A NOVEL METHOD FOR THE PREPARATION OF HIGHLY TRITIATED METHYL IODIDE, AND ITS USE FOR THE SYNTHESES OF METHYL-^{3}H-LABELLED DIAZEPAM AND THE METHIODIDES OF (+)- AND (-)-BICUCULLINE

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SUMMARY

The syntheses of methyl-^{3}H-labelled diazepam (4) and the methiodides of (+)- and (-)-bicuculline (5) labelled with tritium in their methyl groups are described. Also reported here is a novel method for the preparation of tritiated methyl iodide (3) serving as the radioactive precursor for the substances mentioned above.

Key words: methyl iodide, diazepam, bicuculline

INTRODUCTION

According to literature references ^{3}H-methyl iodide (3) is obtained by hydrolysis of tritiated methyl esters, especially methyl naphthoate, with hydroiodic acid. This labelled methyl ester is prepared by exchange of the carboxyl group hydrogen of β-naphthoic acid with tritiated water, followed by esterification of the ^{3}H-labelled acid with diazomethane in ethereal solution [1,2].

In order to avoid the drawbacks of the exchange reaction (loss and ambiguous predictions of specific activity) and to approach the theoretical

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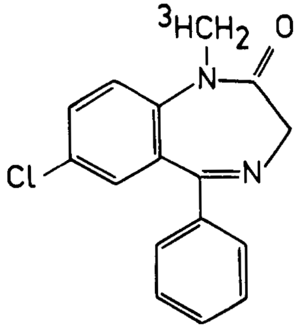
specific activity of 29 Ci/mmole more closely, an alternate synthesis for ^{3}H-methyl iodide (3) has been worked out with bischloromethyl ether (1) as the starting material. It was catalytically hydrogenolyzed with tritium in ethyl acetate in the presence of 10 % Pd on charcoal and two equivalents of triethylamine. Cleavage of the tritiated dimethyl ether (2) with hydroiodic acid gave ^{3}H-methyl iodide (3) in 70 % yield. GC checks (15 % PAG 600) of nonradioactive material, synthesized by the same route, showed a purity of 99%. As the C——I bond is very sensitive to radiolysis, methyl iodide was used immediately after preparation.

$$\underset{(1)}{ClCH_2OCH_2Cl} \xrightarrow[Pd]{^3H_2} \underset{(2)}{^3HCH_2OCH_2{}^3H} \xrightarrow{HI} \underset{(3)}{^3HCH_2I}$$

Any handling of bischloromethyl ether (1) has to be done with caution, since it is known to be a powerful carcinogen. It is easily prepared from formaldehyde and hydrochloric acid by addition of chlorosulfonic acid [3]. The compound is somewhat unstable. Although distillation is recommended for purification, decomposition was observed on warming. Hydrochloric acid, formaldehyde and formaldehyde polymers had to be removed before tritiation by shaking with 20 % $NaHSO_3$ solution and drying with K_2CO_3 and KOH. Otherwise the yield of dimethyl ether dropped to about 50 % or less. Later experiments showed that both low yields and low specific activities were also due to traces of water. Therefore the compound was dried with P_2O_5 after removal of the decomposition products. Reproducible results are obtained only with extremely pure bischloromethyl ether (1) which before tritiation should always be tested for 80 % yield in a hydrogenolysis reaction with ordinary hydrogen.

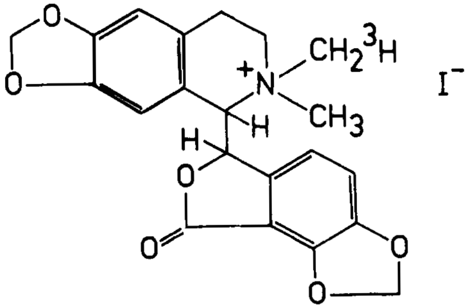
^{3}H-labelled diazepam (4) was synthesized following a description by H.Kägi [4]. 7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one was treated with thallous ethoxide in DMF. The thallous salt of the chloro-lactam upon reaction with ^{3}H-methyl iodide gave the wanted product in a radiochemical yield of 30 %

based on $^3H_2$. The specific activity was 14.5 Ci/mmole. The colourless crystals turned green overnight and black after one week without showing radiolysis products in the TLC scan (radiochemical purity 99.8 %). When dissolved, they gave again colourless solutions.



(4)

^3H-methyl iodide with high specific activity was also used to synthesize the ^3H-methiodides (5) of natural (+)-bicuculline and the (-)-isomer [4]. Equivalent amounts of bicuculline and ^3H-methyl iodide (3) were dissolved in acetone. After two days standing at room temperature the methiodides (5) could be filtered off. The radiochemical yields of 19 % and 34 % based on $^3H_2$ were lower than expected due to impurities in the starting material.



(5)

EXPERIMENTAL

Bischloromethyl ether (1)

72 g of paraformaldehyde (2.4 mole formaldehyde) were poured into 50.0 ml of conc. HCl (0.6 mole) and cooled to 5°C. With good stirring 136 ml of chlorosulfonic acid (2.07 mole) were added dropwise at such a rate that no HCl gas evolved from the reaction mixture, the temperature always being kept below 10°C. The addition was finished after 5 hours. Stirring was kept on for 4 hours in the melting ice bath and afterwards the mixture allowed to stand overnight. The upper layer was separated, washed twice with ice water and with 75 ml of 40 % ice-cooled NaOH. With good cooling the product was dried over solid K_2CO_3 and KOH. After filtration 98.8 g of bischloromethyl ether could be isolated (≙ 72 %).

For hydrogenolysis reactions the product had to be purified by shaking 10ml of bischloromethyl ether with 10 ml of 20 % $NaHSO_3$ solution containing some ice cubes for cooling. The upper layer was separated and treated with K_2CO_3 and KOH to remove traces of acid. After filtration the liquid was dried with P_2O_5 overnight at 0°C.

Bis-^{3}H-methyl ether (2)

27 µl of bischloromethyl ether (0.304 mmole) were dissolved in 2.1 ml of ethyl acetate. 84 µl of triethylamine (0.608 mmole) and 7.7 mg of 10 % Pd on charcoal were added. The reaction flask was fitted with a stopcock, cooled with dry ice, evacuated, and filled with 6.7 ml of $^3H_2$ (≙ 17 Ci) measured on a tritium dosage apparatus. After 1 h shaking at room temperature there was no more $^3H_2$ left. The flask was filled up with ordinary hydrogen and shaken for another 4 hours. By refluxing the solution, bis-^{3}H-methyl ether was removed by a H_2-stream and condensed in a trap cooled with liquid nitrogen, most of ethyl acetate being stripped off in a Liebig condenser (water cooling). For purification the crude material was warmed to -78°C and the volatiles transferred to a gas bulb on the vacuum line at 10^{-3} Torr, giving 11.3 mg of pure bis-^{3}H-methyl

ether (≙ 80 % of theory).

^{3}H-Methyl iodide (3)

10.1 mg of bis-^{3}H-methyl ether (0.22 mmole) and 0.5 g of 57 % HI (2.2 mmole HI) were heated to 150° C in a sealed tube. After 5h of propellerlike rotating in the oilbath the tube was cooled with dry ice, opened and the reaction mixture transferred to a flask fitted with a stopcock. The flask was cooled with liquid nitrogen and evacuated. $^3HCH_2I$ was expelled from the water phase by melting the mixture with good stirring. After refreezing to -30°C the gaseous phase was transferred to a gas bulb. This operation was repeated four times at 10^{-3} Torr. The crude ^{3}H-methyl iodide, containing water, hydroiodic acid and iodine was passed to another bulb through two tubes, the one containing KOH pellets, the other P_2O_5. In a cold run the purity of methyl iodide synthesized by this way was 98.7 % as checked by gas chromatography. From the hot run 41.7 mg of ^{3}H-methyl iodide (≙ 70 %) were isolated.

^{3}H-Diazepam (4)

To a solution of 64 mg of 7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (0.236 mmole) in 0.5 ml of DMF 18 µl of thallous ethoxide (63.4 mg ≙ 0.254 mmole) were added. After stirring for one minute at room temperature, 33.5 mg of ^{3}H-methyl iodide (0.236 mmole) were condensed to the solution on the vacuum line. The reaction mixture was covered with nitrogen and allowed to melt with good stirring. When the formation of thallous iodide had started, stirring was continued for another 20 minutes. Then DMF was removed at 70°C in the vacuum. The residue was dissolved in a small amount of $CHCl_3$ and chromatographed with the same solvent on a column made up from 4 g of silicagel. A first fraction with a yellow colour contained no radioactivity. The main fraction was nearly colourless. The solvent was evaporated and the residue recrystallized from ether/hexane. 50 mg of ^{3}H-diazepam were isolated (≙ 75 %) as colourless crystals which soon turned green and nearly black after one week. The specific activity was 50.47 mCi/mg (≙ 14.37 Ci/mmole), the radiochemical yield, based on $^3H_2$, was 30 %. Investigations on the stability of the labelled compound showed no radiolysis after 4 weeks' storage at 0°C although the crystals were black.

^{3}H-Methiodide of (+)-bicuculline

A solution of 87.5 mg of (+)-bicuculline (0.192 mmole) in 3 ml of acetone was added to 33.1 mg of ^{3}H-methyl iodide (0.192 mmole). A small amount of inactive (+)-bicuculline methiodide was added to initiate crystallisation. After two days' standing at room temperature the solution was yellow. Ether was added to complete crystallisation. The mother liquor was decanted and the residue was washed with acetone/ether (1/1). 87 mg of bicuculline ^{3}H-methiodide with a specific activity of 15.72 mCi/mg were isolated (≙ 80 %). The radiochemical yield, based on $^3H_2$, was 19 %.

^{3}H-Methiodide of (-)-bicuculline

This substance was prepared in the same way from 126 mg of (-)-bicuculline and 47.7 mg of ^{3}H-methyl iodide. 135.4 mg of (-)-bicuculline ^{3}H-methiodide with a specific activity of 25.4 mCi/mg were obtained (≙ 79 %). The radiochemical yield, based on $^3H_2$, was 34 %.

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