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The preparation of ring-labelled tritiated nicotinic acid by exchange in concentrated sulphuric acid

A method is described for the preparation of ^3H-labelled nicotinic acid by exchange of the hydrogens of the pyridine ring. This is a simple and convenient method of preparing cheaply large quantities of radiochemically pure tritiated nicotinic acid. It can also be carried out, if necessary, on a semi-micro scale. The choice of ^3H rather than ^{14}C as the radioactive label is governed by several factors. Not only is ^3H itself a much cheaper material than ^{14}C, but also there are no simple methods available for the preparation of ring-labelled ^{14}C compounds as is the case for ^3H. As ^3H can form stable linkages with carbon it follows that ^3H can be used to label carbon atoms in the organic molecule. This is particularly useful as methods now exist for the detection of ^3H-labelled compounds on paper chromatograms[1].

The hydrogen on an aromatic ring is labile in conc. H_2SO_4 (ref. 2) but non-labile under ordinary biological conditions. It is therefore a simple matter to label an organic molecule in the aromatic ring provided it is chemically stable to conc. H_2SO_4.

A sample of nicotinic acid (5 mg) was placed at the bottom of a dry tube and the tube and its contents were cooled in a dry ice–alcohol or liquid-air bath. Tritiated sulphuric acid (0.05–0.01 ml), prepared by the dilution of fuming H_2SO_4 with tritiated water (4 C/ml) was run onto the sample through a capillary tube. Solution of the compound in H_2SO_4 was required for exchange to occur and it was found that a mole ratio of 10 moles of H_2SO_4 to 1 mole of nicotinic acid was suitable. The reaction tube was sealed and left under the required conditions of temperature and time (~30° for 3–5 weeks, optimum). The contents of the reaction tube were diluted to 2 N H_2SO_4 with distilled water. This dilution prevented any further exchange and lowered the H^+ concentration sufficiently so that the nicotinic acid would not be displaced from the resin. The diluted solution was passed down an ion-exchange column (1 g of the cation-exchange resin Zeo-Carb 225*) and the column was washed with distilled water to remove all SO_4^{2-} and any labile ^3H (in the carboxyl group). The nicotinic acid was eluted from the ion-exchange column with 6 N NH_4OH and the excess NH_3 removed by evaporation on a water bath. The residue was then chromatographed and a radioautograph made to test the radiochemical purity and the activity of the product.

The detection and the identification of the product was carried out by paper chromatography. A preliminary one-dimensional chromatogram, carrying a sample spot of the product and a reference spot of the original compound, was run in one of two solvent systems: *n*-butanol–acetic acid–water (4:1:2, v/v) or *n*-butanol–acetone–water (9:1:10, v/v). The dried chromatogram was viewed under ultraviolet light to locate the product. A radioautograph was then made of the chromatogram[1] to detect and to locate any radioactivity. By these two procedures, the presence of the required compound and of any decomposition products of the compound was detected and whether or not they were active was determined. That the active product was identical to the original nicotinic acid was confirmed by two-dimensional co-chromatography of the product and the original compound[1]. Nicotinic acid assays, by a microbiological method[3], were used to determine the degree of purity of the product.

* Permutit Co., Gunnersbury Ave., London.

The results obtained under three different sets of experimental conditions are shown in Table I.

It was found that the product could be readily purified by vacuum sublimation. The discoloured product from Expt. 3 (Table I), sublimed at 140° and 0.3 mm pressure, gave a white powdery sublimate with the same melting point as the original nicotinic acid sample. A chromatogram of the sublimate showed that the product from Expt. 3, after sublimation, contained only one radioactive compound.

TABLE I

Expt.	*Conditions*			*Results*
	[$^3H_2SO_4$] % (w/w)	*Temp. (°)*	*Time (days)*	
1	65	—30	22	Product contained 70% nicotinic acid. Only one active compound, nicotinic acid, was detected.
2	50	—30	12	Product contained 96% nicotinic acid. One radioactive compound.
3	50	room temperature	7	Product contained 84% nicotinic acid. Two active compounds, nicotinic acid and a second product, not visible under ultraviolet light as is nicotinic acid.

A kinetic experiment was carried out under optimum conditions of temperature (—30°), acid concentration (50%, w/w) and mole ratio (10 moles H_2SO_4 to 1 mole of nicotinic acid) to investigate the rate of exchange. Aliquot samples, taken at suitable intervals, were chromatographed and the activities of the residue from each aliquot were compared by the differences in densities of the images they produced on film[1]. It was found that under these conditions exchange was not completed after 5 weeks and that some decomposition had occurred after 17 weeks. However, a product of sufficiently high specific activity for most purposes can be obtained after 3–5 weeks.

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