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Synthesis of Nitrilotriacetic Acid-2-^{14}C

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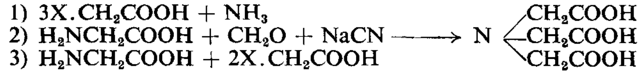
Nitrilotriacetic acid is a well known chelating agent and gives complexes of both theoretical and practical interest with many cations (1, 2). In the framework of a larger investigation the preparation of labelled nitrilotriacetic acid became necessary.

Different syntheses of nitrilotriacetic acid have been reported in the literature (3-12) and may be summarized in three main groups :



$$\begin{array}{l} 1)\ 3X.CH_2COOH + NH_3 \\ 2)\ H_2NCH_2COOH + CH_2O + NaCN \\ 3)\ H_2NCH_2COOH + 2X.CH_2COOH \end{array} \longrightarrow N\begin{array}{l} CH_2COOH \\ CH_2COOH \\ CH_2COOH \end{array}$$

In the first reaction considerable amounts of glycine and iminodiacetic acid were obtained together with the desired nitrilotriacetic acid. Although glycine could be precipitated easily by addition of methanol, fractional recrystallization with considerable loss of material was necessary to isolate nitrilotriacetic acid from the remaining mixture.

The second method gave good yields, as reported (11-12); however the preparation and especially the purification of the product were found rather cumbersome.

The third method, reported by Kazarinova and co-workers (5), gave satisfactory results and was adopted for the preparation of the labelled compound, although some modifications proved necessary.

Thus interaction of glycine-2-^{14}C in alkaline solution with excess chloroacetic acid gave upon acidification a 95% yield of nitrilotriacetic acid-2-^{14}C. After recrystallization, the chemical purity of the (inactive) sample was tested by infrared spectroscopy, thin-layer chromatography and elementary analysis.

The infrared spectrum was in agreement with that reported by Duval and co-workers (13); it showed the carboxyl absorption at 1 740 cm^{-1}. Radioactive purity was assayed by radiochromatogram scanning in thin layer, which gave one peak corresponding to an R*f* value of 0.35-0.42 in agreement with that reported by Hartley and Lawson (14). The same R*f* values were obtained by this method in chemical purity tests.

Nitrilotriacetic acid does not dissolve in toluene, xylene, anisole or dioxane, the common solvents of scintillating solutions. However, it dissolves well in

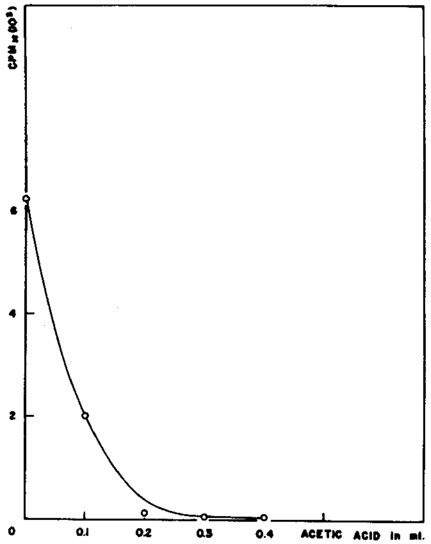


Fig. 1

dimethylformamide, and a solution in toluene : dimethylformamide (1 : 1) gave good counting results. In this solution the quenching effect of dimethylformamide is almost entirely overcome. Counting efficiency was of the order of 47-48 %. Higher efficiencies were obtained with Hyamine-10X hydroxide. To overcome the undesirable photoluminescent effect of this base (Fig. 1),

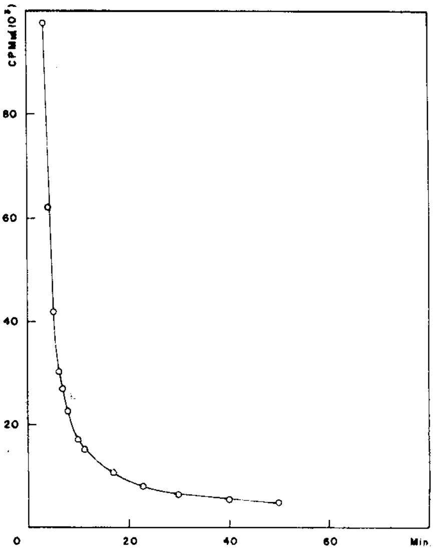


Fig. 2

the counting solution was acidified with acetic acid as reported in the literature (15) (Fig. 2). In this way the counting efficiency was raised to 58-60%.

In a 150 ml three-necked, round-bottomed flask, provided with a dropping funnel, a reflux condenser, a sintered glass funnel and a magnetic stirrer, glycine-2-^{14}C (0.75 g; 10 mMoles) was dissolved in a solution of sodium hydroxide (2.12 g; 53 mMoles) in water (8 ml) and chloroacetic acid (2.17 g; 23 mMoles) added with stirring. The solution was refluxed for four hours in an oil bath, cooled and acidified by addition of hydrochloric acid (3 ml 33%; in 10 ml water) through the dropping funnel. The cold mixture was filtered through the sintered glass and the precipitate dissolved in water (80 ml) by heating and left to crystallize. M.p. 237-240° C (dec.); yield 1.8-1.9 g (95-100%). Recrystallization of the crude material gave a pure product of m.p. 238-241° C (dec.); yield 1.72-1.81 g (90-95%).

Analysis (inactive sample) :
Calc. for $C_6H_9NO_6$: C, 37.7; H, 4.7; N, 7.3.
Found : C, 37.8; H, 4.8; N, 7.3.

Thin-layer chromatography : The purity of the product was assayed by thin-layer chromatography on cellulose, using an ethanol : water : 15% amonia (35 : 13 : 2) mixture as described by Hartley and Lawson (14). The dried plates were scanned in a Packard chromatogram scanner and showed only one peak corresponding to R*f* values of 0.35-0.42.

Radioactivity measurements were performed in a Packard Tricarb liquid scintillation spectrometer.

1) A sample of 1 mg was dissolved in a dimethylformamide : toluene solution (1 : 1; 10 ml) containing 0.6% of PPO and 0.05% of dimethyl-POPOP. Counting efficiencies were 47-48%.

2) A sample of 1 mg was dissolved in 0.5 ml of 1 M Hyamine-10X base solution in methanol, and 10 ml of toluene, containing 0.6% PPO and 0.05% dimethyl-POPOP, and 0.2 ml of acetic acid was added. Counting efficiencies were of the order of 58-60%.

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Self-radiolysis of Tritiated Long-chain Compounds

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Much work has been made by many investigators studying on the self-radiolysis of ^{14}C-labelled organic compounds (1-2). Additional light has recently been thrown on the similar radiolysis of organic compounds labelled with tritium whose beta particles are of rather strong reactivity with matters (3). Guarino *et al.* have thus reported strong self-radiolysis of tritiated methyl stearate, when its specific activity is in the order of magnitude of 10^2 to 10^3 C/mol (4). We have compared gaschromatograms and radiogaschromatograms of tritiated compounds of (A) low specific activity kept for storage for long time, (B) high specific activity for short time, and (C) high specific activity for long time.

All tritiated samples were thoroughly purified (5-7) and stored in glass ampoules or in paraffin-sealed vessels for a given time, *t*. They included (A) octadecane-[1-^{3}H] (16 mC/mol in its specific activity at $t = 0$) (4), (B) dodecan--[2,3-^{3}H, ^{3}H]-ol (15 C/mol) (6), and (C) stearic-[9,10-^{3}H, ^{3}H] acid (9.5 C/mol) (7). Then, all the samples were taken out for examination without further treatment. In the case of stearic acid (C), additional experiments were carried out with its methyl ester obtained by treating the stored sample with diazomethane in ethyl ether.

Mass- and radiogaschromatograms were simultaneously taken with Yanagimoto-Aloka GCG-5DH gaschromatograph which comprised an activated CuO-Fe system to convert the sample gases into tritiated hydrogen for counting in gaseous phase. Practically no memory effect has been observed throughout the experiments.

Practically no self-radiolysis was recognized over the chromatographic charts for (A) and (B), as shown in Figure 1, while the high specific activity sam-