TRITIUM LABELLED COMPOUNDS OF HIGH SPECIFIC ACTIVITY II.* AMITRIPTYLINE

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SUMMARY

Tritium labelled amitriptyline (1) of high specific activity was prepared. The tritium was incorporated by catalysed isotope exchange into the precursor dibenzosuberone, which had been previously converted into a cyclic ketal, in order to protect the oxo-group against reduction. It was demonstrated by degradation that isotope exchange occurred exclusively in specified position.

Key Words: Tritium labelling, Amitriptyline, Isotopic exchange

INTRODUCTION

In our preliminary experiments with ^{14}C-labelled amitriptyline (5-(3-dimethylaminopropylidene)-dibenzo[a,d] [1,4]cycloheptadiene, 1) we have found that it was accumulated in the calliculus inferior of the rat brain. For studying in detail the subcellular distribution of this compound in the brain, 1 specifically labelled with tritium at a specific activity of 10 Ci/mmole = 370 GBq/mmole was required. Evans and coworkers (1) published in 1974 that a wide variety of organic compounds, among them those containing a benzyl group, can be prepared specifically labelled

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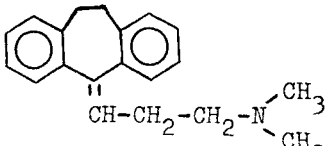
with tritium by catalysed isotope exchange reaction in solution using tritium gas. Two years later Pri-Bar and Buckman (2) announced their investigations concerning the hydrogen exchange between molecular tritium and bibenzyl in solution, catalysed by transition metals. Utilizing the results obtained, they prepared some tricyclic antidepressants labelled with tritium by the aforementioned method (3), but unfortunately the specific activity of amitriptyline was rather low, 1.7 Ci/mmole, because of the undesired reduction of double bond. In order to avoid the disturbing side-reaction we tried to carry out the exchange reaction with the precursor dibenzosuberone (dibenzo[a,d] [1,4]cycloheptadiene--5-one, 2), but in this case the oxo-group underwent reduction. Therefore we had to protect the reducible group in a form from which the compound could be easily regenerated.



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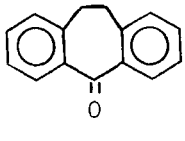

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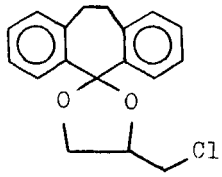

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RESULTS AND DISCUSSION

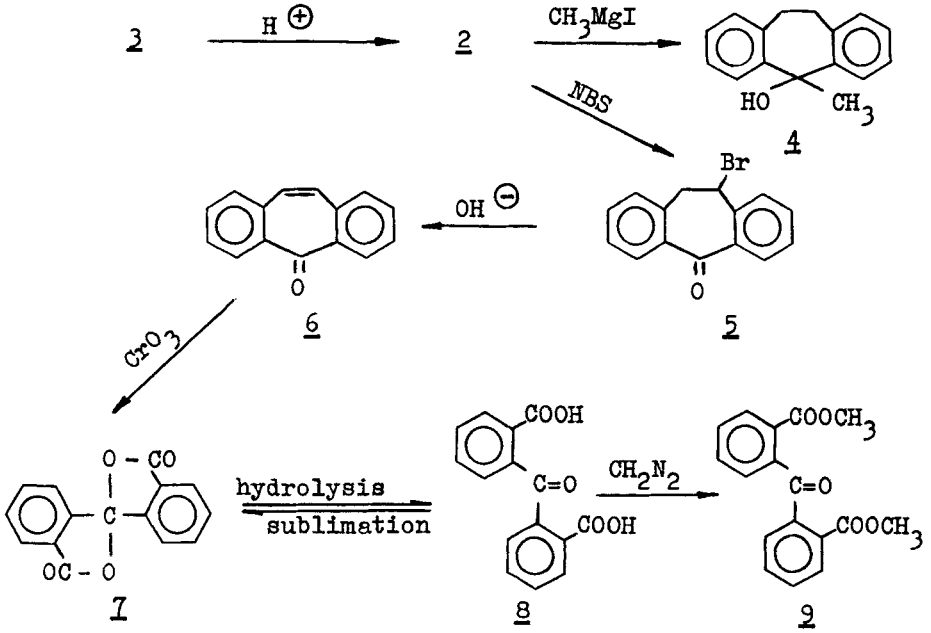
Converting into cyclic ketals is the most common protection of oxo-group against catalytic reduction. The classical method, reacting the ketone (2) with ethylene glycol in the presence of p-toluene-sulfonic acid however did not afford the desired product. The only cyclic ketal of 2 published (4) is the 4'-(bromomethyl)spiro(dibenzo[a,d] [1,4]cycloheptadiene-5,2'-[1,3]dioxolane), prepared from 2 with epibromohydrine using $SnCl_4$ as catalyst. By this method we prepared the chloro analogue of this compound (3) using epichlorohydrine instead of epibromohydrine. (The identity of this compound was verified by mass spectra.) Since 3 did not undergo catalytic halogen-tritium replacement, we have

found it to be suitable as precursor for catalysed isotope exchange.

In a preliminary experiment a small amount of 3 was treated with tritium gas over Pd catalyst, and after removing the labile tritium, a compound with a specific activity of 73.2 Ci/mmole = 2.7 TBq/mmole was obtained in which about 20 % of impurity could be detected by GLC. The tritiated material was mixed with 3 and purified by recrystallization. The specific activity of the pure compound was 78.4 % of the calculated one, agreeing with the GLC analysis.

On the other hand, hydrolysing the mother liquor gave only one radioactive spot by TLC (dibenzosuberone), which indicated that the impurity found by GLC is a derivative of 3, from which, despite of the attempted catalytic reduction, a small part underwent halogen-tritium exchange. After all, the loss of the tritium incorporated into the protecting group was negligable.

The position of the tritium incorporated was established by the degradation outlined in scheme.



The specific activity of 2 was measured in a well-crystallizable form as methyl carbinol (4). Compounds 5 and 6 were prepared according to Arthur and coworkers (5). The oxidation of 6 was carried out with CrO_3 instead of ozonization described, and gave the same product, the lactone of benzophenone-2,2'-dicarboxylic acid (7), which could be hydrolysed to dicarboxylic acid 8. The identity of both compounds was proved by IR spectra.

The specific activities measured (see table 1) show that isotopic exchange occurred in the methylene groups only, consequently this method is suitable for the preparation of amitriptyline specifically labelled with tritium.

Table 1.

Specific activity of degradation products

Compound	mCi/mmole	MBq/mmole
3	14.34	530
4	14.71	544
6	5.53	205
7	0.00	000

So in the next experiment a larger amount of 3 was exposed to tritiating over Pd-catalyst. The specific activity of the product obtained, after removing the labile tritium, was 23.75 Ci/mmole = 882 GBq/mmole. This compound was mixed with inactive 3, to obtain a specific activity of 10 Ci/mmole = 370 GBq/mmole, and hydrolysed to 2, which was reacted with a large excess of Grignard-compound prepared from 3-dimethylaminopropyl chloride as described by Protiva and coworkers (6). On treating with conc. HCl the compound obtained gave the crude amitriptyline (1), which was purified by chromatography on silica gel.

EXPERIMENTAL

GLC analyses were performed on a JEOL-JGC-20K instrument. IR spectra were recorded with a Perkin-Elmer 457 spectrometer. A Specord UV VIS (Carl Zeiss Jena) spectrophotometer was used for

UV measurements.

Radioactivity was measured with a Packard TRI-CARB liquid scintillation spectrometer. TLC was carried out on precoated silica gel 60 F_{254} sheets (Merck), and a Berthold TLC scanner was used for evaluation.

The melting points were determined on a Boëtius hot stage, and are uncorrected. All evaporations were carried out under reduced pressure.

I. 4'-(Chloromethyl(spiro)dibenzo[a,d] [1,4]cycloheptadiene-5,2'--[1,3]dioxolane) (3)

To a solution of 2 (10.4 g, 50 mmole) in $CHCl_3$ (50 ml) a mixture of epichlorohydrine (5.5 g, 60 mmole) and $CHCl_3$ (10 ml) was added at 0 C°. After being stirred for three hours, a solution of NaOH (10 %) was added to the mixture to make it alkaline. The organic phase was separated, washed with water, dried over anhydrous $CaCl_2$ and evaporated. The residue was distilled in vacuum to give viscous oil (10.4 g), (b.p. 177-181 C° at 0.2 Torr or 27 Pa), which was crystallized from alcohol. White crystals, 8.6 g, (28.6 mmole, 57.2 %). M.p. 65-67 C°.

This compound was dissolved in EtOH (100 ml) and hydrogenated over Pd/C catalyst in the presence of triethylamine (3 g, 30 mmole) for 24 hours. A few ml of hydrogen was taken up, but the material recovered was unchanged. Molar weight: 300 (by mass spectrometry) Cl % = 11.7 (calc. = 11.8). GLC analysis showed no impurity.

II. Localization of tritium incorporated by catalysed exchange reaction

1.8 mg (6 /umole) of 2 dissolved in DMF (200 /ul) was exposed to tritium (5 Ci = 185 GBq) over 5 % Pd/C catalyst (8.7 mg) for one hour. Then solution was filtered, evaporated, and MeOH as well as tert-butanol were added and distilled off until no change in the activity could be measured. The residue was taken up in

EtOH. The quantity of the material was 0.77 mg, calculated from the UV spectrum. [EtOH, 267 nm (ε = 1262), 272 nm (ε = 1214)]. Specific activity: 73.2 Ci/mmole = 2.71 TBq/mmole. Total activity: 187.6 mCi = 694 MBq. GLC analysis showed about 20 % of impurities.

This material was mixed with 3.0 g (10 mmole) of 3, and recrystallized from EtOH to give 2.7 g of white crystals, which had a specific activity of 14.34 mCi/mmole = 530 MBq/mmole, 78.4 % of the calculated one. The pure crystalline material was then refluxed in a mixture of EtOH (200 ml), water (100 ml) and conc. H_2SO_4 (10 ml) for three hours. After distilling off the ethanol, the residue was extracted with 50 ml of ether four times. The combined organic phase was dried over anhydrous $CaCl_2$ and evaporated. The yellow oily product obtained was distilled in vacuum resulting in 1.76 g (8.46 mmole, 94.2 %) of dibenzosuberone, which proved to be chemically and radiochemically pure by TLC, using CCl_4:EtOH = 50:1 solvent system.

Hydrolysing similarly the material obtained from the mother liquor of the alcoholic recrystallization, only one radioactive spot was shown by TLC, which was found to be identical with the main product.

For measuring the radioactivity, a small amount of above compound was converted into methyl carbinol (4) as follows: 0.22 g (1.05 mmole) of tritiated dibenzosuberone was reacted with methylmagnesium iodide prepared from methyl iodide (0.3 g, 2.1 mmole) in dry ether (10 ml). After being stirred for one hour, the reaction mixture was decomposed by adding saturated aqueous ammonium chloride (5 ml). The ethereal solution was separated and washed with water. After evaporation, the residue was crystallized from 96 % EtOH (2 ml) to give 0.1 g (0.46 mmole) of 4. M.p.: 136-142 C° (lit.: 135-143.5 C°) (5). Specific activity: 14.7 mCi/mmole = 544 MBq/mmole.

1.55 g (7.4 mmole) of tritiated dibenzosuberone, N-bromo-

-succinimide (1.37 g, 7.7 mmole) and dibenzoyl peroxide (0.015 g) were refluxed in carbon tetrachloride (18.0 ml) for three hours. The mixture was filtered and evaporated. To the residue KOH (3.41 g, 6.1 mmole), water (5.1 ml) and MeOH (8.5 ml) were added and refluxed for three hours. After cooling, the precipitated product was filtered off and recrystallized from MeOH to give 0.94 g (4.56 mmole, 61.6 %) of yellowish crystals. M.p. 85-87 C° (lit.: 88.4-89.2 C°) (5). Specific activity: 5.53 mCi/mmole = 205 MBq/mmole.

0.515 g (2.5 mmole) of this compound was dissolved in acetic acid and a solution of CrO_3 (1.12 g, 11.2 mmole) in acetic acid (7.5 ml) and water (2.5 ml) was added droppwise at 80 C°. After being stirred for six hours, the solution was mixed with water (100 ml) and the precipitated product was filtered off, washed with water and dried. 0.48 g (1.61 mmole) of 7 was obtained as yellow crystals. M.p. 199-206 C°, (lit.: 208-211 C°) (5).

The crude lactone (7) was hydrolysed by refluxing in 5N NaOH solution for ten minutes. The solution was treated with charcoal, filtered and acidified with conc. HCl to give 0.38 g (1.42 mmole) of white crystalline 8. IR: ν C=O acid: 1705 cm^{-1}, ketone: 1680 cm^{-1}. This product was not radioactive.

A small amount of this compound was sublimed in wacuum to give pure 7. M.p. 210-211 C°. IR: ν C=O 1790 cm^{-1}.

A small amount of dicarboxylic acid 8 in ethereal solution was treated with diazomethane to give 9. M.p. 83 C°.

III. 5-(3-dimethylaminopropylidene)-dibenzo[a,d] [1,4]cyclo-heptadiene-(10,11-$^{3}H_4$)

49 mg (0.16 mmole) of 3 in DMF (1.2 ml) was exposed to tritium (~ 20 Ci = 740 GBq) over 5 % Pd/C catalyst (200 mg) for one hour. The mixture was then filtered, the solution was evaporated, and MeOH as well as tert-butanol were added and distilled off until no change in the specific activity was found. At last 31.3 mg

(calculated from UV spectrum), 2.48 Ci = 92 GBq (23.75 Ci/mmole = 878 GBq/mmole) of labelled 3 was obtained, which was mixed with unlabelled material (40 mg), and refluxed in a mixture of EtOH (50 ml), water (25 ml) and conc. H_2SO_4 (2.5 ml) for three hours. The ethanol was distilled off and the residue was extracted three times with ether (10 ml). The ethereal solution was dried over anhydrous $CaCl_2$ and evaporated. The residue was added to a Grignard reagent prepared from 3-dimethylaminopropyl chloride (1.82 g, 15 mmole) and magnesium (0.37 g, 15 mmole) in dry ether (20 ml). After being stirred under reflux for five hours, 6.2 ml of conc. HCl was added to the mixture. The ether was distilled off, and the residue was heated at 150 C^o for one hour. After cooling the reaction mixture was diluted with water (30 ml), washed with benzene (30 ml) and made alkaline with conc. NH_4OH. The crude amitriptilyne (1) was extracted with dichloromethane (30 ml) and after evaporation it was purified by chromatography on silica gel using MeOH- conc. NH_4OH 45:5 eluent. The compound obtained was treated in ethyl acetate (2 ml) with HCl-EtOH to give 0.057 g (0.18 mmole) of white crystals, the hydrochloride of labelled 1. M.p. 190-192 C^o. Total activity: 1.89 Ci (70.2 GBq). Specific activity: 33.3 Ci/g (1.23 TBq/g), 10.4 Ci/mmole (386 GBq/mmole). Not more then 1 % of radioactive impurities could be detected by TLC.

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