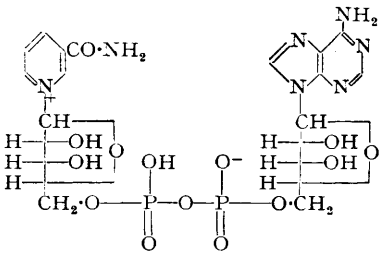
66. *Codehydrogenases. Part I. The Synthesis of Dihydronicotinamide-D-ribofuranoside* [N-D-*Ribofuranosidyl*-1 : 2(*or* 6)-*dihydronicotinamide*].

By L. J. Haynes and A. R. Todd.

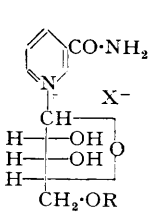
As a first step in a projected synthesis of cozymase, a number of *N*-glycosidyl-3-carbamylpyridinium bromides and their reduction products, the dihydronicotinamide glycosides, have been prepared, including a *dihydronicotinamide*-D-*ribofuranoside* [N-D-*ribofuranosidyl*-1 : 2(or 6)-*dihydronicotinamide*], probably identical with the dihydronicotinamide nucleoside prepared from natural cozymase. The formation of two (probably α- and β-) isomeric glucosides by condensation of α-acetobromoglucose with nicotinamide has been observed, and it is shown that quaternary glycosides can be formed from nicotinamide with both *cis*- and *trans*-acetobromosugars.

In recent years an extensive series of investigations has been undertaken in this laboratory, aimed at the clarification of structural problems in the nucleotide field by application of synthetic methods. One of these problems is the verification of the structures proposed for the so-called nucleotide coenzymes (adenosine di- and tri-phosphates, coenzymes I and II, and flavin-adenine-dinucleotide). Investigations leading to the synthesis of the natural purine and pyrimidine ribonucleosides and the development of methods of phosphorylation and polyphosphorylation have been described in papers published in this Journal (for summarised accounts see Todd, *J.*, 1946, 647; Lythgoe and Todd, *Symp. Soc. Exp. Biol.*, 1947, **1**, 15; Todd, *Bull. Soc. chim.*, 1948, 933). On the basis of these studies it was possible to effect the total synthesis of the cophosphorylases adenosine diphosphate (Baddiley and Todd, *J.*, 1947, 648) and adenosine triphosphate (Baddiley, Michelson, and Todd, *Nature*, 1948, **161**, 761; *J.*, 1949, 582). In

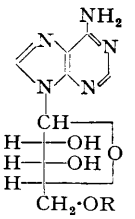
continuation of our studies we have now devoted attention to the problem of a synthesis of cozymase (coenzyme I, codehydrogenase I, diphosphopyridine nucleotide), which acts as coenzyme in a number of biological oxidations. As a result of researches by von Euler, Schlenk, Warburg, and Karrer (for review see Lythgoe, *Ann. Reports*, 1945, **42**, 175) structure (I) has been advanced for cozymase mainly as a result of degradative work on this substance and the related coenzyme II (codehydrogenase II, triphosphopyridine nucleotide). Although the evidence for structure (I) seems conclusive, a total synthesis would be very desirable, not merely for purposes of confirmation, but also because it might make the substance more accessible in a pure condition and because it might be possible to apply the synthetic method to the production of a variety of structural analogues with which some study of the specificity of the coenzyme might be undertaken, or antagonists to it be discovered.



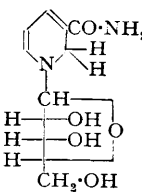
(I.)



(II.)



(III.)



(IV.)

The problem of a total cozymase synthesis in its most elementary form resolves itself into three parts: (1) the synthesis of "nicotinamide nucleoside" (II; R = H) and adenosine (III; R = H), (2) the phosphorylation of (II) and (III) to the corresponding nucleotides [II; R = $PO(OH)_2$] and [III; R = $PO(OH)_2$], and (3) the linkage of these nucleotides to yield cozymase. Of these various parts, adenosine (III; R = H) (Davoll, Lythgoe, and Todd, *J.*, 1948, 967; Kenner, Taylor, and Todd, *J.*, 1949, 1620) and adenosine-5′ phosphate [III; R = $PO(OH)_2$] (Baddiley and Todd, *loc. cit.*) have already been synthesised. The present communication deals with the synthesis of the dihydronicotinamide riboside (IV). In (IV) the formulation as a 1 : 2-dihydronicotinamide derivative is arbitrary; whether it is a 1 : 2- or 1 : 6-dihydro-compound is at present unknown (cf. Karrer, Schwarzenbach, Benz, and Solmssen, *Helv. Chim. Acta*, 1936, **19**, 811; Karrer, Schwarzenbach, and Utzinger, *ibid.*, 1937, **20**, 720).

The first recorded synthesis of a glycosidylpyridinium salt is due to Fischer and Raske (*Ber.*, 1910, **43**, 1750), who prepared *N*-2′ : 3′ : 4′ : 6′-tetra-acetyl D-glucosidylpyridinium bromide by allowing α-acetobromoglucose to react with pyridine in presence of phenol. In the course of the researches leading to the formulation of cozymase as (I) and the recognition that the active centre of the molecule in biological hydrogen transfer is the quaternary ammonium grouping (reversible reduction to a dihydro-compound), Karrer, Ringier, Büchi, Fritsche, and Solmssen (*Helv. Chim. Acta*, 1937, **20**, 55) extended the work of Fischer and Raske to the preparation of several related compounds. Acetobromoglucose, acetobromoarabinose, and acetobromoxylose were condensed with nicotinamide in dioxan solution and, in the case of acetobromoglucose, condensations were also carried out with other derivatives of nicotinic acid. In these experiments the preparation and purification of the pentose derivatives seem to have been troublesome, since analytical values are recorded only for the nicotinamide arabopyranoside. These quaternary salts showed oxidation–reduction properties similar to those of cozymase. That the nicotinamide–ribose linkage in cozymase was similar to that present in the synthetic materials was later shown by enzymic hydrolysis of the coenzyme with the nucleotidase obtained from sweet almond press-cake. This hydrolysis yielded adenosine and a "nicotinamide nucleoside" which, although devoid of coenzyme activity, showed properties analogous to those of Karrer's model substances. The "nicotinamide nucleoside" was an amorphous hygroscopic powder which retained moisture even when dried in a high vacuum (Schlenk, *Naturwiss.*, 1940, **28**, 46; *Svensk Akad. Kemi*, 1941, **14**, *A*, 13; *J. Biol. Chem.*, 1942, **140**, 619; *Arch. Biochem.*, 1943, **3**, 93; Schlenk and Gingrich, *J. Biol. Chem.*, 1942, **143**, 295; *J. Bact.*, 1944, **47**, 535).

We have further extended Fischer's synthesis to include the preparation of quaternary salts from nicotinamide and acetohalogeno-derivatives of D-mannopyranose, D-galactopyranose, and D-ribofuranose and have repeated the preparation of the D-glucose and L-arabinose derivatives already described by Karrer *et al.* (*loc. cit.*). The successful formation of a quaternary salt from α-acetobromo-D-mannopyranose calls for some comment. It has been stated by Micheel

and Micheel (*Ber.*, 1930, **63**, 390; 1932, **65**, 253) that acetohalogeno-sugars, in which the halogen atom on $C_{(1)}$ is in the *trans*-position with respect to the acetyl groups at $C_{(2)}$ and $C_{(3)}$, will not react with tertiary bases to form quaternary salts, and that reaction with a tertiary amine to form a quaternary salt is diagnostic of a *cis*-acetohalogeno-sugar. α-Acetobromo-D-mannopyranose has, of course, a *trans*-configuration, and since it does form a quaternary salt with nicotinamide it is clear that the rule of Micheel and Micheel is not absolute. The German workers based their conclusions on experiments in which they attempted to cause acetobromomannose, acetochloromannose, and other acetohalogeno-sugars to react with trimethylamine in benzene, ethanol, or a mixture of these two solvents at room temperature or in sealed tubes at 100°. Examination of their results shows that, in experiments where no quaternary salt was formed, either starting materials were recovered unchanged (room-temperature experiments) or gross decomposition occurred (sealed-tube experiments at 100°).

In our early attempts to condense nicotinamide with α-acetobromo-D-mannopyranose (which we selected as a model compound in view of the configurational similarity of mannose and ribose), the two compounds were heated together under reflux for some hours in methyl cyanide solution. Although this procedure had given an excellent yield of quaternary salts with α-acetobromo-D-glucopyranose, no quaternary salt was formed, the main reaction being dehydrohalogenation of the sugar derivatives with formation of nicotinamide hydrobromide. Further experiments showed that, if the solution of the reactants was maintained at 40—50°, comparatively little nicotinamide hydrobromide was formed and *3-carbamyl*-N-2′ : 3′ : 4′ : 6′-*tetra-acetyl*-D-*mannopyranosidylpyridinium bromide* was produced in a yield of some 60%. A series of experiments on the general problem of the formation of quaternary salts from nicotinamide and acetohalogeno-sugars showed that acetofluoro-sugars are too unreactive to undergo either quaternary salt formation or dehydrohalogenation, while acetochloro-sugars (including α-acetochloro-D-glucopyranose) react more slowly than the corresponding bromo-compounds and undergo only the dehydrohalogenation reaction. Apparently quaternary salt formation and dehydrohalogenation are simultaneous competing reactions when acetobromo-sugars are used. Higher temperatures favour dehydrohalogenation, and in the case of the *trans*-acetobromo-sugars a difference of 30—40° in temperature may mean that the quaternary salt is formed almost exclusively or not at all. The "rule" of Micheel and Micheel has thus no general significance as a means of determining configuration in an acetohalogeno-sugar. Fischer and Raske (*loc. cit.*) reported that two forms of the quaternary acetylated glucoside were produced by condensation of pyridine with α-acetobromo-D-glucose. These two forms differed markedly in their physical properties, the first ($[\alpha]_D^{20} = -6{\cdot}43°$) being a crystalline compound, insoluble or sparingly soluble in organic solvents, and the second ($[\alpha]_D = +16{\cdot}2°$) an amorphous, very hygroscopic solid, easily soluble in methyl ethyl ketone but insoluble in ether. We have found that the condensation of nicotinamide with α-acetobromo-D-glucopyranose in methyl cyanide solution proceeds similarly; in addition to the crystalline compound ($[\alpha]_D^{19} = -18{\cdot}3°$) isolated by Karrer *et al.* (*loc. cit.*), it is possible to isolate an isomeric substance as a very hygroscopic amorphous solid, easily soluble in methyl cyanide, chloroform, and ethanol, and insoluble in ether ($[\alpha]_D^{19} = +20{\cdot}9°$). These two products are almost certainly α,β-isomers. With other acetobromo-sugars we obtained only amorphous glycosides and in no case were we able to obtain two distinct compounds; the possibility that some of these amorphous products may be mixtures of α- and β-forms cannot be excluded.

Reduction of the quaternary salts prepared from nicotinamide and acetobromo-D-glucopyranose, acetobromo-D-galactopyranose, acetobromo-D-mannopyranose, and acetobromo-L-arabopyranose with sodium dithionite yielded the corresponding acetylated dihydronicotinamide glycosides which could be readily reoxidised. It was found advantageous to carry out these reductions in an inert atmosphere and to avoid undue exposure of the products to light. The acetylated dihydro-glycosides were readily deacetylated with methanolic ammonia. With the exception of the glucose derivative already described by Karrer *et al.* (*loc. cit.*), all the compounds prepared were amorphous and hygroscopic and have so far defied all attempts at crystallisation; in the case of the quaternary salts, variation of the acid radical also failed to yield crystalline products. There can, however, be little doubt of their homogeneity, since repeated fractional precipitations gave products which showed no significant differences in physical or chemical characteristics. Mild acid hydrolysis of the quaternary acetylated galactoside gave *3-carbamyl*-N-D-*galactopyranosidylpyridinium bromide*, which was also amorphous.

Condensation of acetobromo-D-ribofuranose (Howard, Lythgoe, and Todd, *J.*, 1947, 1052) with nicotinamide gave a quaternary salt reduced by sodium dithionite to *dihydronicotinamide-*

(2′ : 3′ : 5′-*triacetyl* D-*ribofuranoside*) and from this product *dihydronicotinamide*-D-*ribofuranoside* [N-D-*ribofuranosido*-1 : 2(or 6)-*dihydronicotinamide*] (IV) was prepared as a pale yellow amorphous hygroscopic powder by deacetylation. It is not yet possible to say with certainty that this synthetic product is identical with Schlenk's dihydronicotinamide nucleoside, since the latter has been rather inadequately characterised, and in the absence of direct comparison the possibility that the natural and synthetic products might be α,β-isomers must be considered. The configuration of the natural product is, of course, unknown, although, since the natural purine and pyrimidine ribonucleosides have the β-configuration at the glycosidic carbon atom, one might well expect that the nicotinamide nucleoside would also be a β-compound. We incline to the view that the synthetic riboside we have obtained is, in fact, identical with the natural product on the following grounds. Schlenk and Gingrich (*loc. cit.*) have shown that nicotinamide nucleoside will replace coenzyme-I as a growth factor (V factor) for *Hæmophilus influenzæ* and *H. parainfluenzæ*, an effect not shown by nicotinamide or nicotinic acid. Tests carried out by Mr. D. A. Hughes of Sheffield University have shown that our synthetic dihydronicotinamide-ribofuranoside shows a similar effect, having an activity roughly half that of coenzyme-I on a molar basis.

Experimental.

(Analytical samples were dried *in vacuo* over phosphoric oxide at room temperature for several days. All evaporations under reduced pressure were effected at a bath temperature not exceeding 35°. Light petroleum refers to the fraction of b. p. 40—60°.)

Acetobromo-sugars.—Acetobromo-D-glucopyranose, -D-mannopyranose, -D-galactopyranose, and -L-arabopyranose were all prepared by the following method. The fully acetylated sugar was stirred with twice its weight of a solution of hydrogen bromide in glacial acetic acid (saturated at 0°) for a period of 2 hours, during which time the solid completely dissolved. Chloroform was then added, and the solution poured on ice, separated, and washed with ice-water until the washings were neutral to Congo-red. The chloroform solution was dried (Na_2SO_4), and the solvent evaporated under reduced pressure. The residual syrup was taken up in dry ether, dry light petroleum added to faint cloudiness, and the solution set aside at 0° for some hours. The acetobromo-sugars crystallised in good yield and were sufficiently pure for further work if used within one or two days.

3-*Carbamyl*-2′-*hydroxyethylpyridinium Bromide.*—Ethylene bromohydrin (10 g., 1 mol.) and nicotinamide (10 g., 0·98 mol.) were refluxed in ethanol during 1 hour. The solvent was evaporated, and the residual gum caused to solidify by rubbing it with a few drops of ethanol. The *pyridinium bromide* (17·5 g., 87·5%) was thus obtained as a white crystalline powder, m. p. 141—144°. On recrystallisation from methanol by addition of ethyl acetate the m. p. rose to 148° (Found: C, 39·2; H, 4·5. $C_8H_{11}O_2N_2Br$ requires C, 38·9; H, 4·5%).

3-*Carbamyl*-N-(2′ : 3′ : 4′ : 6′-*tetra-acetyl* D-*glucopyranosidyl*)*pyridinium Bromide* (cf. Karrer *et al., loc. cit.*).—Acetobromo-D-glucopyranose (15 g.) and nicotinamide (5 g.) were gently refluxed in dry methyl cyanide (70 c.c.) during 3½ hours. A crystalline precipitate separated. The mixture was then set aside overnight and filtered. 3-Carbamyl-*N*-(2′ : 3′ : 4′ : 6′-tetra-acetyl (?β-)D-glucopyranosidyl)pyridinium bromide (13·78 g., 71%) was thus obtained as a white powder, which crystallised from methanol after the addition of ethyl acetate as colourless plates, m. p. 195—200° (decomp.) (Karrer *et al., loc. cit.*, give m. p. 192—200°, decomp.), $[\alpha]_D^{19}$ −18·3° (*c*, 2·5 in water). The filtrate was evaporated to dryness under reduced pressure; the residue, a dark oil, was taken up in chloroform (30 c.c.) and filtered, and anhydrous ether (150 c.c.) added. A white precipitate separated. This was washed well with ether and dried *in vacuo*. From its analysis and properties this material appeared to be an isomeric 3-*carbamyl*-N-(2′ : 3′ : 4′ : 6′-*tetra-acetyl* (?α-)D-*glucopyranosidyl*)*pyridinium bromide* (3 g., 15%) (Found: C, 45·3; H, 5·2. $C_{20}H_{25}O_{10}N_2Br$ requires C, 45·1; H, 4·7%), $[\alpha]_D^{19}$ = +20·9° (*c*, 2·5 in water).

N-(2′ : 3′ : 4′ : 6′-*Tetra-acetyl* (?β-)D-*glucopyranosidyl*)-1 : 2(*or* 6)-*dihydronicotinamide* (cf. Karrer *et al., loc. cit.*).—A solution of sodium hydrogen carbonate (150 g.) in water (2½ l.) was saturated with carbon dioxide. The crystalline quaternary acetylated glucoside (20 g.) was then added. When this had completely dissolved, sodium dithionite (100 g.) was added. A brilliant yellow colour immediately appeared and there was a vigorous effervescence. The solution was set aside overnight in darkness, during which time a bulky precipitate of matted needles separated. On filtration and washing with water, *N*-(2′ : 3′ : 4′ : 6′-tetra-acetyl D-glucopyranosidyl)-1 : 2(or 6)-dihydronicotinamide (12·85 g., 75·5%) was obtained as a pale primrose-yellow solid, m. p. 155—157°, which after recrystallisation from water had m. p. 157° (Karrer *et al., loc. cit.*, give m. p. 157—158°), $[\alpha]_D^{18}$ = −11·1° (*c*, 0·8 in chloroform).

N-(2′ : 3′ : 4′ : 6′-*Tetra-acetyl* (?α-)D-*glucopyranosidyl*)-1 : 2(*or* 6)-*dihydronicotinamide.*—The non-crystalline quaternary acetylated glucoside (1·85 g.), dissolved in an aqueous solution (200 c.c.) of sodium hydrogen carbonate (20 g.) which had been saturated with carbon dioxide, was treated with sodium dithionite (10 g.). A brilliant yellow colour immediately appeared and there was a vigorous effervescence. The solution was set aside overnight in darkness. No precipitate separated. The solution was extracted several times with chloroform, and the combined extracts washed with water and dried (Na_2SO_4). The chloroform solution was concentrated to small bulk under reduced pressure, and a large excess of dry light petroleum then added. A flocculent yellow precipitate formed. The bulk of the mother-liquor was decanted, and the residue dried *in vacuo*. N-(2′ : 3′ : 4′ : 6′-*Tetra-acetyl* D-*glucopyranosidyl*)-1 : 2(or 6)-*dihydronicotinamide* (0·70 g., 46%) was thus obtained as a pale-yellow hygroscopic, amorphous powder, easily soluble in most organic solvents, but insoluble in light petroleum (Found: C, 49·9; H, 5·9; N, 5·7. $C_{20}H_{26}O_{10}N_2$ requires C, 52·8; H, 5·8; N, 6·2. $C_{20}H_{26}O_{10}N_2,1\frac{1}{2}H_2O$ requires C, 50·0; H, 6·0; N, 5·8%), $[\alpha]_D^{19}$ = +19·9° (*c*, 1·0 in chloroform).

3-*Carbamyl*-N-(2′ : 3′ : 4′ : 6′-*tetra-acetyl* D-*galactopyranosidyl*)*pyridinium Bromide.*—Acetobromo-D-

galactopyranose (20 g., 1 mol.) and nicotinamide (6·0 g., 1·01 mols.), dissolved in methyl cyanide (100 g.), were kept at 60° during 6 hours and then at room temperature overnight. The solvent was then evaporated under reduced pressure. The residue, a light brown resin, was taken up in cold chloroform, set aside for some hours, and then filtered to remove separated nicotinamide hydrobromide (0·71 g.). The filtrate was evaporated to dryness under reduced pressure. *3-Carbamyl*-N-(2′ : 3′ : 4′ : 6′-*tetra-acetyl* D-*galactopyranosidyl*)*pyridinium bromide* was thus obtained as a very hygroscopic "solid foam," soluble in chloroform, ethanol, and water, but insoluble in ether, ethyl acetate, and light petroleum; it could not be induced to crystallise. For purification, the quaternary salt was redissolved in dry chloroform, the solution filtered, and excess of dry ether added. A flocculent white precipitate formed. The mother-liquors were decanted, and the precipitate was washed 3 times by decantation with dry ether (Found: C, 44·2; H, 4·8; N, 5·2. $C_{20}H_{25}O_{10}N_2Br$ requires C, 45·1; H, 4·7; N, 5·3. $C_{20}H_{25}O_{10}N_2Br,\frac{1}{2}H_2O$ requires C, 44·4; H, 4·8; N, 5·2%), $[\alpha]_D^{18} = +15·2°$ (*c*, 1·2 in water).

3-Carbamyl-N-D-*galactopyranosidylpyridinium Bromide.*—The quaternary acetylated galactoside (10 g.) was dissolved in water (25 c.c.) containing concentrated hydrobromic acid (0·5 c.c.), and the solution heated in a water-bath at 55—60° for 2 hours. The aqueous solution was evaporated to dryness under reduced pressure to give a colourless gum which was twice evaporated to dryness with ethanol under reduced pressure. The gum was dissolved in a small quantity of dry ethanol, and excess of anhydrous ether added. A precipitate formed which was coagulated by shaking overnight. The precipitate was filtered off and dried in a vacuum-desiccator. *3-Carbamyl*-N-D-*galactopyranosidyl-pyridinium bromide* was thus obtained in good yield as a very hygroscopic amorphous powder, very soluble in water and ethanol, sparingly soluble in acetone, and insoluble in chloroform and ether. It retained solvent tenaciously (Found: C, 41·4; H, 5·5; N, 7·1. $C_{12}H_{17}O_6N_2Br,C_2H_5·OH$ requires C, 41·0; H, 5·6; N, 6·9%).

N-(2′ : 3′ : 4′ : 6′-*Tetra-acetyl* D-*galactopyranosidyl*)-1 : 2(*or* 6)-*dihydronicotinamide.*—The quaternary acetylated galactoside (15·9 g.) was dissolved in a saturated aqueous solution (600 c.c.) of sodium hydrogen carbonate which had been saturated with carbon dioxide. Sodium dithionite was added; a brilliant yellow colour immediately appeared and there was a vigorous effervescence. The solution was set aside overnight in darkness. No precipitate separated. The solution was 4 times extracted with chloroform, and the combined extracts washed with water and dried (Na_2SO_4). The chloroform solution was concentrated to small bulk under reduced pressure, and a large excess of dry light petroleum added. A slightly gummy yellow precipitate formed which broke up to a yellow powder on vigorous shaking of the mixture in a mechanical shaker. The mixture was set aside for 14 days, and the precipitate collected. N-(2′ : 3′ : 4′ : 6′-*Tetra-acetyl* D-*galactopyranosidyl*)-1 : 2(or 6)-*dihydronicotinamide* (10·48 g., 77%) was obtained as a pale yellow, hygroscopic, amorphous powder, easily soluble in chloroform, ethanol, ethyl acetate, acetone, and methyl cyanide, sparingly soluble in ether and water, and insoluble in light petroleum and benzene. It could not be crystallised and became a glass at *ca.* 60° (Found: C, 53·3; H, 6·3; N, 5·9. $C_{20}H_{26}O_{10}N_2$ requires C, 52·8; H, 5·8; N, 6·2%), $[\alpha]_D^{18} = +10·7°$ (*c*, 2·0 in chloroform).

3-Carbamyl-N-(2′ : 3′ : 4′ : 6′-*tetra-acetyl* D-*mannopyranosidyl*)*pyridinium Bromide.*—Acetobromo-D-mannopyranose (10 g., 1 mol.) and nicotinamide (3·4 g., 1·15 mols.) in methyl cyanide (60 c.c.) were kept at 60° for 7 hours and then at room temperature for 48 hours. The solution was then cooled to 0° and filtered from separated nicotinamide hydrobromide (0·85 g.). Evaporation of the filtrate to dryness under reduced pressure gave *3-carbamyl*-N-(2′ : 3′ : 4′ : 6′-*tetra-acetyl* D-*mannopyranosidyl*)*pyridinium bromide* as a very hygroscopic uncrystallisable foam which was soluble in chloroform, alcohol, and water and insoluble in ether, ethyl acetate, and light petroleum. The material was purified as described for the galactose derivative (Found: C, 44·4; H, 4·8; N, 5·4. $C_{20}H_{25}O_{10}N_2Br$ requires C, 45·1; H, 4·7; N, 5·3. $C_{20}H_{25}O_{10}N_2Br,\frac{1}{2}H_2O$ requires C, 44·4; H, 4·8; N, 5·2%).

N-(2′ : 3′ : 4′ : 6′-*Tetra-acetyl* D-*mannopyranosidyl*)-1 : 2(or 6)-*dihydronicotinamide.*—The quaternary acetylated mannoside (5·47 g.) was dissolved in a saturated aqueous solution (750 c.c.) of sodium hydrogen carbonate which had been saturated with carbon dioxide. Sodium dithionite (25 g.) was added, a brilliant yellow colour immediately developed and there was a brisk effervescence. The solution was set aside in darkness for 24 hours and then extracted with chloroform. The combined extracts were washed with water, and then dried (Na_2SO_4), and the solvent was evaporated under reduced pressure. N-(2′ : 3′ : 4′ : 6′-*Tetra-acetyl* D-*mannopyranosidyl*)-1 : 2(or 6)-*dihydronicotinamide* (4·1 g., 88%) separated as a light-yellow hygroscopic solid which could not be crystallised (Found: C, 50·9; H, 5·9; N, 5·6. $C_{20}H_{26}O_{10}N_2$ requires C, 52·8; H, 5·8; N, 6·2. $C_{20}H_{26}O_{10}N_2,H_2O$ requires C, 50·9; H, 5·9; N, 5·9%).

3-Carbamyl-N-(2′ : 3′ : 4′-*triacetyl* L-*arabopyranosidyl*)*pyridinium Bromide.*—Acetobromo-L-arabopyranose (10 g., 1 mol.) and nicotinamide (3·6 g., 1·2 mols.) in methyl cyanide (60 c.c.) were kept at 80° during 5 hours and then overnight at 0°. Separated nicotinamide hydrobromide (1·36 g.) was filtered off, and the solvent evaporated from the filtrate under reduced pressure. The foam so obtained was purified by solution in chloroform and precipitation with ether as previously described. *3-Carbamyl*-N-(2′ : 3′ : 4′-*triacetyl* L-*arabopyranosidyl*)*pyridinium bromide* was obtained as a hygroscopic amorphous powder, very soluble in chloroform, acetone, and water, but insoluble in ether, ethyl acetate, and light petroleum (Found: C, 43·3; H, 4·7; N, 6·0. $C_{17}H_{21}O_8N_2Br$ requires C, 44·3; H, 4·6; N, 6·1. $C_{17}H_{21}O_8N_2Br,\frac{1}{2}H_2O$ requires C, 43·4; H, 4·7; N, 6·0%).

N-(2′ : 3′ : 4′-*Triacetyl* L-*arabopyranosidyl*)-1 : 2(*or* 6)-*dihydronicotinamide.*—The quaternary acetylated arabinoside (1·25 g.) was reduced with sodium dithionite (5 g.) in sodium hydrogen carbonate solution (100 c.c.) by the procedure previously described. N-(2′ : 3′ : 4′-*Triacetyl* L-*arabopyranosidyl*)-1 : 2(or 6)-*dihydronicotinamide* (0·7 g., 66%) was obtained as a light-yellow hygroscopic solid, soluble in ethanol and chloroform, and insoluble in light petroleum; it could not be crystallised (Found: C, 50·7; H, 5·9; N, 6·9. $C_{17}H_{22}O_8N_2$ requires C, 53·5; H, 5·8; N, 7·3. $C_{17}H_{22}O_8N_2,H_2O$ requires C, 50·8; H, 6·0; N, 7·0%).

Dihydronicotinamide Glycosides [N-*Glycosido*-1 : 2(or 6)-*dihydronicotinamides*].—*General method* (cf. Karrer *et al.*, *loc. cit.*). The dihydro-acetylated *N*-glycoside was dissolved in methanol (dried by fractionation using a Fenske column), the solution cooled to 0°, saturated at 0° with anhydrous ammonia, and set aside for 24 hours at 0°. The solvent was evaporated under reduced pressure to leave a yellow

solid residue. This was thoroughly extracted with ethyl acetate to remove acetamide, leaving the dihydro-glycoside as a pale yellow, hygroscopic, amorphous powder, which could not, except in the case of the glucoside described by Karrer, be crystallised, and was very readily oxidised when moist. In this way were obtained: *N*-D-glucosidyl-1 : 2(or 6)-dihydronicotinamide (93%), m. p. 199° (decomp.), after recrystallisation from water (Karrer *et al.*, *loc. cit.*, give m. p. 203—205°, decomp.), $[\alpha]_D^{18} = +4{\cdot}9°$ (*c*, 1·5 in water); N-D-*galactopyranosidyl*-1 : 2(or 6)-*dihydronicotinamide* (96%) (Found: C, 48·9; H, 6·8; N, 9·8. $C_{12}H_{18}O_6N_2$ requires C, 50·3; H, 6·3; N, 9·8. $C_{12}H_{18}O_6N_2,\frac{1}{2}H_2O$ requires C, 48·9; H, 6·5; N, 9·5%), $[\alpha]_D^{18} = +36{\cdot}5°$ (*c*, 2·5 in water); N-D-*mannopyranosidyl*-1 : 2(or 6)-*dihydronicotinamide* (*ca.* 80%) (Found: C, 48·6; H, 6·4; N, 9·0 $C_{12}H_{18}O_6N_2$ requires C, 50·3; H, 6·3; N, 9·8. $C_{12}H_{18}O_6N_2,\frac{1}{2}H_2O$ requires C, 48·9; H, 6·5; N, 9·5); and N-L-*arabopyranosidyl*-1 : 2(or 6)-*dihydronicotinamide* (*ca.* 80%) (Found: C, 50·3; H, 6·5; N, 10·2. $C_{11}H_{16}O_5N_2$ requires C, 51·6; H, 6·3; N, 11·0. $C_{11}H_{16}O_5N_2,\frac{1}{2}H_2O$ requires C, 50·0; H, 6·4; N, 10·6%).

N-(2′ : 3′ : 5′-*Triacetyl* D-*ribofuranosidyl*)-1 : 2(*or* 6)-*dihydronicotinamide*.—A solution of aceto-bromo-D-ribofuranose [from tetra-acetyl D-ribofuranose (2·35 g.) (Howard, Lythgoe, and Todd, *J.*, 1947, 1052] in dry methyl cyanide (15 c.c.) was cooled to 0° and added to a solution of nicotinamide (2 g.) in the same solvent (125 c.c.) at 0°. The solution was kept at 0° for 24 hours and then filtered from precipitated nicotinamide hydrobromide (1·0 g.). The filtrate was evaporated to dryness under reduced pressure at room temperature. The residue was dissolved in dry chloroform (15 c.c.), the solution filtered, and excess of anhydrous ether (80 c.c.) added. The precipitated white powder was washed by decantation 3 times with anhydrous ether. On drying *in vacuo* the crude quaternary glycoside (1·4 g.) was obtained as an extremely hygroscopic, amorphous powder. The quaternary salt (1·4 g.) was dissolved in a saturated aqueous solution (175 c.c.) of sodium hydrogen carbonate which had been saturated with carbon dioxide, and the aqueous solution was again saturated with carbon dioxide. Sodium dithionite (6 g.) was then added, a brilliant yellow colour immediately developed and there was a vigorous effervescence. The solution was set aside for 24 hours in darkness and extracted 6 times with chloroform. A nitrogen atmosphere was maintained wherever possible. The combined chloroform extracts were washed with water, dried (Na_2SO_4), and evaporated under reduced pressure. The residue, a solid foam, was purified by precipitation from chloroform solution with light petroleum. The N-2′ : 3′ : 5′-*triacetyl* D-*ribofuranosidyl*-1 : 2(*or* 6)-*dihydronicotinamide* (0·45 g.) so obtained was a pale yellow, hygroscopic, amorphous solid (Found: C, 52·9; H, 5·9; N, 7·3. $C_{17}H_{22}O_8N_2$ requires C, 53·5; H, 5·8; N, 7·3%), $[\alpha]_D^{20} = -18{\cdot}3°$ (*c*, 0·8 in chloroform).

N-D-*Ribofuranosidyl*-1 : 2(*or* 6)-*dihydronicotinamide*.—The above acetylated glycoside (0·38 g.) was dissolved in anhydrous methanol (50 c.c.), and the solution cooled to 0°, saturated with anhydrous ammonia, and stored overnight at 0°. The solution was then concentrated under reduced pressure to small bulk, and excess of dry ethyl acetate (70 c.c.) added. The mixture was set aside for several days to age the precipitate, which was then washed thoroughly with dry ethyl acetate to remove acetamide. Filtration gave N-D-*ribofuranosidyl*-1 : 2(*or* 6)-*dihydronicotinamide* as a pale yellow amorphous powder, which could not be crystallised (Found: C, 48·3; H, 6·6; N, 9·7. $C_{11}H_{16}O_5N_2$ requires C, 51·6; H, 6·3; N, 10·9. $C_{11}H_{16}O_5N_2,H_2O$ requires C, 48·3; H, 6·6; N, 10·2%), $[\alpha]_D^{21} = -20{\cdot}8°$ (*c*, 1·0 in water). The material was very soluble in water, and insoluble in ethyl acetate, light petroleum, ether, and chloroform; it was very hygroscopic and readily oxidised to a dark brown gum when moist.

We are indebted to Messrs. Imperial Chemical Industries Ltd. and Roche Products Ltd. for grants and gifts of material and to Mr. D. A. Hughes for microbiological assays. One of us (L. J. H.) participated in the investigation during his tenure of a D.S.I.R. Senior Research Award and an I.C.I. Fellowship.

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, October 6th*, 1949.]