The Reaction of Pyridinecarboxylic Acids with Dicyclohexylcarbodiimide and p-Nitrophenol

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To modify the terminal amino groups of lysine and ornithine, we recently needed the p-nitrophenyl esters of 2-pyridinecarboxylic (picolinic) acid, 3-pyridinecarboxylic (nicotinic) acid and 4-pyridinecarboxylic (isonicotinic) acid. We used the method described by Bodanszky and du Vigneaud[2] for the preparation of benzyloxycarbonyl-asparagine and glutamine p-nitrophenyl ester. Their method consists of adding dicyclohexylcarbodiimide (DCC) to a solution of the acid and a 20 % excess of p-nitrophenol in N,N-dimethylformamide (DMF). The results were satisfactory in the case of nicotinic and isonicotinic acid, and the corresponding esters were obtained in a yield of about 60 % after recrystallization.

In the case of picolinic acid (1), however, this procedure was not satisfactory and two major products were formed which could be isolated by fractional crystallization from 2-propanol. p-Nitrophenyl picolinate (2) was the minor product, isolated in a yield of only 16 %. The major product, isolated in a yield of 32 %, was N-picolinoyl-N,N'-dicyclohexylurea (3). When the reaction was carried out in dichloromethane, the desired ester was obtained in a yield of 70 % after recrystallization. Only a minor amount of acylurea was formed in this solvent. This is in accordance with results obtained with methods using chloroform[3] and pyridine[4] as solvents.

The activation of carboxylic acids by DCC is generally thought to proceed as originally proposed by Khorana.[5] The intermediate is presumed to be a N,N'-dicyclohexyl-O-acylisourea which can acylate the substrate directly or react with a second molecule of the acid to give an anhydride and dicyclohexylurea (DCU). The side

reaction is an intramolecular O→N acyl shift to yield an N-acylurea.

In the peptide field, DCC-mediated couplings have been studied extensively, and O→N acyl transfer has been traditionally considered to be promoted by high temperatures[6,7] or by bases such as amines,[6,8] especially when present in excess,[9] and the use of polar solvents such as THF[10,11] and dioxane.[11] Sterically hindered amino acids such as Val or Ile show a pronounced tendency to rearrange due to a slow reaction with a nucleophile.[12]

In our synthesis, high yields of acylurea were formed only in DMF and only with picolinic acid. Both the solvent and the structure of the acid influence the reaction. Possible reaction mechanisms are shown in Scheme 1. In dichloromethane, picolinic acid (1) may react in the normal way, perhaps via an ion pair, as favoured by DeTar and Silverstein[13] for acetic acid, to form the O-acylisourea (4). Compound 4 may then acylate p-nitrophenol or react with a second molecule of the acid to give anhydride and DCU.

In DMF, the reaction may be different from that in dichloromethane. In the polar solvent DMF, picolinic acid may exist to a substantial degree as the zwitterion (1a), as is the case for all pyridinecarboxylic acids in aqueous solution.[14] The zwitterion may react with DCC to form the adduct 5, which is stabilized by intramolecular hydrogen bonding. This adduct may have a lifetime sufficient to allow it to rearrange to the N-acylurea (3). It is plausible to have a negative charge on the DCC nitrogen atoms since bases have been shown to increase the formation of N-acylurea.
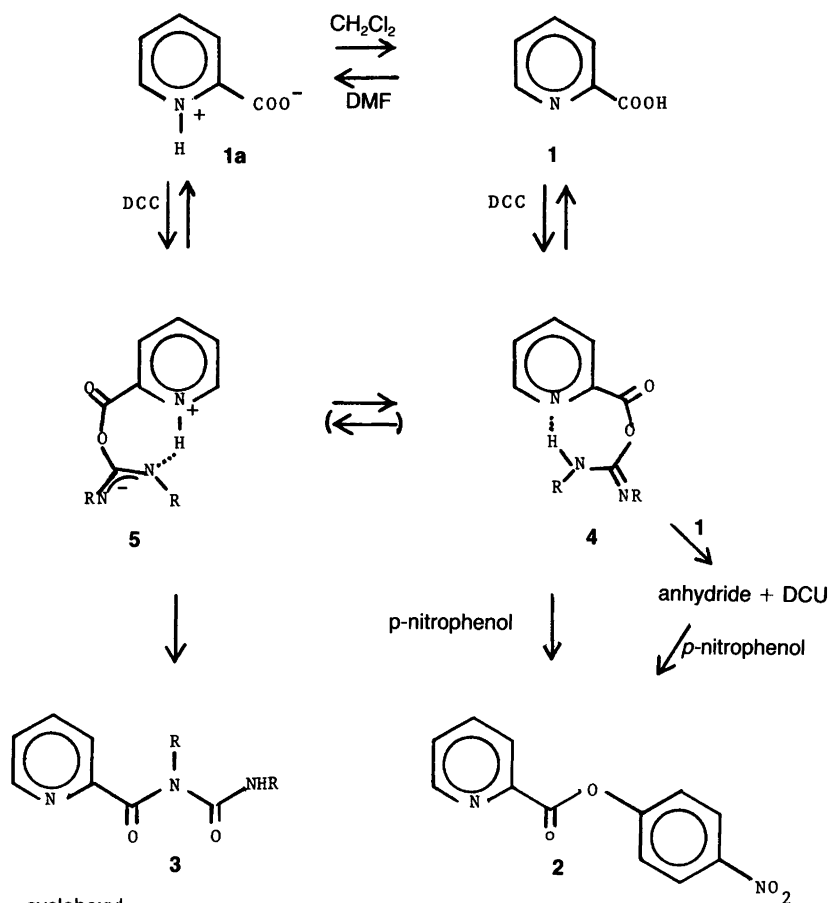
Scheme 1. R = cyclohexyl.

Support for this reaction scheme is provided by a report by Arendt and Kolodziejczyk[15] on the reaction between N-formylphenylalanine and DCC. They suggested that the isomeric acylureas are formed in parallel reactions, and that, consequently, N-acylurea is not formed via O-acylisourea. In this context, N,N-dimethylglycine hydrochloride was reported to react with DCC in ethanol to give a high yield of the ethyl ester; however, the free acid, which most likely exists as the zwitterion in ethanol, gives N-acylurea.[16]

There are other mechanistic alternatives, and the most obvious one is that in DMF, but not in dichloromethane, the transformation 4→5 is reasonably fast compared to reaction with a nucleophile. This alternative seems less likely, because 4 should react rapidly with p-nitrophenol as proposed by DeTar and Silverstein[13] for the corresponding reaction with acetic acid. The possibility of intramolecular base catalysis by the pyridine nitrogen could further facilitate ester formation. We have observed that acylation of the ε-amino group of BOC-lysine with p-nitrophenyl picolinate in DMF is substantially faster than acylation with the isomeric nicotinic and isonicotinic esters.[17]

Experimental

Melting points were measured on a Thomas Hoover capillary melting point apparatus and are uncorrected. NMR data are reported as δ-values downfield from TMS.

General procedure for esterification.[2] Acid (10 g, 81 mmol) and p-nitrophenol (13.4 g, 96 mmol)

were dissolved/suspended in 250 ml of DMF (with ice-cooling for nicotinic and picolinic acids). 16.7 g (81 mmol) of DCC were then added and stirring was continued for 4 h (after 1 h at 0 °C for nicotinic and picolinic acid). The mixture was filtered, the filter cake was washed with 50 ml of cold DMF, and the product was precipitated from the combined filtrate and washings by the addition of 300 ml of water. Filtration and drying *in vacuo* gave the crude product.

Purification. The esters of nicotinic and isonicotinic acids were purified by recrystallization from 2-propanol. The yield of *p*-nitrophenyl nicotinate was 11.3 g, 57 %; m.p. 172–173 °C, lit.[18] 172 °C. The yield of *p*-nitrophenyl isonicotinate was 12.1 g, 61 %; m.p. 139–141 °C, lit.[3] 137–139 °C.

The crude product from the reaction of picolinic acid was dissolved in 250 ml of hot 2-propanol and the solution was kept in a refrigerator overnight. The precipitated product was redissolved in 170 ml of hot 2-propanol. The ester precipitated from this solution while it was still quite warm, and was isolated by filtration. Two more recrystallizations gave 3.15 g, 15.8 %, of pure *p*-nitrophenyl picolinate; m.p. 155–157 °C (decomp.), lit.[4] 144–146 °C (decomp.). We consistently observed melting points of about 155 °C. NMR (CDCl$_3$), 200 MHz: δ 7.48 and 8.34, m, 2H each, AA'BB' pattern from the nitrophenyl group; 7.62, eight lines, 1H, pyridine H^5, $J_{5,4}$ = 8 Hz, $J_{5,6}$ = 5 Hz, $J_{5,3}$ = 1.5 Hz; 7.97, six lines, 1H, pyridine H^4, $J_{4,5}$ = $J_{4,3}$ = 8 Hz, $J_{4,6}$ = 1.8 Hz; 8.30, m. 1H, pyridine H^3, $J_{3,4}$ = 8 Hz, $J_{3,5}$ <1 Hz; 8.87, m. 1H, pyridine H^6, $J_{6,5}$ = 5 Hz. Anal. C$_{12}$H$_8$N$_2$O$_4$: C, H, N.

The mother liquor from the 170 ml of solution was evaporated to give 11 g of crude acylurea. Recrystallization from 50 ml of 2-propanol gave 8.5 g, 32 %, of pure *N*-picolinoyl-*N*,*N*'-dicyclohexylurea; m.p. 144–146 °C, lit.[19] 144–145 °C. NMR (CDCl$_3$), 200 MHz: δ 0.9–2.0, m, 20H, cyclohexyl CH$_2$ groups; 3.52, m, and 4.18, m, 1H each, cyclohexyl CH groups; 6.02, broad d, 1H, NH; 7.35, eight lines, 1H, pyridine H^5; 7.68, m, 1H, pyridine H^3; 7.79, six lines, 1H, pyridine H^4; 8.57, m, 1H, pyridine H^6. The values of the coupling constants for the aromatic protons are the same as for the corresponding protons in the ester.

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