SYNTHESIS OF A TUMOR-GROWTH INHIBITOR–DIHYDROCHLORIDE OF 1–NITRO – 9 –/DIMETHYLAMINO–PROPYLAMINO/–ACRIDINE /C–283/ TRITIUM LABELLED IN THE ACRIDINE RING AND ^{14}C-LABELLED IN THE SIDE CHAIN*

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SUMMARY

1-Nitro-9/dimethylaminopropylamino/-acridine dihydrochloride /C-283, 'Ledacrin'/ is used as an anti-tumor drug. In view of the studies on its mechanism of action, the syntheses of two specimens of this compound labelled with radioisotopes have been carried out. One of them contained C^{14} in the side chain and the other tritium in the acridine ring. 3-Dimethylaminopropylamine /1-C^{14}/ prepared on the basis of $K^{14}CN$ was used as the initial substrate for the synthesis of C-283 labelled with ^{14}C. It was condensed with pyridinium salt of 1-nitro-9-chloroacridine.

In order to introduce tritium to the acridine ring, m-nitroaniline was tritiated in the acidic solution of tritiated water.

Thus obtained tritiated m-nitroaniline was condensed by Ullman's method with o-chlorobenzoic acid.

The N-/3'-nitrophenyl/anthranilic acid was then converted to the derivative of 1-nitro-9-chloroacridine /2,3,4-T_3/. The specific activity of C-283 ^{14}C labelled was 5.5 mCi/mmole and that of C-283 tritium labelled 103 mCi/mmole.

Introduction

The extensive studies of A. Ledóchowski and coll.[1/] on 9-aminoacridine derivatives led in 1966 to the synthesis of dihydrochloride 1-nitro-9-/dimethylamino-propylamino/-acridine /C-283/.

*The researches granted by Zjedn. Przem. Farm. "Polfa".

The biological in vitro and in vivo tests showed its strong antitumor activity[2]. It was then subjected to the thorough biochemical and pharmacological examination and in 1974 it was introduced as a medicament named "Ledacrin".

Hammick and Mason[3] have observed that in an animal cell 9-derivatives of 9-aminoacridines undergo the elimination of the side chain from the position 9. In order to confirm whether the metabolic path is the same in the case of C-283 it was necessary to synthetize this compound labelled with two different radioisotopes so that the quantitative determination of each of those two could be made. The labelling with tritium and carbon ^{14}C meets those requirements. The synthesis of C-283 tritium labelled has not been carried out yet. However two compounds labelled with ^{14}C have been synthetized, one having ^{14}C in position 9 of the acridine ring[4] and the other with isotopic carbon in position 1 of the side chain.[5]

Results

C-283 is unstable in solutions, so the electrophilic substitution of tritium cannot be carried out.

Attempts to tritiate the intermediates of the synthesis: 1-nitroacridone and N-/3'-nitrophenyl/-anthranilic acid in acidified water-T had to be given up due to their insufficient solubility in water. On the other hand, the slight solubility of 1-nitroacridono-5-sulphonic acid in dilute acids eliminated the possibility of its hydrolysis, that is the substitution of sulphonic group by tritium.

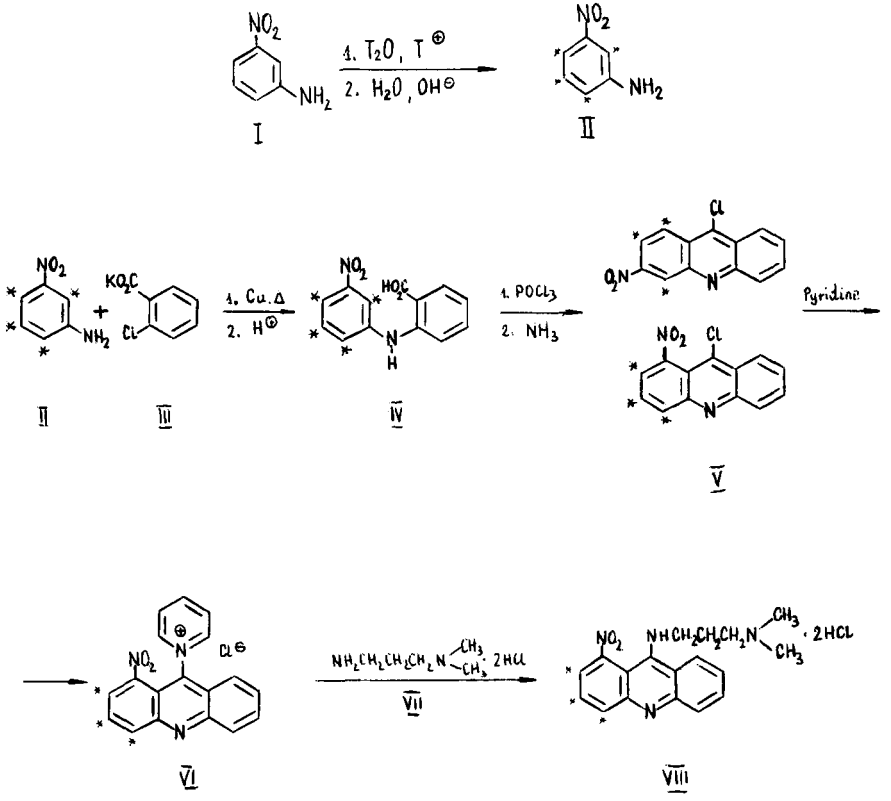
The thermal decarboxylation of 1-nitro-4-carboxy-9-acridonic acid[6] failed too, as the acid appears to be stable up to 250°C. Above mentioned factors forced us to look for a substitution in earlier stages of the synthesis of C-283. As a substrate we used m-nitroaniline /I/ tritiated for 48 hours in 120°C with the help

of tritiated water containing 50% /by weight/ of sulphuric acid. The specific activity of tritiated water was 36 mCi/mval, and that of m-nitroaniline after tritiating was 149 mCi/mmol thus proving the exchange of all four aromatic protons by tritium. Radioactive m-nitroaniline /II/ was condensed by Ullmann's method with potassium o-chlorobenzoate /III/. In the described method[4/] of the preparation of N-/3'-nitrophenyl/-anthranilic acid a considerable excess of m-nitroaniline, being at the same time the reaction medium, is needed.

Thanks to the introduction of DMF as a solvent and the periodical additions of a new catalyst during the reaction it was possible to obtain the product of the condensation with 75% yield with respect to m-nitroaniline /sp. act. - 144 mCi/mmol/.

The action of phosphorus oxychloride causes the cyclization of this compound giving the mixture of 1- and 3-nitro-9-chloroacridine /V/ isomers, with the excess of 1-nitro isomer[4/] /sp. act. 106 mCi/mmol/. The chemical yield of this stage was 96% and the radiochemical yield was only 69% due to the loss of one tritium atom from the molecule during the cyclization. The mixture of the isomers was separated on the basis of the difference of the rate of forming salts with pyridine.[7/] The yield of pyridinium salt of 1-nitro-9-chloroacridine /VI/ was 56% /sp. act. 105,5 mCi/mmol/. It can be further condensed with dimethylamino-propylamine ^{14}C labelled /IX/ in order to obtain the doubly labelled compound. However having on one's disposal two compounds: one tritium labelled in the acridine ring and the other ^{14}C labelled in the side chain is much more convenient as they can be mixed in desired proportions just before "in vivo" tests. For this reason we have used the unlabelled amine /VII/ getting with 57% yield dihydrochloride of 1-nitro-9/dimethylaminopropylamino/-acridine /2,3,4-T_3/ /VIII/, sp. act. 103 mCi/mmol.

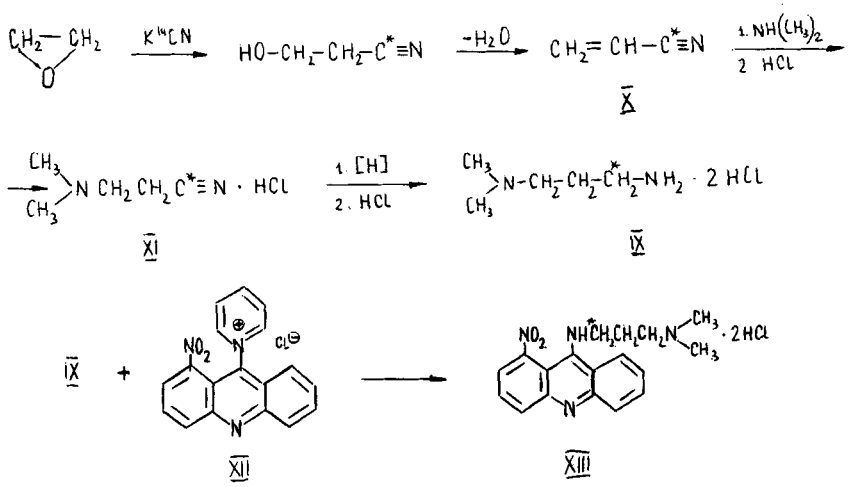
Dimethylaminopropylamine labelled with ^{14}C /IX/ was prepared



from acrylonitrile[8] /X/ which in turn had been prepared from labelled potassium cyanide /sp. act. of $K^{14}CN$-5.6 mCi/mmol, total activity - 40 mCi/. Making use of the addition of dimethylamine to acrylonitrile[9] we obtained β-dimethylaminopropionitrile /XI/, which was isolated with 65% yield as hydrochloride /sp. act. 5,6 mCi/mmol/. This compound was then reduced by Bouveautt-Blanc's method[10] giving 3-dimethylaminopropylamine-1-^{14}C /IX/ with 40% yield /sp. act. 5.5 mCi/mmol/.

After condensation of dihydrochloride of the amine /IX/ with the help of pirydinium salt of 1-nitro-9-chloro-acridine /XII/ we obtained dihydrochloride 1-nitro-9-/dimethylaminopropyl/-amino-1-^{14}C/-acridine /XIII/ of sp. act. 5.5 mCi/mmol. Both la-

belled compounds had physicochemical properties identical with the standard of C-283.



Experimental

All stages of the synthesis were worked out and checked on unlabelled compounds. The progress of the reactions was controlled by TLC on thin-plates coated with silica gel Kieselgel G /Merck/, in the case of C-283 with aluminium oxide **Aluminiumoxid** G /Type E, Merck/. The radioactivity of products and intermediates was measured by the scintillation method with the help of Isocap-300 /Nuclear-Chicago/.

3-Nitroaniline /2,4,5,6-T_4/ /II/

A sealed ampoule containing 798 mg /6 mmoles/ of m-nitroaniline /I/, 1 c.c. /55 mmoles/ of water-T /sp. act. 73 mCi/mmol, total act. 4,6 Ci/ and 0.5 c.c. /9.4 mmoles/ of concentrated sulphuric acid has been heated for 48 hours at 120°C. After cooling the contents were dissolved in 100 c.c. of water and evaporated under reduced pressure. The dry residue was then dissolved in 50 c.c. of water and made alkaline with 10% aqueous solution of

NaOH. After cooling, crystalline m-nitroaniline was filtered off, rinsed with cold water and dried. The yield was 701 mg /88% theor./ of the chromatographically pure product. Sp. act. 149 mCi/mmol, total act. 766 mCi, m.p. 113.5 - 114°C.

N-/3'-nitrophenyl/-anthranilic acid /2,4,5,6-T_4/ /IV/

690 mg /5 mmoles, 745 mCi/ of labelled m-nitroaniline /II/, 1980 mg /10 mmoles/ of potassium salt of o-chlorobenzoic acid /III/ and 50 mg of freshly precipitated copper in 2 ml of DMF were heated to boiling under reflux. After 15 minuts, 50 mg of copper powder was added and then after 30 minuts the same portion was again added. The heating was completed after 45 minuts, the contents were poured to 50 c.c. of 5% solution of K_2CO_3, heated to boiling with charcoal and filtered off. After acidifying to pH=5,2 with dilute hydrochloric acid, the precipitated N-/3'-nitrophenylanthranilic/ acid /IV/ was filtered off. The yield was 979 mg /75% of theor. chem., 73% theor. radiochem./, sp. act. 144 mCi/mmole total act. 549 mCi, m.p. 216-218°C /ref.[11] m.p. 218°C/.

1-Nitro-9-chloroacridine /2,3,4,5-T_3/ and 3-nitro-9-chloroacridine 1,2,4-T_3 /V/[4]

The mixture of 976 mg /3,8 mmoles, 547 mCi/ of /IV/ and 6 c.c. of $POCl_3$ was heated under reflux for 15 minuts at 80°C and then 30 minuts at 120°C. The excess of phosphorus oxychloride was removed by distillation under reduced pressure. The remainder was dissolved in 4 c.c. of acetone and poured to the mixture of 5 c.c. of 25% solution of ammonium hydroxide with 15 g of ice. The precipitate was filtered off, rinsed with 2% solution of ammonium hydroxide and dried. 928 mg /96%/ of the mixture of the isomers /V/ was obtained. Sp. act. 106 mCi/mmole, total act. 380 mCi /radioact. yield - 69%/.

Pirydinium salt of 1-nitro-9-chloroacridine /2,3,4-T_3/ /VI/ [7]

920 mg /3.5 mmoles, 378 mCi/ of the mixture of nitrochloroacridine isomers /V/ was mixed with 4 c.c. of pirydine and the insoluble 3-nitro-isomer was filtered off. The precipitate was rinsed thrice with 1 c.c. of pirydine. The combined filtrates were left for a night at room temperature and pirydine salt of 1-nitro-9-chloroacridine /VI/ was filtered off. It was then rinsed once with 1 c.c. of pirydine and thrice with 0.75 c.c. of acetone. 676 mg /56%/ of the product was obtained, sp. act. 105.5 mCi/mmole, total act. 211 mCi, radiochemical yield - 56% m.p. 152-153°C /ref.[7] 154-155°C/.

Dihydrochloride of 1-nitro-9-/dimethylaminopropylamino/-acridine /2,3,4 - T_3/ /C-283/ /VIII/ [7,12]

670 mg /2 mmoles, 209 mCi/ of pirydine salt /VI/ was heated with 1.5 g of phenol for 15 minuts at 80°C. After cooling 350 mg /2 mmoles/ of dihydrochloride of dimethylaminopropylamine[13] was added and the mixture was heated for 30 minuts more. After cooling the contents were dissolved in 10 c.c. of benzene and poured to 15 c.c. of chilled 15% aqueous solution of KOH. The organic layer was separated and the aqueous layer was extracted thrice with 10 c.c. of benzene. The combined organic solutions were dried with anhydrous $MgSO_4$ and the excess of etherical solution of hydrogen chloride was added. After 30 minuts the solvents were decanted, the remainder was dissolved in absolute methanol and bleached with charcoal. The product was precipitated, by absolute ether. 451 mg /57%/ /VIII/ of the product was obtained with sp. act. 103 mCi/mmole and total act. 117 mCi /radiochem. yield - 56%/, mp. 222-3°C /ref. [7,12] - 223-4°C/. The product gave only one chromatographic spot on thin plates with R_f values identical as the standard. The spot of the product contained total radiochemical activity.

Acrylonitrile /1-^{14}C/ /X/ [8]

230 mg /60% theor./ of acrylonitrile /X/ with b.p. 76-78° /ref.[8] 60-70% theor., b.p. 76-78°/ was obtained from 470 mg /7.23 mmoles/ of K^{14}CN of sp. act. 5.6 mCi/mmol and total act. 40 mCi.

Hydrochloride of N,N-dimethylaminepropionitrile /1-^{14}C/ /XI/ [9]

1 c.c. of 50% aqueous solution of dimethylamine was added to the prepared acrylonitrile /X/ cooled at ice-bath. The mixture was left for a night at room temperature. Then a small stream of argon was bubbled for 20 minuts through the solution in order to remove the remaining dimethylamine. After acidifying the solution with concentrated HCl /0.5 c.c., 6 mmoles/, it was evaporated under reduced pressure. The remainder was dried in a vaccum desiccator over KOH and P_2O_5. It was crystallized from absolute methanol giving 380 mg /65% theor./ of the product, sp. act. 5.6 mCi/mmole, total activity - 15.4 mCi, m.p. 197-199° /ref.[9] - 199°/.

Dihydrochloride of 3-dimethylaminopropylamine/ 1-^{14}C/ /IX/ [10]

Desiccated aminonitrile hydrochloride /XI/, 376 mg /2.8 mmoles/, was dissolved in 10 c.c. of absolute ethanol, and 450 mg of sodium was added. After the end of the reaction the contents were diluted with water and the amine was removed by steam distillation. The distillate was acidified with dilute HCl do pH=1 and the solution was evaporated to dryness under reduced pressure. The product was dried in a vaccum desiccator over KOH and P_2O_5. 196 mg /40% theor./ of the product was obtained. Sp. act. 5.5 mCi/mmole, total act. 6.2 mCi, m.p. 181-183°C /ref. 184° [9], 182-4 [10].

Dihydrochloride of 1-nitro-9-/3-dimethylaminopropylamino-1-^{14}C/ acridine /C-283/ /XIII/

192 mg /1.1 mmoles/ of the amine hydrochloride /IX/ was

condensed with 370 mg /1.1 mmoles/ of the pirydinium salt of 1-nitro-9-chloroacridine[13] /XII/ in 750 mg of phenol by the same method as in the synthesis of C-283 tritium labelled. 250 mg of the product /57% theor./ was obtained. Sp. act. 5.5 mCi/mmole, total act. 3.5 mCi, m.p. 222-223° /ref.[7,12] 223-224°C/.

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