THE SYNTHESIS OF BUTYLNITROSOUREA - 1 - ^{14}C .

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SUMMARY

The synthesis of butylnitrosourea-1-^{14}C is described starting from sodium cyanide-^{14}C. Nucleophilic displacement of bromine in 1-bromopropane with labelled cyanide ion gave butyronitrile-1-^{14}C which was reduced with lithium aluminium hybride to butylamine-1-^{14}C. The amine was isolated as its hydrochloride salt which was converted to butylurea-1-^{14}C by reaction with urea. Nitrosylation of the resulting substituted urea, without its isolation, gave the title compound in an overall yield of 32% based on sodium cyanide-^{14}C. The reaction between butylamine and silicon tetraisocyanate was investigatd as a possible alternative synthesis of butylurea. This approach gave unsatisfactory results and was not utilised in the radiochemical synthesis. Butylnitrosourea in solution has been found to be very unstable to light.

INTRODUCTION

The carcinogenic activity of N-nitroso compounds has been well established[1,2]

A possible mechanism for their mode of action invokes an enzymatic or purely chemical conversion of these compounds to an "active" electrophillic alkylating species capable of reaction with the genetic material (DNA and RNA) of a cell. As a result of investigations in these laboratories concerning the carcinogenicity of butylnitrosoures, we required the compound with a radio-

active label on the α-carbon atom of butyl group for metabolic studies. The synthesis devised is outlined in fig.1. and represents the first chemical synthesis, as far as we are aware, of butylnitrosourea-1-^{14}C.

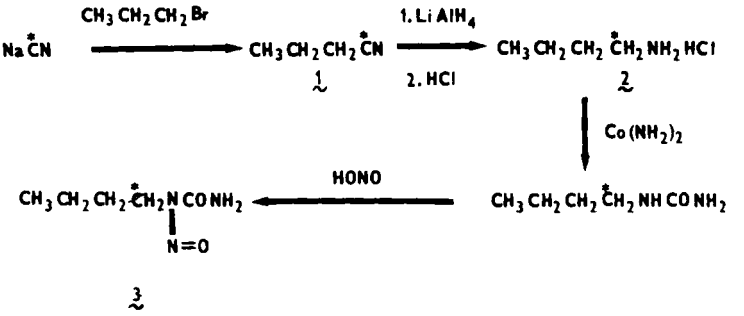

FIG.1 SYNTHESIS of BUTYLNITROSOUREA-1-^{14}C

DISCUSSION AND RESULTS

Sodium cyanide-^{14}C mixed with inactive sodium cyanide was converted to butyronitrile-1-^{14}C by heating it under reflux with a slight excess of 1-bromopropane in ethane-1,2-diol. The nitrile was isolated in 87% yield after fractional distillation and was shown to be pure by gas liquid chromatography. Reduction of butyronitrile-1-^{14}C with lithium aluminium hydride in refluxing ether gave butylamine-1-^{14}C which was isolated as its crystalline hydrochloride in 78% yield. A dry ice-acetone condenser was incorporated on top of the usual double surface water condenser normally employed, thus minimising losses of the volatile amine during the refluxing period and in the decomposition of excess reagent phase. The yield of butylamine obtained is a significant improvement on that described previously [3] for the reduction and is most

likely a result of the more efficient condensing system. Reaction of butylamine-1-^{14}C-hydrochloride with urea was carried out by heating them together under reflux in aqueous solution, the initially acidic solution (methyl red) rapidly became basic due to the ammonia liberated during the reaction. Thin layer chromatographic (tlc) examination of the reaction mixture revealed the formation of only one product whose mobility was identical with that of authentic butylurea. The butylurea was not isolated but was nitrosylated directly by reaction with nitrous acid, generated from sodium nitrite and sulfuric acid, to give butylnitrosourea-1-^{14}C which was purified by recrystallisation from petroleum ether. The synthetic, labelled material was identical (m.p., mixed m.p., u/v spectrum, and tlc) with an authentic sample of butylnitrosourea. The specific activity as determined using internal liquid scintillation counting techniques with a toluene/PPO/POPOP scintillation system, was 2.24×10^8 dpm/mg or 100.9μCi/mmol and was unchanged by further recrystallisation. The product was shown to be chemically and radiochemically pure by a combination of tlc, radiochromatogram scanning, and autoradiographic techniques. On fluorescent silica gel it migrated to give a discrete spot which was easily detected by quenching of background fluorescence under u/v irradiation and was indistinguishable from an authentic sample in two solvent systems; with R_f 0.47 (ether-petroleum ether, 1.1) and R_f 0.42 (ethyl acetate-petroleum ether 1.1). Radiochromatogram scanning as well as autoradiographic examination of the tlc plates revealed only one radioactive region (fig.2) corresponding to the position at which quenching of fluorescence occurred.

The stability of butylnitrosourea-1-^{14}C towards light was examined by exposing a 1% solution in toluene to daylight and examining the solution by tlc at varying time intervals. Subsequent examination of the chromatograms by radiochromatogram scanning and autoradiography revealed the formation of more polar, non u/v absorbing products which occurred at the origin of the chromatogram

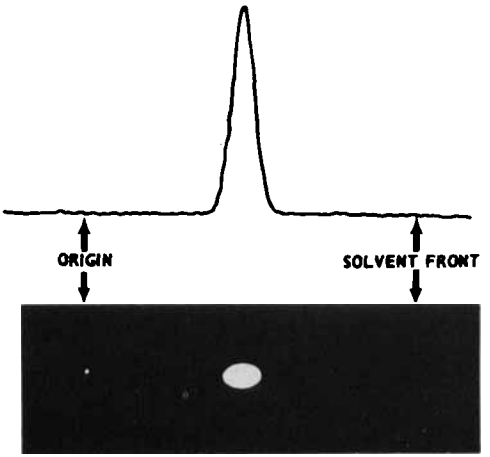

FIG.2 THIN LAYER RADIOCROMATOGRAPH and AUTORADIOGRAPH of BUTYLNITROSOUREA-1-^{14}C in ETHER-PETROLEUM ETHER 1:1

using the solvent system, ether-petroleum ether 1:1. After exposure for 1h, approximately 10% of the radioactivity could be found at the chromatogram origin; this amount had increased to ca. 40% after 48h. Further tlc investigation of the solution revealed that a complex mixture of photochemical products was formed.

A fairly recent report[4], listing the preparation in almost quantitative yield of a range of urea derivatives from primary and secondary amines, describes the synthesis of butylurea in 98% yield from butylamine and silicone tetraisocyanate in benzene. The reaction is reported to proceed via the intermediate formation of a silylurea derivative which undergoes facile hydrolysis on treatment with water to give pure butylurea. In our hands, however, this procedure proved

to be unsatisfactory. In addition to the desired urea an approximately equal amount of another, as yet unidentified product is also produced which has been isolated pure by column chromatography on silica gel. The consequent low yield of butylurea obtained by this approach prompted us to abandon it.

EXPERIMENTAL

Melting points were determined on a Gallenkamp apparatus and are uncorrected. Tlc was carried out on silica gel GF254 on pre-coated aluminium sheets (Merck). The compounds were located either by direct visualisation under u/v light or by spraying the plates with one of the following reagents; i) 1% solution of potassium permanganate in sulphuric acid (2N), ii) 20% solution of ammonium molybdate in sulphuric acid (5N). Gas liquid chromatography (Varian Aerograph, autoprep, model 705) was carried out on a column of 15% (W/W) carbowax 20m on 80-100 mesh acid washed, DMCS treated chromosorb W (6'xl/8th") at 50° and with a flow rate of 65ml/min of nitrogen. Specific activity determinations were made using a Tracer-Lab Corumatic 100a liquid scintillation spectrometer. Radiochromatographic scanning was performed with a Packard (model 7201) radiochromatograph scanner. The ultraviolet spectrum was measured with a Unicam SP 1800 spectrophotometer. The term petroleum ether refers to the fraction of b.p. 60-80°.

Butyronitrile-1-^{14}C (1).

Sodium cyanide-^{14}C (5 mCi, 52.8 mCi/mmol, obtained from the Radiochemical Centre, Amersham, England) was transferred from the delivery vial to a 50ml round bottom flask with the aid of an aqueous solution of sodium cyanide (50mg in 10ml) and the resulting solution was freeze dried. Sodium cyanide (2.4g) was added together with ethane-1,2-diol (15ml) and 1-bromopropane (6.5g, 53mmol). The mixture was heated under reflux for 2h. and then fraction-

ally distilled through a Vigreux column (10cm). The fraction, b.p. 114-118° (mainly 116°) was collected (3.0g, 87%). It was pure on examination by gas liquid chromatography and had the same retention time as authentic butyronitrile, lit.,[5] b.p. 117.28°.

Butylamine-1-^{14}C-hydrochloride (2)

Butyronitrile-1-^{14}C (3.0g, 43.5 mmol) was dissolved in sodium dried ether (50ml) and the solution was added dropwise to a suspension of lithium aluminium hydride (3.5g, 92 mmol) in dry ether (100ml). A dry ice/acetone condenser was fitted on top of the usual ether condenser and the reaction mixture was heated under refluxing for 8h. Excess of lithium aluminium hydride was decomposed by the cautious addition of water (5ml) and sodium hydroxide (4N, 10ml) and the resulting mixture was refluxed for a further 0.5h. The reaction mixture was cooled to -70° and the inorganic salts were removed by filtration. Dry hydrogen chloride was passed through the filtrate until no more precipitate was produced and the solvent was removed in vacuo. The resulting semi-solid residue was concentrated from ethanol (3 x 100ml) to remove moisture and the product was crystallised by the addition of ether (200ml) to give butylamine-1-^{14}C-hydrochloride (3.7g, 79%) as a hygroscopic white solid.

Butylnitrosourea-1-^{14}C (3)

Butylamine-1-^{14}C-hydrochloride (3.7g, 34 mmol) was dissolved in water (20ml) to which urea (7.2g, 120 mmol) was added. The mixture was heated under reflux for 3h and after cooling to room temperature, sodium nitrite (5.5g, 80 mmol) was added and the resulting solution added to a solution of concentrated sulphuric acid (5ml) in water (10ml) with cooling to below 0°. The precipitated product was collected by filtration and washed well with cold water. Recrystallisation of the dried material from petroleum ether gave pure butylnitrosourea-1-^{14}C as pale yellow prisms, (2.35g, 48%), m.p. 82.5-84°, λ max (methanol) 240mμ (ε 4900), lit.[2], m.p. 83-84°, λ max (methanol) 237mμ (ε 4650),

specific activity, 2.24×10^8 dpm/mmol or 100.9μCi/mmol, unchanged on further recrystallisation. The synthetic compound showed no depression in m.p. on admixture with an authentic sample and the two samples were indistinguishable by tlc (Rf 0.47, ether-petroleum ether 1:1, Rf 0.42 ethyl acetate-petroleum ether 1:1).

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REFERENCES

1. MAGEE, P.N. and BARNES, J.M., Adv.Cancer Res., 10 : 167 (1967).

2. DRUCKREY, H., PREUSSMANN, R., IVANOVICK, S., and SCHMAHL, D. Z.Krebsforsch., 69, 103, (1967).

3. AMUNDSEN, L.H. and NELSON, L.S. J.Am.Chem.Sco., 73, 242, (1951).

4. NEVILLE, R.G. and McGEE, J.J. Can.J.Chem., 41, 2123, (1969).

5. TIMMERMANS, "Physico-chemical Constants of Pure Organic Compounds", Elsevier Publishing Co., Inc., New York, N.Y. 1950.