SYNTHESIS OF ^{14}C- AND ^{3}H-LABELLED 1,6-DIMETHYL-3-CARBETHOXY-4-OXO-6,7,8,9-TETRAHYDROHOMOPYRIMIDAZOLE SALTS

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SUMMARY

For pharmacological investigations tetrahydrohomopyrimidazole salts were synthetized which were labelled by ^{14}C and ^{3}H isotopes in definite positions. The 1-methyl-^{14}C, carbonyl-^{14}C, 3-carbethoxyethyl-^{14}C, 1-methyl-^{3}H, 6,7,8,9-^{3}H and 3-carbethoxyethyl-^{3}H-homopyrimidazole isotope isomers were prepared.

INTRODUCTION

In order to carry out pharmacological investigations with isotope-labelled specimens, the isotope isomers of the analgetic drug[1,2,3,4] 1,6-dimethyl-3-carbethoxy-4-oxo-6,7,8,9-tetrahydrohomopyrimidazolium methosulphate[+++] (PROBON^{R})[++++] (MZ-144) were synthetized. For a more precise evaluation of isotope distribution, detoxication, metabolism as well as micro- and macroautoradiographic studies, ^{14}C and ^{3}H were incorporated in various sites of the drug molecule.

[+++] 1,6-Dimethyl-3-carbethoxy-6,7,8,9-tetrahydro-4H-pyrido-(1,2-a)--pyrimidine-4-one methosulphate.

[++++] PROBON^{R} registered name; Rimazolium methylsulphate, generic name.

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The compound 1,6-dimethyl-3-carbethoxy-(carboxyl-^{14}C)-4-^{14}C--oxo-6,7,8,9-tetrahydrohomopyrimidazolium methosulphate was obtained according to the synthetic pathway shown in Figure 1[7,8].

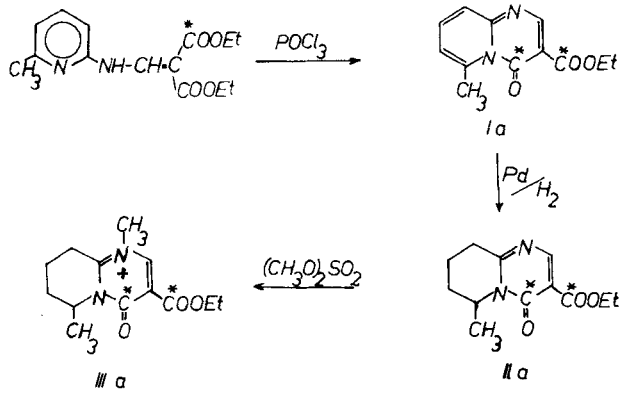

Figure 1.

The substance ethyl 2-methylpyridyl-6-aminomethylenemalonate--(carboxyl-^{14}C) was prepared from K^{14}CN[5] according to Figure 2[6].

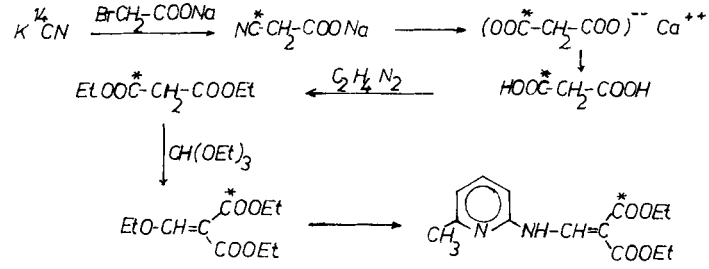

Figure 2.

α-Picolylaminomethylenemalonate, used as starting material, was obtained in the following way: labelled malonate was reacted with ethyl orthoformate and then with 6-amino-2-picoline.

The intermediate containing ^{14}C-carbethoxy group was condensed with 3-carbethoxy-(carboxyl-^{14}C)-4-^{14}C-oxo-6-methylhomopyrimidazole (Ia) by means of the Bischler-Napieralsky reaction (Figure 1). This latter substance took up two moles of hydrogen to

give IIa on hydrogenation in the presence of a palladium catalyst. This reduced base was quaternised with methyl sulphate to yield 1,6-dimethyl-3-carbethoxy-(carboxyl-^{14}C)-4-^{14}C-oxo-6,7,8,9-tetrahydrohomopyrimidazolium methosulphate (IIIa).

When the catalytic reduction of 3-carbethoxy-4-oxo-6-methylhomopyrimidazole was carried out in a microhydrogenating apparatus by using tritium gas, then 3-carbethoxy-4-oxo-6-methyl-6,7,8,9-^{3}H--tetrahydrohomopyrimidazole (IIb) was obtained (Figure 3). Quaternisation with methyl sulphate of IIb gave the ^{3}H-labelled end-product (IIIb).

When the quaternisation of 3-carbethoxy-4-oxo-6-methyl--6,7,8,9-tetrahydrohomopyrimidazole was effected by using ^{14}C or ^{3}H-labelled methyl iodide, then 1-^{14}C-methyl or 1-^{3}H-methyl derivatives (IIIc and IIId, respectively) were obtained. Methyl iodide labelled with radiocarbon was prepared from $^{14}CO_2$ (10) via the methanol intermediate(9). The tritium-labelled methyl iodide was prepared by means of isotope exchange reaction, through the thermal decomposition of ^{3}H-trimethylsulphoxonium iodide(11).

Labelling of the carboxyethyl group bound to the carbon atom in position 3 was based on ethanol intermediate, labelled by ^{14}C or ^{3}H, respectively. Ethanol labelled on carbon was obtained by reduction of 1-^{14}C ethyl acetate with mixed metal hydride(12). Ethanol labelled with tritium was prepared from ethyl bromoacetate subjected to reductive dehalogenation with tritium on a palladium catalyst and subsequently to reduction by mixed metal hydride. The labelled ethanol was introduced during the condensation reaction of ethyl 2-methylpyridyl-6-aminomethylenemalonate when the phosphoric acid complex arising from cyclisation was decomposed with the radioactive ethanol. The products IIIe and IIIf were reached by catalytic reduction and quaternisation with methyl sulphate following the ring closure.

Chemical and radiochemical purity of the labelled substances

were checked by thin layer autoradiographic chromatography. Radioactivity of the samples was determined in an ionisation chamber (Type Dinacon 6000) and by liquid scintillation method (TRI-CARB type β -spectrometer).

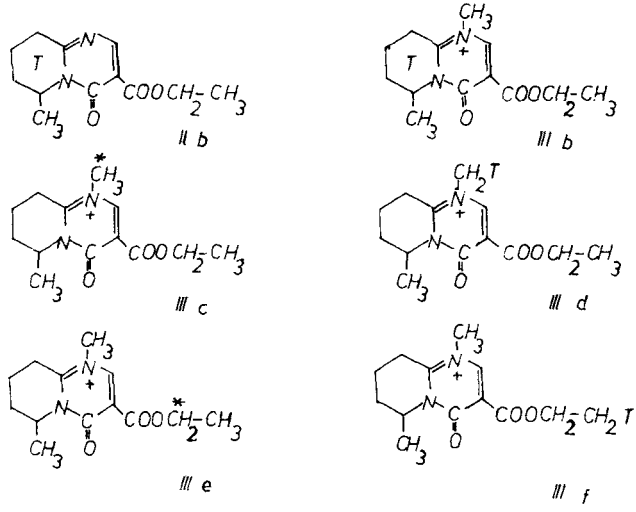

Figure 3.

EXPERIMENTAL

Malonic acid (^{14}C-carboxyl)

A solution of 3.84 (27.5 mmoles) of bromoacetic acid in 3 to 4 ml of water was neutralised by 2N NaOH (pH 7 to 8) and 1.625 g (25 mmoles, 95 mCi) of $K^{14}CN$ was added. The mixture was heated at 100° for one hour, 2.5 ml of 12.5N NaOH was added and hydrolysed at 100° for further 2 hours. After hydrolysis, a solution of 3.7 g (30 mmoles) of $CaCl_2$ in 5 ml of water was dropped to the mixture. After standing overnight, the precipitated, white crystals were filtered, washed with water and dried. The calcium salt was dissolved in 8 ml of 20% HCl and extracted with ether continuously for 24 hours. After evaporation of the solvent, white, crystalline

malonic acid (m.p. 134-139°) was obtained in a yield of 2.3 g (22 mmoles), i.e. 88% as calculated for $K^{14}CN$.

Ethyl (^{14}C-carboxyl) malonate

A freshly prepared, ethereal solution of diazoethane, dried previously over KOH, was dropped in portions to 2.3 g (22 mmoles) of malonic acid until reaching a stable yellow colour. After one hour the solvent was evaporated and the radioactive malonic ester was obtained as a residual colourless oil, in a yield of 2.76 g (17 mmoles).

Ethyl 2-methylpyridyl-6-aminomethylene-(^{14}C-carboxyl)-malonate

A mixture of 2.76 g (17 mmoles) of crude malonic ester, 4.1 ml (25 mmoles) of ethyl orthoformate, 5 ml (50 mmoles) of acetic anhydride and 10 mg of freshly fused $ZnCl_2$ was refluxed in a bath of 130-140° for 2 hours. The ethyl acetate formed was evaporated and this procedure was repeated after addition of 0.4 ml of inactive malonic ester, 1.5 ml of ethyl orthoformate and 2.2 ml of acetic anhydride; finally, the unreacted components were removed at 200°. The residual, yellow oily radioactive ethyl ethoxymethylenemalonate was mixed with 2.2 g (20 mmoles) of α-aminopicoline and refluxed at 120-130° for 30 minutes. After cooling 5 ml of 50% aqueous ethanol was added, the crystalline product was filtered off, washed with ice-cold aqueous ethanol and dried over P_2O_5 to give 3.93 g (14.2 mmoles) of product (m.p. 107-108°), 50 mCi, 12.6 mCi/g.

3-Carbethoxy-(^{14}C-carboxyl)-4-^{14}C-oxo-6-methylhomopyrimidazole Ia hydrochloride

4.2 ml (about 45 mmoles) of $POCl_3$ and 0.5 ml of polyphosphoric acid were added dropwise into 3.92 g (14.2 mmoles) of radioactive ethyl 2-methylpyridyl-6-aminomethylenemalonate in a round bottom glass bottle fitted with stirrer and reflux condenser. The mixture

was boiled for 3 hours, cooled to 0° and 15 ml of abs. ethanol was added. The crystals that separated overnight were filtered off, washed with ice-cold ethanol and chloroform and dried to give 3.1 g (11.5 mmoles) of product (m.p. 210-212°).

3-Carbethoxy-(^{14}C-carboxyl)-4-^{14}C-oxo-6-methyl-6,7,8,9-tetrahydrohomopyrimidazole IIa

A suspension of 3.1 g (11.5 mmoles) of the above product in abs. ethanol was hydrogenated in the presence of Pd-C catalyst. When hydrogen was no longer absorbed the catalyst was filtered off and solvent evaporated. The residue was IIa hydrochloride (3.1 g, 11.5 mmoles, m.p. 167-169°) that was dissolved in 50 ml of 50% ethanol and carried through a column filled with an OH^- form anion-exchange resin. The chloride-free eluate was evaporated and the residue dissolved in 3-4 ml of ethyl acetate. The base IIa crystallized at -60° (m.p. 55-58°) in a yield of 1.88 g (7.1 mmoles), 26.8 mCi, 14.28 mCi/g, 3.37 mCi/mmole.

1,6-Dimethyl-3-carbethoxy-(^{14}C-carboxyl)-4-^{14}C-oxo-6,7,8,9-tetrahydrohomopyrimidazolium methosulphate IIIa

0.63 g (5 mmoles) of methyl sulphate was added to the solution of 1.07 g (4.5 mmoles) of IIa base (containing 2.48 mCi/mmole molar activity) in 4-5 ml of abs. acetone. After standing 2 days, the precipitated crystals were filtered off and recrystallized from 5 ml of isopropanol to give 1.42 g (3.9 mmoles) of product (m.p. 166-167°), 7 mCi/g, 2.5 mCi/mmole.

3-Carbethoxy-4-oxo-6-methyl-6,7,8,9-^{3}H-tetrahydrohomopyrimidazole IIb

A solution of 21 mg of inactive 3-carbethoxy-6-methyl-4-oxo-homopyrimidazole in 0.5 ml of abs. ethyl acetate was reduced with T_2 in the presence of Pd-C catalyst in a microhydrogenator. After

a consumption of 0.6 ml, the reduction was completed with inactive hydrogen, the mixture was diluted with methanol, the catalyst filtered off and the solvent evaporated. After addition of 2.36 g (10 mmoles) of inactive base, the distillation residue was recrystallized from 8 ml of ethyl acetate to give 1.83 g (7.7 mmoles) of product (m.p. 56-58°), 232.9 mCi, 127.29 mCi/g, 30 mCi/mmole.

1,6-Dimethyl-3-carbethoxy-4-oxo-6,7,8,9-^{3}H-tetrahydrohomopyrimidazolium methosulphate IIIb

The base IIb was methylated with methyl sulphate. From 1.07 g (4.5 mmoles) of 3-carbethoxy-4-oxo-6-methyl-6,7,8,9-^{3}H-tetrahydrohomopyrimidazole (containing 30 mCi/mmole specific activity) 1.32 g (3.6 mmoles) of product (m.p. 165-166°) was obtained, 108.6 mCi, 82.29 mCi/g, 29.8 mCi/mmole.

1,6-Dimethyl-3-carbethoxy-4-oxo-6,7,8,9-tetrahydrohomopyrimidazolium-(1-methyl-^{14}C) iodide IIIc

To the abs. acetone solution of 1.18 g (5 mmoles) of IIb base, 0.64 g (4.5 mmoles) of methyl iodide (containing 8 mCi/mmole) in 2 ml of abs. acetone was added at 0°. The mixture was slowly heated to 20°, after 2 days the yellowish crystals were separated and recrystallized from 15 ml of abs. ethanol to give 0.7 g (1.8 mmoles) of product (m.p. 198-200°), 14.8 mCi, 21.3 mCi/g, 8 mCi/mmoles.

1,6-Dimethyl-3-carbethoxy-4-oxo-6,7,8,9-tetrahydrohomopyrimidazolium-(1-methyl-^{3}H) iodide IIId

When the above procedure was repeated with ^{3}H-methyl iodide in a scale of 15 mmoles, 2.33 g (6.1 mmoles) of product (m.p. 199°) was obtained, 122 mCi, 52.42 mCi/g, 19.76 mCi/mmole.

1,6-Dimethyl-3-carbethoxy-(ethyl-1-^{14}C)-4-oxo-6,7,8,9-tetrahydrohomopyrimidazolium methosulphate IIIe

4.83 g (12 mmoles) of benzyl 6-methylpyridyl-2-aminomethylenemalonate was condensed by phosphorus oxychloride and polyphosphoric acid according to preparation of Ia and the phosporic acid complex was then decomposed by 1 g of ethanol-1-^{14}C (containing 0.5 mCi/mmole). The obtained 1.2 g (4.5 mmoles) of 3-carbethoxy-(ethyl-1-^{14}C)-4-oxo-6-methylhomopyrimidazole was dissolved in a little abs. acetone and hydrogenated in the presence of Pd-C catalyst. When the consumption had ceased, the catalyst was filtered off and the solvent was evaporated. The remaining hydrochloride was freed of chloride ions by means of an anion-exchange column in OH^- form, the solvent was evaporated and the base obtained was alkylated with methyl sulphate as described above for the preparation of IIIa. A yield of 0.4 g (1.1 mmoles) was obtained (m.p. 167°), 0.2 mCi, 0.5 mCi/g, 0.18 mCi/mmole.

1,6-Dimethyl-3-carbethoxy-(ethyl-2-^{3}H)-4-oxo-6,7,8,9-tetrahydrohomopyrimidazolium methosulphate IIIf

5.5 g (20 mmoles) of α-picolylaminomethylenemalonate was cyclised by means of phosphorus oxychloride and polyphosphoric acid as described above for the preparation of Ia. The phosphoric acid complex was decomposed by 1 g of ethanol-2-^{3}H (containing 50 mCi/mmole). The obtained 1,6-dimethyl-3-carbethoxy-(ethyl-2-^{3}H)-4-oxo-6,7,8,9-tetrahydrohomopyrimidazole hydrochloride was transformed into the end-product IIIf as described for preparation of IIIe. A yield of 1.45 g (4 mmoles) was obtained, 99 mCi, 7 mCi/g, 2.5 mCi/mmole.

REFERENCES

1. Knoll J., Mészáros Z., Szentmiklósi P. and Fürst S. - Arzneimittel-Forschung, 21 : 717 (1971).

2. Knoll J., Fürst S. and Mészáros Z. - Arzneimittel-Forschung, 21 : 719 (1971).
3. Knoll J., Fürst S. and Mészáros Z. - Arzneimittel-Forschung, 21 : 727 (1971).
4. Knoll J., Magyar K. and Bánfi D. - Arzneimittel-Forschung, 21 : 733 (1971).
5. Bánfi D., Mlinkó S. and Palágyi T. - J. Labelled Comp. 7 : 221 (1971).
6. Calvin M., Heidelberger C., Reid J. C., Tolbert B. M. and Yankwich P. F. - Isotopic Carbon, Wiley and Sons, 1960 p. 191.
7. Mészáros Z., Knoll J. and Szentmiklósi P. - Hung. Pat. No. 156 119; Hung. Pat. 158 085; Brit. Pat. 1 209 946.
8. Mészáros Z., Knoll J., Szentmiklósi P., Dávid Á., Horváth G. and Hermecz I. - Arzneimittel-Forchung, 22 : 815 (1972).
9. Cox J. D., Turner H. S. and Warne R. J. - J. Chem. Soc. 1950, 3167.
10. Calvin M., Heidelberger C., Reid J. C., Tolbert B. M. and Yankwich P. F. - Isotopic Carbon, Wiley and Sons, 1960 p. 169.
11. Private comunication of Tanács B.
12. Cox J. C., Turner H. S. and Warne R. J. - J. Chem. Soc. 1950, 3176.