734. *Codehydrogenases. Part II.* A Synthesis of Nicotinamide Nucleotide.*

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The acyl groups can be satisfactorily removed from 3-*N*-(poly-*O*-acylglycosyl)carbamoylpyridinium halides with methanolic ammonia. Thus, nicotinamide nucleoside (I; R = H, Hal = Cl) has been synthesised from 1-acetyl-2 : 3 : 5-tri-*O*-benzoyl-D-ribose, and has been converted by means of phosphoryl chloride in methyl cyanide or nitromethane into nicotinamide nucleotide (II). Comparison of the synthetic nucleotide with that derived from cozymase shows that the former contains minor amounts of the isomeric nucleotide which has the α-configuration at the glycosidic carbon.

Nicotinamide nucleotide (II) has been produced from diphosphopyridine nucleotide (cozymase) by the action of a specific pyrophosphatase.[1] It is correspondingly a valuable starting material for the synthesis of diphosphopyridine nucleotide,[2] and we have therefore undertaken its synthesis from nicotinamide and D-ribose through the nucleoside (I; R = H).

The synthesis of a dihydro-derivative of the nucleoside (I; R = H) † was described in Part I,[3] but its great instability, particularly towards acid, rendered it an unsuitable starting point for further elaboration of the nucleotide structure. This dihydro-derivative had been prepared from the triacetyl quaternary riboside bromide (I; R = Ac, Hal = Br) by reduction with sodium dithionite and subsequent deacetylation with methanolic ammonia. Hitherto ammoniacal deacylation of the quaternary glycosides themselves has been regarded as prohibited by their notorious instability under alkaline conditions. Consequently acidic hydrolysis has been employed and, when most of our work had been completed, the application of this method to the acetylated ribofuranoside was described.[4] However, in our experience, there is also considerable hydrolysis of the glycosidic link, particularly with the ribofuranoside. Therefore our observation that ammoniacal deacetylation can indeed be applied satisfactorily to quaternary glycosides, provided that care is taken, has been of the utmost assistance to us. By this means the quaternary glucopyranoside of nicotinamide has been obtained crystalline for the first time, and the preparation of the ribofuranoside is also satisfactory.

For the routine preparation of nicotinamide nucleoside (I; R = H) some improvements in our earlier condensation method [3] were made. Tri-*O*-acetylribofuranosyl chloride has been preferred to the bromide as a reagent for the synthesis of purine nucleosides,[5] and it has proved superior in the present work. The acetylated nucleoside chloride (I; R = Ac, Hal = Cl) was obtained crystalline and in better yield than the amorphous bromide. Meanwhile Fletcher and his colleagues showed that 1-*O*-acetyl-2 : 3 : 5-tri-*O*-benzoyl-D-ribose can be obtained from ribose through methyl ribofuranoside in one series of operations.[6] Therefore we adopted this beautifully crystalline substance as our starting material in preference to 1 : 2 : 3 : 5-tetra-*O*-acetyl-D-ribose,[7] although, as described in the Experimental section, the latter can indeed be prepared in a similar fashion but lower yield. The benzoylated nucleoside (I; R = Bz, Hal = Cl) was obtained in good yield

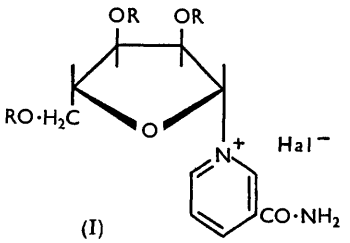
* Part I, *J.*, 1950, 303.

† The majority, if not all, of the synthetic materials described here are mixtures of β- and α-anomers, but generally the β-form (as shown in the structural formulæ) predominates. This point is discussed later in the text.

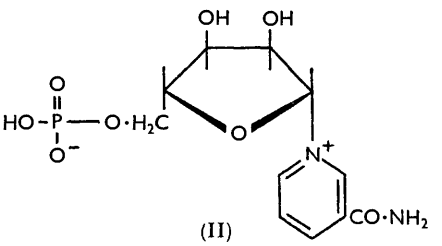
[1] Kornberg and Pricer, *J. Biol. Chem.*, 1950, **186**, 557.
[2] Hughes, Kenner, and Todd, following paper.
[3] Haynes and Todd, *J.*, 1950, 303.
[4] Viscontini, Marti, and Karrer, *Helv. Chim. Acta*, 1954, **37**, 1373.
[5] Davoll, Lythgoe, and Todd, *J.*, 1948, 967.
[6] Ness, Diehl, and Fletcher, *J. Amer. Chem. Soc.*, 1954, **76**, 763.

from the chloro-sugar in either methyl cyanide or, slightly better, nitromethane solution, but it could not be crystallised. The benzoyl groups were removed by methanolic ammonia without difficulty.

The nicotinamide nucleoside (I; R = H, Hal = Cl) prepared through either the acetylated or the benzoylated intermediate was a hygroscopic amorphous powder with the correct elementary composition. Its furanoside nature followed from its mode of synthesis and from its consumption of scarcely more than 1 mol. of periodate during 20 hr. at 0°. Viscontini, Marti, and Karrer [4] concluded that a nucleoside prepared from 1 : 2 : 3 : 5-tetra-*O*-acetyl-D-ribose in a different way [4] had a pyranose structure because it consumed 2 mols. of periodate during several days at room temperature. However, we have found that quaternary nicotinamide derivatives, such as *N*-benzyl-3-carbamoylpyridinium chloride and 3-*N*-(2′ : 3′ : 4′ : 6′-tetra-*O*-acetyl-D-glucopyranosyl)carbamoylpyridinium bromide, but not nicotinamide itself, are oxidised by periodate at room temperature, while they are stable at 0°. The evident need to work at the lower temperature recalls the similar case of riboflavin derivatives.[8] A later examination [9] of the initial oxidation product by reduction, hydrolysis, and paper chromatography convinced the Swiss workers that their material was actually furanoside.



(I)



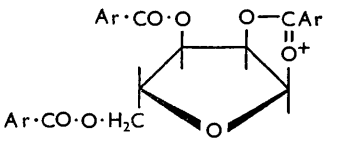
(II)

A much more difficult question is whether the synthetic and the natural nucleoside have the same configuration at the glycosidic centre. Unfortunately the optical rotation of the nucleoside obtained by enzymic degradation of cozymase [10] has not been recorded. The synthetic nucleotide (II) has $[\alpha]_D$ −38° (in H_2O) while the anomer, obtained in an ingenious way from an impurity in some samples of cozymase, has $[\alpha]_D$ +58°.[11] These values support the customary assumption that cozymase is a derivative of β-D-ribofuranose (cf. formulæ I and II), and it may be reasonably supposed that the anomeric nucleosides have rotations similar to those of the nucleotides. These considerations encouraged us to believe that our synthetic nucleoside, having $[\alpha]_D$ −28°, consisted at any rate mainly of the natural β-anomer. Recently a contrary view has been taken, for Viscontini and Hürzeler-Jucker [12] have deduced from the velocities of periodate oxidation of various nicotinamide nucleosides that the ribofuranoside [4] having $[\alpha]_D$ +49° is the β-anomer. Their conformational arguments appear to have several defects, which it would be out of place to examine here. We prefer to attach the usual significance to the sign of rotation even though there are considerable variations among the glycosylamines due to their differing light absorptions.[13] We recall that, for example, the anomeric ribofuranosides of 5 : 6-dimethylbenziminazole obey the rule (α- having $[\alpha]_D$ +14°, β- $[\alpha]_D$ −44°),[14,15] and that their configurations are assured by the *X*-ray analysis [16] of vitamin B_{12}. If we are wrong,

[7] Howard, Lythgoe, and Todd, *J.*, 1947, 1052.
[8] Forrest and Todd, *J.*, 1950, 3295.
[9] Viscontini, Hoch, Marti, and Karrer, *Helv. Chim. Acta*, 1955, **38**, 646.
[10] Schlenk, *Arch. Biochem.*, 1943, **3**, 93.
[11] Kaplan, Ciotti, Stolzenbach, and Bachur, *J. Amer. Chem. Soc.*, 1955, **77**, 815.
[12] Viscontini and Hürzeler-Jucker, *Helv. Chim. Acta*, 1956, **39**, 1620.
[13] Cf. Howard, Kenner, Lythgoe, and Todd, *J.*, 1946, 861.
[14] Holly, Shunk, Peel, Cahill, Lavigne, and Folkers, *J. Amer. Chem. Soc.*, 1952, **74**, 4521.
[15] Johnson, Miller, Mills, and Todd, *J.*, 1953, 3061.
[16] Hodgkin *et al.*, *Proc. Roy. Soc.*, 1957, *A*, in the press.

then cozymase is an α-glycoside in the nicotinamide moiety because our synthetic nucleoside has been correlated with it, first through phosphorylation to the nucleotide (II) and, secondly, through synthesis of cozymase itself.[2]

Phosphorylation of the nucleoside presented difficulties. It was insoluble in organic solvents and sufficiently unstable to render improbable schemes for protection of the 2'- and 3'-hydroxyl groups with, say, an *iso*propylidene residue. Therefore, in collaboration with Dr. R. L. Hinman, we explored direct routes to nucleosides from ribose derivatives already carrying at the 5'-position a reactive group, such as iodo, or even a protected phosphoryl residue. The results were in general disappointing and we turned to the æsthetically less satisfying direct phosphorylation of the nucleoside. Some preliminary experiments with phosphoryl chloride in moist pyridine, a reagent used successfully in the riboflavin field,[8] were encouraging, but the nucleoside had a half-life of only 18 hr. in pyridine at room temperature, being converted into another glycoside, identified with quaternary pyridine ribofuranoside by paper chromatography (*i.e.*, there had been an exchange of nicotinamide and pyridine). The corresponding reaction with 2 : 6-lutidine was much slower, but lutidine reacted with phosphoryl chloride forming a yellow precipitate. The nucleoside however was stable when dissolved in anhydrous *m*-cresol or dimethylformamide or when suspended in methyl cyanide or nitromethane. The last provided the best medium for phosphorylation, although methyl cyanide was almost as good; about 20% of the nucleoside was phosphorylated during four days with phosphoryl chloride at room temperature. The nucleotide fraction was isolated by passage through cation- and anion-exchange resins. It was retained by the latter sufficiently long to effect separation from ribose, but a more efficient technique, cation-exchange chromatography in formic acid solution,[2] was discovered after completion of this work. Fortunately the only nucleotide produced was the 5'-phosphate (II), so far as could be detected by our methods of analysis, *viz.*, treatment with snake-venom 5'-nucleotidase (which caused complete breakdown to



(III)

nucleoside), periodate titration, and both chromatography and electrophoresis on paper in presence of borate ions. Moreover, the paper chromatographic behaviour of the synthetic nucleotide was identical with that of a sample of the degradation product of natural cozymase, generously supplied by Dr. A. Kornberg of the National Institutes of Health, Bethesda.

The optical rotation (−24°) of our synthetic nucleotide corresponded to the presence of β- and α-anomers in 4 : 1 ratio, and this composition was confirmed by the further synthetic experiments described in the following paper.[2] As no satisfactory technique for separating the anomers was available, we considered the possibility of steering the nucleoside synthesis towards the β-anomer. It seemed possible that formation of the latter could arise through nucleophilic attack on the cation (III); if so then an increase in neighbouring-group participation might well offer a solution of the problem. Accordingly the synthesis was repeated with *p*-anisoyl instead of benzoyl residues, and indeed the nucleoside obtained had $[\alpha]_D$ −36° instead of the previous −28°. However, this result was not consistently reproduced and in fact there appeared to be little advantage, apart from the crystallinity of methyl 2 : 3 : 5-tri-*O*-*p*-anisoyl-D-ribofuranoside, in working with anisoyl derivatives. Lack of consistency in the proportions of anomers was also encountered in the benzoyl series. Bearing in mind the aglycone-interchange reaction observed when the nucleoside is dissolved in pyridine (see above), we examined the course of the quaternisation polarimetrically. The optical rotation fell steadily, but it never became negative despite the negative rotation of the isolated product. It may be noted

that the acetylated amorphous nucleoside of Viscontini, Marti, and Karrer [4] had $[\alpha]_D$ +43°, although our crystalline material (I; R = Ac, Hal = Cl) had $[\alpha]_D$ −59°; the chief apparent difference between the preparations was that the Swiss workers used the bromo- instead of the chloro-sugar and there was acetic acid in their reaction medium. At present the factors controlling the proportions of anomers are regrettably obscure.

Experimental

All analytical samples were dried at 20°/10^{-3} mm.

3-(N-β-D-*Glucopyranosylcarbamoyl*)*pyridinium Bromide*.—The crystalline acetylated glucoside [17,3] (20 g.) was dissolved in boiling methanol (500 c.c.), and the solution cooled to 0°, saturated with ammonia gas, and then kept overnight at 0°. The orange solution was then concentrated to 80 c.c. under reduced pressure and set aside. Next day the microcrystalline solid (12 g., 88%) was collected. The colourless *glucoside* (60%), m. p. 157—158° (decomp.), was obtained by charcoal-treatment of an aqueous solution, which was evaporated twice with ethanol; the residue crystallised from hot methanol although the recrystallised material was very sparingly soluble in this solvent. The glucoside had $[\alpha]_D^{18}$ +32·2° (*c* 1·8 in H_2O) and $\varepsilon_{max.}$ 5060 at 265 mμ (Found: C, 40·0; H, 5·1; N, 7·5. $C_{12}H_{17}O_6N_2Br$ requires C, 39·5; H, 4·7; N, 7·7%).

1 : 2 : 3 : 5-*Tetra*-O-*acetyl*-D-*ribose*.—A solution of D-ribose (5 g., dried at 1 mm.) in dry methanol (120 c.c.), containing 1% of hydrogen chloride, was kept for 45 min. at 20° before addition of dry pyridine (6 c.c.). The solvents were then evaporated, finally at 1 mm. The residual syrup was dissolved in dry pyridine (80 c.c.), which was cooled to 0° and treated with acetic anhydride (25 c.c.). The mixture was kept overnight at room temperature and it was then stirred with ice-water for 1 hr. The neutral product was isolated by the usual chloroform-extraction and evaporation. A 50% w/v solution (20 c.c.) of hydrogen bromide in acetic acid was added to a solution of the resulting syrup in acetic acid. After being kept for 45 min. at room temperature the mixture was treated with chloroform (200 c.c.) and ice (200 g.). The chloroform extract was washed at 0° with water, sodium hydrogen carbonate solution, and again water before being run into a stirred suspension of silver carbonate (5 g.) in acetone (90 c.c.) and water (2 c.c.). After 30 min. the solution was filtered and evaporated. The residue was thoroughly dried by two-fold dissolution in pyridine and evaporation, before being kept overnight with dry pyridine (40 c.c.) and acetic anhydride (10 c.c.). The resulting solution was stirred with ice-water for 30 min. and the neutral product isolated by the usual chloroform-extraction. Recrystallisation from ethanol afforded 1 : 2 : 3 :5-tetra-*O*-acetyl-D-ribose, m. p. and mixed m. p. 82°, $[\alpha]_D^{20}$ −12·4° (*c* 4·5 in $CHCl_3$), in agreement with the literature [18] (yield: 2·0 g., 19%).

3-N-(2′ : 3′ : 5′-*Tri*-O-*acetyl*-D-*ribofuranosyl*)*carbamoylpyridinium Chloride*.—A solution of acetochlororibofuranose [5] (from 5 g. of 1 : 2 : 3 : 5-tetra-*O*-acetyl-D-ribose) in methyl cyanide (50 c.c., freshly distilled from phosphoric oxide) was added to a solution of nicotinamide (2·5 g., dried overnight at 120°) in methyl cyanide (400 c.c.) at 0°. A colourless precipitate of nicotinamide hydrochloride started to separate in 10 min., and the mixture was set aside at 0° for 42 hr. The solution was evaporated and chloroform (25 c.c.) added. Next day the precipitate was removed and washed with chloroform (15 c.c.), and the combined filtrate and washings were concentrated to 25 c.c. Rapid addition of dry ether (150 c.c.) gave a fine white precipitate which coagulated when shaken. The liquors were decanted and the precipitate was washed by decantation with dry ether. Nicotinamide was extracted with ethyl acetate from the solid, which was evaporated several times with methanol or chloroform. The residue, which originally gave an emulsion with chloroform, could then be dissolved in that solvent (40 c.c.). Dry ethyl acetate was added cautiously to the pale yellow solution until a sticky precipitate started to appear on the walls of the vessel. There ensued a rapid crystallisation of the colourless acetylated *nucleoside* (2·35 g., 62%), $[\alpha]_D^{18}$ −58·7° (*c* 1·6 in H_2O) (Found: C, 48·6; H, 5·4; N, 6·8. $C_{17}H_{21}O_8N_2Cl$ requires C, 49·0; H, 5·1; N, 6·7%).

3-N-(2′ : 3′ : 5′-*Tri*-O-*benzoyl*-D-*ribofuranosyl*)*carbamoylpyridinium Chloride*.—1-*O*-Acetyl-2 : 3 : 5-tri-*O*-benzoyl-D-ribose, m. p. 130—131°, $[\alpha]_D^{20}$ +44·0° (*c* 1·5 in $CHCl_3$), was prepared

[17] Karrer, Ringier, Büchi, Fritzsche, and Solmssen, *Helv. Chim. Acta*, 1937, **20**, 55.
[18] Zinner, *Chem. Ber.*, 1950, **83**, 153.

essentially according to Kissmann *et al.*[19] The 1-chloro-derivative, $[\alpha]_D^{20}$ +69·7° (*c* 1·9 in nitromethane), was prepared from 8·3 g. in the usual way [5] and dissolved in either nitromethane or methyl cyanide (80 c.c.). This solution was added to a cold solution of nicotinamide (2·8 g.) in the same solvent (200 c.c.). The mixture was kept at 0° for 36 hr. before the nicotinamide hydrochloride was filtered off. Evaporation yielded a foam which was dissolved in ether (100 c.c.) and water (100 c.c.). The aqueous layer was washed with ether (2 × 50 c.c.) and then extracted with chloroform (2 × 50 c.c.). The chloroform extracts were dried (Na_2SO_4) and concentrated to small bulk (20 c.c.). Dropwise addition of this solution (20 c.c.) to dry ether (300 c.c.) precipitated the amorphous benzoylated *nucleoside* (4·0 g., 40%), $[\alpha]_D^{20}$ −44·0° (*c* 3·4 in MeOH) (Found: C, 62·6; H, 4·8; N, 4·7. $C_{32}H_{27}O_8N_2Cl,\frac{1}{2}H_2O$ requires C, 62·8; H, 4·6; N, 4·6%). It was advisable to allow the precipitate to age for several hours before attempting filtration.

3-(N-D-*Ribofuranosylcarbamoyl*)*pyridinium Chloride* (*Nicotinamide Nucleoside*).—(a) *From the benzoyl derivative.* A solution of the foregoing compound (2·0 g.) in dry methanol (200 c.c.) was saturated at 0° with dry ammonia and then set aside at 0° for 18 hr. The yellow gum left on evaporation was dissolved in dry methanol (10 c.c.) and added to dry ethyl acetate (500 c.c.). The flocculent precipitate was collected by centrifugation, dissolved in methanol, and reprecipitated. The *nucleoside* (0·71 g., 73%) was obtained as a very hygroscopic, yellow, amorphous powder, $[\alpha]_D^{20}$ −28·6° (*c* 2·78 in MeOH), consuming 1·14 mols. of periodate during 14 hr. at 0° (Found: C, 43·9; H, 6·2; N, 9·5. $C_{11}H_{15}O_5N_2Cl,\frac{1}{2}H_2O$ requires C, 44·1; H, 5·4; N, 9·4%).

(b) *From the acetyl derivative.* The crystalline 3-*N*-(2′ : 3′ : 5′-tri-*O*-acetyl-D-ribofuranosyl)-carbamoylpyridinium chloride was deacetylated in the same way (Found: C, 44·0; H, 5·9; N, 9·2%).

Nicotinamide Nucleotide.—A suspension of 3-*N*-D-ribofuranosylcarbamoylpyridinium chloride (nicotinamide nucleoside) (7·44 g., dried to constant weight at 20°/1 mm.) in dry nitromethane (400 c.c.) was shaken vigorously for 12 hr. To this fine suspension was added water (1 c.c.), followed by phosphoryl chloride (11 c.c., freshly distilled). The mixture was shaken vigorously with exclusion of moisture at 20° during 4 days. The solution was then concentrated to 30 c.c. and diluted with ice-water (500 c.c.). The pH was adjusted to 5·0 with 10% aqueous ammonia, and the solution was concentrated to 250 c.c. before being passed through a column (30 cm. × 20 cm.²) of Dowex-2 anion-exchange resin (acetate form). The column was washed with 0·001N-acetic acid (300 c.c.) to remove the last traces of nucleotide. The combined filtrate and washings, which were free from phosphate and chloride ions, were concentrated to 250 c.c. before being passed through a column (30 cm. × 20 cm.²) of Dowex-50 cation-exchange resin (hydrogen form). The last traces of nucleotide were washed through with water (300 c.c.), and the combined solutions, which were free from nicotinamide and nicotinamide nucleoside, were concentrated to 20 c.c. and freeze-dried. The resulting gummy solid (1·3 g.) contained about 90% of nicotinamide nucleotide, most of the remainder being water; it was used directly for further stages of synthesis.[2]

A solution of this material (0·1 g.) in water (2 c.c.) was brought to pH 7 by addition of ammonia solution and then added to a column (15 cm. × 10 cm.²) of Dowex-2 resin (acetate form). The column was washed with water (1 c.c. per min.), which was collected in 10 c.c. fractions. Generally the first ten tubes contained a little nicotinamide and ribose, and then the nucleotide (detected by absorption at 266 mμ) was eluted in the next ten; sometimes 0·001N-acetic acid was needed to elute the nucleotide at a reasonable rate. The *nicotinamide nucleotide* was recovered by concentration and freeze-drying as a colourless amorphous hygroscopic solid with $[\alpha]_D^{20}$ −24° (*c* 2·0 in water) (Found: C, 36·0; H, 4·5; N, 7·7; P, 8·35. $C_{11}H_{15}O_8N_2P,2H_2O$ requires C, 35·6; H, 5·1; N, 7·6; P, 8·4%). It consumed 1·1 mols. of periodate during 14 hr. at 0°. A mixture of the nucleotide (1 mg.), 0·25M-glycine buffer of pH 8·5 (0·3 c.c.), 0·1M-magnesium chloride (0·1 c.c.), and a solution of *Crotalus atrox* venom (2 mg.) in 0·1M-potassium chloride (0·1 c.c.) was incubated at 37° for 4 hr.; paper chromatography then revealed complete hydrolysis of the nucleotide to nucleoside and inorganic phosphate.

Paper Chromatography.—The nicotinamide derivatives were detected by viewing or photographing the chromatograms in the light of a mercury lamp. Quaternary pyridinium derivatives could also be detected [20] by fuming the paper with ammonia and ethyl methyl ketone for 30 min., which produced fluorescent spots. Ascending chromatography on Whatman No. 1

[19] Kissmann, Pidacks, and Baker, *J. Amer. Chem. Soc.*, 1955, **77**, 18.
[20] Kodicek and Reddi, *Nature*, 1951, **168**, 475.

paper was carried out with butan-1-ol–acetic acid–water in the ratios either 5 : 2 : 3 (system A) or 20 : 3 : 7 (system B). R_F values were (given first for system A): ribose 0·40, 0·27; nicotinamide 0·67, 0·65; nicotinamide nucleoside chloride 0·28, 0·15; *N*-D-ribofuranosylpyridinium chloride 0·47, 0·36; 2 : 6-dimethyl-*N*-D-ribofuranosylpyridinium chloride 0·58, 0·41; nicotinamide nucleotide 0·18, 0·05; inorganic phosphate 0·30 (in A). The benzoyl and *p*-anisoyl derivatives of the nucleoside both had R_F 0·83 in system B. The comparison of the synthetic nucleotide with Dr. Kornberg's sample was also made by descending chromatography during 5 days in propan-2-ol (100 c.c.)–water (50 c.c.)–boric acid (0·5 g.)–ammonia (to pH 9); R_F values were very small.

Methyl 2 : 3 : 5-*Tri*-O-p-*anisoyl*-D-*ribofuranoside*.—A solution of D-ribose (6 g., dried at 20°/1 mm.) in dry methanol (120 c.c.), containing 1% of hydrogen chloride, was kept for 45 min. at 20° before the addition of dry pyridine (6 c.c.). The solvents were evaporated, finally at 1 mm., and the residual syrup was taken up in dry pyridine (75 c.c.). *p*-Anisoyl chloride (32 g.) was added slowly to the stirred solution at 0°. A precipitate of pyridine hydrochloride was formed almost immediately. The solution was kept overnight at room temperature and then poured into ice-water (4 l.). The usual chloroform extraction yielded a neutral product, which crystallised from methanol in needles. Recrystallisation afforded *methyl* 2 : 3 : 5-*tri*-O-p-*anisoyl*-D-*ribofuranoside* (12·5 g., 55%), m. p. 86—88°, $[\alpha]_D^{20}$ +109·5° (*c* 2·0 in $CHCl_3$) (Found, in material dried at 60°: C, 63·6; H, 5·2. $C_{30}H_{30}O_{11}$ requires C, 63·7; H, 5·3%).

1-O-*Acetyl*-2 : 3 : 5-*tri*-O-p-*anisoyl*-D-*ribofuranose*.—The liquors, from which the foregoing substance had been crystallised, were evaporated, finally at 1 mm. The resulting syrup was dissolved in acetic acid (16 c.c.) and treated at 20° with a 50% w/v solution (1 c.c.) of hydrogen bromide in acetic acid. After 20 min. the precipitate of anisic acid was filtered off and the filtrate was diluted with acetic acid (100 c.c.). Water (25 c.c.) was added gradually to the ice-cooled solution, and the neutral product was then isolated by the customary treatment with ice-water and chloroform-extraction. Crystallisation and recrystallisation from ethanol afforded colourless needles of 1-O-*acetyl*-2 : 3 : 5-*tri*-O-p-*anisoyl*-D-*ribofuranose* (3·0 g., 13%), m. p. 123—125°, $[\alpha]_D^{20}$ +84·4° (*c* 2·0 in $CHCl_3$) (Found, in material dried at 70°: C, 62·4; H, 5·0. $C_{31}H_{30}O_{12}$ requires C, 62·6; H, 5·1%). From the liquors there were obtained, by acetylation with acetic anhydride in pyridine and the usual isolation procedure, a further 2·0 g. (9%) of the same compound and another more soluble substance (0·65 g., 3%), m. p. 117—119°, $[\alpha]_D^{20}$ +80·5° (*c* 0·75 in $CHCl_3$) (Found, in material dried at 60°: C, 62·9; H, 5·3%). This substance, which had a very similar infrared spectrum to 1-*O*-acetyl-2 : 3 : 5-tri-*O*-*p*-anisoyl-D-ribofuranose, was probably 2-*O*-acetyl-1 : 3 : 5-tri-*O*-*p*-anisoyl-D-ribofuranose; treatment of it with hydrogen bromide in acetic acid liberated almost 1 mol. of anisic acid.

3-(N-2′ : 3′ : 5′-*Tri*-O-p-*anisoyl*-D-*ribofuranosyl*)*carbamoylpyridinium Chloride*.—Either methyl 2 : 3 : 5-tri-*O*-*p*-anisoyl-D-riboside or the corresponding 1-acetate was converted into the 1-chloro-derivative and brought into reaction with nicotinamide, in the same way as 1-*O*-acetyl-2 : 3 : 5-tri-*O*-benzoyl-D-ribose. The anisoylated *nucleoside* (27%) was obtained as a colourless amorphous powder, $[\alpha]_D^{20}$ −38·4° (*c* 2·5 in MeOH) (Found, in material dried at 50°: C, 60·6; H, 4·8; N, 4·0. $C_{35}H_{33}O_{11}N_2Cl$ requires C, 61·1; H, 4·8; N, 4·0%). Nicotinamide nucleoside chloride with $[\alpha]_D^{20}$ −36° (*c* 2·3 in MeOH) was obtained in 40% yield by the standard ammoniacal deacylation.

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