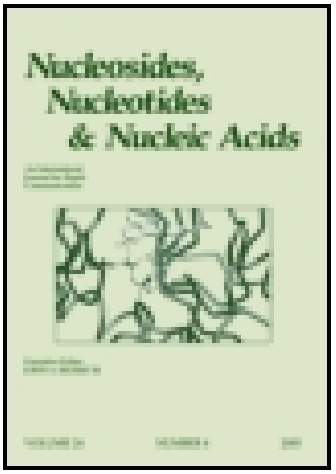

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Simple Synthesis of the 5-O-Benzoylriboside of 1,4-Dihydronicotinic Acid; a Cofactor for DT Diaphorase and Nitroreductase Enzymes

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SIMPLE SYNTHESIS OF THE 5-O-BENZOYLRIBOSIDE OF 1,4-DIHYDRONICOTINIC ACID; A COFACTOR FOR DT DIAPHORASE AND NITROREDUCTASE ENZYMES

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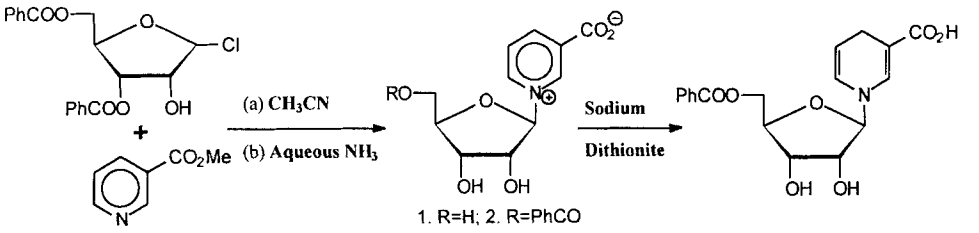
Abstract: A simple one flask synthesis of the 5-O-benzoylriboside and riboside of nicotinic acid has been developed. Reduction of the benzoylriboside with sodium dithionite gives the 1,4-dihydronicotinic acid in good yield.

The published syntheses of the riboside (1)[1] and 5-O-benzoylriboside (2)[2] of nicotinic acid involve four steps, starting from nicotinic acid, and give overall yields of 25% and 10% respectively. A convenient one-flask synthesis has been developed in which methyl nicotinate and 3,5-dibenzoylribosyl chloride[3] are condensed in acetonitrile. Addition of aqueous ammonia gave, depending on hydrolysis conditions, either the riboside (1) or benzoylriboside (2) in 25% yield.



1. R=H; 2. R=PhCO

The 5-O-benzoylriboside of 1,4-dihydronicotinic acid is a cofactor for the reduction of 5-(aziridin-1-yl)-2,4-dinitrobenzamide (CB 1954) by the enzyme DT Diaphorase.[2] The cofactor has been synthesised by reduction of benzoylriboside (2) with alkaline sodium dithionite. The product was then extracted into ethyl acetate. Multiple extractions (between five and ten) were necessary to extract all the product giving a yield of 60%.

EXPERIMENTAL

NMR spectra were determined in DMSO-d_6 (relative to TMS) using a Bruker AC250 spectrometer. Thin layer chromatograms were run on fluorescent cellulose (Merck 5574) using butan-1-ol:acetic acid:water (5:2:3) as solvent. Preparative chromatography was performed using a Axxial Modulprep column (20 mm) coupled to a UV monitor operating at 254 nm. Mass spectrometry was performed on a TSQ 700 triple quadrupole system equipped with an electrospray ion source (Finnigan API).

1-(D-Ribofuranosyl)pyridinium-3-carboxylate (1) and 1-(5-O-Benzoyl-D-ribofuranosyl)pyridinium-3-carboxylate (2).

A solution of methyl nicotinate (0.21 g, 1.5 mmol) and 3,5-dibenzoylribosyl chloride (0.57 g, 1.5 mmol) in dry acetonitrile (10 mL) was stirred at room temperature. Twenty hours later aqueous ammonia (1.4 N, 30 mL) was added gradually (2 hours).

(1) - After 24 hours tlc showed that riboside 1 was the major product. The oily suspension was evaporated to dryness, dissolved in aqueous acetic acid (1%, 15 mL) and applied to a preparative reverse-phase column (RP8, Merck 9324) eluted with the same solvent. The fractions containing 1 were evaporated to dryness and the residue triturated with ethanol to give 94 mg (25%) of white crystals which were pure on nmr and tlc and identical with an authentic sample of 1. The full nmr spectra was not previously reported. δ 3.66 and 3.82 (2H, 2 x ddd, $J_{5'5'}$ = 13 Hz, $J_{5'\text{-OH},\,5'\text{-CH}_2}$ = $J_{4'\text{-CH},\,5'\text{-CH}_2}$ = 4 Hz, 5'-CH_2), 4.10 (1H, q, J = 4 Hz and 5 Hz, 3'-CH), 4.22 (2H, m, 2'+4'-CH), 5.32 (1H, t, J = 5 Hz, 5'-OH), 5.40 (1H, d, J = 5 Hz, 3'-OH), 5.95 (1H, d, J = 6 Hz, 2'-OH), 6.13 (1H, d, J = 5 Hz, 1'-CH), 8.10 (1H, dd, J = 6 Hz and 8 Hz, 5-CH), 8.84 (1H, d, J = 8 Hz, 4 or 6-CH), 9.13 (1H, d, J = 6 Hz, 4 or 6-CH), 9.34 (1H, s, 2-CH).

(2) - After 3 hours tlc showed that benzoylriboside 2 was the major product. The solution was evaporated until neutral and then applied to a preparative reverse phase column (RP18, Merck 9303) which was eluted successively with water, 5% aqueous CH_3CN and 10% aqueous CH_3CN. The latter fractions were evaporated to a few ml and methanol added to give after cooling 124 mg of pure product, a second crop gave 9 mg. Total yield was 133 mg (25%) of material identical with an authentic sample of 2.

1,4-Dihydro-1-(β-D-ribofuranosyl)-3-pyridinecarboxylic acid. All procedures were carried out under nitrogen. A solution of sodium dithionite (0.25 g), sodium bicarbonate (0.25 g) and sodium carbonate (0.25 g) in water (10 mL) was added with stirring to benzoylriboside 2 (0.1 g, 0.28 mmol). After two and three hours further portions of dithionite (0.25 g) were added, and after a total of five hours the solution was extracted with ethyl acetate (10 x 15 ml). The combined extracts were evaporated to dryness, redissolved in ethyl acetate, evaporated and dried in vacuo in the dark to yield 60 mg (60%) of gummy product which was pure on nmr. δ 2.92 (2H, m, 4-CH_2), 3.94 (2H, m, 2' + 3'-CH), 4.0 (1H, m, 4'-CH), 4.40 (2H, m, 5'-CH_2), 4.69 (1H, dt, $J_{4,5}$ = 3 Hz, $J_{5,6}$ = 9 Hz, 5-CH), 4.75 (1H, d, J = 5 Hz, 1'-CH), 5.21 + 5.27 (2H, 2 x d, J = 5 Hz, 2'+3'-OH), 6.01 (1H, dd, $J_{4,6}$ = 1 Hz, $J_{5,6}$ = 9 Hz, 6-CH), 7.12 (1H, s, 2-CH), 7.54 (2H, t, 7 Hz, benzoyl H-3), 7.68 (1 H, t, J = 7 Hz, benzoyl H-4), 7.97 (2H, d, J = 7 Hz, benzoyl H-2, 11.4 (1H, bs, CO_2H).

M/Z 362 $(M+H)^+$, 237 $(benzoylribose)^+$, 126 $(dihydronicotinic\ acid + H)^+$. Anal. $C_{18}H_{19}NO_7$. $EtAc_{0.25}$ requires C 59.52, H 5.52, N 3.65; found C 59.38, H 5.21, N 3.29. (Nmr confirms the presence of EtAc).

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