4-Substituted Nicotinic Acids and Nicotinamides. Part II.[1] The Preparation of 4-Methylnicotinamide Riboside

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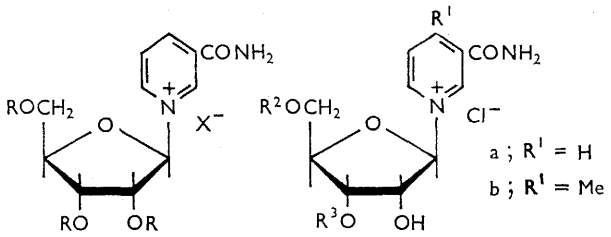
An improved method for the synthesis of nicotinamide ribosides is described. Nicotinamide and 4-methylnicotinamide condense with 3,5-di-*O*-benzoyl-D-ribofuranosyl chloride to give the dibenzoylribofuranosides in good yield. The ribosides obtained by mild hydrolysis contain predominantly the β-anomer.

Ross has discussed the possible utility of 4-substituted nicotinamide derivatives as antitumour agents.[1] A prerequisite for such activity is their *in vivo* conversion into the corresponding 4-substituted NAD † analogues. One biosynthetic pathway by which nicotinamide is converted into NAD involves its riboside as an intermediate.[2] A 4-substituted nicotinamide riboside would be potentially useful, both as a possible *in vivo* precursor of the corresponding 4-substituted NAD and as an intermediate in the chemical synthesis of such a derivative.

Of previous synthetic routes to nicotinamide ribosides,[3-6] only that of Todd and his co-workers [4] has been proved to give the necessary high proportion of the natural β-anomer in the product. In this procedure, reaction between 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride and nicotinamide in acetonitrile at 0° for 36 hr. gave the protected nucleoside (I; R = Bz, X = Cl)



(I) (II)

(40%). The 5-phosphate of the riboside (I; R = H, X = Cl) was shown to contain the α- and β-anomers in the ratio 1 : 4 by comparison of its specific optical rotation with those of α- and β-anomers derived from natural sources. If a similar composition is assumed for the riboside, $[\alpha]_D$ −28°, a knowledge of the rotation of the α-anomer (+46°) [5] gives a value of −46° for the pure β-anomer.

The low solubility of nicotinamide in acetonitrile limits its concentration under the reaction conditions described to only 1% w/v. Whilst a higher concentration is attainable at elevated temperatures, a poor yield of acetylated nicotinamide glucoside has been obtained from nicotinamide and 2,3,4-tri-*O*-acetyl-D-glucopyranosyl chloride in acetonitrile under reflux.[7] Even at 37°, Atkinson and his co-workers observed quite rapid decomposition in this solvent of the α-anomer of the tribenzoylated nicotinamide riboside (I; R = Bz, X = Cl) in the presence of nicotinamide.[5] It was concluded that lower reaction temperatures should be retained, and that an increase in the yield of protected nucleoside should be sought by the use of a more reactive ribosyl halide.

Initially, the reaction between nicotinamide and 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide was investigated. Comparable reaction conditions resulted in a higher yield (61%) of protected riboside (I; R = Bz, X = Br) than in the preparation of the corresponding chloride.[4] Very recently, similar results were claimed by Mel'nikova and Berezovskii,[6] but they gave no optical or analytical data for this product.

The possible utility of the crystalline 3,5-di-*O*-benzoyl-D-ribofuranosyl halides in nucleoside synthesis has been proposed,[8] and the preparation of 1-(3,5-di-*O*-benzoyl-D-ribofuranosyl)pyridinium chloride described.[9] 3,5-Di-*O*-benzoyl-D-ribofuranosyl chloride [10] was found to have several advantages over the corresponding tribenzoate as a reagent for the preparation of a protected nicotinamide riboside. Its reaction with nicotinamide was rapid and quantitative whilst retaining the advantage of stereospecificity. Moreover, crystallisation of nicotinamide from a supersaturated (5% w/v) solution in acetonitrile is not immediate at room temperature, and its reaction with an excess of 3,5-di-*O*-benzoyl-D-ribofuranosyl chloride, being complete within 2 hr., was sufficiently rapid to avoid the separation which occurs during the slower reaction with tri-*O*-benzoyl-D-ribofuranosyl halides.

The complete reaction of nicotinamide greatly simplified the isolation of the product, 3-carbamoyl-1-(3,5-di-*O*-benzoyl-D-ribofuranosyl)pyridinium chloride (IIa; $R^2 = R^3 = Bz$) in 91% yield. Its predominantly β-configuration was implicit in the specific rotation (−37°) of the nucleoside (IIa; $R^2 = R^3 = H$) obtained from it by treatment with methanolic ammonia. The

† Nicotinamide adenine dinucleotide.

[1] W. C. J. Ross, *J. Chem. Soc.* (*C*), 1966, 1816 is taken to be Part I of this series.
[2] H. Grünicke, M. Liersch, M. Hinz, B. Puschendorf, E. Ritcher, and H. Holzer, *Biochim. Biophys. Acta*, 1966, **121**, 228.
[3] M. Visconti, M. Marti, and P. Karrer, *Helv. Chim. Acta*, 1954, **37**, 1373.
[4] L. J. Haynes, N. A. Hughes, G. W. Kenner, and Sir Alexander Todd, *J. Chem. Soc.*, 1957, 3727.
[5] M. R. Atkinson, R. K. Morton, and R. Naylor, *J. Chem. Soc.*, 1965, 610.
[6] L. M. Mel'nikova and V. M. Berezovskii, *Zhur. obschei. Khim.*, 1967, **37**, 1507.
[7] C. Woenckhaus, M. Volz, and G. Pfleiderer, *Z. Naturforsch.*, 1964, **196**, 467.
[8] R. K. Ness and H. G. Fletcher, *J. Org. Chem.*, 1957, **22**, 1465.
[9] R. K. Ness and H. G. Fletcher, *J. Org. Chem.*, 1957, **22**, 1470.
[10] R. K. Ness and H. G. Fletcher, *J. Amer. Chem. Soc.*, 1956, **78**, 4710.

larger negative value was indicative of an even higher content of β-anomer than in the product obtained from the corresponding tribenzoate.[4]

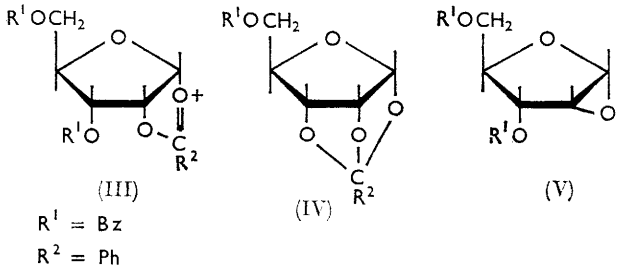
The 4-methyl analogue (IIb; $R^2 = R^3 = H$) was similarly prepared from its dibenzoate (IIb; $R^2 = R^3 =$ Bz), obtained in quantitative yield from 4-methylnicotinamide and 3,5-di-*O*-benzoyl-D-ribofuranosyl chloride. The specific optical rotation (−47°) again indicates a high proportion of the β-anomer.

N.m.r. spectroscopy was used for assessing the proportion of the required β-anomer in the nucleosides (IIa and b; $R^2 = R^3 = H$). The spectrum of each nucleoside contained a unique doublet, attributable to the anomeric proton, H(1), coupled to the adjacent hydrogen atom, H(2), of the ribose moiety. The absence of a second doublet in this region provided strong evidence for the essential anomeric purity of the nucleosides, since the anomeric protons of the two anomers (α and β) would probably have different chemical shifts and coupling constants, owing to their different electronic environments, and to the different dihedral angles between H(1) and the adjacent H(2).

The ratio between the mean amplitudes of the doublet components, and the amplitude of neighbouring background signals gives the minimum ratio of β- to α-anomer in each case. This method of estimating the relative proportions of two components in a mixture by comparing the heights of corresponding signals has been successfully used by Griffin *et al.*,[11] who showed that, when a low proportion of one component is present, the estimated proportion of that component is higher than that actually present. Hence the proportions of β-anomer, 85 and 88%, estimated for the respective nucleosides (IIa and b; $R^2 = R^3 = H$) by the peak-height method represent the minimum contents of this anomer.

The stereospecificity of the reactions between 3,5-di-*O*-benzoyl-D-ribofuranosyl chloride and nicotinamide or its 4-methyl derivative is implicit in the high proportion of β-anomer present in the nucleosides (IIa and b; $R^2 = R^3 = H$) derived from the products of these reactions. The predominance of the β-anomer in the products of the reaction between nicotinamide and tri-*O*-benzoyl-D-ribofuranosyl chloride has been attributed to the formation of the intermediate (III).[4] This stereospecificity is an example of the general observation,[12,13] that the glycosidic linkage formed in the reaction between an acylated glycosyl halide and an aglycone has a *trans*-configuration relative to the 2-*O*-acyl group, whatever the configuration of the reacting halide. Participation of the acyl group *via* an intermediate such as (III) could account for this phenomenon.

The formation of a similar type of intermediate could account for the stereospecificity of the reaction between 3,5-di-*O*-benzoyl-D-ribofuranosyl chloride and nicotinamide, despite the absence of a 2-*O*-acyl group. It is known that the orthoester 5-*O*-benzoyl-1,2,3-*O*-benzylidyne-α-D-ribofuranose (IV) can be formed from 3,5-di-*O*-benzoyl-D-ribofuranosyl chloride.[8] Moreover, both these



compounds react with benzyl alcohol to give a β-glycoside, benzyl 3,5-di-*O*-benzoyl-β-D-ribofuranoside,[8] implying that the orthoester could be an intermediate in the formation from the ribosyl halide of a β-glycoside. Cleavage of the hypothetical intermediate (IV) accompanying attack by nicotinamide could, however, give either a 3,5-dibenzoate (IIa; $R^1 = R^2 =$ Bz) or the isomeric 2,5-dibenzoate. Moreover, it is possible that the 2,5- and 3,5-dibenzoates might interconvert by acyl migration.

The anomeric proton signals in the n.m.r. spectrum (dimethyl sulphoxide–deuterium oxide) of the product of the reaction between 3,5-di-*O*-benzoyl-D-ribofuranosyl chloride and nicotinamide consisted of a pair of doublets, of intensity ratio 1 : 4. The more intense doublet, at higher field, could confidently be assigned to a dibenzoate of the β-configuration, since this configuration predominated in the derived nucleoside (IIa; $R^2 = R^3 = H$).

The product of the reaction between the isomeric 2,5-di-*O*-benzoyl-D-ribofuranosyl chloride [9] and nicotinamide showed, as the sole anomeric proton signal, a singlet at lower field than the doublet ascribed to the β-anomer described above. The β-anomer should also predominate in this new product, since the 2-*O*-benzoyl group could participate by either of the mechanisms embodied in the structures (III) and (IV).

The relative chemical shifts of the anomeric proton signals from the major products of the two reactions could be attributed to the deshielding effect of a neighbouring 2-*O*-benzoyl group, relative to a 2-hydroxy-group. This relationship has been observed among isomeric 2- and 3-*O*-acyl derivatives of purine and pyrimidine ribonucleosides.[11] Hence nucleoside formation from nicotinamide and the two di-*O*-benzoyl-D-ribofuranosyl chlorides was not accompanied by the reorientation of the benzoyl groups between the 2- and 3-positions.

The lower field anomeric proton signal for the product derived from nicotinamide and 3,5-di-*O*-benzoyl-D-ribofuranosyl chloride, since it was a doublet, could not be attributed to the 2,5-dibenzoate of the β-configuration. It was reasonably assigned to the anomeric proton signal for the α-anomer of the 3,5-dibenzoate. Signals attribut-

[11] B. E. Griffin, M. Jarman, C. B. Reese, J. E. Sulston, and D. R. Trentham, *Biochemistry*, 1966, **5**, 3638.
[12] R. S. Tipson, *J. Biol. Chem.*, 1939, **130**, 55.
[13] B. R. Baker, J. P. Joseph, R. E. Shaub, and J. H. Williams, *J. Org. Chem.*, 1954, **19**, 1786.

able to the α-anomer of the 4-methyl analogue were not observed, and a lower limit of *ca.* 86% could be estimated for the content of β-anomer (IIb; $R^2 = R^3 = Bz$) from the relative heights of the anomeric proton signals and the neighbouring background, compared with *ca.* 80% for the β-anomer of the nicotinamide derivative (IIa; $R^2 = R^3 = Bz$) estimated form the relative heights of the two pairs of anomeric proton signals. These values resemble those for the derived nucleosides (IIa and b; $R^2 = R^3 = H$).

Since the reactions between the isomeric di-*O*-benzoyl-D-ribofuranosyl chlorides and nicotinamide yielded no common products, acyl migration could not have occurred, nor could a common intermediate (IV) be invoked. Since its formation from either halide was theoretically possible, one of the reactions may have involved the intermediacy of (IV), provided that subsequent ring opening occurred in the direction dictated by the known structure of the products.

The n.m.r. spectra (deuterium oxide) showed evidence for benzoyl group migration between the 2- and the 3-position. The spectrum of the 2,5-dibenzoate, determined 15 min. after solution, showed two signals, that at lower field being the more intense. After 4 hr., the higher field signal was the major component. The spectrum of the 3,5-dibenzoate showed, after 15 min., only one significant signal, coincident with the higher field signal in the spectrum from the 2,5-isomer. After 4 hr. the corresponding lower field signal had appeared; the relative intensities of the two signals were similar to those observed in the spectrum from the 2,5-dibenzoate after this time. These observations provided further evidence for the structural relationship between the supposed 3,5-dibenzoate (IIa; $R^2 = R^3 = Bz$) and its 2,5-isomer. The equilibrium favoured the 3-isomer.

The intermediate production of a 1,2-epoxide (V) following abstraction of the 2-hydroxy-proton by nicotinamide is an alternative mechanism which would account for the formation of a β-anomer. 1,2-Epoxide formation has been invoked to explain the faster base catalysed hydrolysis of methyl-β-D-glucopyranoside compared with its 2-*O*-methyl derivative.[14] The second step in the present scheme would require attack by the 3-carbamoylpyridinium ion formed in the intermediate step on the epoxide (V). This is reminiscent of the known reaction between pyridinium salts and cyclohexene oxide to form a *trans*-2-hydroxycyclohexylpyridinium salt.[15]

Removal of both benzoyl groups from the protected nucleosides (IIa and b; $R^2 = R^3 = Bz$) required 4 days under the very mild conditions (M-methanolic ammonia at −15°) designed to minimise cleavage of the glycosidic linkage. However, the 3-*O*-benzoyl group was selectively removed in 4 hr.; the 5-*O*-benzoylnucleosides (IIa and b; $R^2 = Bz$, $R^3 = H$) were the only appreciable components after this time. The crystalline nicotinamide derivative (IIa; $R^2 = Bz$, $R^3 = H$) separated from concentrated solutions of the dibenzoate (IIa; $R^2 = R^3 = Bz$) in methanolic ammonia or triethylamine, but its 4-methyl analogue, though formed, remained in solution. The previously observed selective removal of a 3-*O*-acyl group from a ribonucleoside [16] has been attributed to anchimeric assistance of hydrolysis by the neighbouring 2-hydroxy-group.

The conversion of 4-methylnicotinamide riboside (IIb; $R^2 = R^3 = H$) into its 5-phosphate is the next stage, both in the hypothetical biosynthetic pathway to 4-methyl-NAD, and in its chemical synthesis. Protection of the *cis*-diol functions is highly desirable if selective phosphorylation at the 5-position is to be achieved. The riboside (IIb; $R^2 = R^3 = H$) is readily converted into its 2,3-*O*-isopropylidene derivative, 3-carbamoyl-1-(2,3-*O*-isopropylidene-D-ribofuranosyl)-4-methylpyridinium chloride. Since acidic removal of the isopropylidene groups was not accompanied by cleavage of the glycosidic linkage, this compound is a potential intermediate for the preparation of the required 5-phosphate.

EXPERIMENTAL

U.v. absorption spectra for solutions in methanol were measured with a Unicam SP 800 spectrometer. N.m.r. spectra were measured with a Perkin-Elmer R10 spectrometer, operating at 60 Mc./sec., with t-butyl alcohol (in deuterium oxide) and tetramethylsilane (in dimethyl sulphoxide) as internal standards. Ascending paper chromatography was conducted on Whatman no. 1 paper in freshly prepared butan-1-ol–acetic acid–water (5 : 2 : 3). U.v.-absorbing material on paper chromatograms was detected with a Hanovia Chromatolite u.v. lamp. Compounds containing *cis*-diol groups gave a positive reaction to the periodate–rosaniline spray.[17] Quaternary nicotinamide derivatives lacking the 4-methyl group gave fluorescent spots under the u.v. lamp after exposure to the vapour of ammonium hydroxide–methyl ethyl ketone.[18]

T.l.c. was conducted with Kieselgel G (Merck). Spots were detected by exposure to iodine vapour.

Acetonitrile was dried by distillation (P_2O_5). Dimethylformamide was heated under reflux with calcium hydride, then distilled. Ether was dried over sodium. M.p.s were determined with a Kofler hot-stage apparatus, and are corrected.

2,3,5-Tri-O-benzoyl-D-ribofuranosyl Bromide.—1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (1 g., 0·002 mole) was suspended in dry ether (100 ml.) and dry hydrogen bromide was passed into the stirred solution at 0° for 1 hr. After a further 1 hr. at 0°, t.l.c. (ethyl acetate–benzene, 1 : 20) showed no remaining starting material. The solution was evaporated at 25° under reduced pressure, then co-evaporated with dry benzene (4 × 25 ml.) to remove the remaining hydrogen bromide. The product, a pale yellow oil, was identical on t.l.c. (ethyl acetate–benzene, 1 : 20)

[14] J. Jansen and B. Lindberg, *Acta Chem. Scand.*, 1959, **13**, 138.
[15] F. Hayes, L. C. King, and D. E. Peterson, *J. Amer. Chem. Soc.*, 1956, **78**, 2527.
[16] B. E. Griffin and C. B. Reese, *Proc. Nat. Acad. Sci. U.S.A.*, 1964, **51**, 440.
[17] J. G. Buchanan, C. A. Decker, and A. G. Long, *J. Chem. Soc.*, 1950, 3162.
[18] E. Kodicek and K. K. Reddi, *Nature*, 1951, **168**, 475.

with that obtained by treatment of methyl 2,3,5-tri-*O*-benzoyl-D-ribofuranoside with hydrogen bromide in acetic acid.[19]

3-*Carbamoyl*-1-(2,3,5-*tri*-O-*benzoyl*-D-*ribofuranosyl*)-*pyridinium Bromide*.—A solution of nicotinamide (1 g., 0·0082 mole) in dry acetonitrile (200 ml.) was added at 0° to 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide [from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (1 g., 0·002 mole)]. After 48 hr. at −5°, the solvent was removed at 25° under reduced pressure. The residue was partitioned between ether (20 ml.) and water (20 ml.); any unchanged tribenzoylribosyl bromide passed into the ether layer. The aqueous layer was extracted with chloroform (2 × 10 ml.); the water retained unchanged nicotinamide (R_F 0·71). The extract was dried (Na_2SO_4) and concentrated to 5 ml. at 25° under reduced pressure. The solution was added dropwise with stirring to dry ether (50 ml.). The precipitated *tribenzoate* was collected by centrifugation, washed with dry ether, and stored *in vacuo* over phosphorus pentoxide–paraffin wax to give an amorphous powder (R_F 0·94, negative *cis*-diol test, positive methyl ethyl ketone–ammonia test) (0·78 g., 61%), $[\alpha]_D^{25}$ −42° (*c* 1·15 in methanol), $\lambda_{max.}$ 270 mμ (ε 7000), $\lambda_{min.}$ 260 mμ (ε 6400) (Found: C, 59·0; H, 4·4; Br, 12·7; N, 4·11. $C_{32}H_{27}BrN_2O_8$ requires C, 59·35; H, 4·2; Br, 12·35; N, 4·35%).

3-*Carbamoyl*-1-(3,5-*di*-O-*benzoyl*-D-*ribofuranosyl*)*pyridinium Chloride*.—A solution of nicotinamide (1·65 g., 0·0135 mole) in hot, dry acetonitrile (80 ml.) was cooled to room temperature and treated, before crystallisation commenced, with 3,5-di-*O*-benzoyl-D-ribofuranosyl chloride [10] (5·6 g., 0·015 mole). After 2 hr., paper chromatography revealed no unchanged nicotinamide (R_F 0·71). The solution was concentrated to 20 ml. at 25° under reduced pressure, and added dropwise with stirring to dry ether (200 ml.). Unchanged dibenzoylribosyl chloride remained in solution, and the precipitated *dibenzoate* was filtered off, washed with ether, and kept *in vacuo* over phosphorus pentoxide–paraffin wax to give an amorphous powder (R_F 0·86, negative *cis*-diol test, positive methyl ethyl ketone–ammonia test) (6·1 g., 91%), $[\alpha]_D^{25}$ −35° (*c* 1·0 in chloroform), $\lambda_{max.}$ 268 mμ (ε 5500), $\lambda_{min.}$ 258 mμ (ε 5100) (Found: C, 59·55; H, 4·7; Cl, 7·35; N, 5·4. $C_{25}H_{23}ClN_2O_7$ requires C, 60·2; H, 4·65; Cl, 7·1; N, 5·6%), τ [dimethyl sulphoxide–D_2O, 9 : 1 (20% w/v)] 3·06 (d, *J* 5·3 c./sec.) and 3·41 (d, *J* 5·7 c./sec.), assigned respectively to the anomeric H(1) of the α- and the β-anomer, coupled to H(2) of the ribose. The lower field doublet never exceeded 25% of the intensity of the doublet at higher field, and was not clearly seen in the following spectrum. The n.m.r. spectrum [D_2O (15% w/v)] showed (*a*) after 15 min. at room temperature a doublet at τ 3·64 assigned to the anomeric proton H(1) of the 3,5-dibenzoate ($J_{1,2}$ 4·5 c./sec.); (*b*) after 4 hr. an incompletely resolved signal at τ 3·40, assigned to the anomeric proton of the 2,5-dibenzoate, and a doublet at τ 3·66 (*J* 4·5 c./sec.), intensity ratio 1 : 2·4.

3-*Carbamoyl*-1-(2,5-*di*-O-*benzoyl*-D-*ribofuranosyl*)*pyridinium Chloride*.—A solution of nicotinamide (0·1 g., 0·00082 mole) in hot dry acetonitrile (4·5 ml.) was cooled to room temperature and treated immediately with 2,5-di-*O*-benzoyl-D-ribofuranosyl chloride [9] (0·33 g., 0·00088 mole). After 5 min., separation of nicotinamide commenced. The mixture was treated at two 30 min. intervals with a supersaturated solution of nicotinamide (0·1 g.) in acetonitrile (1·5 ml.). After a further 2 hr. the filtered solution was added dropwise with stirring to dry ether (75 ml.); unchanged nicotinamide and dibenzoylribosyl chloride remained in solution. The product was collected by centrifugation, washed with ether, and stored *in vacuo* (P_2O_5–paraffin wax) to give an amorphous powder (R_F 0·86, negative *cis*-diol test, positive methyl ethyl ketone–ammonia test) (0·182 g., 42%), $[\alpha]_D^{25}$ −4° (*c* 1·0 in chloroform) (Found: C, 60·2; H, 4·9; Cl, 7·45; N, 5·8. $C_{25}H_{23}ClN_2O_7$ requires C, 60·2; H, 4·15; Cl, 7·1; N, 5·6%), τ [dimethyl sulphoxide–D_2O, 9 : 1, (20% w/v)] 3·13 [s anomeric proton H(1)], τ [D_2O (15% w/v)] (*a*) (after 15 min. at room temperature) 3·38 [d, $J_{1,2}$ 1·5 c./sec., H(1) of 2,5-dibenzoate] and 3·65 [d, $J_{1,2}$ 4·5 c./sec., H(1) of 3,5-dibenzoate], intensity ratio 1·4 : 1; (*b*) (after 4 hr.) 3·4 (incompletely resolved) and 3·65 (d, *J* 4·5 c./sec.), intensity ratio 1 : 2·8.

3-*Carbamoyl*-1-(5-O-*benzoyl*-D-*ribofuranosyl*)*pyridinium Chloride*.—A solution of 3-carbamoyl-1-(3,5-di-*O*-benzoyl-D-ribofuranosyl)pyridinium chloride (2 g., 0·004 mole) in triethylamine–methanol (1 : 20, v/v; 5 ml.) was kept at 0°. After 4 hr. the *monobenzoate* was the only significant component. Crystallisation commenced after 8 hr. and was complete in 48 hr. The product, which contained traces of dibenzoate and fully debenzoylated material, was filtered off, washed with ethanol, and stored *in vacuo* ($CaCl_2$) (yield 1·06 g., 67%). It gave needles, m.p. 166—168° (from methanol), R_F 0·69, positive *cis*-diol test, positive methyl ethyl ketone–ammonia test, $[\alpha]_D^{25}$ −110° (*c* 2·5 in water), $\lambda_{max.}$ 268 mμ (ε 5500), $\lambda_{min.}$ 256 mμ (ε 4900) (Found: C, 54·65; H, 4·85; Cl, 9·35; N, 7·05. $C_{18}H_{19}ClN_2O_6$ requires C, 54·75; H, 4·85; Cl, 9·0; N, 7·1%).

3-*Carbamoyl*-1-(D-*ribofuranosyl*)*pyridinium Chloride*.—A solution of 3-carbamoyl-1-(3,5-di-*O*-benzoyl-D-ribofuranosyl)pyridinium chloride (3 g., 0·006 mole) in M-methanolic ammonia (75 ml.) was kept at −15° for 4 days. The solution then contained 3-*carbamoyl*-1-(D-*ribofuranosyl*)-*pyridinium chloride* and strongly fluorescent materials (R_F 0·53 and 0·74). The solution was evaporated at 25° under reduced pressure. A solution of the residue in methanol (5 ml.) was added dropwise with stirring to butan-1-ol (50 ml.). All the fluorescent material remained in solution, and the pale yellow nucleoside was collected by centrifugation, washed with the same solvent (3 × 20 ml.), and freed of solvent *in vacuo* ($CaCl_2$) to give a hygroscopic, amorphous yellow powder (0·6 g., 34%) (R_F 0·39, positive *cis*-diol test, positive methyl ethyl ketone–ammonia test) $[\alpha]_D^{25}$ −37° (*c* 1·88 in water), $\lambda_{max.}$ 265 mμ (ε 5400), $\lambda_{min.}$ 247 mμ (ε 4200) (Found: C, 45·5; H, 5·5; Cl, 12·0; N, 9·5. $C_{11}H_{15}ClN_2O_5$ requires C, 45·45; H, 5·2; Cl, 12·2; N, 9·65%), τ [D_2O (15% w/v)] 0·38 [1H, pyridine H(2)], 0·69 [1H, sextet, $J_{6,2}$ 1·5, $J_{6,4}$ 1·5, $J_{6,5}$ 6·3 c./sec., pyridine H(6)], 0·97 [1H, sextet, $J_{4,2}$ 1·5, $J_{4,5}$ 8·1 c./sec., pyridine H(4)], 1·68 [1H, q, H(1)], and 3·74 [1H, d, $J_{1,2}$ 4·2 c./sec., sugar H(1)].

3-*Carbamoyl*-1-(3,5-*di*-*O*-*benzoyl*-D-*ribofuranosyl*)-4-*methyl*-*pyridinium Chloride*.—A solution of 4-methylnicotinamide (2·5 g., 0·018 mole) in hot, dry acetonitrile (125 ml.) was cooled to room temperature and treated, before crystallisation commenced, with 3,5-di-*O*-benzoyl-D-ribofuranosyl chloride (7·6 g., 0·0202 mole). After 2 hr., paper chromatography revealed no remaining 4-methylnicotinamide (R_F 0·68). The solution was concentrated

[19] R. K. Ness, H. W. Diehl, and H. G. Fletcher, *J. Amer. Chem. Soc.*, 1954, **76**, 763.

to 50 ml. at 25° under reduced pressure, then added dropwise with stirring to dry ether (500 ml.). Unchanged dibenzoylribosyl chloride remained in solution, and the precipitated *dibenzoate* was filtered off, washed with ether, and kept *in vacuo* (P_2O_5–paraffin wax) to give an amorphous powder (R_F 0·90, negative *cis*-diol test, negative methyl ethyl ketone–ammonia test) (9·3 g., 100%), $[\alpha]_D^{25}$ −46° (*c* 2·5 in chloroform), λ_{infl} 265 mμ (ε 5000) (Found: C, 61·05; H, 5·15; Cl, 7·2; N, 5·4. $C_{26}H_{25}ClN_2O_7$ requires C, 60·9; H, 4·9; Cl, 6·9; N, 5·45%), τ [dimethyl sulphoxide–D_2O, 9 : 1 (20% w/v)] 3·50 [d, $J_{1,2}$ 5·4 c./sec., sugar H(1)].

3-Carbamoyl-4-methyl-1-(D-ribofuranosyl)pyridinium Chloride.—A solution of 3-carbamoyl-1-(3,5-di-*O*-benzoyl-D-ribofuranosyl)-4-methylpyridinium chloride (6·45 g., 0·0126 mole) in M-methanolic ammonia (64·5 ml.) was kept at −15° for 4 days. The solution then contained *3-carbamoyl-4-methyl-1-(D-ribofuranosyl)pyridinium chloride* as the only significant component. The solution was evaporated at 25° under reduced pressure, to leave a brown residue which solidified on trituration with ethanol (1·5 ml.). A solution of the crude product (3·16 g.) in methanol (50 ml.) was decolorised by passage through a charcoal column (4 cm. × 3 cm.²) and washed through with methanol (200 ml.). Evaporation of the colourless solution at 25° under reduced pressure left a solid residue which was triturated with ethanol (15 ml.), filtered off, washed with ethanol (3 × 0·5 ml.), and stored *in vacuo* ($CaCl_2$) to give the pale yellow, crystalline nucleoside (R_F 0·42, positive *cis*-diol test, negative methyl ethyl ketone–ammonia test) (2·82 g., 73%), m.p. 138—139°, $[\alpha]_D^{25}$ −47° (*c* 2·0 in water). λ_{infl} 264 mμ (ε 4300) (Found: C, 47·1; H, 5·15; Cl, 11·3; N, 9·4. $C_{12}H_{17}ClN_2O_5$ requires C, 47·3; H, 5·6; Cl, 11·65; N, 9·2%), τ [D_2O (20% w/v)] 0·76 [1H, pyridine H(2)]. 0·99 (1H, q, $J_{6,2}$ 1·5, $J_{6,5}$ 6·6 c./sec., pyridine H(6)], 1·90 [1H, d, pyridine H(5)], 3·86 [1H, d, $J_{1,2}$ 3·9 c./sec., sugar H(1)], and 7·27 (3H, s, Me).

The chemical shifts and coupling constants for the pyridinium ring proton signals in 3-carbamoyl-1-(D-ribofuranosyl)pyridinium chloride and its 4-methyl analogue are comparable to the corresponding values for 1-benzylpyridinium chloride and its 4-deuterio-derivative.[20]

3-Carbamoyl-1-(2,3-O-isopropylidene-D-ribofuranosyl)-4-methylpyridinium Chloride.—A suspension of 3-carbamoyl-4-methyl-1-(D-ribofuranosyl)pyridinium chloride (0·5 g., 0·00164 mole) in 2,2-dimethoxypropane (1·25 ml.), dry dimethylformamide (5 ml.) and 6M-hydrogen chloride in dioxan (0·25 ml.) was stirred at room temperature for 8 hr. The resulting clear solution was left for a further 16 hr.; paper chromatography then revealed no unchanged starting material. It was neutralised with M-methanolic ammonia (1·5 ml.) then concentrated at 65° under reduced pressure. Residual dimethylformamide was removed by co-evaporation with butan-1-ol. The residue was triturated with chloroform (25 ml.) and the insoluble material (NH_4Cl) was filtered off. After concentration to 5 ml. the solution was added dropwise with stirring to dry ether (50 ml.). The pale yellow *isopropylidene derivative* was filtered off, washed with dry ether, and stored *in vacuo* ($CaCl_2$–paraffin wax) to give a hygroscopic pale yellow amorphous powder (R_F 0·75, negative *cis*-diol test, negative methyl ethyl ketone–ammonia test) (0·495 g., 88%) (Found: C, 51·8; H, 6·15; Cl, 10·1; N, 8·25. $C_{15}H_{21}ClN_2O_5$ requires C, 52·25; H, 6·15; Cl, 10·3; N, 8·13%).

The isopropylidene derivative (0·02 g.) was dissolved in 0·1N-hydrochloric acid (1 ml.) at room temperature. Paper chromatography of 0·1 ml. aliquot portions, neutralised with 0·1N-ammonium hydroxide before spotting, showed complete conversion into 3-carbamoyl-4-methyl-1-(D-ribofuranosyl)pyridinium chloride in 48 hr. No 4-methylnicotinamide was formed.

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