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Preparation of bis-/2-chloroethyl-1,2-^{3}H/methylamine hydrochloride

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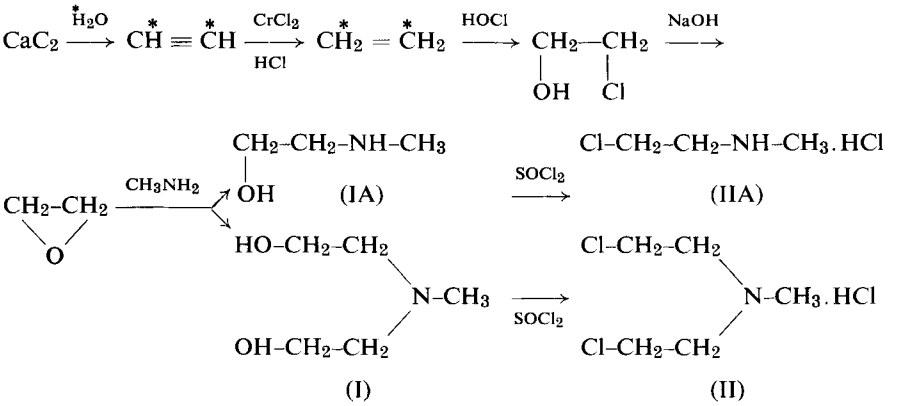
SUMMARY

Bis-(2-chloroethyl-1,2-^{3}H)methylamine hydrochloride was prepared. The starting product was acetylene-1,2-^{3}H which was selectively hydrogenated to ethylene-1,2-^{3}H. It was observed that on reduction with chromium (II) chloride solution in dilute hydrochloric acid the isotopic exchange with acetylene-1,2-^{3}H is negligible. Ethylene was converted to ethyleneoxide-1,2-^{3}H by the usual method. Ethyleneoxide-1,2-^{3}H can be used for synthesis of several labelled halogenalkylamines and as the starting compound for other syntheses.

INTRODUCTION

Considerable interest has been given in the last 20 years to the N-yperite derivatives which proved to possess certain cancerostatic properties. Research in the field of these compounds at present proceeds in two directions. The effect of simple N-yperites as alkylating agents on nucleic acid molecules is being studied on one hand, and on the other more complex yperite derivatives are being synthesized as compounds with potential cancerostatic activity [1, 2]. For studies of the mechanism of the activity of these compounds it is useful to label them with a suitable radioisotope. Bis-/2-chloroethyl/methyl-^{14}C-amine hydrochloride has been prepared by condensing methylbromide-^{14}C with diethanolamine[3]. Bis-/2-chloroethyl-1,2-^{14}C/methylamine hydrochloride has been prepared by treating ethanolamine with ethyleneoxide-^{14}C [4]. Bis-/2-chloroethyl-1,2-^{3}H/methylamine hydrochloride whose synthesis has not yet been described, was prepared by us. The starting acetylene-1,2-^{3}H was obtained by the reaction of calcium carbide with water-^{3}H and converted to ethylene-1,2-^{3}H by reduction with chromium (II) chloride in dilute hydrochlorid acid solution. Ethyleneoxide-1,2-^{3}H was prepared from 2-chloroethanol-1,2-^{3}H and by its reaction with methylamine yielded bis-/2-hydroxy-ethyl-1,2-^{3}H/methylamine [I] and 2-hydroxyethylmethylamine [IA]. By treatment with thionyl chloride I and

IA were converted to bis-/2-chloroethyl-1,2-^{3}H/methylamine hydrochloride [II] and 2-chloroethylamine hydrochloride. [IIA]



For the reduction of acetylene-1,2-^{3}H a chromium (II) chloride solution in dilute hydrochloric acid (I:I) was used. This method has been employed by Cox and Warn [5] for preparing ethylene-1,2-^{3}H. We observed that on reduction of acetylene-1,2-^{3}H in the above mentioned medium the isotopic exchange of hydrogen proceeds only in negligible extent (less than 1%) and that this method can be used for the preparation of ethylene-1,2-^{3}H of high specific activity (of the order of 10-100 mCi/mmol). For the preparation of ethyleneoxide the method described by Cox and Warne for the synthesis of ethyleneoxide-1,2-^{14}C [5] was used. Ethyleneoxide-1,2-^{3}H was used without further purification for the reaction with methylamine in aqueous medium. The reaction mixture formed was evaporated above phosphoric pentoxide and the residue dissolved in anhydrous chloroform. The compounds I and IA were then converted to II and IIA by means of thionylchloride. From the reaction mixture compound II was isolated in pure form by crystallisation from acetone at −20 °C and sublimation.

EXPERIMENTAL

The melting points were not corrected. The radioactivity of the compounds labelled with tritium was determined by means of liquid scintillators using a single channel scintillation counter NE 5503 (Nuclear Enterprises, England). The liquid scintillator SLD 31 (dioxane, naphtalene) manufactured by Tesla Pardubice, ČSSR, was used. The counting efficiency was determined for each sample by means of an internal standard (standard EK 1, toluene-T, spec. act. 1.97 μCi/g, product of ÚVVVR, ČSSR).

The radiochemical purity of compound I was determined by paper chromatographic analysis in the descending arrangement in the system *n*-butanol saturated with 3N hydrochloric acid and isopropanol-2N hydrochloric acid

(65:35). The paper chromatograms were measured by a radiochromatogram scanner, designed in our Institute, with a windowless proportional flowcounter, slot width 5 mm.

The spectra of N-yperite were measured on the photometer UR 10 (VEB Carl Zeiss, Jena) using the KBr technique.

Acetylene-1,2-^3H

The compound was prepared by treating finely ground calcium carbide with 0.10 ml of tritium labelled water of specific activity 4.1 C/ml. Acetylene-1,2-^3H was collected in a flask of 1 litre volume. 74 ml of acetylene-1,2-^3H of the total activity 154 mCi were obtained.

Ethylene-1,2-^3H

Into the flask containing acetylene-1,2-^3H, 220 ml of the hydrogenating solution (NOTE 1) were introduced. The flask with the hydrogenating solution was shaken for 6 hours. The hydrogenation solution changed its transparent blue colour during the reaction, becoming blue-green. After termination of the reaction a small gas sample was withdrawn. A test with a 2% copper (II) chloride solution in aqueous ammonia showed that no excess acetylene is present in the gas. 69 ml of ethylene-1,2-^3H of a total activity of 141 mCi were obtained.

NOTE 1

Preparation of the hydrogenating solution [7]

To 120 ml of a saturated chromium (III) sulphate solution in dilute hydrochlorid acid (1:1) were added about 65 g of zinc amalgam. Reduction proceeded in CO_2 atmosphere. After 16 hours the azur blue solution was used directly for the reduction.

Ethylenechlorhydrin-1,2-^3H

24.5 ml (1.09 mmol) of ethylene-1,2-^3H of an activity of 49 mCi were transferred into an evacuated vessel cooled with liquid nitrogen and containing 1.9 ml of a 1.5 M solution of freshly prepared hypochloric acid solution (see NOTE 2). The addition of hypochloric acid to ethylene-1,2-^3H was followed by the pressure decrease in the reaction vessel. 38.2 mCi (76%) of the product were obtained.

NOTE 2

Preparation of hypochloric acid

Hypochloric acid of the concentration 1.5-4.OM was prepared by the reaction of chlorine monoxide with water. The solution of chlorine monoxide was obtained by oxidizing chlorine dissolved in carbon tetrachloride with mercury (II) oxide. The concentrations of chlorine and hypochloric acid were determined by titrating the released iodine with thiosulphate solution.

Ethyleneoxide-1,2-^{3}H

The solution of 66.8 mg (0.83 mmol) of ethylenechlorhydrin-1,2-^{3}H of the activity 38.2 mCi was pipetted into a small ampoule, frozen with liquid nitrogen and transferred into test-tube A, Figure 1, containing solid KOH. The apparatus was evacuated. The melting reaction solution reacted with the solid potassium hydroxide and ethyleneoxide-1,2-^{3}H was released which was condensed in the capillary part of the trap cooled with liquid nitrogen. The yield was 0.65 mmol of ethyleneoxide, i.e. 78.5%, having a total activity of 28.1 mCi.

Addition of ethyleneoxide-1,2-^{3}H to methylamine

To the solution of methylamine (0.35 mmol) in 0.2 ml of water were condensed 0.65 mmoles of ethyleneoxide-1,2-^{3}H. The reaction space of 5 ml volume was closed and the reaction vessel was immerged into a water bath at a temperature of 20 °C. The reaction mixture was pipetted into a small test-tube after 16 hours and allowed to dry for 24 hours in vacuo above phosphorus pentoxide.

Bis-(chloroethyl-1,2-^{3}H)methylamine hydrochloride [II]

Into a test-tube with a flat bottom, provided with a small iron stirrer sealed in glass, were placed 0.3 ml of thionylchloride and 0.2 ml of anhydrous chloroform. The reaction mixture after the addition of ethyleneoxide-1,2-^{3}H in 0.2 ml of anhydrous chloroform was added dropwise under stirring to this solution within 12 minutes. The reaction mixture was heated to boiling under a reflux cooler connected with a washing flask containing sulphuric acid. The reaction was terminated after three hours and the reaction mixture was evaporated under stirring in vacuo. To the solid residue were added three times 0.5 ml of anhydrous chloroform and evaporated in vacuo.

Chromatography in the system *n*-butyl alcohol saturated with 3N hydrochloric acid showed the presence of the compounds II and IIA. The reaction mixture was dissolved in anhydrous acetone and allowed to crystallize for

24 hours at −20 °C. The precipitated crystals were separated by centrifuging and washed with dry acetone and ether. The product after crystallisation had the melting point 96 °C. By sublimation at 100 °C and 5 mm Hg a product was obtained, melting at 106 °C (ref. [3] m.p. 109 °C) as the authentic N-yperite sample. 21.1 mg (0.109 mmol) of the product of spec. activity 96.3 mCi/mmol were obtained.

Chromatography in the systems *n*-butyl alcohol saturated with 3N hydrochloric acid and isopropanol-2N hydrochloric acid (65:35) showed a single active spot (R_F 0.56 and 0.81). The infrared spectrum of the product was also identical with that of a standard sample of N-yperite.

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