

308. *The Epimerization of 2-Acetamido-2-deoxy-D-pentoses.*¹

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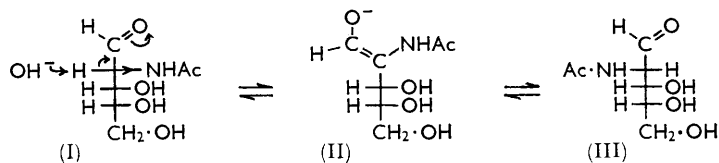
2-Acetamido-2-deoxy-D-ribose and -D-arabinose are equilibrated in aqueous ammonia, an effect ascribed to enolisation of the 2-hydrogen atom of the open-chain form under the influence of the acetamido-group.

THE Lobry de Bruyn-Alberda van Ekenstein isomerization of reducing sugars in dilute base² proceeds through intermediary enediols. Thus, one carbon-bound deuterium atom is introduced into glucose in alkaline heavy water.³ Reaction of 2-acetamido-2-deoxy-aldoses^{4,5} is not complicated by the formation of 2-ketoses or 2-ketimines, in contrast to that of normal aldoses and 2-amino-2-deoxyaldoses. Rapid equilibration of 2-acetamido-2-deoxy-D-glucose or -mannose in dilute aqueous base was observed to give in each case a mixture containing these epimers in the proportion ~4 : 1 respectively.⁴

When 2-acetamido-2-deoxy-D-ribose⁶ (I), $[\alpha]_D -36^\circ$, was dissolved in dilute aqueous ammonia at 21°, the specific rotation decreased to -59° in 12 hr., and then slowly increased. Paper chromatography of the mixtures at various times suggested that 2-acetamido-2-deoxy-D-arabinose (III) was formed, and that equilibration was complete after about 48 hr., giving a proportion of about 2 : 1 of D-arabino- to D-ribo-epimer. The major product was separated and characterized as 2-acetamido-2-deoxy-D-arabinose (III), $[\alpha]_D -94^\circ$.^{6,7} This monosaccharide gave a similar mixture in dilute aqueous ammonia, showing that the epimerization was a true equilibration.

The above discrepancy between the rotational evidence and the paper-chromatographic results for the maximum concentration of epimer, is probably due to side-reactions, and paper chromatography of an epimerized reaction mixture after 8 days revealed extensive degradation to other products.

In a comparative experiment, the epimerization of D-ribose to D-arabinose and D-erythro-pentulose was insignificant and hence the epimerization is facilitated by the acetamido-group. The rate-determining step in this process is the rate of ionization of the 2-hydrogen atom, which leads to the enolate ion (II). The electrophilic inductive



effect of the acetamido-group is greater than that of the hydroxyl group by virtue of the fractional positive charge on the nitrogen atom which is produced by mesomerism [cf. acetamidoacetic acid (pK 3.65) which is a stronger acid than glycollic acid (pK 3.83)⁸]. Thus, the 2-acetamido-2-deoxy-aldoses will epimerize *via* the acyclic form (*e.g.*, I) and the electron-attracting effect of the acetamido-group will accelerate the ionization of the 2-hydrogen atom and hence increase the rate of formation of the enolate anion (*e.g.*, II), with consequent destruction of asymmetry at position 2 and later formation of the two epimers.

¹ For a preliminary communication, see Coxon and Hough, *Chem. and Ind.*, 1960, 374.

² Lobry de Bruyn and Alberda van Ekenstein, *Rec. Trav. chim.*, 1895, **14**, 156, 203; 1896, **15**, 92; 1897, **16**, 241, 257, 262, 274, 282; 1899, **18**, 147; 1900, **19**, 1; Wolfrom and Lewis, *J. Amer. Chem. Soc.*, 1928, **50**, 837.

³ Sowden and Schaffer, *J. Amer. Chem. Soc.*, 1952, **74**, 505.

⁴ Comb and Roseman, *J. Amer. Chem. Soc.*, 1958, **80**, 3166; Spivak and Roseman, *ibid.*, 1959, **81**, 2403.

⁵ Brug and Paerels, *Nature*, 1958, **182**, 1159.

⁶ Coxon and Hough, *Chem. and Ind.*, 1959, 1249; Kuhn and Baschang, *Annalen*, 1959, **628**, 193.

⁷ Baer and Fischer, *J. Amer. Chem. Soc.*, 1960, **82**, 3709.

⁸ "International Critical Tables," McGraw-Hill Book Co., New York, 1929, Vol. VI, p. 259.

Although the rate of carbanion formation is not necessarily linearly correlated with the acidic dissociation constant of the ionizing proton,⁹ the idea is well substantiated that electrophilic substituents on a carbon atom to which a potentially acidic hydrogen is attached can increase the rate of carbanion formation. Thus, Hine *et al.*¹⁰ found that deuteriochloroform underwent base-catalysed exchange of deuterium for hydrogen about as readily as did deuterated acetaldehyde and acetone.

With 2-acetamido-2-deoxy-pentoses and -hexoses, although the epimerization will proceed *via* the acyclic forms, the resting state of the molecules will probably be the pyranose ring form.¹¹ Hence the proportion of each epimer in the equilibrium mixture will be governed by the relative stabilities of their pyranose chair conformations.¹

EXPERIMENTAL

Evaporations were under reduced pressure. Paper chromatography was performed by the descending method at room temperature on Whatman No. 1 filter paper with butan-1-ol-pyridine-water (10:3:3 v/v) as mobile phase. The following sprays were used: *a*, 0.02M-sodium metaperiodate followed after 5 min. by 4% w/v ammoniacal silver nitrate reagent (for detection of polyols); *b*, *p*-anisidine hydrochloride in butan-1-ol-ethanol-water;¹² *c*, Elson-Morgan reagents.¹³ Rates of movement of compounds are quoted relative to that of rhamnose (R_{Rh}) or the solvent front (R_F). M. p.s were determined on a Kofler micro-heating stage. Infrared absorption maxima are for Nujol mulls.

Preliminary Investigation of the Effect of Dilute Aqueous Ammonia on 2-Acetamido-2-deoxy-D-ribose.—A solution of 2-acetamido-2-deoxy-D-ribose (0.116 g.) in 0.73N-aqueous ammonia (10.0 ml.) was examined at various times (*t*) by polarimetry and by paper chromatography. The specific rotation (calculated as 2-acetamido-2-deoxy-pentose) changed as follows:

<i>t</i> (hr.) ...	0.08	0.5	1.5	2.5	3.5	5.5	11.0	24.0	48.0	72.0	108.0
$[\alpha]_D^{21}$...	-40.1°	-41.6°	-45.7°	-48.5°	-51.6°	-56.1°	-58.9°	-55.2°	-50.0°	-45.7°	-41.2°

Paper chromatography after 2 hr. revealed that, in addition to 2-acetamido-2-deoxy-D-ribose (R_{Rh} 1.0), a slower-moving reducing sugar was present which had R_{Rh} 0.78 [sprays *a* and *b* (orange spot)]. The latter spot rapidly increased in intensity and reached a maximum after *ca.* 48 hr. Meanwhile, the intensity of the spot corresponding to 2-acetamido-2-deoxy-D-ribose diminished in intensity until it was *ca.* half that of the other. After 48 hr. a streak of slower-moving degradation products was detectable with spray *a* and this increased in intensity until after 8 days, it was clear that considerable degradation had occurred.

2-Acetamido-2-deoxy-D-arabinose.—A solution of 2-acetamido-2-deoxy-D-ribose (0.163 g.) in 0.73N-aqueous ammonia (10 ml.) was kept at 21° for 96 hr. Concentration then gave a pale yellow syrup, which was separated into two fractions (R_{Rh} 1.0 and 0.78) by chromatography on Whatman 540 (acid-washed) paper run in the pyridine solvent for 4.5 days. Zones were detected with spray *b*; the faster-moving one (R_{Rh} 1.0) corresponded to starting material and was not further investigated; the slower-moving zone (R_{Rh} 0.78) was eluted with methanol (Soxhlet) for 18 hr. and concentration of the eluate then gave a syrup (0.068 g.) which crystallized from methanol-ether, yielding hexagonal plates of 2-acetamido-2-deoxy-D-arabinose (0.033 g.), m. p. 158—160°. Recrystallized from methanol-ether and washed with methanol, they had m. p. 161—163°, $[\alpha]_D^{27}$ -102° (5.5 min.) → -94° (final; 15 min.; *c* 1.04 in water), R_F 0.34 [sprays *a*, *b* (orange), and *c* (purple)] (Found: C, 43.7; H, 7.0; N, 7.3; Ac, 15.6. Calc. for $C_7H_{13}O_5N$: C, 44.0; H, 6.9; N, 7.3; Ac, 22.5%), ν_{max} 3430m, 3300m (OH), 3220w, 1550m (NH), 1645s (*N*-Ac). Baer and Fischer⁷ give m. p. 159—160° (decomp.), $[\alpha]_D$ -138° (2 min.) → -97.3° (30 min.). Kuhn and Baschang⁸ record m. p. 160—163°, $[\alpha]_D$ -149° → 97° (2 hr.).

Effect of Dilute Aqueous Ammonia on 2-Acetamido-2-deoxy-D-arabinose.—2-Acetamido-2-deoxy-D-arabinose (2.5 mg.) was dissolved in 0.73N-aqueous ammonia (0.15 ml.) and kept at 21°. Paper chromatography after 12 hr. revealed 2-acetamido-2-deoxy-D-ribose (R_{Rh} 1.0) in addition to starting material. The intensity of the spot corresponding to the former slowly

⁹ Hine, "Physical Organic Chemistry," McGraw-Hill Book Co., New York, 1956, p. 227.

¹⁰ Hine, Peek, and Oakes, *J. Amer. Chem. Soc.*, 1954, **76**, 827.

¹¹ Ferrier and Overend, *Quart. Rev.*, 1959, **13**, 272.

¹² Hough, Jones, and Wadman, *J.*, 1950, 1702.

¹³ Kent and Whitehouse, "Biochemistry of the Amino-sugars," Butterworths, London, 1955, 164, and references therein.

increased to *ca.* one-half of that of the spot due to the starting material. After *ca.* 40 hr. an equilibrated mixture of the *ribo*- and *arabino*-derivatives was obtained, similar to that prepared by the action of dilute aqueous ammonia on 2-acetamido-2-deoxy-D-ribose.

Effect of Dilute Aqueous Ammonia on D-Ribose.—D-Ribose (0.163 g.) was dissolved in 0.73N-aqueous ammonia (10.0 ml.), and the solution was kept at 21° and examined at intervals by polarimetry and paper chromatography. The specific rotation (calc. as pentose) changed slightly in a direction opposite to that of the equilibrium value (−106°) of D-arabinose: ¹⁴

<i>t</i> (hr.)	0	2	23	49	96	624
$[\alpha]_D^{21}$	−20°	−20°	−20°	−20°	−19°	−17°

D-Arabinose was not detectable on paper chromatograms with spray *b* after 50, 72, and 96 hr. There was, however, a very faint streak towards the starting line, presumably due to degradation products.

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¹⁴ Hough and Taylor, *J.*, 1956, 970.
