolic solution of oxalic acid dihydrate (66 mg) was added to an ethanolic solution of the dithioacetal (173 mg) to give the neutral oxalate which crystallised after addition of a little dry ether; m.p. 175–178°.

Anal. Calc. for $C_{12}H_{24}N_2O_6S_4$: C, 34.27; H, 5.75; N, 6.66. Found: C, 34.31; H, 5.85; N, 6.57.

2-Acetamido-3-hydroxypropionaldehyde ethylene dithioacetal (**14**). — A solution of the above dithioacetal (1 g) in dry methanol (10 ml) was treated with acetic anhydride (1.4 ml) for 6 h at room temperature. The solution was evaporated, and the residue was triturated with toluene, which was then evaporated. The crystalline product (quantitative yield) had m.p. 137–138°; $\nu_{max}^{CHCl_3}$ 3430, 3280 (NH and OH); 3010; 2930; 1665 (amide I); 1510 (amide II); 1425; 1373; 1280; 1120; 1080; 1045 cm^{-1}. λ_{max}^{water} 234 nm (ε 320); and was soluble in chloroform. The mass spectrum exhibited the following principal peaks as a percentage of the base peak: *m/e* 148 (84); 134 (6); 120 (4); 107 (6); 106 (6); 105 (100); 102 (20); 92 (4); 87 (3); 84 (3); 74 (3); 61 (14); 60 (84); 59 (6); 58 (3); 45 (13); 43 (43); 42 (8); 31 (4); 30 (9). Parent peak at 207 (0.15).

Anal. Calc. for $C_7H_{13}NO_2S_2$: C, 40.58; H, 6.32; N, 6.76; S, 30.89. Found: C, 40.40; H, 6.24; N, 6.73; S, 30.62.

1-Amino-3-hydroxypropan-2-one semicarbazone hydrochloride. — A solution of 1-amino-3-hydroxypropan-2-one hydrochloride (250 mg, 2 mmoles) in methanol was treated with equivalent amounts of semicarbazide hydrochloride and sodium acetate trihydrate under reflux for 30 min. The solution was evaporated to dryness, and the

residue was crystallized from methanol to give the product, m.p. 161–162° (dec.). N.m.r. data: δ 4.83 (2-proton singlet) and δ 4.35 (2-proton singlet).

Anal. Calc. for $C_4H_{11}ClN_4O_2$: C, 26.31; H, 6.07; N, 30.68; Cl, 19.42. Found: C, 26.39; H, 6.11; N, 30.68; Cl, 19.37.

2-Benzylamino-3-hydroxypropionaldehyde diethylacetal (**5**). — A solution of ethyl 2-benzylamino-3,3-diethoxy-propionate[28] (20 g) in dry ether (200 ml) was added dropwise during 3 h to a stirred suspension of lithium aluminium hydride (5.6 g) in dry ether (200 ml). Water (11 ml) was added to the cooled (−15°) mixture which was then filtered. The residue was washed three times with boiling ethanol, and the combined organic filtrates were evaporated. Distillation of the residue (15.7 g) gave an initial fraction (0.7 g), b.p. 30–50°/0.2 mm, which was discarded, and then compound **5** as a light-yellow oil (10 g), b.p. 130–135°/0.2 mm, n_D^{25} 1.5020, which gave a picrate, m.p. 91–92° (from aqueous ethanol).

Anal. Calc. for $C_{20}H_{26}N_4O_{10}$: C, 49.79; H, 5.43; N, 11.61. Found: C, 49.72; H, 5.51; N, 11.85.

2-Benzylamino-3-hydroxypropionaldehyde hydrochloride, and reduction of its phenylhydrazone. — Acetal **5** (1.5 g) was added dropwise to conc. hydrochloric acid (6 ml) at −15°. The solution was kept for 4 h at room temperature, and then evaporated to give a syrup (1.2 g) which was dissolved in water (10 ml) and treated with phenylhydrazine (640 mg) dissolved in 50% aqueous acetic acid (0.25 ml). The hydroxypyruvaldehyde 1,2-bis(phenylhydrazone) (270 mg, m.p. 130–135°) was removed, and the filtrate was hydrogenated in the presence of Raney nickel at room temperature and atmospheric pressure. Hydrogen (1.1 equiv.) was absorbed after 18 h. The filtered solution was washed with benzene and then evaporated. The residue was dissolved in a little alcohol and treated with picric acid (1.53 g) dissolved in alcohol to give the dipicrate of 3-amino-2-benzylaminopropan-1-ol, m.p. 186–187° (dec.) (from ethanol–light petroleum).

Anal. Calc. for $C_{22}H_{22}N_8O_{15}$: C, 41.39; H, 3.47; N, 17.55. Found: C, 41.52; H, 3.62; N, 17.78.

2-(2,4-Dinitrophenylamino)-3-hydroxypropionaldehyde diethyl acetal (**6**). — The acetal **1** (1.63 g, 10 mmoles) was stirred for 24 h at room temperature with 1-fluoro-2,4-dinitrobenzene (1.86 g, 10 mmoles) and sodium carbonate decahydrate (1.43 g, 5 mmoles) in 50% aqueous acetone (80 ml). The mixture was evaporated, the residue was dissolved in ethanol, and, after removal of sodium fluoride, the filtrate was evaporated. The residue was recrystallized from ethanol–light petroleum to give acetal **6** as yellow needles (90%), m.p. 68–69°.

Anal. Calc. for $C_{13}H_{19}N_3O_7$: C, 47.41; H, 5.82; N, 12.76. Found: C, 47.60; H, 5.89; N, 12.82.

The hydrolysis of the above compound in a solution of acetone–5M hydrochloric acid (4:1) gave only 2,4-dinitroaniline, m.p. 179°, with a quantitative yield if the hydrolysis time was long enough.

2-(2,4-Dinitrophenylamino)-3-methoxypropionaldehyde dibenzyl acetal (**9**). — The phthalimido aldehyde[23] **7** (23.3 g) was heated under reflux in a Dean and Stark

apparatus with benzene (800 ml) in the presence of benzyl alcohol (25.2 g) and toluene-*p*-sulphonic acid (0.8 g). The cooled solution was washed with water, dried (Na_2SO_4), and evaporated. The syrupy residue (38 g) was dissolved in ethanol (350 ml) and treated under reflux for 3 h with an ethanolic solution (350 ml) of M hydrazine hydrate. On cooling, some of the phthalohydrazide precipitated, and the rest, after the addition of dichloromethane. The filtrate was evaporated to yield a yellow oil. This oil (600 mg) was characterized as 2-amino-3-methoxypropionaldehyde dibenzyl acetal by its reaction with 1-fluoro-2,4-dinitrobenzene (372 mg, 2 mmoles) and sodium carbonate (106 mg, 1 mmole) in 50% aqueous acetone (20 ml). The mixture was evaporated to dryness and the residue taken up several times in alcohol to yield bright-yellow crystals of acetal **9**, m.p. 85–86°.

Anal. Calc. for $C_{23}H_{23}N_3O_7$: C, 61.66; H, 5.39; N, 8.99. Found: C, 61.85; H, 5.54; N, 8.80.

3-Methoxy-2-phthalimidopropionaldehyde diethyl acetal (**8**). — The aldehyde **7** (11.5 g) was dissolved in the minimal amount of ethanol, and kept for a week in the presence of ethyl orthoformate (8 g) and anhydrous ammonium chloride (26 mg). The solution was evaporated, and the residue was taken up in water (100 ml) containing a few drops of conc. ammonia. The product was extracted by ether, and, after removal of the solvent, the residue was distilled to give the acetal **8** (12 g, 80%), b.p. 150°/0.1 mm, m.p. 44–45°.

Anal. Calc. for $C_{16}H_{21}NO_5$: C, 62.52; H, 6.88; N, 4.56. Found: C, 61.85; H, 6.80; N, 4.95.

2-Amino-3-methoxypropionaldehyde diethyl acetal (**10**). — An ethanolic solution (90 ml) of acetal **8** (12 g) was refluxed for 30 min with a M ethanolic solution (48.5 ml) of hydrazine hydrate. The cooled solution was filtered and evaporated, and distillation of the residue gave acetal **10** (5.6 g, 88%), b.p. 85°/12mm, which rapidly turned red in the atmosphere.

Anal. Calc. for $C_8H_{19}NO_3$: C, 54.21; H, 10.81; N, 7.90. Found: C, 54.21; H, 10.63; N, 8.40.

The 2-(2,4-dinitrophenyl) derivative **11** (74%) was prepared from acetal **10** by the method already described for **5**, to give golden-yellow needles, m.p. 72°.

Anal. Calc. for $C_{14}H_{21}N_3O_7$: C, 48.97; H, 6.17; N, 12.24; Found: C, 49.16; H, 6.10; N, 12.35.

2-Acetamido-3-hydroxypropionaldehyde diethyl acetal (**12**). — Acetic anhydride (0.9 ml) was added dropwise to a stirred solution of the acetal **1** (1 g) in dry methanol (10 ml). After 24 h at room temperature, the solution was evaporated, and the last traces of acetic acid were removed by addition of water and evaporation. The syrupy residue crystallised *in vacuo* over P_2O_5 at 0°. The crystals were washed with ice-cold light petroleum; m.p. 66–70°. The product decomposed on attempted distillation.

Anal. Calc. for $C_9H_{19}NO_4$: C, 52.66; H, 9.33; N, 6.82. Found: C, 51.83; H, 9.59; N, 7.16.

Preparation and reactions of 2-acetamido-3-hydroxypropionaldehyde. — (*a*) *Aqueous solution.* The acetal **12** (1.025 g) was heated for 5 min at 95° in 0.1N

sulphuric acid (25 ml), and the cooled solution was neutralized with barium carbonate and centrifuged.

(*b*) *Reduction.* The above supernatant was cooled to 0° and stirred with sodium borohydride (690 mg) dissolved in cold water (25 ml) for 30 min, and then for 2 h at room temperature. The solution was neutralized with dilute acetic acid and demineralized on Dowex-50 (H^+, 32 mequiv.) and Amberlite IR-45 (OH^-, 120 mequiv.) resin columns. The eluate was evaporated to dryness, and traces of boric acid were removed by repeated evaporation of methanol. The crystalline residue (0.5 g, 75%), m.p. 80–84°, was recrystallized from methanol–ethyl acetate (1:1) by adding light petroleum to give 2-acetamidopropane-1,3-diol, m.p. 87–88°; lit.[8], m.p. 89–80°.

Anal. Calc. for $C_5H_{11}NO_3$: C, 45.10; H, 8.32; N, 10.52. Found: C, 45.21; H, 8.27; N, 10.66.

(*c*) *2,4-Dinitrophenylhydrazone* **13**. The acetal **12** (205 mg, 1 mmole) in ethanol (2.5 ml) was added to a solution of the reagent (198 mg) in alcohol (50 ml) and 5% hydrochloric acid (15 ml). The reaction mixture was heated at 100° until the appearance of a precipitate, and then kept at 0° to yield yellow crystals, m.p. 196–201°.

Anal. Calc. for $C_{11}H_{13}N_5O_6$: C, 42.44; H, 4.21; N, 22.50. Found: C, 42.33; H, 4.22; N, 22.54.

2-Acetamido-3-methoxypropionaldehyde diethyl acetal (**15**). — (*a*) A mixture containing acetal **12** (1.23 g), methyl iodide (1.5 ml), and barium oxide (1.84 g) in *N,N*-dimethylformamide (20 ml) was stirred for 7 h at 20°. The product was extracted with chloroform (3 × 20 ml), and the chloroform solution was washed with water (3 × 10 ml) and evaporated. Distillation of the residue in a micro-distilling tube at 150°(bath)/0.1 mm gave acetal **15**, m.p. 49–54°.

Anal. Calc. for $C_{10}H_{21}NO_4$: C, 54.77; H, 9.65; N, 6.39. Found: C, 54.57; H, 9.36; N, 6.38.

(*b*) Treatment of the diethyl acetal **10** with acetic anhydride–methanol, as described for acetal **12**, gave acetal **15**, b.p. 89°/0.2 mm, m.p. 67°.

Anal. Calc. for $C_{10}H_{21}NO_4$: C, 54.77; H, 9.65; N, 6.39. Found: C, 54.70; H, 9.65; N, 6.47.

2-Benzamido-3-benzoyloxypropionaldehyde 2,4-dinitrophenylhydrazone (**17**). — Compound **16** (100 mg) was dissolved in alcohol and treated for a few minutes at 100° with the reagent (53 mg) dissolved in ethanol (10 ml) and 2N hydrochloric acid (3 ml) to give the title compound, m.p. 195° (from ethyl acetate).

Anal. Calc. for $C_{23}H_{19}N_5O_7$: C, 57.86; H, 4.01; N, 14.67. Found: C, 57.65; H, 4.18; N, 14.77.

2-Benzamido-3-benzoyloxypropionaldehyde ethylene dithioacetal (**18**). — A solution of the dithioacetal **4** (10 mmoles) in chloroform (25 ml) and pyridine (3.3 ml) was treated with benzoyl chloride (40 mmoles), in the usual way, to give compound **18**, m.p. 166°.

Anal. Calc. for $C_{19}H_{19}NO_3S_2$: C, 61.10; H, 5.13; N, 3.75. Found: C, 60.58; H, 5.11; N, 3.51.

2-Benzamido-3-benzoyloxypropionaldehyde diethyl dithioacetal (**19**). — The acetal **1** (3.26 g) was dissolved in ice-cold water (1.6 ml). To this solution was added, with vigorous stirring, conc. hydrochloric acid (14 ml), followed by ethanethiol (4.9 ml). After 24 h at room temperature, the reaction mixture was poured on to 40% sodium hydroxide (35 ml) and crushed ice (90 g). The product was extracted by chloroform, and the extract was dried (Na_2SO_4) and evaporated. A solution of the syrupy residue (3.72 g, 95%) in chloroform (50 ml) and pyridine (6.6 ml) was treated with benzoyl chloride (4 equiv.) by the usual method. The product was recrystallized from ethanol; yield, 5.1 g (63% total yield); m.p. 131–133°.

Anal. Calc. for $C_{21}H_{25}NO_3S_2$: C, 62.50; H, 6.24; N, 3.47. Found: C, 62.41; H, 6.22; N, 3.40.

Degradation of the ovalbumin glycopeptide. — The glycopeptide[26] (410 mg) was kept for 24 h at 5° in the dark in 0.05M sodium metaperiodate (50 ml). The excess of metaperiodate was then destroyed by treatment with ethylene glycol (1.4 ml) for 1 h at room temperature. Barium chloride dihydrate (611 mg) was then added, and the filtered solution was treated with sodium borohydride (4.5 g) for 24 h at 4–5°, brought to pH 7 with dilute hydrochloric acid, and evaporated at 30°. The residue (containing a great amount of sodium chloride and borate) was taken up in conc. hydrochloric acid (100 ml) and stirred for 48 h at room temperature with 1,2-ethanedithiol (5 ml). The hydrochloric acid and the excess of 1,2-ethanedithiol were rapidly evaporated at room temperature/0.1 mm. The residue was dried *in vacuo* over KOH and then taken up in dry methanol. The insoluble sodium salts were discarded, and the filtrate evaporated to dryness. A solution of the dry residue in chloroform was filtered and evaporated. The residue was chromatographically identical with racemic 2-acetamido-3-hydroxypropionaldehyde ethylene dithioacetal **14** in the following three systems: (*a*) paper chromatography (Whatman No. 1 paper) with butyl alcohol–acetic acid–water (5:2:3) and detection by iodine vapour; R_F 0.87. (*b*) t.l.c. (fluorescent silica gel) with isopropyl alcohol–water–ethyl acetate (24:12:65) and u.v. detection (254 nm); R_F 0.68; and benzene–ethanol (100:15); R_F 0.33.

The mercaptolysis product was purified by the last chromatographic system, using a 0.5-mm layer and eluting the band with boiling alcohol, to give a product (17 mg) having a mass spectrum that was identical with that of compound **14**. Although the racemic compound was only slightly soluble in chloroform, the i.r. spectra of the two compounds in chloroform were identical except that the NH and OH bands (3430 and 3280 cm^{-1}) were not resolved for the D-isomer.

2-Acetamido-2-deoxy-D-glucose ethylene dithioacetal. — 1,2-Ethanedithiol (1.9 ml, 22.7 mmoles) was added to a stirred solution of 2-acetamido-2-deoxy-D-glucose (4.42 g, 20 mmoles) in conc. hydrochloric acid (20 ml) at 0°. The stirring was continued for 20 h at room temperature. The solution was diluted with methanol (200 ml), neutralized with lead carbonate, filtered, concentrated, and again filtered. Light petroleum was added until turbidity, and the product was recrystallized from ethyl acetate–methanol (1:1) to give the title compound, m.p. 168–169°, which was soluble in methanol and hardly soluble in ethyl acetate and chloroform. The product

had R_F (t.l.c.) 0.37 in isopropyl alcohol–water–ethyl acetate (24:12:65) and 0.04 in benzene–ethanol (100:15).

Anal. Calc. for $C_{10}H_{19}NO_5S_2$: C, 40.38; H, 6.44; N, 4.71; S, 21.56. Found: C, 40.67; H, 6.33; N, 4.57; S, 21.91.

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