LABELLING OF 2-HYDROXY-5-CARBOMETHOXYBENZYLOXYAMINE HYDROCHLORIDE WITH ^{14}C ISOTOPE.

J. Engler and E. Kasztreiner.

Research Institute for Pharmaceutical Chemistry, Budapest - Hungary.

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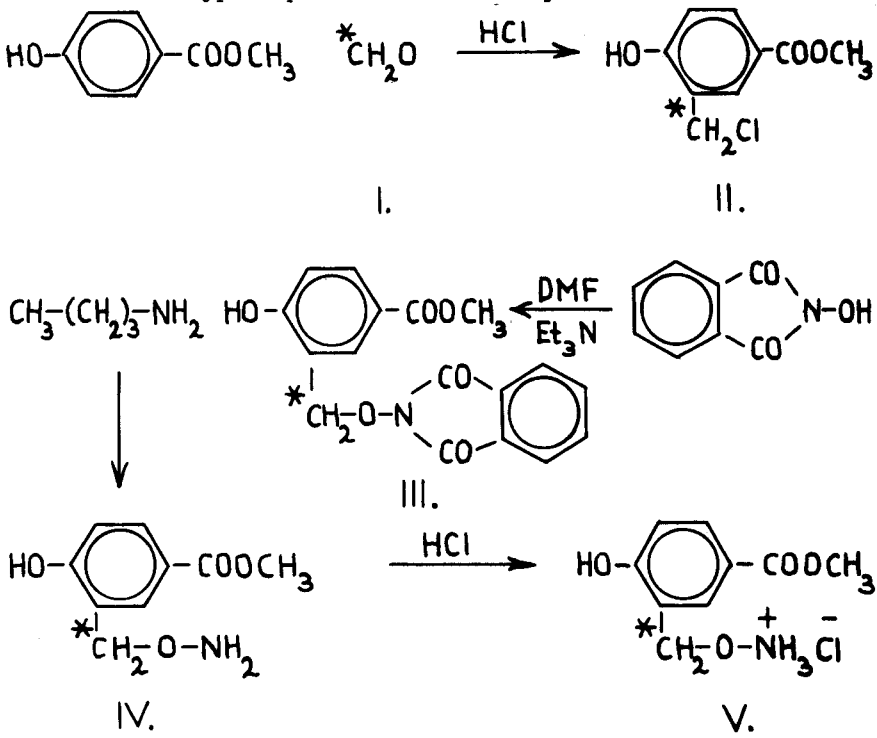
SUMMARY

Radioisotopic labelling of a novel histamine level reducing and antiinflammatory agent is described. The 2-hydroxy-5-carbomethoxybenzyl-^{14}C-oxyamine hydrochloride was prepared for the purpose of radioisotopic indication and further for pharmacological and action mechanism studies.

INTRODUCTION.

2-Hydroxy-5-carbomethoxybenzyloxyamine hydrochloride was synthesized by Kasztreiner at al.[1]. In course of the pharmacological investigations, this compound exhibited a powerful histidine decarboxylase inhibiting action both in vitro and in vivo experiments and further on it possessed histamine level reducing and antiinflammatory effects. In order to study the mode of action by means of radioisotopic labelling, the ^{14}C-labelled model compound was synthesized by the route demonstrated in the synthetic scheme.

As follows, paraformaldehyde-^{14}C (I) was first prepared from $Ba^{14}CO_3$ and then used to the chloromethylation of methyl 4-hydroxybenzoate. The methyl 3-(chloromethyl-^{14}C)-4-hydroxybenzoate (II) obtained was reacted with N-hydroxyphthalimide to give N-(2-hydroxy-5-carbomethoxy-benzyl-^{14}C-oxy)-phthalimide (III) which, on heating with n-butylamine yielded crude 2-hydroxy-5-carbomethoxy-benzyl-^{14}C-oxyamine (IV). The hydrochloride (V) of IV, i.e. the end-product was prepared by means of conc. hydrochloric acid (dens. 1.19) in acetic acid solution. An overall radiochemical yield of 23% as calculated for $Ba^{14}CO_3$ was reached. The chemical and radiochemical purity of the end-product was checked by thin layer chromatography. The chromatograms were evaluated radioautographically and radiometrically by means of a Tri-Carb type liquid scintillation spectrometer.



EXPERIMENTAL.

Paraformaldehyde-^{14}C (I).

Radioactive paraformaldehyde was prepared by the $LiAlH_4$ reduction of $^{14}CO_2$ in dry tetrahydrofuran [2,3,4]. A yield of 0.214 g (7.15 mmoles) of paraformaldehyde-^{14}C was obtained a total activity of 31.50 mCi (147.19 mCi/g, 4.40 mCi/mmole).

Radiochemical yield: 68% based on $Ba^{14}CO_3$.

Methyl 3-(chloromethyl-^{14}C)-4-hydroxybenzoate (II).

0.214 g of I was added in little portions to the mixture of 0.989 g (6.50 mmoles) of finely pulverized methyl 4-hydroxybenzoate and 4.5 ml of conc. hydrochloric acid (dens. 1.19) at -20° under stirring, during one hour. The mixture was then heated to 55° during the course of 40 minutes, stirred at the same temperature for 2.5 hours and cooled in ice-water for 3 hours. The precipitate was filtered by suction, washed with 5 x 3 ml of water and dried to constant weight in a vacuum desiccator over P_2O_5 and NaOH (7 to 10 days are necessary for complete drying; the water contamination of the product strongly reduces the yield of the following step).

Yield: 1.181 g (5.89 mmoles), 90.6% based on methyl 4-hydroxybenzoate, m.p. 140-142°C.

Radiochemical yield: 25.65 mCi (21.71 mCi/g, 4.35 mCi/mmoles), 81.4% based on I.

N-(2-hydroxy-5-carbomethoxy-benzyl-^{14}C-oxy)-phthalimide (III).

0.960 g (5.89 mmoles) of finely pulverized N-hydroxyphthalimide was added to the mixture of 1.181 g of II and 3 ml of dry dimethylformamide and the whole was stirred at 0°C for 5 minutes. Then the solution of 0.620 g (6.12 mmoles) of dry triethylamine in 0.62 ml of dry dimethylformamide was added at 5°C during 25 minutes, the mixture was stirred at 0°C for 3 hours, set aside at room temperature for 5 days, then cooled at 0°C for 3 hours and filtered by suction. The precipitate was first washed with one ml of cold methanol, then with 5 x 2 ml of water and dried to constant weight in a

vacuum desiccator over P_2O_5 and KOH.

Yield: 1.312 g (4.01 mmoles), m.p. 195-196°C.

Radiochemical yield: 16.63 mCi (12.67 mCi/g, 4.14 mCi/mmole), 64.8% based on II.

2-Hydroxy-5-carbomethoxy-benzyl-^{14}C-oxy-amine (IV).

A solution of 0.879 g (12.03 mmoles) of dry n-butylamine in 2.0 ml of dry methanol was added to the mixture of 1.312 g of III and 18.0 ml of dry methanol at 10°C under stirring, during 30 minutes. The mixture was stirred at room temperature for 2 hours, boiled for 3 hours and set aside at room temperature overnight. The solvent was removed under reduced pressure and the residue was thoroughly triturated with 12.0 ml of 1 N hydrochloric acid. The precipitate was filtered off, washed with 3 x 1 ml of 1 N hydrochloric acid, the filtrate was made alkaline with $KHCO_3$ and set aside in a refrigerator overnight. The precipitate was filtered by suction, washed with 3 x 3 ml of cold water and dried in a vacuum desiccator over P_2O_5 at room temperature. The crude product was recrystallized from 50 ml of dry ethanol.

Yield: 0.575 g (2.92 mmoles), m.p. 150-151°C.

Radiochemical yield: 12.09 mCi (21.00 mCi/g, 4.14 mCi/mmole), 72.6% based on III.

2-Hydroxy-5-carbomethoxy-benzyl-^{14}C-oxyamine hydrochloride (V).

0.3 ml of conc. hydrochloric acid (dens. 1.19) was added to a solution of 0.575 g of IV in 3.4 ml of acetic acid at 40°C. After cooling to room temperature, 3.4 ml of dry ethyl acetate was added and the mixture was set aside in a refrigerator overnight. The precipitate was filtered off, washed with 3 x 1 ml of cold, dry ethyl acetate and dried in a vacuum desiccator, over P_2O_5 at room temperature.

Yield: 0.595 g (2.55 mmoles), m.p. 170-171°C.

Radiochemical yield: 10.55 mCi (17.73 mCi/g, 4.14 mCi/mmoles), 87.3% based on IV, 22.7% based on $Ba^{14}CO_3$.

Chromatographic determinations.

Adsorbent: Kieselgel G. Solvent (IV): n-butanol : acetic acid : water = 8 : 2 : 10. Rf: 0.85. Solvent (V): benzene : acetone = 9 : 1 . Rf: 0.69. Autoradiogram: contact exposure on a Forte-type X-ray film for 24 hours.

REFERENCES

1. Kasztreiner, E., Vargha, L., Huszti, Zs., Borsy, J., Szilágyi, G., Szakály-Kosáry, J., Elek, S. and Polgári, I. - Hung. Pat. Appln. GO-1152 (1970) and Kaszreiner, E., Szilágyi, G., Szakály-Kosáry, J. and Sohár, P. - Hung. Pat. Appln. GO-1155 (1971).
2. Weygand, F. and Linden, H. - Angew. Chem., 66 : 174 (1954); Z. Naturforsch., 9b : 682 (1954).
3. Bubner, M. and Schmidt, L. - " Die Synthese Kohlenstoff-14-markierter Organischer Verbindungen". Veb Georg Thieme, Leipzig 1966, p. 98-100.
4. Vollmer, K. O. and Koss, F. W. - Arzneim.-Forsch.(Drug Res.), 20 : 990-992 (1970).
