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Note

The formation of arabino nucleosides from 3-acetamido-1,2-di-*O*-acetyl-3,5-dideoxy-D-ribofuranose during the fusion synthesis

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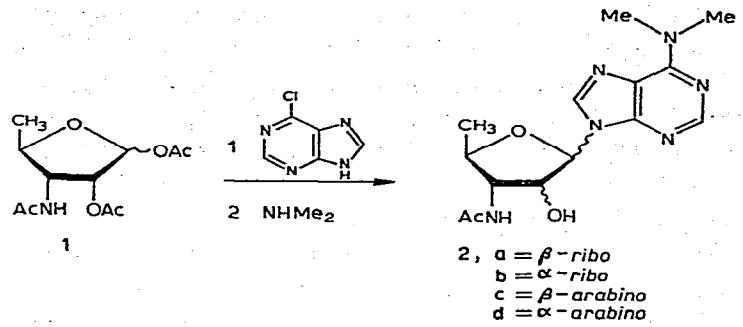
The fusion reaction[1] for synthesis of nucleosides from fully acetylated sugars and purine or pyrimidine heterocycles has gained wide application in recent years[2]. In addition to its convenience, this synthesis avoids the use of mercuric salts[3]—a potential source of contaminating mercuric ions which can interfere with the biological interpretation of the properties of nucleosides[4].

We report here the formation of *arabino* nucleosides during the fusion of a new amino sugar, 3-acetamido-1,2-di-*O*-acetyl-3,5-dideoxy-D-ribofuranose[5] (**1**) (1 α:2.2 β anomeric mixture)[5] with 6-chloropurine. Fusion of **1** (1.0 g, 3.86 mmole) with 6-chloropurine (0.60 g, 3.88 mmole) and 10 mg of chloroacetic acid at 120° gave a mixture of four 6-chloropurine nucleosides, which were readily separated after conversion into the 6-dimethylaminopurine nucleosides. The mixture of 6-dimethylamino nucleosides was separated into the *arabino* (15%) and *ribo* (25%) isomers by crystallization of the *ribo* mixture from ethyl acetate. The purine nucleosides were subsequently isolated by separation on a column of silica gel with chloroform–methanol as the eluent. The u.v. spectra of each nucleoside exhibited maxima at 267 nm (pH 1), 275 (pH 7), 275 (pH 13), excluding the presence of 7-substituted purines[6–8]. The mass spectra of all four nucleosides showed the molecular ion at *m/e* 320, the purine-base ion at *m/e* 162, and the sugar ion at *m/e* 158.

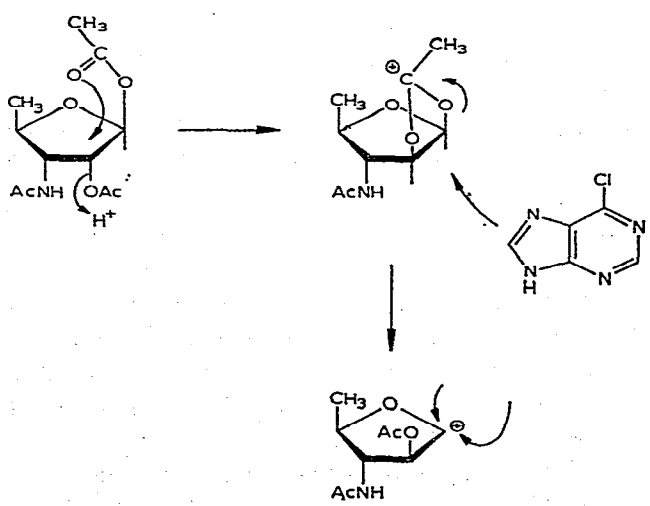
Nucleosides **2a** (m.p. 205–206°) and **2b** (m.p. 177–180°) were identified as the *β-ribo* and *α-ribo* isomers, respectively. Both nucleosides underwent N→O acyl migration in ethanolic hydrogen chloride, confirming that there was retention of the *ribo* configuration[9]. The anomeric assignments were established by p.m.r. spectroscopy. The anomeric proton of *cis* nucleosides always resonates at lower field (usually δ 0.5) than that of *trans*-nucleosides[10]. In addition, the Karplus equation[11] predicts that the observed $J_{1',2'}$ coupling constants can vary from 3.5–8.0 Hz for the α-nucleoside, and 0.0–8.0 Hz for the β-nucleoside. Thus, when both anomers are available, assignment of the β-configuration can be made if the coupling constant is less than 3.5 Hz. The observed chemical shifts of δ 6.00 (J 1.6 Hz) for the β-nucleoside



2a and δ 6.47 (J 3.5 Hz) for the α-nucleoside **2b** are consistent with the predicted values.

As expected for a 2′,3′-*trans*-system, nucleosides **2c** (m.p. 180–184°) and **2d** (m.p. 197–198°) did not undergo N → O acyl migration as did the *ribo* nucleosides. A possible epimerization at C-3′ can be ruled out by observation of the chemical shifts of the C-4′ protons. Epimerization of an acetamido group from the *ribo* to the *xylo* configuration would cause a downfield shift of H-4′. Downfield shifts of ~0.7 Hz have been observed in conversions from the *ribo* to the *xylo* configuration in other 3-acetamidofuranoses[12]. The C-4′ proton resonates at δ 4.29–4.25 in the starting sugar **1** and also in the four products, **2a–2d**. In addition, the downfield chemical shift of δ 6.23 for the anomeric proton of **2c**, compared with δ 5.98 for **2d**, established configurations of 1′,2′-*cis* for **2c** and 1′,2′-*trans* for **2d**. Thus **2c** was assigned as the *β-arabino* nulceoside and **2d** was assigned the *α-arabino* configuration.

It is noteworthy that similar results were also obtained with pure *β*-**1**, either in the presence or absence of acid catalyst. Epimerization of monosaccharides through



Scheme 1.

a postulated ortho ester intermediate has been observed in systems employing 95% acetic acid and elevated temperatures[13]. A possible mechanism for the formation of four nucleoside products could involve the formation of a C-1 carbonium ion via the ortho ester intermediate from the β-acetoxyl sugar (Scheme 1). Attack by the purine would favor formation of the α-nucleoside, which is consistent with the 2α–1β ratio of *arabino* nucleosides formed.

The fusion reaction is generally applied for the preparation of ribofuranosyl nucleosides from 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose, and no reports of epimerization have appeared. An isolated case, in which epimerization was observed with ethyl 1,2,3-tri-*O*-acetyl-5,6-dideoxy-D-*ribo*-heptofuranuronate, has been reported[14]. The authors suggested the possibility that the unique structure of their sugar might have been responsible for the epimerization. It now appears that epimerization during the fusion reaction is not unique to any particular sugar, and that any 1,2-diacetoxyl sugar may be a potential substrate for this reaction. The utilization of this reaction in preparing other *arabino* aminonucleosides from aminoribofuranoses is being pursued.

EXPERIMENTAL

General methods. — N.m.r. spectra were obtained in methanol-d_4 solution with a Varian A-60 D spectrometer; tetramethylsilane was the internal reference. The R_F values were obtained with silica gel Eastman chromatogram sheets developed with 7.5% methanol in chloroform. All melting points were determined on a Mel-Temp apparatus and are uncorrected.

Isolation of 6-dimethylamino-9-(3-acetamido-3,5-dideoxy-β-D-ribofuranosyl)-purine (**2a**), *6-dimethylamino-9-(3-acetamido-3,5-dideoxy-α-D-ribofuranosyl)purine* (**2b**), *6-dimethylamino-9-(3-acetamido-3,5-dideoxy-β-D-arabinofuranosyl)purine* (**2c**), and *6-dimethylamino-9-(3-acetamido-3,5-dideoxy-α-D-arabinofuranosyl)purine* (**2d**) *from the fusion reaction.* — A mixture of 1.00 g (3.86 mmoles) of **1** (α:β ratio of 1:2.2) and 0.600 g (3.88 mmoles) of 6-chloropurine was heated rapidly to 110°. Chloroacetic acid (10 mg) was then added and the temperature was raised slowly to 120° to produce a melt. The melt was placed under vacuum (0.2 mm) and heated at 118–120° until evolution of acetic acid ceased (30 min). The dark-brown melt was then mixed with chloroform (100 ml) and filtered. The filtrate was washed with saturated sodium hydrogen carbonate (25 ml) and saturated sodium chloride (2 × 25 ml), dried ($MgSO_4$), and evaporated *in vacuo* to a foam (1.21 g). The solid foam was dissolved in 40% aqueous dimethylamine (20 ml) and kept for 30 min at room temperature. The volatile materials were removed *in vacuo* and the residue was dissolved in saturated sodium chloride (60 ml) and extracted with ethyl acetate (3 × 100 ml). Evaporation of the combined organic extracts gave a light-yellow solid (0.447 g). Crystallization from ethyl acetate (10 ml) gave a white, solid mixture containing the β- and α-ribofuranosyl isomers **2a** and **2b**. These were separated by passing the mixture (in chloroform) through 17 g silica gel while eluting with chloroform (50 ml), 5% methanol in

chloroform (50 ml), and 7.5% methanol in chloroform, successively. Elution was then continued with 10% methanol in chloroform and 50-ml fractions were collected. The β-ribofuranosyl nucleoside (**2a**) was obtained as a white solid (107 mg) by evaporation of the fourth fraction. The next two fractions were evaporated to give the α-ribofuranosyl nucleoside **2b** as a white solid (58.7 mg). The filtrate from the original solid mixture of **2a** and **2b** was passed through 35 g of silica gel in a cclumn packed in chloroform, with elution by chloroform (100 ml), 2.5% methanol in chloroform (100 ml), 5% methanol in chloroform (100 ml), and 7.5% methanol in chloroform (100 ml), successively. Elution was then continued with 10% methanol in chloroform. The first 150 ml of this effluent was discarded, and evaporation of the next 85 ml gave an additional 99.6 mg of **2a**. The next 100 ml of effluent gave 116 mg of the α-arabinofuranosyl nucleoside **2d**. Finally, evaporation of the next 125 ml of effluent gave 55.5 mg of the β-arabinofuranosyl nucleoside **2c**.

Crystallization of the combined crops of **2a** from ethyl acetate gave analytical material as white crystals, m.p. 205–206°, $[\alpha]_D^{22}$ −11.0°, (*c* 1, methanol); R_F 0.61; λ_{max} pH 1, 267; pH 7, 274; pH 13, 275 nm; p.m.r. δ 8.18 and 8.07 (2 s, 2 × 1, H-2 and H-8), 6.00 (d, 1, $J_{1',2'}$ 1.6 Hz, H-1'), 4.60 (dd, 1, $J_{1',2'}$ 1.6 Hz, $J_{2',3'}$ 5.2 Hz, H-2'), 4.29 (dd, 1, $J_{2',3'}$ 5.2 Hz, $J_{3',4'}$ 10.7 Hz, H-3'), 3.48 (s, 6, NMe_2), 2.05 (s, 3, $NCOCH_3$), 1.43 (d, 3, $J_{4',5'}$ 5.5 Hz, 5'-CH_3).

Anal. Calc. for $C_{14}H_{20}N_6O_3$: C, 52.49; H, 6.29; N, 26.24. Found: C, 52.43; H, 6.23; N, 26.08.

Crystallization of **2b** from methanol gave an analytical sample as a white, crystalline monohydrate, m.p. softened at 155°, melted at 178–180°; R_F 0.4 g; λ_{max} pH 1, 267; pH 7, 275; pH 13, 275 nm; p.m.r. δ 8.17 and 8.10 (2s, 2 × 1, H-2 and H-8), 6.47 (d 1, $J_{1',2'}$ 3.5 Hz, H-1'), 3.50 (s, 6, NMe_2), 2.02 (s, 3, $NCOCH_3$), 1.33 (d, 3, $J_{4',5'}$ 5.5 Hz, 5'-CH_3).

Anal. Calc. for $C_{14}H_{22}N_6O_4$: C, 49.69; H, 6.55; N, 24.84. Found: C, 50.00; H, 6.66; N, 24.55.

Crystallization of **2c** from chloroform gave an analytical sample as the white crystalline monohydrate, m.p. softened at 149°, melted at 180–184°, R_F 0.45; λ_{max} pH 1, 267; pH 7, 274; pH 13, 274 nm; p.m.r. δ 8.15 and 8.10 (2 s, 2 × 1, H-2 and H-8), 6.28 (d, 1, $J_{1',2'}$ 5.0 Hz, H-1'), 4.35 (dd, 1, H-2'), 4.25 (m, 1, H-4'), 3.85 (dd, 1, H-3'), 3.48 (s, 6, NMe_2), 2.03 (s, 3, $NCOCH_3$), 1.45 (d, 3, $J_{4',5'}$ 5.5 Hz, H-1').

Anal. Calc. for $C_{14}H_{22}N_6O_4$: C, 49.69; H, 6.55; N, 24.84. Found: C, 49.89; H, 6.64; N, 25.01.

Crystallization of **2d** from ether–chloroform gave an analytical sample as a white crystalline solid, m.p. 197–198°; R_F 0.57; λ_{max} pH 1, 267; pH 7, 275; pH 13, 273 nm; p.m.r. δ 8.18 and 8.10 (2 s, 2 × 1, H-2 and H-8), 5.98 (d, 1, $J_{1',2'}$ 5.0 Hz, H-1'), 4.35 (dd, 1, H-2'), 4.25 (m, 1, H-4'), 3.9 (dd, 1, H-3'), 3.48 (s, 6, NMe_2), 2.02 (s, 3, $NCOCH_3$), 1.35 (d, 3, $J_{4',5'}$ −5.5 Hz, 5'-CH_3).

Anal. Calc. for $C_{14}H_{20}N_6O_3$: C, 52.49; H, 6.29; N, 26.24. Found: C, 52.25; H, 6.35; N, 26.15.

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