The Preparation of Some 4-Substituted Nicotinic Acids and Nicotinamides

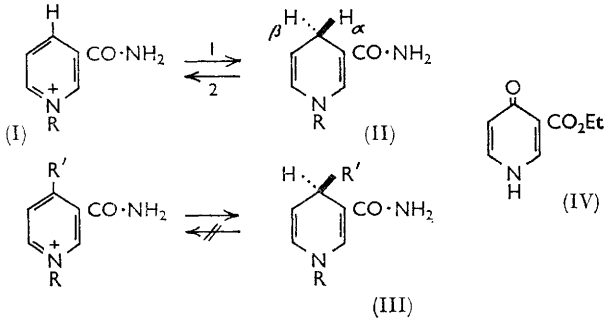
By W. C. J. Ross

Nicotinamide adenine dinucleotide (NAD) analogues in which the 4-position of the pyridine ring carries a substituent are potential inhibitors of the glycolytic process by which many cancer cells derive an appreciable proportion of their energy requirements. Such analogues should be produced *in vivo* by the administration of 4-substituted nicotinic acid derivatives.

The preparation of the following derivatives is now described: 4-hydroxy-, 4-methoxy-, 4-butoxy-, 4-(2-hydroxyethylthio)-, 4-*S*-cysteinyl-, 4-amino-, 4-anilino-, and 4-benzylamino-nicotinic acid and -nicotinamide. 4-Ethoxy- and 4-(2-bromoethylthio)-nicotinic acid and 4-benzylthionicotinamide were also obtained.

A preliminary report on the biological activity of some of the derivatives is given.

Some neoplastic cells are more dependent on glycolytic energy than normal cells.[1] The stereospecificity of NAD-mediated hydrogen-transfer reactions suggests a means by which relatively specific inhibition of gycolysis could be achieved. The first oxidative step in glycolysis is the conversion of glyceraldehyde phosphate into diphosphoglyceric acid, in which the pyridine ring of NAD (I) accepts a hydrogen atom in the β-configuration. In the later reduction of pyruvate to lactate NAD is regenerated from NADH (II) by the transfer of the α-hydrogen atom (step 2).



Normally the NADH produced in step 1 can be utilised in step 2. If the 4-hydrogen atom in (I) is replaced by some other group step 1 would produce a dihydropyridine derivative (III) which cannot be utilised in step 2 since it does not contain an α-hydrogen atom. If the 4-substituted NAD is an active coenzyme in step 1 the glycolytic process which involves recycling of NAD should be competitively inhibited by the presence of the substituted co-factor. Since numerous nicotinic acid derivatives are incorportated *in vivo* into the NAD molecule [2] it should be sufficient to administer suitably substituted nicotinic acid derivatives to produce the effect.

The present Paper reports the preparation of a number of substituted nicotinic acids and amides. Special attention has been given to the incorporation of groups which would facilitate intracellular transport. Thus attempts have been made to introduce longer-chain alkoxy-groups and substituted amino- and thiol-groups that would increase lipoid solubility and also amino-acid containing groups which are known to increase carcinostatic potency.[3] A 4-(2-bromoethylthio)-derivative has also been prepared as potential irreversible antagonist.[4]

The known 4-methylnicotinamide was prepared by hydrolysing 4-methylnicotinonitrile [5] with IRA-400 resin [6] but all the other 4-substituted derivatives were

[1] A. C. Aisenberg, "The Glycolysis and Respiration of Tumours," Academic Press, New York, 1961.

[2] S. R. Humphreys, J. M. Vendetti, C. J. Ciotti, I. Kline, A. Goldin, and N. O. Kaplan, *Cancer Res.*, 1962, **22**, 483.

[3] W. C. J. Ross, *Biochem. Pharmacol.*, 1964, **13**, 969.

[4] B. R. Baker, *Cancer Chemotherapy Rep.*, 1959, **4**, 1.

[5] J. M. Bobbit and D. A. Scola, *J. Org. Chem.*, 1960, **25**, 560.

[6] A. Galat, *J. Amer. Chem. Soc.*, 1948, **70**, 3945.

obtained by using 4-chloro-nicotinic acid or -nicotinamide as starting material.

4-Chloronicotinic acid was prepared by oxidation of 4-chloro-3-methylpyridine with potassium permanganate, essentially by the method of Herz and Murty;[7] however, it was necessary to modify the published method for preparing the chloropicoline.

3-Methylpyridine 1-oxide was nitrated by a modification of Herz and Tsai's method[8] giving 3-methyl-4-nitropyridine 1-oxide. The action of phosphorus trichloride in dry chloroform below 10° on the nitro-1-oxide was originally stated to give a 65% yield of 3-methyl-4-nitropyridine.[8] Later these conditions were reported to give a mixture of 4-nitro- and 4-chloro-3-methylpyridines and if the mixture was heated for a few minutes only the chloro-compound was obtained.[7] It has now been found that when dry purified chloroform is used as solvent the only product is the nitro-compound even after heating. Some 4-chloro-3-methylpyridine was obtained when chloroform containing 2% of ethanol was used but the best yields were achieved when this chloroform was first saturated with hydrogen chloride. It would appear that free hydrogen chloride is necessary for the replacement of the nitro-group by a chlorine atom.

In the anionic form of 4-chloronicotinic acid the halogen atom is relatively unreactive, for an aqueous solution of the sodium salt can be boiled for several hours without decomposition. However, if the free acid is heated in water, replacement of the chlorine atom by a hydroxyl group rapidly occurs. This is due partly to the increased electron-withdrawing effect of the carboxyl group as compared with the carboxylate ion and partly to the electron attraction of the ring-nitrogen atom when protonated by the liberated hydrogen chloride. Similarly when the chloro-acid is heated with dry methanol 4-methoxynicotinic acid is formed in good yield. If the methanol is first saturated with hydrogen chloride, methyl 4-methoxynicotinate is obtained. When the chloro-acid is heated with ethanol, 4-ethoxynicotinic acid is formed with about 10% of the pyridone ester (IV). When isopropyl, butyl, or isopentyl alcohol are used the corresponding pyridone esters are the only products that can be isolated.

The formation of the pyridone esters is probably due partly to the more rapid reaction of the chloro-acid with any traces of water present and also to the known lability of 4-alkoxy-groups under conditions where the ring-nitrogen atom becomes protonated (see below). Subsequent esterification of the hydroxy-acid thus formed will occur under the catalytic influence of the liberated hydrogen chloride. On heating the chloro-acid in butyl alcohol saturated with hydrogen chloride butyl 4-butoxynicotonate and butyl 1,4-dihydro-4-oxonicotinate are formed in the ratio 2 : 1. 4-Butoxynicotinic acid was obtained by alkaline hydrolysis of the former ester.

[7] W. Herz and D. R. K. Murty, *J. Org. Chem.*, 1961, **26**, 122.
[8] W. Herz and L. Tsai, *J. Amer. Chem. Soc.*, 1954, **76**, 4184.

The pyridone ester structure of the products has been confirmed by hydrolysis to the acid, by conversion into the oxo-amide on treatment with methanolic ammonia and by the characteristic pyridone absorption shown in the infrared spectrum at 1640—1650 cm.$^{-1}$ (see ref. 9). A characteristic property of these pyridone esters is their sublimation without decomposition below the melting point. Further confirmation of structure (IV) is afforded by the presence of a single group, pK_a about 9·7, on titration in the range pH 4—12. The electron-attracting alkoxycarbonyl groups have the expected effect on dissociation, for the pK_a of the hydroxyl group in 4-hydroxypyridine is 11·1. An ionised carboxyl group has relatively little effect, for the pK_a of the hydroxyl group in 4-hydroxynicotinic acid is 10·8. Expected electronic effects are also observed in the case of the 4-substituted nicotinic acids for the pK_a of nicotinic acid (4·8) is lowered by a chlorine atom (to 3·75) and raised by alkoxy-groups (to 6·2).

S-Substituted 4-mercaptonicotinic acids are formed when 4-chloronicotinic acid is allowed to react with thiophenol, 2-mercaptoethanol, and cysteine. With the last two compounds, reaction is entirely with the more nucleophilic thiol group. 4-(2-Hydroxyethylthio)-nicotinic acid is converted into 4-(2-bromoethylthio)-nicotinic acid hydrobromide when heated with concentrated aqueous hydrobromic acid showing that the 4-alkylthio-linkage is more stable than a 4-alkoxy-linkage.

4-Aminonicotinic acid is conveniently prepared by the action of concentrated aqueous ammonia under pressure on 4-chloronicotinic acid.[10] The amino-acid was characterised by the preparation of its acetyl derivative, which had previously been obtained by oxidation of 4-acetamido-3-methylpyridine,[11] and its methyl ester.

4-Anilinonicotinic acid has been prepared by heating 4-chloronicotinic acid with aniline.[7] A compound of higher melting point has now been prepared by this method and 4-benzylaminonicotinic acid has been similarly prepared in good yield.

4-Chloronicotinamide has been prepared by the action of ammonia on 4-chloronicotinoyl chloride or more conveniently on the mixed anhydride formed from 4-chloronicotinic acid and isobutyl chloroformate. The chloro-amide readily decomposed but it forms a stable hydrochloride and picrate.

4-Chloronicotinamide reacts with 2-mercaptoethanol, toluene-ω-thiol, cysteine, aniline, and benzylamine to give 4-(2-hydroxyethylthio)-, 4-benzylthio-, 4-*S*-cysteinyl-, 4-anilino- and 4-benzylamino-nicotinamide, respectively, but this method cannot be adapted for the preparation of the other required amides.

The corresponding nicotinamide is readily obtained by the action of saturated methanolic ammonia at 90° on

[9] M. St. C. Flett, "Characteristic Frequencies of Chemical Groups in the Infra Red," Elsevier, London, 1963, p. 73.
[10] E. C. Taylor and J. A. Crovetti, *J. Org. Chem.*, 1954, **19**, 1633.
[11] T. Itai and H. Ogura, *J. Pharm. Soc. Japan*, 1955, **75**, 292.

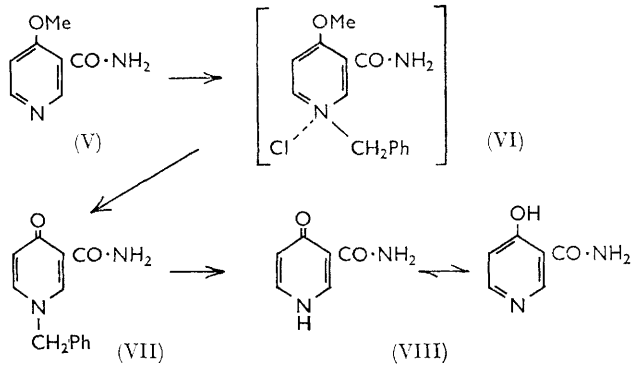
the following esters: butyl 4-hydroxy-, methyl 4-methoxy-, butyl 4-butoxy-, and methyl 4-amino-nicotinate. 4-Methoxynicotinamide had previously been prepared by hydrolysis of 3-cyano-4-methoxypyridine [12] and by reducing its 1-oxide, obtained by the action of sodium methoxide on 4-nitronicotinamide 1-oxide.[13] 4-Aminonicotinamide has been prepared by the prolonged action of liquid ammonia under high pressure on methyl 4-aminonicotinate: [14] the modification now described is more convenient.

Alkaline hydrolysis of 4-butoxynicotinamide gives 4-butoxynicotinic acid in high yield but acid hydrolysis affords only 4-hydroxynicotinic acid. This is another instance of the effect of a charge on the ring-nitrogen atom on the stability of 4-alkoxy-groups (see below). 4-Methoxynicotinic acid was stable in boiling water at pH 4; at this pH the ring-nitrogen atom (pK_a about 2·5) will not be appreciably protonated.

Wieland *et al.*[12] considered a number of possible routes for the synthesis of 4-hydroxynicotinamide and eventually prepared the amide by the action of ammonia on the mixed anhydride formed from 4-hydroxynicotinic acid and ethyl chloroformate. The yield was only 30% and purification by chromatography on cellulose powder was tedious and not suitable for the preparation of larger amounts. This route has now been repeated with isobutyl chloroformate but similar difficulties of purification were encountered. An improved method was described by Biener and Wieland.[13] 4-Benzyloxynicotinic acid 1-oxide was converted successively into the methyl ester and the amide and this was reduced to give the hydroxy-amide.

Two new routes have now been devised. Butyl 4-hydroxynicotinate (see above) is readily converted into the hydroxy-amide in 75% yield by the action of methanolic ammonia. This method is suitable for larger-scale preparations since purification by crystallisation from water is readily achieved. The ready displacement of the methoxyl group by a hydroxyl group in quaternary derivatives of 4-methoxynicotinamide [12] suggested an alternative route to the hydroxy-amide. 4-Methoxynicotinamide (V) was treated with benzyl chloride in aqueous methanol. Under these conditions the quaternary derivative (VI) first formed interacts with water affording *N*-benzyl-1,4-dihydro-4-oxonicotinamide (VII). Catalytic hydrogenation of the *N*-benzyl derivative gives the hydroxy-amide (VIII) in almost quantitative yield.

Preliminary Biological Data.—There is a wide variation in toxicity between the derivatives so far examined. While the LD_{50} (in rats) is >1000 mg./kg. for 4-methyl- and 4-hydroxy-nicotinic acid and -nicotinamide the values for 4-methoxynicotinamide, 4-aminonicotinic acid, and 4-aminonicotinamide are 140, 113, and 18 mg./kg., respectively. Moderate inhibition of the transplanted Walker rat tumour is exhibited by 4-(2-hydroxyethylthio)nicotinamide (66% inhibition of growth), 4-(2-bromoethylthio)nicotinic acid (50%) and 4-anilinonicotinamide (50%). Up to 20% extension of survival time of mice bearing the L1210 leukæmia is produced



by 4-amino- and 4-(2-hydroxyethylthio)-nicotinic acid and by 4-methyl-, 4-chloro-, 4-amino-, and 4-anilinonicotinamide. Such life extension would be produced by about a 90% reduction in the number of circulating leukæmic leucocytes.[15] The results quoted were achieved by intraperitoneal injections of the various agents.

EXPERIMENTAL

Melting points were determined with a Townson and Mercer heated metal block apparatus and are corrected. The activated alumina used was Spence type H.

3-Methyl-4-nitropyridine 1-Oxide.—Aqueous hydrogen peroxide (80 ml.; 30%) was added to a stirred solution of 3-methylpyridine (50 g.) in glacial acetic acid (150 ml.) during 30 min., the temperature being kept below 10°. After being heated at 70° for 24 hr. the solution was evaporated under reduced pressure. The resulting pale yellow viscous oil was cooled below 5° and a mixture of concentrated sulphuric acid (158 ml.) and fuming nitric acid (124 ml.; *d* 1·5) was added dropwise with vigorous stirring. After being heated cautiously to 100—105° for 2 hr. the mixture was poured on crushed ice and the pH adjusted to 3 (Na_2CO_3). The solid was filtered off and extracted with hot acetone. This extract was combined with material obtained by extracting the filtrate with chloroform. After removal of solvents under reduced pressure the product was crystallised from acetone. The yield of nitro-1-oxide, m. p. 137° (lit.,[8] 136—138°), was 58 g.

4-Chloronicotinic Acid.—3-Methyl-4-nitropyridine 1-oxide (50 g.) was dissolved in chloroform (1 l.), and the solution was saturated with dry hydrogen chloride at room temperature. Phosphorus trichloride (80 ml.) was added dropwise to the stirred solution (at 0—5°). After the solution had been allowed to reach room temperature reaction was initiated by cautious warming on a steam-bath. The reaction then proceeded without heating and was moderated as necessary by cooling. When the reaction subsided the mixture was heated under reflux for 20 min. and then evaporated under reduced pressure. The residue was dissolved in iced water (800 ml.) and after the addition of an excess of saturated aqueous sodium carbonate the solution was steam-distilled giving 4-chloropicoline (36 ml.). This

[12] T. Wieland, C. Fest, and G. Pfleiderer, *Annalen*, 1961, **642**, 163.
[13] H. Biener and T. Wieland, *Chem. Ber.*, 1962, **95**, 277.
[14] H. H. Fox, *J. Org. Chem.*, 1952, **17**, 547.
[15] H. E. Skipper, F. M. Schabel, jun., and W. S. Wilcox, *Cancer Chemotherapy Rep.*, 1964, **35**, 1.

was dispersed in water (500 ml.) and potassium permanganate (119 g.) was added. The stirred mixture was heated at 80—90° for 4 hr. and then steam-distilled to remove any unchanged chloropicoline. The precipitated manganese dioxide was filtered off through "Hyflo" filter aid and washed well with hot water. After the filtrates had been concentrated to about 100 ml. the pH was adjusted to 3 with concentrated hydrochloric acid. The precipitated 4-chloronicotinic acid was quickly filtered off and pressed as dry as possible before being washed with acetone (3 × 30 ml.) and then with dry ether. The yield was 29 g. A specimen of the acid was purified by dissolution in the theoretical amount of N-sodium hydroxide solution and reprecipitation by slow addition of an equivalent of dilute hydrochloric acid. The acid formed prismatic needles, decomp. 175—177° (lit., m. p. 162—163°,[7] 164°[10]) (Found: C, 45·8; H, 2·7; Cl, 22·4; N, 8·8%; Equiv., by titration, 157·5. Calc. for $C_6H_4ClNO_2$: C, 45·7; H, 2·6; Cl, 22·5; N, 8·9%; Equiv., 157·5), pK_a (COOH) 3·75. All equivalents and pK_a values described in this Paper were determined by potentiometric titration of 0·001 mole of the compound in aqueous ethanol (1 : 1; 20 ml.) with aqueous sodium hydroxide (0·1N).

3-Methyl-4-nitropyridine.—Phosphorus trichloride (30 ml.) was added dropwise to a solution of 3-methyl-4-nitropyridine 1-oxide (5 g.) in dry chloroform (100 ml.), cooled below 3°. After being heated under reflux for 10 min. the mixture was poured on ice containing a slight excess of sodium hydroxide. On passing a chloroform extract of the solution down a column of activated alumina and eluting with fresh chloroform a series of oily fractions were obtained all of which gave picrates, m. p. 125—126° (lit.,[8] m. p. 128—129° for 3-methyl-4-nitropyridine picrate).

4-Hydroxynicotinic Acid.—4-Chloronicotinic acid (1 g.) was heated for 1 hr. in water (20 ml.). After adjusting the pH of the solution to 4 with sodium hydroxide, evaporation to half bulk and cooling afforded the hydroxy-acid (750 mg.), m. p. 260° (decomp.) (lit.,[16] m. p. 250°); $\nu_{max.}$ (Nujol) 1640 cm.$^{-1}$, pK_a (COOH) 6·2 and (OH) 10·8.

4-Methoxynicotinic Acid.—A solution of 4-chloronicotinic acid (10 g.) in dry methanol (200 ml.) was heated under reflux for 2½ hr. After removal of the methanol under reduced pressure the residue was dissolved in water and the pH was adjusted to 3·5 with sodium hydroxide. The residue obtained on evaporation to dryness was repeatedly extracted with ethanol (4 × 100 ml.). Concentration of the extracts gave the *methoxy-acid* as thin pearly plates (80%), m. p. 178—179° (decomp.) (Found: C, 54·5; H, 4·5; N, 9·3. $C_7H_7NO_3$ requires C, 54·9; H, 4·6; N, 9·1%), pK_a (COOH) 6·15. The *picrate*, m. p. 175—178°, formed plates from ethanol (Found: C, 40·6; H, 2·4; N, 15·1. $C_{13}H_{10}N_4O_{10}$ requires C, 40·9; H, 2·4; N, 14·7%). The acid was unchanged when its aqueous solution (0·1 molar; initial pH 4) was boiled for 1 hr.

Methyl 4-Methoxynicotinate.—A solution of the chloro-acid (2 g.) in dry methanol (20 ml.) was saturated with dry hydrogen chloride and heated under reflux for 3 hr. After removal of the solvent under reduced pressure the residue was dissolved in water and an excess of sodium carbonate was added. A dried chloroform extract of the mixture was passed through a short column of activated alumina. Early eluates contained *methyl 4-methoxynicotinate* (1·4 g.) which formed long prismatic needles, m. p. 83—84°, from light petroleum (b. p. 60—80°) (Found: C, 57·5; H, 5·5; N, 7·9. $C_8H_9NO_3$ requires C, 57·4; H, 5·4; N, 8·4%).

The Action of Ethanol on 4-Chloronicotinic Acid.—A solution of the chloro-acid (1 g.) in ethanol (40 ml.) was heated under reflux for 1 hr. On concentration under reduced pressure prisms, m. p. 178° (decomp.), separated. Potentiometric titration indicated that this product was *4-ethoxynicotinic acid, hydrochloride* pK_a (COOH) 6·25 (Found: C, 47·3; H, 5·1; Cl, 16·9; N, 6·8. $C_8H_{10}ClNO_3$ requires C, 47·2; H, 5·0; Cl, 17·4; N, 6·9%). The acid forms a *picrate*, flattened needles, m. p. 172—173°, from ethanol (Found: C, 42·7; H, 3·3; N, 14·4. $C_{14}H_{12}N_4O_{10}$ requires C, 42·4; H, 3·1; N, 14·1%).

Potentiometric titration of the mixture, prepared as above, indicated the presence of about 50% of product of pK_a 6·25 and about 10% of material of pK_a about 10. When this mixture was evaporated to dryness after adjustment of the pH to 4 and the residue was extracted with ethanol a small quantity of insoluble material remained. This formed prisms (from acetone), m. p. 229—230°, subliming in the capillary. Titration of this compound indicated that it contained a single dissociating group of pK_a 9·7. It is probably *ethyl 1,4-dihydro-4-oxonicotinate* (Found: C, 57·3; H, 5·5; N, 8·4. $C_8H_9NO_3$ requires C, 57·4; H, 5·4; N, 8·4%), $\nu_{max.}$ (Nujol) 1640 cm.$^{-1}$. The *picrate* forms large plates, m. p. 158—160°, from ethanol (Found: C, 42·4; H, 3·1; N, 14·5. $C_{14}H_{12}N_4O_{10}$ requires C, 42·4; H, 3·1; N, 14·1%).

The Action of iso*Propyl and Isopentyl Alcohol on 4-Chloronicotinic Acid*.—A solution of 4-chloronicotinic acid (2 g.) in *iso*propyl alcohol (10 ml.) was heated under reflux for 7 hr. Water was added to the cooled solution and the pH was adjusted to 4 with sodium hydroxide. The solid which separated formed prismatic needles (from ethanol), m. p. 228—229°, subliming in the capillary (Found: C, 59·6; H, 6·1; N, 7·7. $C_9H_{11}NO_3$ requires C, 59·7; H, 6·1; N, 7·7%); pK_a (OH) 9·7, $\nu_{max.}$ (Nujol) 1650 cm.$^{-1}$, indicated that the product is iso*propyl 1,4-dihydro-4-oxonicotinate*.

Isopentyl 1,4-dihydro-4-oxonicotinate, plates (from ethanol), m. p. 219—221° with sublimation, was similarly prepared (Found: C, 63·0; H, 7·5; N, 6·8. $C_{11}H_{15}NO_3$ requires C, 63·1; H, 7·2; N, 6·7%), pK_a (OH) 9·7, $\nu_{max.}$ (Nujol) 1650 cm.$^{-1}$. When this ester (200 mg.) was heated under reflux with aqueous sodium hydroxide (5 ml.; N) for 2 hr. 4-hydroxynicotinic acid, m. p. 255—260°, was formed. This was identical (mixed m. p. and i.r. spectrum) with the product described above.

The Action of Butanolic Hydrogen Chloride on 4-Chloronicotinic Acid.—A solution of 4-chloronicotinic acid (10 g.) in n-butanol (500 ml.) was saturated with dry hydrogen chloride and heated under reflux for 2 hr. The residue obtained after removal of the solvent under reduced pressure was dissolved in water and the pH adjusted to 7 with solid sodium hydrogen carbonate. Extraction with chloroform then afforded an oil which on rubbing with light petroleum (b. p. 40—60°) gave a solid (4·5 g.); the petroleum contained an oil (8·1 g.).

The solid formed plates, m. p. 199—200° with sublimation, from methanol (Found: C, 61·5; H, 7·0; N, 7·2. $C_{10}H_{13}NO_3$ requires C, 61·5; H, 6·7; N, 7·2%), $\nu_{max.}$ (Nujol) 1650 cm.$^{-1}$; that this was *butyl 1,4-dihydro-4-oxonicotinate* was proved by the pK_a (9·7) of the titratable group and by the formation of 4-hydroxynicotinamide when heated with methanolic ammonia (see below).

The oily product formed a *picrate*, m. p. 137—139°, plates from ethanol (Found: C, 50·4; H, 5·3; N, 11·9.

[16] A. Kirpal, *Monatsh.*, 1902, **23**, 243.

$C_{20}H_{24}N_4O_{10}$ requires C, 50·0; H, 5·1; N, 11·7%). That this neutral product was butyl 4-butoxynicotinate was confirmed by its conversion into 4-butoxynicotinamide (see below) and by hydrolysis with N-sodium hydroxide to *4-butoxynicotinic acid*, m. p. 135—137°, prismatic needles from acetone (Found: C, 61·6; H, 6·6; N, 7·2%; Equiv., 196. $C_{10}H_{13}NO_3$ requires C, 61·5; H, 6·7; N, 7·2%; Equiv., 195·2).

4-Phenylthionicotinic Acid.—4-Chloronicotinic acid (1 g.), thiophenol (4 ml.), and acetone (10 ml.) were heated on a steam-bath for 1 hr. The product was collected by filtration of the cooled solution and dissolved in an excess of aqueous ammonia. When this solution was boiled *4-phenylthionicotinic acid* separated. It formed prisms, m. p. 239—240° (decomp.), from acetone (Found: C, 62·2; H, 4·1; N, 6·0; S, 14·1%; Equiv., 232; pK_a (COOH) 5·38. $C_{12}H_9NO_2S$ requires C, 62·3; H, 3·9; N, 6·1; S, 13·9%; Equiv., 231·3).

4-(2-Hydroxyethylthio)nicotinic Acid.—4-Chloronicotinic acid (15·7 g.), 2-mercaptoethanol (14 ml.), sodium acetate trihydrate (13·6 g.), and water (10 ml.) were heated on a steam-bath for 1 hr., a clear solution being obtained. The solid which separated on cooling was filtered off and washed with a little iced water (yield, 15·8 g.). Crystallisation from water gave the *thio-acid* as flattened needles, m. p. 190° (decomp.). This material is apparently a hydrate for water is rapidly lost above 100° and slowly at room temperature, the crystals crumbling to a powder. The equivalent weight of a freshly filtered sample is 213, pK_a (COOH) 5·35, the monohydrate requires equiv. 217. The analytical specimen was dried at 100°/0·5 mm. for 4 hr. (Found: C, 48·1; H, 4·8; N, 7·0; S, 15·9. $C_8H_9NO_4S$ requires C, 48·2; H, 4·6; N, 7·0; S, 16·1%).

4-(2-Bromoethylthio)-3-carboxypyridinium Bromide.—A solution of the thio-acid (4 g.) in hydrobromic acid (20 ml.; *d* 1·5) was heated under reflux for 1 hr. On cooling, the solution yielded the *bromoethylthio*-derivative (4·2 g.) as flattened needles. Slow heating results in decomposition but when the compound is placed in the apparatus at 225° a clear melt forms just before charring (Found: C, 28·0; H, 2·7; Br, 45·7; N, 4·3; S, 9·4. $C_8H_9Br_2NO_2S$ requires C, 28·0; H, 2·6; Br, 46·6; N, 4·1; S, 9·4%).

4-(S-Cysteinyl)nicotinic Acid.—4-Chloronicotinic acid (12·56 g.), cysteine hydrochloride (12·56 g.), sodium acetate trihydrate (21·76 g.), and water (80 ml.) were heated on a steam-bath. After 20 min. the solution was clear but soon afterwards a solid separated. Heating was continued for 1½ hr. and then the solution was cooled and the product (16·0 g.) collected by filtration and washed with acetone. The *cysteinylnicotinic acid* was purified by dissolving it in an excess of aqueous ammonia and then adjusting the pH to 2·5 with dilute hydrochloric acid. The acid separated as prisms, m. p. 225—230° (decomp.) (Found: C, 44·9; H, 4·0; N, 11·8; S, 13·6. $C_9H_{10}N_2O_4S$ requires C, 44·6; H, 4·2; N, 11·6; S, 13·2%).

4-Aminonicotinic Acid and its Methyl Ester.—4-Chloronicotinic acid (20 g.) in concentrated aqueous ammonia (800 ml.) was heated in an autoclave at 175—180° for 5 hr. Evaporation to low bulk, dilution with water (1 l.), and re-evaporation until no more ammonia was evolved gave a solution which on cooling deposited the amino-acid as prismatic needles (15 g.), m. p. 330—340° (decomp.). The melting/decomposition point is very dependent on the rate of heating; literature values range from 328° [10] to 357°.[17]

A solution of the amino-acid (12 g.) in acetic anhydride (100 ml.) was left at room temperature for 5 days. Removal of the solvent under reduced pressure afforded the acetyl derivative which formed prismatic needles (9 g.), m. p. 257° (lit.,[11] m. p. 255°), from water.

Some difficulty was experienced in the esterification of the amino-acid; only the following procedure gave satisfactory results. The amino-acid (13 g.) was slowly added to ice-cooled concentrated sulphuric acid (35 ml.), the temperature being kept below 20°. Methanol (100 ml.) was then added slowly to the stirred solution (temp. below 30°). After being heated on a steam-bath for 4 hr. the solution was cooled and poured on ice and then neutralised with solid sodium carbonate (temp. below 10°). The methyl ester was extracted with chloroform and crystallised from benzene, forming long prismatic needles, m. p. 176°, soon resolidifying due to betaine formation (lit., m. p. 173°,[16] 173·5° [11]).

4-Anilino- and 4-Benzylamino-nicotinic Acid.—4-Chloronicotinic acid (1 g.) and aniline (2 g.) were heated on a steam-bath for 2 hr. Water (10 ml.) and aqueous sodium hydroxide (6·4 ml.; N) were added and the solid (1·2 g.) collected by filtration. The anilino-acid formed needles, m. p. 285—287° (lit., m. p. 268—269°), from a large volume of methanol (Found: C, 67·3; H, 4·8; N, 12·9. Calc. for $C_{12}H_{10}N_2O_2$: C, 67·2; H, 4·7; N, 13·1%).

An ethereal suspension of the anilino-acid was treated with an excess of ethereal diazomethane and when solution was complete sufficient acetic acid to decolourise was added. The eluates obtained when the solution was passed through a column of activated alumina contained *methyl 4-anilinonicotinate*, prisms, m. p. 85—86°, from light petroleum (b. p. 60—80°) (Found: C, 68·5; H, 5·5. $C_{13}H_{12}N_2O_2$ requires C, 68·4; H, 5·3%).

4-Benzylaminonicotinic acid, similarly prepared in 75% yield, formed slender needles, m. p. 265—268°, from water (Found: C, 68·3; H, 5·2; N, 12·2. $C_{13}H_{12}N_2O_2$ requires C, 68·4; H, 5·3; N, 12·3%).

4-Chloronicotinamide.—(*a*) 4-Chloronicotinic acid (3 g.) was heated under reflux with thionyl chloride (30 ml.) for ¾ hr. After distillation of the thionyl chloride under reduced pressure the residue was suspended in dry benzene (100 ml.) and dry ammonia was passed in for 1 hr. (temp. below 20°). Aqueous ammonia (15 ml.; *d* 0·88) was then added with vigorous stirring. The solid product (1·75 g.) was filtered off and crystallised from acetone or ethyl acetate. 4-Chloronicotinamide formed prismatic needles which sintered at 130° (decomp.). If placed in the melting-point apparatus at 165° it formed a clear melt before decomposing: no m. p. is recorded in the literature. The crystalline amide becomes resinous when kept for a few days at room temperature (Found: C, 45·8; H, 3·4; Cl, 22·6; N, 18·0. Calc. for $C_6H_5ClN_2O$: C, 46·0; H, 3·2; Cl, 22·7; N, 17·9%). By dissolving the amide in concentrated hydrochloric acid and then adding a large volume of acetone the *hydrochloride* is obtained as flattened needles, decomp. above 250° (Found: C, 37·6; H, 3·3; Cl, 36·5; N, 14·6. $C_6H_6Cl_2N_2O$ requires C, 37·3; H, 3·1; Cl, 36·7; N, 14·5%). The *picrate* forms plates, m. p. 168° (decomp.), from ethanol (Found: C, 37·8; H, 2·3; Cl, 9·4. $C_{12}H_8ClN_5O_8$ requires C, 37·4; H, 2·1; Cl, 9·2%).

(*b*) A solution of 4-chloronicotinic acid (10·48 g.) and

[17] E. C. Taylor and J. S. Driscoll, *J. Amer. Chem. Soc.*, 1960, **82**, 3141.

triethylamine (9·25 ml.) in dry tetrahydrofuran (250 ml.) was cooled to 0° and isobutyl chloroformate (9 ml.) was added dropwise with stirring. After 1 hr. at 0° dry ammonia was passed into the vigorously stirred solution for 2½ hr. (temp. below 15°). The solution was then filtered and evaporated under reduced pressure, and the residue was dissolved in ice-cooled concentrated hydrochloric acid (60 ml.). Addition of acetone (500 ml.) gave the hydrochloride of 4-chloronicotinamide (7·8 g.), identical with that obtained by method (*a*).

4-(2-*Hydroxyethylthio*)*nicotinamide*.—Sodium hydrogen carbonate (16·7 g.) was slowly added to a solution of 4-chloronicotinamide hydrochloride (12·7) and 2-mercaptoethanol (11·5 ml.) in water (30 ml.). The clear solution was heated on a steam-bath for ½ hr., solid then separating. The cooled solution was filtered and the product (14·7 g.) was washed with a little ice–water. 4-(2-*Hydroxyethylthio*)-*nicotinamide* formed long prismatic needles, m. p. 188—190°, from water (Found: C, 48·0; H, 5·2; N, 14·0; S, 16·6. $C_8H_{10}N_2O_2S$ requires C, 48·5; H, 5·1; N, 14·1; S, 16·2%).

4-*Benzylthionicotinamide* (Margaret A. Webster).—Toluene-ω-thiol (3·2 g.) in ethanol (50 ml.) was added to a solution of 4-chloronicotinamide hydrochloride (5 g.) in water (10 ml.) containing aqueous sodium hydroxide (15·4 ml.; N), and the mixture was heated under reflux for 1½ hr. The cooled solution was filtered and the filtrate evaporated giving 4-*benzylthionicotinamide* (5·1 g., 82%), which formed pale yellow plates, m. p. 194—195°, from ethanol (Found: C, 64·0; H, 5·1; N, 11·8; S, 13·5. $C_{13}H_{12}N_2OS$ requires C, 63·9; H, 5·0; N, 11·5; S, 13·1%).

4-(S-*Cysteinyl*)*nicotinamide*.—4-Chloronicotinamide hydrochloride (1·92 g.), cysteine hydrochloride (1·57 g.), sodium acetate trihydrate (4·08 g.), and water (10 ml.) were heated on a steam-bath for ½ hr. The cooled solution deposited solid which was collected and crystallised by dissolution in an excess of aqueous ammonia and then heating until no more ammonia was evolved. 4-(S-*Cysteinyl*)*nicotinamide* (1·4 g.) separated as lozenges, m. p. 215° (decomp.) (Found: C, 44·9; H, 4·6; N, 17·6; S, 13·5. $C_9H_{11}N_3O_3S$ requires C, 44·8; H, 4·6; N, 17·4; S, 13·3%).

4-*Anilino- and* 4-*Benzylamino-nicotinamide*.—4-Chloronicotinamide hydrochloride (5 g.) was heated with aniline (15 ml.) for 2 hr. on a steam-bath. After cooling, water (150 ml.) was added and the solid collected and washed successively with water, acetone, and ether. 4-*Anilinonicotinamide* (6 g.) formed platelets, m. p. 198—199°, from benzene (Found: C, 67·2; H, 5·5. $C_{12}H_{11}N_3O$ requires C, 67·5; H, 5·2%).

4-*Benzylaminonicotinamide*, similarly prepared in 50% yield, formed platelets, m. p. 161—162°, from benzene (Found: C, 68·9; H, 6·1; N, 18·5. $C_{13}H_{13}N_3O$ requires C, 68·7; H, 5·8; N, 18·5%).

4-*Hydroxy-*, 4-*Methoxy-*, 4-*Butoxy-*, *and* 4-*Amino-nicotinamide*.—Butyl 4-hydroxynicotinate (5 g.) in methanol (50 ml.), saturated with dry ammonia at 0°, was heated in a pressure bottle at 80—90° for 2 days. The residue obtained after evaporation was recrystallised from water. 4-Hydroxynicotinamide (3·75 g.) formed prismatic needles, m. p. 277—280° (lit.,[12,13] m. p. 263°) (Found: C, 52·6; H, 4·9; N, 20·2. Calc. for $C_6H_6N_2O_2$: C, 52·2; H, 4·4; N, 20·3%).

The following nicotinamides were similarly prepared from the corresponding esters.

4-Methoxynicotinamide, prisms, m. p. 153—155°, from benzene (lit., m. p. 151—153°,[12] 151°[13]); yield 75%. These crystals contain benzene of crystallisation which is lost on exposure to air. The analytical specimen was heated at 100°/10 mm. for 4 hr. (Found: C, 55·0; H, 5·3; N, 18·5. Calc. for $C_7H_8N_2O_2$: C, 55·3; H, 5·3; N, 18·4%). The *picrate* formed plates, m. p. 183—186°, from ethanol (Found: C, 40·4; H, 2·7; N, 18·3. $C_{13}H_{11}N_5O_9$ requires C, 40·9; H, 2·9; N, 18·4%).

4-*Butoxynicotinamide*, prisms, m. p. 109—110·5°, from benzene; yield 65% (Found: C, 61·5; H, 7·4; N, 14·7. $C_{10}H_{14}N_2O_2$ requires C, 61·8; H, 7·3; N, 14·4%).

4-Aminonicotinamide, needles, m. p. 235—236°, from water (lit.,[14] m. p. 229—230·5°) (Found: C, 52·5; H, 5·1; N, 30·5. Calc. for $C_6H_7N_3O$: C, 52·5; H, 5·2; N, 30·6%). In this case the product was extracted with hot chloroform or benzene and 50% of the original ester was recovered. The residue contained a 50% yield of the amino-amide.

Hydrolysis of 4-*Butoxynicotinamide*.— (*a*) 4-Butoxynicotinamide (1 g.) was heated under reflux with aqueous sodium hydroxide (10 ml.; N) until evolution of ammonia ceased—about 4 hr. Hydrochloric acid (10 ml.; N) was added and the solution was evaporated to dryness. On cooling a hot acetone extract of the residue, 4-butoxynicotinic acid (750 mg.), m. p. 135—137°, was obtained.

(*b*) The amide (600 mg.) in concentrated hydrochloric acid (5 ml.) was heated under reflux for 1 hr. The residue obtained on evaporation to dryness was dissolved in water and the pH adjusted to 4 with aqueous sodium hydroxide. When this solution was concentrated and cooled 4-hydroxynicotinic acid (300 mg.), m. p. 252—254°, was obtained.

N-*Benzyl*-1,4-*dihydro*-4-*oxonicotinamide*.—4-Methoxynicotinamide (1·67 g.), benzyl chloride (2 ml.), calcium carbonate (1·0 g.), methanol (30 ml.), and water (5 ml.) were heated under reflux for 5 days. The residue obtained after evaporation under reduced pressure was extracted with chloroform and the extracts were passed through activated alumina. The eluates contained the N-*benzyl* derivative, plates, m. p. 172—175°, from acetone; yield 1·9 g. (Found: C, 68·4; H, 5·6; N, 12·2. $C_{13}H_{12}N_2O_2$ requires C, 68·4; H, 5·3; N, 12·3%); $\nu_{max.}$ (Nujol) 1660 cm.$^{-1}$.

4-*Hydroxynicotinamide*.—A solution of the *N*-benzyl derivative (1·9 g.) in ethanol (80 ml.) containing palladium–charcoal catalyst (200 mg.; 5% Pd) was shaken in an atmosphere of hydrogen at 40—50°. The theoretical amount of hydrogen (186 ml.) was taken up during 5 hr. The catalyst was filtered off and repeatedly washed with hot water. Evaporation of the combined filtrates gave the hydroxy-amide (1·0 g.), m. p. 277—280°, identical with the product obtained from butyl 4-hydroxynicotinate.

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