823. *Preparation of ^{14}C-Labelled Dialkylnitrosamines, and an Improved Preparation of* N-*Methyl*-N-*t-butylamine.*

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^{14}C-Labelled dialkylnitrosamines have been prepared with the following *N*-alkyl groups: [1-^{14}C]diethyl; butyl[^{14}C]methyl; [1-^{14}C]butylmethyl; [^{14}C]methyl-t-butyl; methyl-[1-^{14}C]-t-butyl; methyl-[2-^{14}C]-t-butyl. An improved method has been found for preparing *N*-methyl-*N*-t-butylamine.

Several ^{14}C-labelled dialkylnitrosamines RR'N·NO have been prepared for toxicological studies.

The [1-^{14}C]diethyl and butyl[^{14}C]methyl compounds were prepared in the same way as [^{14}C]dimethylnitrosamine.[1] *N*-Ethyl- or *N*-n-butyl-toluene-*p*-sulphonamide was treated with [^{14}C]methyl or [^{14}C]ethyl iodide, the *NN*-dialkyl-sulphonamides were hydrolysed, and the amines nitrosated with sodium nitrite in 50% acetic acid. [^{14}C]Methyl-t-butylnitrosamine could not be prepared in the same way. *N*-t-butyltoluene-*p*-sulphonamide with methyl iodide [1] gave only toluene-*p*-sulphonamide and starting material, and with methyl sulphate gave toluene-*p*-sulphonamide and its *NN*-dimethyl derivative. Sabatier and Mailhe's method,[2] modified in that t-butyl isocyanide was reduced with lithium aluminium hydride [3] instead of catalytically, gave poor yields of methyl-t-butylamine. On a large scale, treatment [4] of t-butylamine with an excess of formic acid and reduction of the product with lithium aluminium hydride gave excellent yields of the amine, which was separated as its hydrochloride and nitrosated as described above (when hydrochloric acid was used instead of acetic acid the yield of nitrosamine fell from 90% to 16%).

The [^{14}C]methyl compound was prepared by the reactions,

$$H\cdot{}^*CO_2H \longrightarrow Bu^t\cdot NH_3{}^+H\cdot{}^*CO_2{}^- \longrightarrow Bu^t\cdot NH\cdot{}^*CHO \longrightarrow Bu^t\cdot NH{}^*Me.$$

Carrier sodium formate and t-butylamine formate were added at the appropriate stages; here, because an excess of formic acid could not be used for the condensation, the reaction was carried out in three stages, the product being withdrawn in ether at each stage.

[1,3-^{14}C]- or [2-^{14}C]-Acetone was converted (Grignard) into t-butyl alcohol and thence into t-butylformamide [5] from which the amine and nitrosamine were prepared as above. The Grignard reaction gave the t-butyl alcohol in ether; even when carrier alcohol had been added it proved difficult to concentrate the ethereal solution without losing much of the alcohol (b. p. 85°); a recovery of about 85% was achieved by using a long, narrow, plain tube as a fractionating column, provided that about 1 ml. of ether was allowed to remain undistilled (this did not interfere with the succeeding reactions).

[1-^{14}C]Butylmethylnitrosamine was prepared from sodium [1-^{14}C]butyrate by the reactions:

$$Pr\cdot{}^*CO_2H \longrightarrow Pr\cdot{}^*COCl \longrightarrow Pr\cdot{}^*CO\cdot NHMe \longrightarrow Pr\cdot{}^*CH_2\cdot NHMe \longrightarrow {}^*BuMeN\cdot NO.$$

The radiochemical yields were calculated from the specific activities of specimens of barium carbonate got by wet oxidation of known portions of the products after dilution with carrier.[6] The t-butyl compounds were obtained as solids and were weighed. The rest were prepared as dilute aqueous solutions, from which they were not easily separated

[1] Dutton and Heath, *J.*, 1956, 1892.
[2] Sabatier and Mailhe, *Bull. Soc. chim. France*, 1907, **1**, 614.
[3] Cf. Amundsen and Nelson, *J. Amer. Chem. Soc.*, 1951, **73**, 242.
[4] Hamlin and Freifelder, *J. Amer. Chem. Soc.*, 1953, **75**, 369; Wawzonek and Culbertson, *ibid.*, 1960, **82**, 441.
[5] Ritter and Kalish, *J. Amer. Chem. Soc.*, 1948, **70**, 4048.
[6] Heath and Dutton, *Biochem. J.*, 1958, **70**, 619.

and in which they were estimated polarographically. The method developed for dimethylnitrosamine [7] proved applicable to all the nitrosamines described in this paper. It was assumed that the starting compounds contained the amounts of ^{14}C stated by the vendors. The yields are listed in Table 1.

The [1-^{14}C]diethyl and the butyl[^{14}C]methyl compound were prepared without carrier and were therefore assumed to be as active as the starting materials. The specific activities of the other compounds were calculated from the specific activities of barium carbonate samples prepared from them. The specific activities are listed in Table 1.

A portion of each product was diluted with about 2 g. of carrier and its specific activity then determined. Most of the mixture was dissolved in water, and extracted with an equal volume of methylene dichloride. The extract was fractionated, a small fraction (0·2—0·4 g.) of purified nitrosamine was collected, and its specific activity was found. In Table 1 the specific activities of each compound before and after purification are given. They are expressed in counts/min. obtained from barium carbonate preparations counted at infinite thickness. Each figure is the average of duplicate determinations. Duplicates usually agreed within 1%. Purification, therefore, only affected significantly the specific activities of the [1-^{14}C]- and [2-^{14}C]-t-butyl compounds, both of which apparently contained about 5% of some active impurity. Probably this impurity was [^{14}C]-t-butyl alcohol, some of which may have been unchanged in the various reactions and separations.

TABLE 1.

Specific activities, yields and purities of [^{14}C]dialkylnitrosamines, RR'N·NO.

RR'	Mc/mmole	Scale (mmole)	Yield (%), based on ^{14}C	Specific activities and purification (c.p.m.) before	after	diff. (%)
[1-^{14}C]Et$_2$	1·8	0·6	64	2455	2440	0·6
Bu[^{14}C]Me	1·0	2·0	56	3154	3181	0·9
[1-^{14}C]BuMe	0·5	3·0	44	1781	1756	1·4
Bu^t[^{14}C]Me	0·21	6·0	48	4285	4359	−1·7
[1-^{14}C]Bu^{t}Me	0·12	6·0	40	3722	3530	5·4
[2-^{14}C]Bu^{t}Me	0·09	5·5	29	3032	2866	5·5

EXPERIMENTAL

N-*t-Butyltoluene*-p-*sulphonamide*.—t-Butylamine (15 g.), benzene (15 ml.), toluene-*p*-sulphonyl chloride (20 g.), and 3N-sodium hydroxide (50 ml.) were stirred together for 1½ hr. The benzene layer was washed with water and concentrated, and light petroleum (b. p. 80—100°) was added. The product (19 g., 80%) crystallised on cooling, having m. p. 110—112° (lit.,[8] m. p. 113—114°).

Action of Methyl Iodide and Sulphate on N-*t-Butyltoluene*-p-*sulphonamide*.—(a) *Methyl iodide*. The sulphonamide was held at 70° for 72 hr. in a sealed tube with potassium hydroxide and methyl iodide in aqueous ethanol.[1] Only toluene-*p*-sulphonamide (0·025 g.) and unchanged starting material (0·187 g.) were separated from the product.

(b) *Methyl sulphate*. The sulphonamide (5 g.) and methyl sulphate (2·2 ml.) in benzene (20 ml.) were refluxed for 1½ hr., cooled, and shaken with 6N-sodium hydroxide. On acidification the aqueous layer yielded toluene-*p*-sulphonamide (2·8 g.), m. p. and mixed m. p. 134° (from benzene). The benzene layer yielded *NN*-dimethyltoluene-*p*-sulphonamide (0·4 g.), m. p. and mixed m. p. 79—80°.

N-*Formyl-t-butylamine*.—t-Butylamine (90 g.) was added to 98—100% formic acid (180 ml.) with cooling. The mixture was heated under reflux for 3 hr., and then fractionated, finally under reduced pressure. The fraction of b. p. 65°/1 mm. was collected (lit.,[5] b. p. 202°/1 atm.) (yield, 81 g., 65%).

N-*Methyl-t-butylamine*.—(*a*) To t-butyl isocyanide [9] (0·89 g.) at 0°, lithium aluminium hydride (0·6 g.) suspended in ether (30 ml.) was added. The mixture was stirred for 1 hr., left

[7] Heath and Jarvis, *Analyst*, 1955, **80**, 613.
[8] Briscoe, Challenger, and Duckworth, *J.*, 1956, 1755.
[9] Nef, *Annalen*, 1899, **309**, 154.

overnight, and treated with 3N-sodium hydroxide (10 ml.). The amine was extracted with ether, from which it was removed in 2N-hydrochloric acid. On evaporation to dryness and recrystallisation from ethanol–water, 0·145 g. of the chloride, m. p. 252—254° was obtained.

(*b*) *N*-Formyl-t-butylamine (23 g.) in ether (50 ml.) was added in ½ hr. to lithium aluminium hydride (22 g.) in ether (300 ml.). The mixture was refluxed for 3 hr., left overnight at room temperature, and worked up essentially as before. *Methyl-t-butylammonium chloride* (25 g., 89%), after several recrystallisations from ethanol, had m. p. 254° (Found: C, 48·7; H, 11·4; N, 11·4; Cl, 29·1. $C_5H_{14}ClN$ requires C, 48·6; H, 11·3; N, 11·3; Cl, 28·7%). The *picrate* of the amine crystallised from ethanol as rectangular yellow leaflets, m. p. 194° (Found: C, 42·2; H, 5·3; N, 17·7. $C_5H_{13}N,C_6H_3N_3O_7$ requires C, 41·8; H, 5·1; N, 17·7%).

N-*Methyl*-N-*t-butylnitrosamine*.—Sodium nitrite (60 g.) in water (100 ml.) was added slowly with stirring to *N*-methyl-t-butylammonium chloride (40 g.) in water (70 ml.) and acetic acid (30 ml.) at 25—35°. After ½ hr. the mixture was cooled in ice and treated with 10N-sodium hydroxide (120 ml.). The *nitrosamine* was extracted with ether (5 times), dried, recovered (34 g., 91%), and fractionated, having b. p. 66°/5 mm. and m. p. 22—23° (pale yellow prisms) (Found: C, 51·8; H, 10·7; N, 24·3. $C_5H_{12}N_2O$ requires C, 51·7; H, 10·3; N, 24·2%).

On a small scale a similar yield was obtained, but this was greatly reduced if hydrochloric acid was added.

N-[^{14}C]*Methyl*-N-*t-butylnitrosamine*.—Sodium [^{14}C]formate (22·2 mg., 2 mc) was diluted to 2 mM with carrier sodium formate. Free formic acid was got by acidification with 1·3N-sulphuric acid (3 ml.) and separated by continuous extraction with ether for 2 hr. The extract was dried (Na_2SO_4) and treated at $<0°$ with an excess of t-butylamine (about 0·25 g.) in dry ether (4 ml.) with shaking, to give t-butylammonium formate. The ether was evaporated in a stream of dry nitrogen, and the residue diluted with carrier t-butylammonium formate (4·3 mM, prepared by mixing cooled ethereal solutions of formic acid and an excess of amine, evaporating the product to dryness, and drying the residue *in vacuo* over phosphorus pentoxide). To convert the formate into the amide it was held under reflux for 1 hr. at 193° in a glycerol bath heated with octan-1-ol vapour. After cooling, the formyl compound was extracted with ether (3 × 5 ml.), the first lot being run down the condenser. The residue was re-heated and re-extracted twice more in the same way. The combined ethereal extracts were dried (Na_2SO_4) and added slowly to lithium aluminium hydride (1·5 g.) in ether (10 ml.) at about −80°. The mixture was refluxed for 4 hr. and cooled to −80°. Water (24 ml.) was added slowly, and the mixture was allowed to warm to room temperature and acidified with acetic acid (35 ml.). The ether was driven off with a stream of nitrogen, sodium nitrite (20 g.) was added, and the mixture was held at 20—30° for 15 min. and treated with sodium hydroxide (40 g.) in water (100 ml.). The nitrosamine was extracted with ether (6 times), dried (Na_2SO_4), and recovered by distillation through a short column. The last solvent was removed by evaporation for 1 min. at room temperature at ~1 mm. The nitrosamine crystallised on cooling (3·9 mmole, 62·2% of overall chemical yield).

N-*Methyl*-N-[1^{14}C]- *and* N-[2-^{14}C]-*t-butylnitrosamine*.—The [2-^{14}C]-compound was prepared as follows. [1,3-^{14}C]Acetone (2 mmoles, 1 mc) was added *in vacuo* to methylmagnesium iodide (4·8 mmoles) in ether (2·8 ml.), previously de-gassed to removed methane. The mixture was held at room temperature for 3½ hr. and cooled below 0°. Saturated ammonium chloride solution (1 ml.) was added with shaking to release t-butyl alcohol. The ether layer and 6 ether washings were transferred to carrier t-butyl alcohol (3·52 mmole) in ether (50 ml.) and dried (Na_2SO_4). The ethereal solution was heated in a 50 ml. round-bottomed flask fitted with a thick-walled air-jacketed tube (50 cm. long × 0·5 cm. internal diameter; to act as a fractionating column) at ~50°, so that the distillation and reflux rates were about equal to 30 drops/min., and distillation was stopped when the residue totalled about 3 ml. By this method about 80% of the t-butyl alcohol remained in the residue. Several other methods gave much lower recoveries. The product was converted into *N*-formylbutylamine as follows. Acetic acid (2·5 ml.) and 95% sodium cyanide (0·5 g.) were added, and then 1 : 1 v/v acetic and sulphuric acid (2·5 ml.) dropwise with shaking during ½ hr. The mixture was left overnight, then cooled to 0°, and water (15 ml.) and enough 6N-sodium hydroxide (about 18 ml.) were added to make the product just alkaline. The product was extracted with ether and the combined extracts were dried (Na_2SO_4). The amide was reduced to *N*-methyl-t-butylamine with lithium aluminium hydride, from which the nitrosamine was prepared as was the [^{14}C]methyl compound in a yield of 3·2 mmole (58% overall chemical yield).

The [1-^{14}C]-compound was prepared similarly from [2-^{14}C]acetone (2 mmoles, 1 mc), except that methylmagnesium bromide was used instead of the iodide, and 4·18 mmoles of carrier t-butyl alcohol were added. An unknown amount of acetone was lost during the transfer *in vacuo*. The yield was 3·36 mmole (54·3% overall chemical yield).

In a trial run (no labelling) the product had m. p. 21—23°.

N-*Butyl*-N-*methyltoluene*-p-*sulphonamide*.—*N*-Butyltoluene-*p*-sulphonamide [10] (20 g.) was dissolved in 8% sodium hydroxide solution (500 ml.), and methyl sulphate (40 g.) was added during ½ hr. at 35—40° with stirring. After a further 1½ hours' stirring at room temperature, the product was extracted with benzene (17·1 g., 80% yield) and distilled [b. p. 190° (bath)/4 mm. (lit.,[11] 200—202°/12 mm.)] (Found: C, 59·7; H, 7·9; N, 6·0. Calc. for $C_{12}H_{19}NO_2S$: C, 59·3; H, 7·9; N, 5·8%).

This sulphonamide (10 g.) was hydrolysed with boiling 60% sulphuric acid for 1 hr., to give *N*-methylbutylamine, which was isolated as the hydrochloride (4·8 g., 94%).

N-*Butyl*-N-*methylnitrosamine*.—This was prepared from commercial *N*-methylbutylamine (50 g.) as described for *N*-methyl-*N*-t-butylnitrosamine. The ether extract containing the nitrosamine was washed with water, dilute hydrochloric acid, water, 3N-sodium hydroxide, and twice more with water, and dried (Na_2SO_4). After removal of the solvent, the residue was fractionated through a column of glass helices (height 6 in.). Almost the whole of the material distilled at 55·5° ± 0·5°/1·5 mm. (lit.,[12] 107·1—107·7°/40 mm.) (44 g., 67%) (Found: N, 24·2. Calc. for $C_5H_{12}N_2O$: N, 24·2%).

N-[1-^{14}C]*Butyl*-N-*methylnitrosamine*.—Sodium [1-^{14}C]butyrate (23·4 mg., 1 mc) was diluted to 320 mg. (2·91 mmoles) with inactive sodium butyrate and dissolved in water (1 ml.) and 2N-sulphuric acid (2 ml.). The free butyric acid was extracted with ether (20 + 5 ml.). The combined ether extracts were dried (Na_2SO_4) and most of the ether was removed. Thionyl chloride (0·5 ml.) was added to the butyric acid at −70° and the mixture was then warmed to room temperature during 5 min. and held at room temperature for 10 min. at 35° for 10 min. *N*-Methylbutyramide was prepared from the product by treating it in dry ether (5 ml.) at −70° with anhydrous methylamine (2 ml.) in ether (5 ml.). The reaction was complete after 10 min. at room temperature, with shaking. Water was added, ether and the excess of methylamine were removed in a stream of nitrogen, and the product was neutralised (Neutral Red) with 2N-sulphuric acid. The product was separated by continuous extraction with ether for 45 min. and dried (Na_2SO_4). Its ethereal solution was run into a suspension of lithium aluminium hydride (0·5 g.) in dry ether (10 ml.) at below 0°. The mixture was refluxed for 4 hr., then cooled to <0°. Water (8 ml.) and glacial acetic acid (8 ml.) were added in turn with shaking, and the ether was evaporated. The butylmethylammonium acetate was converted into the nitrosamine, which was separated by distillation from 3N-sodium hydroxide as an aqueous solution, as described elsewhere.[1]

The yield estimated polarographically was 2·32 mmoles (79·8%).

Paper Chromatography of Amine Picrates.—Descending chromatograms were run for approximately 18 hr. on Whatman No. 1 paper in the non-aqueous layer from 1 : 1 butyl alcohol : 5% v/v aqueous acetic acid. The amines, in ethanol, were neutralised with picric acid and spotted on the paper. Separations were good enough to prove that samples of *N*-methylbutylamine and *N*-methyl-t-butylamine were not contaminated with dimethylamine. The R_F values for picrates of the amines were: butylamine, 0·85; *N*-methylbutylamine, 0·85; t-butylamine, 0·83; methyl-t-butylamine, 0·81; dimethylamine, 0·71; ethylamine, 0·73; ammonium picrate, 0·67; picric acid, 0·70.

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WOODMANSTERNE ROAD, CARSHALTON, SURREY. [*Received, May 9th*, 1961.]

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