98. *Synthesis of Glycosylpyridinium Compounds from Glycosylamines and from Glycosyl Halides.*

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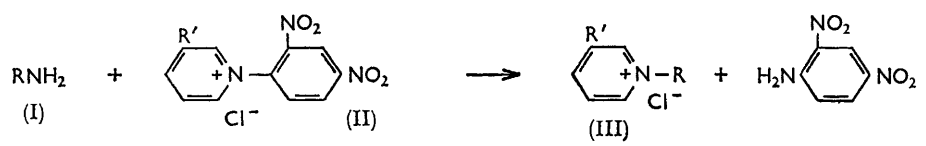
Glycosylpyridinium compounds were prepared from 1-(2,4-dinitrophenyl)-pyridinium chlorides and glycosylamines. Pyranosides were obtained from unprotected pentosylamines and hexosylamines. The ribofuranosyl derivative of nicotinamide, prepared from 2,3,5-tri-*O*-benzoyl-D-ribofurosylamine, contained less than 3% of the natural β-anomer. A number of glycosylpyridinium halides have been prepared from pyridines in reactions with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride and with 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl bromide.

Pyridine nucleosides obtained from glycosyl halides and substituted pyridines are anomeric mixtures;[1-4] the corresponding mixtures of nucleotides obtained by phosphorylation [1] are unsuitable for kinetic studies with adenosine triphosphate-nicotinamide mononucleotide adenylyltransferase, which is specific for the β-anomer of nicotinamide nucleotide.[5]

Shaw *et al.*[6] prepared β-D-ribofuranosylpyrimidines from 2,3,5-tri-*O*-benzoyl-D-ribofuranosylamine and it seemed likely that corresponding reactions would afford single anomers in the pyridine series. This Paper describes the synthesis of glycosylpyridinium chlorides from glycosylamines (I; R = D-ribosyl, D-xylosyl, L-arabinosyl, D-glucosyl, and D-galactosyl) and 1-(2,4-dinitrophenyl)pyridinium chlorides (II; R′ = CO·NH_2 and CO_2Et).



A similar reaction has been used for the preparation of 1-arylpyridinium chlorides.[7] The nicotinamide nucleosides (III; R′ = CO·NH_2) obtained from the unprotected pentosylamines were anomeric mixtures of pyranosides, as indicated by their specific rotations, their chromatographic behaviour, and their conversion into ethylene glycol by acid hydrolysis after treatment with periodate and then with borohydride.

The protected nicotinamide nucleoside (III; R = 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl, R′ = CO·NH_2), obtained from the corresponding amine, was debenzoylated with methanolic ammonia and the nucleoside was purified by paper chromatography. The product ($[\alpha]_D^{24}$ +46°) closely resembled a ribosyl derivative ($[\alpha]_D^{20}$ +49°) obtained [8] from the products of the reaction of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride with nicotinamide. This compound is anomeric [8-10] with the nicotinamide nucleoside that is a constituent of the codehydrogenase nicotinamide adenine dinucleotide.[1] On phosphorylation the product from the ribosylamine was converted into a nucleotide that was shown by enzymic assay [5] to contain less than 1% of β-nicotinamide nucleotide. The nucleoside was identical with a sample of α-nicotinamide nucleoside prepared from α-nicotinamide adenine dinucleotide.[11]

[1] Haynes, Hughes, Kenner, and Todd, *J.*, 1957, 3727.
[2] Fischer and Raske, *Ber.*, 1910, **43**, 1750.
[3] Viscontini, Leutenegger, and Karrer, *Helv. Chim. Acta*, 1955, **38**, 909.
[4] Hughes, Kenner, and Todd, *J.*, 1957, 3733.
[5] Atkinson, Jackson, and Morton, *Biochem. J.*, 1961, **80**, 318.
[6] Shaw, Warrener, Maguire, and Ralph, *J.*, 1958, 2294.
[7] Lettré, Haede, and Ruhbaum, *Annalen*, 1953, **579**, 123.
[8] Viscontini, Marti, and Karrer, *Helv. Chim. Acta*, 1954, **37**, 1373.
[9] Viscontini, Hoch, Marti, and Karrer, *Helv. Chim. Acta*, 1955, **38**, 646.
[10] Viscontini and Hürzeler-Jucker, *Helv. Chim. Acta*, 1956, **39**, 1620.
[11] Kaplan, Ciotti, Stolzenbach, and Bachur, *J. Amer. Chem. Soc.*, 1955, **77**, 815.

van der Veen [12] attributed the absence of β-anomer in solutions of *N*-acetyl-D-glucosamine, to stabilisation of the α-anomer by hydrogen bonding. A corresponding stabilisation of an intermediate in the reaction described here might account for the high yield of α-anomer.

A number of protected ribosylpyridinium chlorides (III; R = 2,3,5-tri-*O*-ribofuranosyl, R′ = H, CO_2Et, $CS{\cdot}NH_2$, $CO{\cdot}NHMe$, $CO{\cdot}NMe_2$, and $CO{\cdot}NEt_2$) were prepared from 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride and the corresponding pyridines, essentially as described by Todd and his co-workers.[1]

In the synthesis of the D-ribofuranosyl derivative of nicotinic acid from the tribenzoyl ethyl ester (III; R = 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl, R′ = CO_2Et), methanolic ammonia could not be used to remove benzoyl groups as the nicotinamide derivative would have been formed. Both ethyl and benzoyl groups were removed in dry methanol with an anion-exchange resin which had been converted into the HCO_3^- form and dried at 65°.

If pure β-anomers are needed, enzymic methods [13] still seem to be more convenient than direct chemical methods for the synthesis of pyridine nucleotides.

Experimental

Solvents for chromatography were: A, butan-1-ol–acetic acid–water (20 : 3 : 7), and B, ethanol–M-ammonium acetate, pH 7·5 (7 : 3); Whatman No. 1 paper was used.

3-Carbamoyl-1-glycosylpyridinium Salts from Unprotected Glycosylamines.—D-Ribosylamine (2·2 g.) in water (20 ml.) was added during 10 min. to a solution of 3-carbamoyl-1-(2,4-dinitrophenyl)pyridinium chloride [7] (2·2 g.) in ethanol (100 ml.) at 70—80°. A red precipitate formed shortly after addition was complete and then redissolved. After 1 hr. at 80°, ethanol was removed under reduced pressure. The residue was mixed with water (50 ml.) and cooled to 0°. 2,4-Dinitroaniline was removed by filtration and washed with cold water (3 × 10 ml.). The combined filtrate was passed through a column (20 cm. × 2 cm.^{2}) of Amberlite CG-50 (NH_4^+ form) which was then washed with water (1 l.). The glycosylpyridinium compound was eluted with 0·1M-ammonium formate (100 ml.). Ammonium formate was completely removed by freeze-drying for 3 days. A solution of the residue in methanol (15 ml.) was filtered into dry ethyl acetate (250 ml.); *3-carbamoyl-1-D-ribopyranosylpyridinium formate* (1·3 g.) was obtained as a hygroscopic amorphous powder, $[\alpha]_D^{20}$ −21·5° (*c* 2·0 in H_2O) (Found: C, 45·0; H, 5·7; N, 9·1. $C_{12}H_{16}N_2O_7,H_2O$ requires C, 45·3; H, 5·7; N, 8·8%), $\lambda_{max.}$ (0·2M-phosphate; pH 7·0) 266 mμ (ε 4700), $\lambda_{max.}$ (M-KCN) 324 mμ (ε 6600). The compound consumed 1·95 mols. of periodate during 3 days at 0°; the product had $[\alpha]_D^{24}$ +1° (*c* 0·5 in H_2O). A solution of the formate (1·5 mg.) in 0·1M-sodium metaperiodate (0·1 ml.) was kept at 20° for 4 hr. and then mixed with sodium borohydride (2 mg.) in water (0·1 ml.). After 16 hr. the solution was heated at 100° for 15 min. with N-hydrochloric acid (0·2 ml.). After chromatography in ethyl acetate–pyridine–water (7 : 2 : 1), the presence of ethylene glycol (R_F 0·61) and the absence of glycerol (R_F 0·36) was shown with a periodate–benzidine spray.[14] The formate had R_F 0·15 (solvent A) and, although chromatographically homogeneous, the specific rotation of the formate and of its product after periodate oxidation shows it to be an anomeric mixture since the specific rotations of the oxidation products of the pure anomeric 3-carbamoyl-1-D-pentopyranosylpyridinium chlorides are known [3] to be +54 and −49°.

Other 3-carbamoyl-1-glycosylpyridinium salts prepared in this way from unprotected glycosylamines are shown in Table 1.

Reaction of 2,3,5-Tri-O-benzoyl-D-ribosylamine with 3-Carbamoyl-1-(2,4-dinitrophenyl)pyridinium Chloride.—A solution of the amine (from 5·5 g. of the azide) [15] in ethyl acetate (50 ml.) was filtered into a solution of the dinitrophenyl compound (1·5 g.) in 80% aqueous ethanol (200 ml.). After 1 hr. at 80° the solvent was removed at 30°/15 mm. The residue was dissolved in 80% aqueous ethanol (150 ml.) and kept at 80° for 1 hr. and at 20° overnight. The

[12] van der Veen, *J. Org. Chem.*, 1963, **28**, 564.
[13] Kaplan, in "The Enzymes," ed. Boyer, Lardy, and Myrback, 2nd edn., Academic Press Inc., New York, 1960, Vol. III, p. 105.
[14] Viscontini, Hoch, and Karrer, *Helv. Chim. Acta*, 1955, **38**, 642.
[15] Baddiley, Buchanan, Hodges, and Prescott, *J.*, 1957, 4769.

solvent was removed at 30°/15 mm., and the residue was partitioned between ether (200 ml.) and water (200 ml.). The aqueous phase was extracted with ether (4 × 25 ml.) and ethyl acetate (4 × 25 ml.), and freeze-dried to yield the crude tribenzoate (0·9 g.) $[\alpha]_D^{24}$ +25° (*c* 1·1 in MeOH). The residue was dried over phosphoric oxide at 20°/0·1 mm. for 7 days and dissolved in dry methanol (10 ml.). The solution was saturated with dry ammonia at 2° and kept at this temperature for 2 days. The brown gum left on removal of solvent was dissolved in dry methanol (10 ml.) and the solution was filtered into dry ethyl acetate (200 ml.) to precipitate the nucleoside. The yield (calculated from the extinction at 329 mμ in M-KCN) was 0·66 mmole. Half of this material was chromatographed on four sheets (30 × 24 cm.) of washed [16] Whatman 3 MM paper (solvent A) (R_F 0·12). After elution with water, filtration, and removal of solvent, 3-carbamoyl-1-α-D-ribofuranosylpyridinium chloride was obtained as an amorphous hygroscopic powder $[\alpha]_D^{24}$ +46° (*c* 1·0 in H_2O) (lit.,[8-10] $[\alpha]_D^{20}$ +49°) (Found: C, 44·5; H, 5·3; Cl, 12·1; N, 9·6. Calc. for $C_{11}H_{15}ClN_2O_5,\frac{1}{2}H_2O$: C, 44·1; H, 5·4; Cl, 11·8; N, 9·3%), $\lambda_{max.}$ (H_2O) 267 mμ (ε 4800). In M-potassium cyanide the nucleoside had $\lambda_{max.}$ 329·5 mμ (ε 6100) 3 min. after mixing; after 1 hr. at 25° the extinction at 329 mμ had decreased by 25% and the absorption curve had inflexions

TABLE 1.

3-Carbamoyl-1-glycosylpyridinium formates from unprotected glycosylamines.

No.	Generating amine	Yield (%)	Found (%) C	H	N [a]	Formula	Requires (%) C	H	N
1	D-Xylosylamine [b]	72	44·8	5·9	8·4	$C_{12}H_{16}N_2O_7,H_2O$	45·3	5·7	8·8
2	L-Arabinosylamine [c]	50	44·9	6·1	— [d]	$C_{12}H_{16}N_2O_7,H_2O$	45·3	5·7	—
3	D-Galactosylamine [e]	74	44·5	6·1	9·1	$C_{13}H_{18}N_2O_8,H_2O$	44·8	5·8	8·0
4	D-Glucosylamine	60	43·8	5·8	8·4	$C_{13}H_{18}N_2O_8,1\frac{1}{2}H_2O$	43·7	5·9	7·8

No.	$[\alpha]_D^{24}$	*c* in H_2O	Mols. periodate consumed	$[\alpha]_D^{24}$ after periodate [f]	R_F in system A	Absorption in 0·01M-phosphate (pH 7) $\lambda_{max.}$ (mμ)	ε × 10^{-3}	Absorption in M-KCN $\lambda_{max.}$ (mμ)	ε × 10^{-3}
1	−2·6°	3·4	1·9 [g]	+58°	0·12,[h] 0·18	266	4·7	323·5	6·4
2	+31·7	3·1	2·0 [i]	+51·5	0·11,[j] 0·16	266	4·7	325	5·59
3	+58·5	1·3	2·2 [i]	+87	0·09	266	4·9	325	6·3
4	+32·5	2·3	1·9 [g]	+71	0·09	266	4·75	324	6·6

[a] Nitrogen analyses of the hygroscopic formates were variable. [b] The *chloride*, obtained by freeze-drying a solution in 0·1N-hydrochloric acid, crystallised from methanol as needles, m. p. 160—163°, $[\alpha]_D^{24}$ +5·3° (*c* 1·6 in H_2O) (Found: C, 45·5; H, 5·4; Cl, 12·5; N, 10·2. $C_{11}H_{15}ClN_2O_5$ requires C, 45·45; H, 5·2; Cl, 12·2; N, 9·6%); R_F 0·12 in solvent A. [c] The *chloride*, $[\alpha]_D^{24}$ +31° (*c* 1·0 in H_2O), had R_F 0·12 and 0·18 in solvent A (Found: C, 42·6; H, 5·7; Cl, 11·5; N, 9·5. $C_{11}H_{15}ClN_2O_5,H_2O$ requires C, 42·8; H, 5·55; Cl, 11·5; N, 9·1%). [d] Found: O, 40·3. $C_{12}H_{16}N_2O_7,H_2O$ requires O, 40·2%. [e] The *chloride*, $[\alpha]_D^{24}$ +59·4° (*c* 1·3 in H_2O), had R_F 0·09 in solvent A (Found: C, 42·5; H, 6·0; Cl, 10·4; N, 8·2. $C_{12}H_{17}ClN_2O_6,H_2O$ requires C, 42·55; H, 5·65; Cl, 10·5; N, 8·3%). [f] Based on the initial concentration of the glycosyl compound. [g] After 4 days at 0°. [h] About 90% of the total material. [i] After 2 hr. at 0°. [j] About 80% of the total material.

at 277 and 307 mμ and a minimum at 281·5 mμ. The nucleoside consumed 2·1 mols. of periodate during 3 days at 0°; the product had $[\alpha]_D^{24}$ −65° (*c* 0·45 in H_2O) (lit.,[8] $[\alpha]_D^{20}$ −71°, after consumption of 1·94 mols. of periodate during 2 days). After oxidation with periodate, reduction with borohydride, and hydrolysis with hydrochloric acid as described previously, the nucleoside afforded glycerol and no ethylene glycol. Enzymic analysis of the nucleoside with β-nicotinamide riboside phosphorylase [17] showed the presence of <3% of β-anomer; this is the approximate experimental error of the assay.

A sample of α-nicotinamide nucleoside (prepared enzymically from α-nicotinamide adenine dinucleotide [11] by the combined action of nucleotide pyrophosphatase and 5′-nucleotidase of *Crotalus adamanteus* venom at pH 8·0 in the presence of 0·05M-magnesium chloride) had $[\alpha]_D^{24}$ +52°. It consumed 1·65 mols. of periodate during 3 days at 0°, and afforded glycerol after successive treatments with periodate, borohydride, and hydrochloric acid. The absorption of

[16] Connell, Dixon, and Hanes, *Canad. J. Biochem. Physiol.*, 1955, **33**, 416.
[17] Rowen and Kornberg, *J. Biol. Chem.*, 1951, **193**, 497.

this nucleoside in M-potassium cyanide closely resembled that of the nucleoside derived from the amine, with a decrease of extinction at 329 mμ and the appearance of inflexions at 277 and 307 mμ 1 hr. after mixing.

Phosphorylation of the Foregoing Nucleoside.—The remaining half of the crude nucleoside was dissolved in water (5·5 ml.), put on a column (5 cm. × 1 cm.²) of Amberlite CG-50 (NH_4^+ form), and eluted with 0·1M-ammonium formate. Part of the eluate, containing 0·15 mmole of nucleoside formate, was freeze-dried for 3 days, and the residue was dried over phosphoric oxide at 20°/0·1 mm. for 7 days. The dry nucleoside formate was phosphorylated and the nucleotide fraction was isolated as described by Haynes, Hughes, Kenner, and Todd.[1] After further purification by paper chromatography (solvent A), the nucleotide (R_F 0·07) was obtained as a colourless powder (15 mg.), $[\alpha]_D^{24}$ +60° (*c* 0·36 in H_2O) (lit.,[11] $[\alpha]_D^{23}$ +58°). The product was not separated from β-nicotinamide nucleotide [5] on chromatography in solvent A (R_F 0·07) or in solvent B (R_F 0·61). On treatment with 5′-nucleotidase from potato [18] at pH 8·8 in the presence of 0·05M-magnesium chloride, 80% of the phosphorylated product was converted into nicotinamide nucleoside. On electrophoresis in 0·05M-borate (pH 9·2) the product was separated into a fraction with the same anionic mobility at β-nicotinamide nucleotide (80—85% of total material absorbing light at 267 mμ) and a minor fraction with 36% of the mobility of the 5′-phosphate. The product had $\lambda_{max.}$ (H_2O) 267 mμ (ε 4800, calculated from the phosphorus content). In M-potassium cyanide, 3 min. after mixing, the product had $\lambda_{max.}$ 330 mμ (ε 6200); after 1 hr. the extinction at 330 mμ had decreased by 23% and the absorption curve had inflexions at 257 and 307 mμ. The concentration of β-nicotinamide nucleotide, measured by a coupled assay with adenosine triphosphate nicotinamide nucleotide adenylyltransferase and alcohol dehydrogenase [5] was 0·8% of the total nucleotide, indicating the presence of α- and β-anomers in proportions of approximately 99 : 1.

1-*Glycosylpyridiniumcarboxylates from Unprotected Glycosylamines.*—D-Ribosylamine (9 g.) and 1-(2,4-dinitrophenyl)-3-ethoxycarbonylpyridinium chloride (9 g.) were reacted essentially as described for the corresponding amide. After removal of dinitroaniline the combined filtrate was passed through a column (18 cm. × 4·5 cm.²) of Amberlite CG-400 resin ($HCOO^-$ form) to remove nicotinic acid and to hydrolyse ethoxycarbonylpyridinium compounds to the corresponding pyridiniumcarboxylates. The column was washed with water and the combined eluant was passed through a column (10 cm. × 2 cm.²) of Amberlite CG-50 (NH_4^+ form) to remove unhydrolysed esters. The combined eluate at 2° was passed through a column (20 cm. × 4·5 cm.²) of Amberlite CG-120 resin (H^+ form) and the column was washed with water (2 l.) to remove ribose. The carboxylate was eluted with 0·1N-ammonium hydroxide at 2° and was detected by its absorption at 266 mμ after 1·6 l. of eluate had been collected; all the carboxylate was contained in the next 400 ml. Fractions containing nucleoside were combined, concentrated to 100 ml. at 30°/15 mm., and then freeze-dried. The product was dissolved in dry methanol (40 ml.) and the solution was filtered into dry ether (500 ml.). 1-D-*Ribopyranosylpyridinium 3-carboxylate* (5·0 g.), $[\alpha]_D^{24}$ −21·4° (*c* 2·0 in water) was obtained as a white hygroscopic powder (Found: C, 50·0; H, 5·6; N, 5·4. $C_{11}H_{13}NO_6,\frac{1}{2}H_2O$ requires C, 50·0; H, 5·3; N, 5·3%), $\lambda_{max.}$ (H_2O) 266 mμ (ε 5050), $\lambda_{max.}$ (M-KCN) 316 mμ (ε 4600). The carboxylate had R_F 0·14 (solvent A) and consumed 1·95 mols. of periodate during 90 hr. at 0°. Other 1-*glycosylpyridiniumcarboxylates* prepared in this way from unprotected glycosylamines are shown in Table 2.

In one experiment the red intermediate *ethyl* 2-(2,4-*dinitroanilinomethylene*)-5-*oxopent*-3-*enoate* was isolated as orange needles, m. p. 163° (from 80% ethanol) (Found: C, 49·8; H, 3·9; N, 12·1. $C_{14}H_{13}N_3O_7$ requires C, 50·15; H, 3·9; N, 12·5%).

Reaction of D-*Ribosylamine with* 1-(2,4-*Dinitrophenyl*)*pyridinium Chloride.*—When D-ribosylamine (1·5 g.) was treated with 1-(2,4-dinitrophenyl)pyridinium chloride (2·5 g.) in the conditions described above 1-(2,4-dinitroanilino)penta-1,3-dien-5-al (1·9 g.) precipitated and did not redissolve. After recrystallisation from acetone the compound had m. p. 176—178° (lit.,[19] 180°) (Found: C, 49·9; H, 3·95; N, 15·7. Calc. for $C_{11}H_9N_3O_5$: C, 50·2; H, 3·45; N, 16·0%). No glycosylpyridinium compound could be detected on chromatography of the aqueous phase.

1-(2,3,5-*Tri*-O-*benzoyl*-D-*ribofuranosyl*)*pyridinium Chlorides.*—These *salts* (Table 3) were prepared from 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride and the pyridines as described for the corresponding reaction with nicotinamide [1] or by a modification in which the reaction was

[18] Kornberg and Pricer, *J. Biol. Chem.*, 1950, **186**, 557.
[19] Zincke, Heuser, and Möller, *Annalen*, 1904, **333**, 296.

carried out in methyl cyanide at 38°. An attempt to prepare a similar derivative from 3-cyanopyridine in nitromethane only afforded traces of a quaternary compound which was detected by its absorption in 0·5M-methanolic potassium cyanide ($\lambda_{max.}$ 333 mμ).

Decomposition of 3-*Carbamoyl*-1-(2,3,5-*tri*-O-*benzoyl*-D-*ribofuranosyl*)*pyridinium Chloride in*

TABLE 2.

1-Glycosylpyridiniumcarboxylates from unprotected glycosylamines.

			Found (%)				Requires (%)		
No.	Generating amine	Yield (%)	C	H	N	Formula	C	H	N
1	D-Xylosylamine [a]	60	48·2	5·6	5·6	$C_{11}H_{13}NO_6,H_2O$	48·35	5·5	5·1
2	D-Galactosylamine	57	47·5	5·7	4·8	$C_{12}H_{15}NO_7,H_2O$	47·5	5·65	4·6
3	D-Glucosylamine	77	46·5	6·1	4·9	$C_{12}H_{15}NO_7,1\frac{1}{2}H_2O$	46·2	5·8	4·5

				Absorption in H_2O		Absorption in M-KCN	
No.	$[\alpha]_D^{24}$	(*c* in H_2O [b])	R_F in system A	$\lambda_{max.}$ (mμ)	$\varepsilon \times 10^{-3}$	$\lambda_{max.}$ (mμ)	$\varepsilon \times 10^{-3}$
1	+5°	2·2	0·1,[c] 0·16	265·5	4·9 [d]	314	5·3 [d]
2	+65	2·0	0·08,[e] 0·11	265	4·8	315	4·8
3	+41	1·5	0·08	265·5	4·65	315	5·2

[a] The *anhydrous compound* had m. p. 176—178° (from methanol), $[\alpha]_D^{24}$ +6·4° (*c* 2·5 in H_2O), R_F 0·1 in solvent A (Found: C, 51·9; H, 5·7. $C_{11}H_{13}NO_6$ requires C, 51·8; H, 5·1; N, 5·5%). [b] The product from xylosylamine consumed 1·5 mols. of periodate in 90 hr. at 0° and the product from galactosylamine consumed 1·2 mols. of periodate in 90 hr. at 0°. The product from glucosylamine consumed 1·0 mols. of periodate in 18 hr. at 0° and 1·6 mols. in 90 hr. at 0°. [c] More than 90% of the total material. [d] Crystalline material was used for these measurements [e] More than 95% of the total material.

the Presence of Nicotinamide.—A solution of the chloride (49 μmoles) and nicotinamide (100 μmoles) in methyl cyanide (4 ml.) was kept at 37°. The nucleoside content (calculated from the extinction at 325 mμ of 0·01 ml. of the solution in 5·0 ml. of 0·5M-methanolic potassium cyanide) was 39, 30, and 16 μmoles, respectively, after 3, 7, and 14 days.

TABLE 3.

1-(2,3,5-Tri-*O*-benzoyl-D-ribofuranosyl)pyridinium chlorides (I; R′ = tribenzoylribofuranosyl) from tribenzoylribosyl chloride and pyridines.

				Found (%)					Required (%)			
R	Yield (%)	$[\alpha]_D^{24}$	*c* in MeOH	C	H	Cl	N	Formula	C	H	Cl	N
H	20 [a]	−17° [a]	1·2	64·1	5·0	5·8	2·5	$C_{31}H_{26}ClNO_7,H_2O$	64·4	4·9	6·1	2·4
	23 [b]											
CO_2Et	60 [a, c]	−8 [a]	1·5 [a]	62·8	5·1	5·9	2·2	$C_{34}H_{30}ClNO_9,H_2O$	62·8	5·0	5·45	2·15
	20 [b, c]	−1 [b]	1·6 [b, d]									
$CS{\cdot}NH_2$	13 [a]	−90	1·2	61·2	4·7	—	4·8	$C_{32}H_{27}ClN_2O_7S,\frac{1}{2}H_2O$ [e]	61·2	4·5	—	4·5
$CO{\cdot}NHMe$ [f]	26 [g]	−63	0·54	62·7	5·0	5·5	4·6	$C_{33}H_{29}ClN_2O_8,H_2O$	62·4	4·9	5·6	4·4
$CO{\cdot}NMe_2$ [h] ...	14 [g]	−33	0·8	62·9	5·1	5·4	4·5	$C_{34}H_{31}ClN_2O_8,H_2O$	62·9	5·1	5·5	4·3
$CO{\cdot}NEt_2$ [i] ...	16 [a]	−12·6	1·3	63·9	5·6	5·0	5·0	$C_{36}H_{35}ClN_2O_8,H_2O$	63·85	5·5	5·2	4·1
	36 [j]	−2·3	1·5	—	—	5·1	—		—	—	5·2	—

[a] Reaction in nitromethane at 2°. [b] Reaction in methyl cyanide at 38° for 5 days. [c] λ_{max} (0·5M-methanolic KCN) 330 mμ (ε 6600). [d] In $CHCl_3$. [e] Found: S, 5·1. $C_{32}H_{27}ClN_2O_7S,\frac{1}{2}H_2O$ requires S, 5·1%. [f] $\lambda_{max.}$ (MeOH) 270 mμ (ε 8150). [g] Reaction in methyl cyanide at 2° for 5 days. [h] $\lambda_{max.}$ (MeOH) 274 mμ (ε 7500). [i] $\lambda_{max.}$ (MeOH) 272·5 mμ (ε 7300). [j] Reactants kept at 80° for 3 hr. without solvent.

3-*Carbamoyl*-1-(2,3,5-*tri*-O-*benzoyl*-D-*arabinofuranosyl*)*pyridinium Bromide.*—2,3,5-Tri-*O*-benzoyl-α-D-arabinofuranosyl bromide (20 g.) and nicotinamide (7 g.) were kept at 25° for 2 days in methyl cyanide (700 ml.). After isolation by the usual method [1] the *pyridinium bromide* (17·5 g.) was obtained as an amorphous powder $[\alpha]_D^{24}$ +24° (*c* 1·8 in MeOH) (Found: C, 57·9; H, 4·2; N, 4·4. $C_{32}H_{27}BrN_2O_8,H_2O$ requires C, 57·75; H, 4·4; N, 4·2%), $\lambda_{max.}$ (MeOH) 267 mμ (ε 7200), $\lambda_{max.}$ (0·5M-methanolic KCN) 323 mμ (ε 6700).

1-D-*Ribofuranosylpyridinium Chloride.*—A solution of the tribenzoate (2·2 g.) (see Table 3) in dry methanol (250 ml.) was saturated with ammonia at 0° and kept at 2° for 48 hr. After purification by the method described for nicotinamide nucleosides,[1] 1-D-*ribofuranosylpyridinium chloride* (0·75 g.) was obtained as a colourless hygroscopic glass (Found: C, 44·8; H, 5·3; Cl, 13·0. $C_{10}H_{14}ClNO_4,H_2O$ requires C, 45·2; H, 6·1; Cl, 13·3%), $\lambda_{max.}$ (0·01M-phosphate; pH 7·0) 260 mμ (ε 4850). The compound had R_F 0·26 in system A and R_F 0·63 in system B.

This nucleoside (0·7 g.) was phosphorylated with phosphoryl chloride, and the monophosphate was isolated and purified by ion-exchange as described previously for the preparation of nicotinamide nucleotide.[1] The product was obtained as an amorphous hygroscopic solid (0·09 g.), $[\alpha]_D^{24}$ +30° (*c* 0·7 in H_2O), and had a phosphorus–ribose ratio of 1·00 : 0·97; $\lambda_{max.}$ 260 mμ (ε 4500) in 0·1N-hydrochloric acid, 0·01M-phosphate (pH 7·0), or M-potassium cyanide. On treatment with 5′-nucleotidase (from potato), under the conditions described for the hydrolysis of nicotinamide nucleotide, conversion into the nucleoside was complete in 24 hr. at 37°. The nucleotide had R_F 0·1 in system A and R_F 0·34 in system B, and remained at the origin on electrophoresis in 0·04M-citrate, pH 4·1.

1-D-*Ribofuranosyl*-3-*thiocarbamoylpyridinium Chloride.*—3-Thiocarbamoyl-1-(2,3,5-tri-*O*-benzoyl-D-ribofuranosyl)pyridinium chloride (1·5 g.) was treated with methanolic ammonia in the usual way to remove the benzoyl groups. The *ribofuranosyl chloride* (0·5 g.) was obtained as a hygroscopic, amorphous brown powder (Found: C, 38·4; H, 5·8; S, 9·1. $C_{11}H_{15}ClN_2O_4S,2H_2O$ requires C, 38·5; H, 5·6; S, 9·35%), $\lambda_{max.}$ (0·01M-phosphate; pH 7·0) 264·5 mμ (ε 6000), $\lambda_{max.}$ (M-KCN) 345 mμ (ε 5600). The nucleoside had R_F 0·29 in system A and R_F 0·57 in system B, as did the nucleoside prepared from thionicotinamide adenine dinucleotide [13,20] with nucleotide pyrophosphatase and 5′-nucleotidase from potato.

Phosphorylation of the chloride (0·25 g.) [1] afforded the impure nucleotide (16 mg.) as a colourless amorphous solid which had a molar phosphorus–ribose ratio of 1·00 : 1·14. The nucleotide was completely converted into nucleoside by 5′-nucleotidase from potato at pH 8·8 in the presence of 0·02M-magnesium chloride. The nucleotide had R_F 0·09 in system A and R_F 0·28 in system B, as did the nucleotide obtained from thionicotinamide adenine dinucleotide on treatment with nucleotide pyrophosphatase. The phosphorylated product was converted into thionicotinamide adenine dinucleotide on treatment with adenosine triphosphate and adenosine triphosphate nicotinamide mononucleotide adenylyl transferase.[20]

1-D-*Ribofuranosylpyridinium-3-carboxylate.*—3-Ethoxycarbonyl-1-(2,3,5-tri-*O*-benzoyl-D-ribofuranosylpyridinium chloride (0·8 g.) in methanol (40 ml.) was mixed with 8 g. of Dowex-2 (HCO_3^- form, dried at 65°/10 mm. for 2 hr.) in methanol (40 ml.). After 3 hr. the mixture was added to a column (4 cm. × 2 $cm.^2$) of the same resin. The column was washed with methanol (100 ml.), solvent was removed, and the residual syrup was dissolved in water (20 ml.). The solution was passed through a column (15 cm. × 2 $cm.^2$) of Amberlite CG-120 (H^+ form) which was then washed with water (500 ml.). The impure nucleoside (0·18 g.) was displaced with 0·5N-ammonium hydroxide and was collected in an 80 ml. fraction after the passage of 300 ml. of eluant. Traces of ribose and nicotinic acid were removed by a second fractionation in the same system. The *pyridiniumcarboxylate* was obtained as a colourless amorphous powder (0·15 g.), $[\alpha]_D^{24}$ −14° (*c* 1·3 in H_2O) (Found: C, 48·4; H, 5·2; N, 4·8. $C_{11}H_{13}NO_6,H_2O$ requires C, 48·35; H, 5·5; N, 5·1%), $\lambda_{max.}$ (H_2O) 265 mμ (ε 4600), $\lambda_{max.}$ (M-KCN) 316 mμ (ε 4700). The nucleoside had R_F 0·16 in system A as did the nucleoside prepared by treatment of nicotinic acid nucleotide [21] with 5′-nucleotidase.

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[20] Jackson, Ph.D. Thesis, University of Adelaide, 1962.
[21] Atkinson and Morton, *Nature*, 1960, **188**, 58.