493. *Preparation of 2-Acyl Derivatives of 2-Amino-2-deoxy-D-glucose.*

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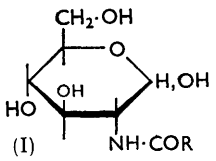
Difficulties in the preparation of certain 2-acylamino-derivatives (I) of 2-deoxy-D-glucose from the corresponding tetra-acetates (II) have been investigated and methods have been developed for the isolation of the de-*O*-acetylated compounds (I).

A series of 2-acylamino-2-deoxy-D-glucoses (I) was required for other studies: such compounds have been prepared by the action of an acid anhydride or chloride on 2-amino-2-deoxy-D-glucose [1] or its hydrochloride in the presence of a strong base; [2,3] dicyclohexylcarbodi-imide and a carboxylic acid have also been used.[4] These methods depend on specific acylation of the amino-group. Since acylamino-groups are usually stable under the conditions used for catalytic de-*O*-acetylations,[5] compounds (I) should, in principle, be prepared less equivocally by acylation of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucose followed by de-*O*-acetylation of the products (II). Although this route has been used,[2,6,7] some workers [8] have had difficulty in obtaining crystalline products by this deacetylation.

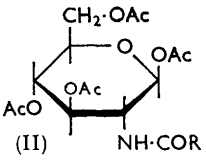
Since recrystallisation of compounds of type (I) is often difficult it is advantageous

[1] Inouye, Onodera, Kitaoka, and Hirano, *J. Amer. Chem. Soc.*, 1956, **78**, 4722; Kuhn and Haber, *Chem. Ber.*, 1953, **86**, 722.
[2] Bergmann and Zervas, *Ber.*, 1931, **64**, 979.
[3] Roseman and Ludowieg, *J. Amer. Chem. Soc.*, 1954, **76**, 301.
[4] Finn and Pitt, *Fed. Proc.*, 1961, **20**, 78; Bonner and McNamee, *J. Org. Chem.*, 1961, **26**, 2554; Kochetkov, Derevitskaya, Likhosherstov, Molodtsev, and Kara-Murza, *Tetrahedron*, 1962, **18**, 273.
[5] Greig, Leaback, and Walker, *J.*, 1961, 879.
[6] Bergmann and Zervas, *Ber.*, 1932, **65**, 1201.
[7] Maley and Lardy, *J. Biol. Chem.*, 1955, **214**, 765.
[8] Doherty, Popenoe, and Link, *J. Amer. Chem. Soc.*, 1953, **75**, 3466; Popenoe, Doherty, and Link, *ibid.*, p. 3469.

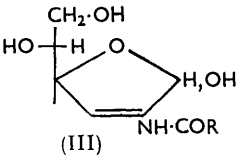
if they crystallise from the deacetylation medium.[9] In the present work 2-formamido-, 2-acetamido-, 2-propionamido-, 2-n-butyramido-, and 2-benzamido-derivatives of 2-deoxy-D-glucose crystallised when the corresponding tetra-acetates (II) were treated with



(I)



(II)



(III)

catalytic amounts of sodium methoxide in relatively small volumes of dry methanol. The benzyloxycarbonylglycylamido-, chloroacetamido-, and fluoroacetamido-compounds did not crystallise when prepared similarly and attempts to isolate them by using other media, *e.g.*, aqueous alkali–acetone, dry ammoniacal methanol, or dry chloroform–sodium methoxide, also led to syrups. On paper chromatograms, these syrups showed the presence of approximately equal quantities of two components: in each case, the slower-moving component was later shown to be the required compound (I) and the faster gave a colour directly with an acid spray of *p*-dimethylaminobenzaldehyde. Paper chromatograms of filtrates from the deacetylation of other tetra-acetates (II) gave similar results (cf. Table 1) but with smaller proportions of the more mobile components.

Kuhn and Kruger [10] showed that, under basic conditions, penta-acetates of 2-amino-2-deoxy-D-glucose gave mixtures of 2-acetamido-2-deoxy-D-glucose (I; R = Me) and ~ 10% of an unsaturated derivative which probably had structure (III; R = Me): the latter compound gave a colour directly with *p*-dimethylaminobenzaldehyde. It therefore seemed probable that, in the present work, deacetylation of the various tetra-acetates (II) gave mixtures of acylamino-compounds (I and III) but that generally insufficient of the

TABLE 1.

Acylamidodeoxy-sugars (I).

R	Method of prep.	Yield (%)	M. p.	$[\alpha]_D^{20}$ *	Required (%) C	H	N	Formula	Found (%) C	H	N	R_F of acylamido-derivative I	III	II
H	a,‡ c	40—50	122—124°	+24°	40·5	6·3	6·8	$C_7H_{13}NO_6$	40·5	6·5	6·6	0·16	0·34	0·85
Me ...	a, c	60—70	200—204	+40	43·4	6·8	6·3	$C_8H_{15}NO_6$	43·4	6·7	6·2	0·22	0·42	0·90
CH_2F	c, d	10—20	189—192	+31	40·0	5·9	5·9	$C_8H_{14}FNO_6$	40·0	5·9	5·8	0·23	0·40	0·90
CH_2Cl	d	10—35	165—177	+25	37·6	5·5	5·5	$C_8H_{14}ClNO_6$	37·6	5·6	5·6	0·44	0·65	0·95
Et ...	a	80	176—178	+38	46·0	7·2	6·0	$C_9H_{17}NO_6$	46·0	7·2	6·0	0·35	0·58	0·94
Pr^n ...	a, c	40—50	190—193	+36	48·2	5·7	5·7	$C_{10}H_{19}NO_6$	47·9	5·7	5·7	0·43	0·70	0·95
Ph ...	a, c	30—40	197—200	+28	55·1	6·0	4·9	$C_{13}H_{17}NO_6$	54·8	6·1	4·8	0·6	0·74	0·87
X † ...	b, c	50—60	186—187	+40	5·20	6·0	7·6	$C_{16}H_{22}N_2O_6$	51·9	5·9	7·6	0·56	0·69	0·91

* Reading after 2 hr. for 1% solution in H_2O (but in MeOH for the last compound). † The 2-benzyloxycarbonylglycylamino-compound. ‡ 5·14 mmoles of tetra-acetate in 10 ml. of medium.

unsaturated compound (III) was formed to interfere with isolation of the required compounds (I).

The formation of the unsaturated derivative was then studied in the hope of minimising it. The pure derivative (III; R = Me) was isolated and a colorimetric method developed for its estimation in solution.

For catalytic deacetylation of the acetamido-compound (II) by sodium methoxide the following results were found: (i) There was a rapid initial formation of the unsaturated derivative and chromatography and polarimetry indicated that this was probably concomitant with the deacetylation. (ii) De-*O*-acetylation was complete within a few

[9] Leaback, *J.*, 1960, 3166.
[10] Kuhn and Kruger, *Chem. Ber.*, 1956, **89**, 1473.

minutes at room temperature and was followed by further but slower formation of the unsaturated derivative; the rate of this slower reaction was similar to that of the reaction of sodium methoxide with a saturated solution of the de-*O*-acetylated compound (I; R = Me). (iii) Formation of the unsaturated derivative from the acetamido-compound (I) was slower than from an equimolar solution of the penta-acetyl derivative (II; R = Me). This is consistent with finding that anhydrosugar formation of an unsaturated compound takes place more readily from 3-*O*-substituted derivatives [11] and suggests the well-known β-elimination as a likely mechanism.[12] (iv) The same amount (2·2%) of unsaturated compound was formed from the α- and β-penta-acetyl derivatives; it will probably be similar from other 1,3,4,6-tetra-*O*-acetyl-2-acylamino-2-deoxy-α-D-glucoses.[13] (v) De-*O*-acetylation of the 2-acetamido-compound (II; R = Me) with sodium methoxide or barium methoxide in dry methanol, with sodium hydroxide in aqueous acetone, and with dry methanolic ammonia afforded, respectively, 2·2, 2·1, 2·1, and 0·1% of unsaturated compound after 30 min. at room temperature, and similar amounts at 5°.

Although the action of methanolic ammonia on the acetamido-derivative (II; R = Me) gave little of the unsaturated derivative, yet neither in this nor in analogous cases did the required product crystallise from the medium, nor was the yield of required compound (I) greater than was obtained by the sodium methoxide procedure: the poor yields may be due to losses incurred in separating the required compounds from acetamide and other compounds [14] which paper chromatograms indicated were present.

It was concluded that special procedures were unnecessary when compounds (I) crystallised from the medium (procedure a). When the extent of the side-reaction led to difficulty, the action of the catalyst was arrested soon after de-*O*-acetylation was complete; earlier work [15] suggested that this might be done by addition of water or a cation-exchange resin; these procedures (b and c) led to the crystalline benzyloxycarbonylglycylamido-derivative (I). With certain acylamino-derivatives (II; R = $CH_2Cl{\cdot}CO$ or $CH_2F{\cdot}CO$) such large amounts of unsaturated compound were formed that chromatographic separation was necessary (procedure d).

Experimental

Paper chromatograms were carried out on Whatman No. 1 paper by the descending method with the non-polar phase of butan-1-ol–ethanol–water (4 : 1 : 5 v/v), and the separated substances were detected with alkaline silver nitrate [16] or acid dimethylaminobenzaldehyde.[17] Solutions were evaporated under reduced pressure.

*De-*O*-acetylation of* 1,3,4,6-*Tetra*-O-*acetyl*-2-*acylamino*-2-*deoxy*-β-D-*glucose*.—The 1,3,4,6-tetra-*O*-acetyl-2-acylamino-2-deoxy-β-D-glucose (2·57 mmoles) [2,5,6] was suspended in dry methanol (9 ml.), and N-sodium methoxide (1 ml.) was added with shaking: the solid dissolved rapidly and the solution was treated by one of the following methods:

(*a*) The solution was left at 5° until crystallisation was complete (usually 24 hr.); the solid was filtered off, washed with a small volume of ethanol, and dried.

(*b*) Water (0·05 ml.) was added to the solution 30 min. after the sodium methoxide; after a further 60 min. at room temperature the solvent was evaporated, and the residue kept over P_2O_5 (16 hr.) and then recrystallised from a small volume of ethanol.

(*c*) 30 min. after the addition of the sodium methoxide, the solution was shaken for a few minutes with dried ZeoKarb 225 resin (250 mg.; H^+ form); the resin was filtered off and the solvent was evaporated, to leave a residue which was recrystallised or triturated with ethanol.

(*d*) The syrupy residue from an acylamino-compound (II) (25·7 mmoles) was dissolved in

[11] Jeanloz and Tremege, *Fed. Proc.*, 1956, **15**, 282.
[12] Kenner and Richards, *J.*, 1954, 278; 1956, 2921.
[13] Leaback and Walker, *J.*, 1957, 4754.
[14] Possibly similar to those described by Ellis and Honeyman, *Adv. Carbohydrate Chem.*, 1955, **10**, 121, or by BeMiller and Whistler, *J. Org. Chem.*, 1962, **27**, 1161.
[15] Anderson and Leaback, *Chem. and Ind.*, 1961, 1451.
[16] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[17] Partridge, *Biochem. J.*, 1948, **42**, 238.

methanol (10 ml.) and adsorbed on a charcoal–Celite 535 column (2 : 1; 4 × 45 cm.). The column was eluted with 50% v/v ethanol. Fractions of the eluate containing reducing material, but not giving a colour reaction with acid *p*-dimethylaminobenzaldehyde,[18] were evaporated to dryness, a solution of the residues in water was filtered and evaporated to dryness again, and the residue was recrystallised from ethanol.

Other De-O-acetylation Techniques.—The amide tetra-acetate (II) (2·57 mmoles) was dissolved in dry methanolic 0·4N-barium methoxide (10 ml.), a N-solution of sodium hydroxide in 1 : 1 aqueous acetone, or a 9 : 1 mixture of dry chloroform with methanolic sodium methoxide (N) or ammonia (11N) in dry methanol.

No crystalline product was obtained when the benzyloxycarbonylglycylamido-, chloroacetamido-, fluoroacetamido-, or formamido-derivative (II) was treated with methanolic ammonia for 30 min. before evaporation at room temperature and treatment of the residue with ethanol. Under similar conditions the acetamido-, propionamido-, n-butyramido-, and benzamido-derivatives (II) gave, respectively, 45, 41, 38, and 22% yields of products (I).

2-*Acetamido*-2-*deoxy*-D-*glucose*-2-*en* (III; R = Me). The acetamido-derivative (II; R = Me) (0·026 mole) was treated as under (*a*), and the crystalline residue filtered off. The filtrate was evaporated to dryness and treated with ethanol (20 ml.); the solution was filtered and added to a washed column described in paragraph (*d*), and the column was eluted with 50% ethanol. Fractions of the eluate containing material giving a colour with *p*-dimethylaminobenzaldehyde were examined by paper chromatography and those showing only the presence of the compound (III; R = Me) were evaporated at room temperature and then freeze-dried. The resulting syrup was dissolved in ethanol; this solution was filtered and evaporated in a high vacuum, to leave a syrup which was homogenous on paper chromatograms and gave ultraviolet and infrared spectra similar to those reported for the unsaturated compound.[10]

TABLE 2.

The action of sodium methoxide on acetamido-compounds.

The following were dissolved at room temperature in dry methanol (10 ml.) containing sodium methoxide (0·1N) and samples analysed at intervals for anhydrosugar content: (*a*) 34·9 μ moles of 2-acetamido-2-deoxy-D-glucose; (*b*) and (*c*) 34·9 μ moles and 2·57 mmoles, respectively, of compound (II; R = Me); and (*d*) 2·57 mmoles of the anomer of compound (II; R = Me).

μ moles of anhydrosugar per 10 ml. of reaction mixture.

Time (min.)	*a*	*b*	*c*	*d*	Time (min.)	*a*	*b*	*c*	*d*
1	—	0·58	—	20·1	30	0·16	0·79	54·8	53·2
5	0·02	0·65	30·7	37·4	45	0·23	0·84	—	54·7
10	0·07	0·69	43·2	46·0	60	0·26	0·93	53·8	54·7
20	—	0·79	54·8	51·8					

An aqueous solution (0·5 ml.) of the syrupy derivative (III; R = Me), standardised by nitrogen estimation, was added to acetic acid (4 ml.) and *p*-dimethylaminobenzaldehyde reagent (0·5 ml.):[18] colour was maximal after 40—50 min. and showed bands at 545 and 584 mμ (ε 2·16 × 10^4 and 2·5 × 10^4, respectively; calc. from the molarity of the glucoseen). The optical density at 584 mμ was used for estimating the glucoseen (III; R = Me) in deacetylation media and in solutions of 2-acetamido-2-deoxy-D-glucose (Table 2).

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[18] Aminoff, Morgan, and Watkins, *Biochem. J.*, 1952, **51**, 379.