SYNTHESIS OF ^{14}C-LABELLED COMPOUNDS OF BIOLOGICAL INTEREST: 4-METHYL-2-PYRIDYL-2-FURAMIDE HYDROCHLORIDE /MEPAF/.

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SUMMARY

A method of carbon-^{14}C introduction into the molecule of a new drug 4-methyl-2-pyridyl-2-furamide hydrochloride /MEPAF/ has been elaborated and furan-2-^{14}C-carboxylic acid has been synthesized. Radioactivity distribution and chemical yields in this synthesis were established.

Key Words: Furfural-2-^{14}C, Furylcarbinol-2-^{14}C, Furan-2-^{14}C-carboxylic acid, 4-Methyl-2-pyridyl-2-^{14}C-furamide hydrochloride, Dimethylformamide-1-^{14}C.

INTRODUCTION

4-Methyl-2-pyridyl-2-furamide hydrochloride /MEPAF/ is a new drug exhibiting a biological activity on the circulatory system /1/. Continuing our investigations /2,3/ on labelling new compounds in order to follow its metabolism in vivo - a method of preparing ^{14}C-labelled 4-methyl-2-pyridyl-2-^{14}C-furamide hydrochloride, <u>8</u>, has been worked out.

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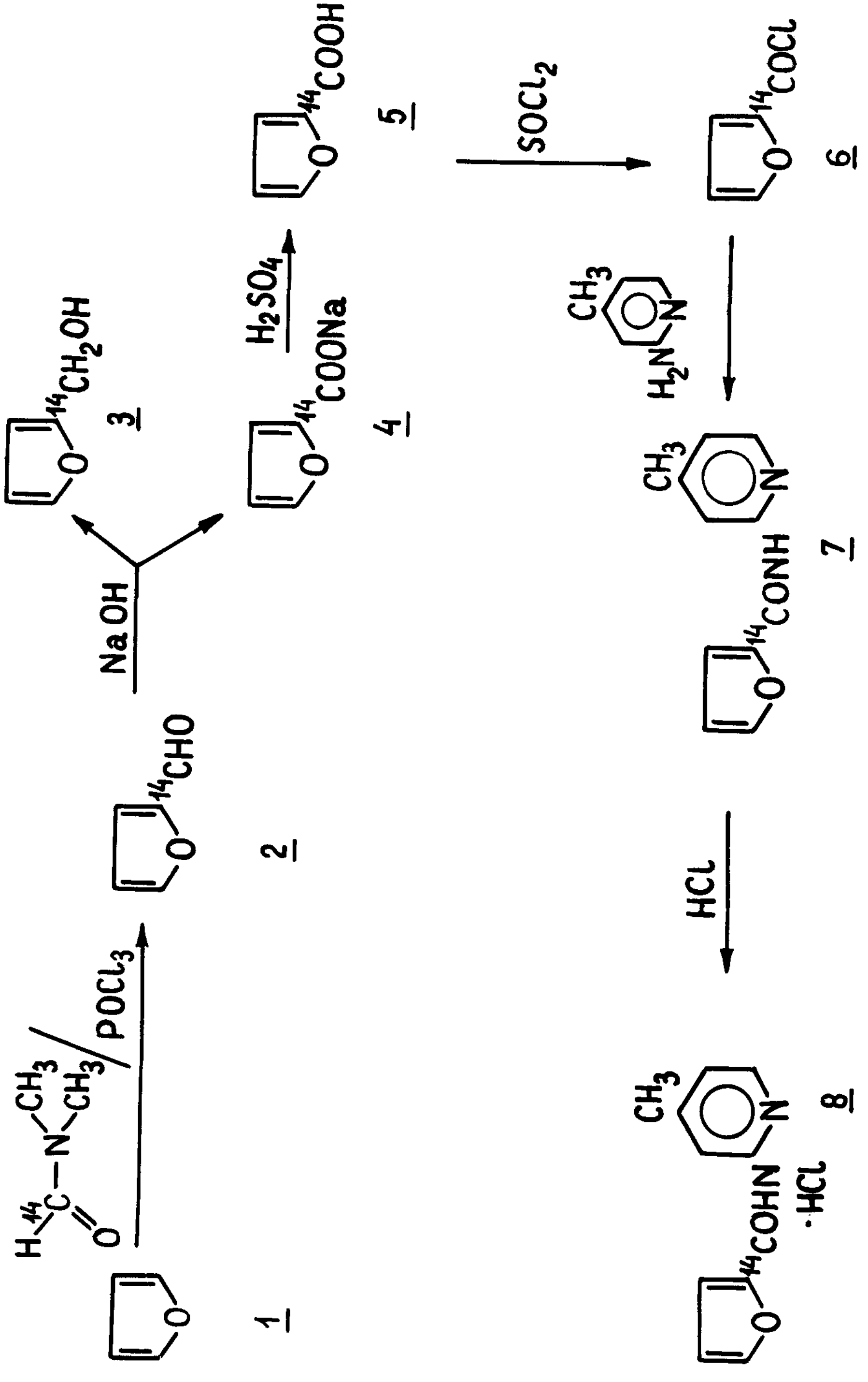
RESULTS

Radioactive isotope of carbon ^{14}C was introduced into the molecule of 4-methyl-2-pyridyl-2-furamide hydrochloride in the reaction of furan, 1, with dimethylformamide-1-^{14}C in the presence of phosphorus oxychloride according to the Scheme.

Furfural-2-^{14}C, obtained in this manner, was treated with sodium hydroxide solution to give furylcarbinol-2-^{14}C, 3, and sodium furan-2-^{14}C-carboxylate, 4, in Canizzaro reaction. Furylcarbinol-2-^{14}C, 3, was extracted from the mixture with ethyl ether; the solid residue was acidified with sulphuric acid, cooled, and furan-2-^{14}C-carboxylic acid, 5, was isolated. This acid, 5, was twice crystallized from water, dried and treated with thionyl chloride to give furan-2-^{14}C-carboxylic acid chloride, 6. It was then used with 2-amine-4-methylpyridine in the synthesis of 4-methyl-2-pyridyl-2-^{14}C-furamide, 7, and its hydrochloride, 8.

Each stage of this process was worked out and checked on unlabelled compounds and two following radioactive syntheses /I and II/ were carried out, using dimethylformamide-1-^{14}C of total activity: 0,35 mCi /I/ and 45 mCi /II/.

The chemical and radiochemical yield and purity were carefully controlled in both cases. It was observed that the chemical yields of the two syntheses were equal, but the radiochemical yields were different in two stages: in the reaction of furan with dimethylformamide-1-^{14}C and phosphorus oxychloride /I-60%, II-66%/; in the Canizzaro reaction of furfural-2-^{14}C with sodium hydroxide solution /I-80%, II-60%/.
All of the labelled compounds have physicochemical properties identical with those of inactive standards.


1
H–14C(=O)–N(CH3)CH3 / POCl3
14CHO
2
NaOH
14CH2OH
3
14COONa
4
H2SO4
14COOH
5
SOCl2
14COCl
6
H2N, CH3, N
14CONH
CH3
N
7
HCl
14COHN
·HCl
CH3
N
8

EXPERIMENTAL

The radioactivity were measured by the scintillation method on "Packard - 3380" apparatus. The chemical purity of these compounds and the reaction progress were checked by TLC on thin-layer plates coated with silica gel "Kieselgel GF 254" /Merck/. Developed zones were visualized under UV /254 nm/ light illumination. The radiochemical purity was determined by counting of the collected and extracted silica gel from the radioactive spots of the chromatogram. IR spectra were measured in KBr on Zeiss-Jena UR-10 spectrometer, NMR spectra were measured on JEOL-60 MHz spectrometer using TMS as internal standard. Melting points were uncorrected. All chemicals and solvents were of analytical grade. The biological tests were carried out at the Metabolism Department of the Institute of Pharmaceutical Industry, Warszawa, Poland.

Furfural-2-^{14}C 2

Freshly distilled phosphorus oxychloride /I-1,7 ml, 18,6 mmoles; II-2,5 ml, 27,3 mmoles/ was added for 20 minutes at the temperature of 0-5^{o} and under stirring to dimethylformamide-1-^{14}C /I-1,5 ml, 19,5 mmoles, spec.activ.-18 μCi/mmole; II-2,2 ml, 28,5 mmoles, spec.activ.-1,58 mCi/mmole/. The reaction mixture solidified forming a pink complex DMF/$POCl_3$; it was kept at 0^{o} for 40 minutes and then a freshly distilled furan, 1, /I-1,3 ml, 17,9 mmoles, II-1,8 ml, 24,7 mmoles/ was added under stirring. The mixture was heated at 100-120^{o} for 30 minutes, cooled, poured into ice-water and 20% solution of potassium carbonate was added to make its pH=6; after 15 min. pH was controlled and corrected. Then, the mixture was extracted with ethyl ether /150-180 ml/, the extract was dried over sodium sulphate, and ether was distilled off.

The chemical yields of furfural-2-^{14}C were: I-1 ml, 12 mmoles /67%/, II-1,4 ml, 16,9 mmoles /68%/.

The radiochemical yields were: I - spec. activ, - 17,5 µCi/mmole, total activ. - 210 µCi/60%/; II - spec. activ: - 1,77 mCi/mmole, total activ. - 30,0 mCi /66%/.

The radiochemical purity: 94,5 - 95,5%.

Furylcarbinol-2-^{14}C 3 and Furan-2-^{14}C-carboxylic acid 5

A solution 33% of sodium hydroxide was added slowly /for 30 min./ to furfural-2-^{14}C, 2, placed in an ice-bath /I-1,4 ml NaOH aq., II-1,9 ml NaOH aq./. The mixture was kept at room temperature for 1,5 hour. Furylcarbinol-2-^{14}C, 3, was extracted with ethyl ether /I-100 ml, II-150 ml/ and the ether was evaporated.

The chemical yields of carbinols were: I-0,34 ml, 3,9 mmoles, /65%/; II-0,5 ml, 4,7 mmoles /67%/.

The radiochemical yields were: I - spec activ. - 16 µCi/mmole, /30%/; total activ. - 62 µCi /30%/; II - spec activ. - 1,87 mCi/mmole, /32%/, total activ. - 10,65 mCi.

The radiochemical purity: 95-96%.

The solid residue of sodium furan-2-^{14}C-carboxylate, 4, was acidified with 40% sulphuric acid to Congo red paper, After cooling, the crude furan-2-^{14}C-carboxylic acid, 5, was filtered off and twice crystallized from distilled water with an addition of decolorizing active carbon, to give 380 mg, 3,39 mmoles /56,5%/ of I and 500 mg, 4,46 mmoles /53%/ of II pure products, 5.

The radiochemical yields of the labelled acids were: I - spec.activ. - 30,2 µCi/mmole, total activ. - 102,6 µCi /48,8%/; II - spec. activ. - 1,85 mCi/mmole, total activ. 8,25 mCi /27,5%/.

The residual solutions from the crystallizations of I and II were evaporated under reduced pressure and second products were isolated, which after two crystallization from water gave 28 mg /0,25 mmole/ of I' spec.activ. - 27 µCi/mmole, /3%/, and 52 mg /0,46 mmole/ of II' furan-2-^{14}C-carboxylic acid, spec.activ. - 1,8 mCi/mmole /3%/.

Melting points of all these acids were 128-130^{o}, and their Rf values in the system of n-butanol+acetic acid+water /2+1+1/ was 0,35. The radiochemical purity 98-98,5%.

Furan-2-^{14}C-carboxylic acid chloride 6

A solution of furan-2-^{14}C-carboxylic acid, 5, /I-380 mg, 3,39 mmole; II-500 mg, 4,46 mmole/ and freshly distilled thionyl chloride /I-3 ml, II-4 ml/ were refluxed for 2,5 hours, and the solution was evaporated. Then, three 20 ml portions of dry benzene were added to remove thionyl chloride and the crude product was used in the next reaction.

4-Methyl-2-pyridyl-2-^{14}C-furamide hydrochloride 8

A solution of furan-2-^{14}C-carboxylic acid chloride, 6, and 4-methyl-2-pyridylamine /I-800 mg, 7,4 mmoles, II - 1100 mg, 10 mmoles/ in dry dioxane was refluxed for 3 hours and the solution was filtered in the hot. The solid residue was washed with dioxane and discarded /it was identified as 4-methyl-2-pyridylamine hydrochloride/. 4-Methyl-2-pyridyl-2-^{14}C-furamide hydrochloride, 8, was precipitated from the filtrate by an addition of HCl/ethyl ether mixture and it was then crystallized from ethanol to give 449 mg, 1,9 mmole of I /56% chem. yield/ and 675 mg, 2,83 mmoles, of II /63% chem. yield/.

The radiochemical yields were: I - spec.activ. - 20,7 µCi/mmole, total activ. - 49,4 µCi /48,5%/; II - spec.activ. - 1,62 mCi/mmole, total activ. - 4,6 mCi /53,5%/.

Combined furan-2-^{14}C-carboxylic acids /I'+II'/ were worked up as above to give 60 mg /0,25 mmole/ of 4-methyl-2-pyridyl-2-^{14}C-furamide hydrochloride, 8, of spec.activ. - 1,1 mCi/mmole, total act. - 0,27 mCi.

Melting points of these products were 224-226^{o}, and their Rf values in the system of n-butanol + acetic acid + water /2+1+1/ were 0,67 at 20^{o} and 0,7 at 25^{o}.

The radiochemical purity: 99,8%.

NMR /D_2O/, δ : 2,52/s, 3,CH_3/, 4,78 /m,2,NH.HCl/,
6,65/m,1,CH/, 7,45 /m,1,CH/,
7,5/m,2,2xCH/, 7,8 /m,1,CH/, 8,25/m,1,CH/

IR /KBr/ $v^{C=O}$ - 1670 cm^{-1}
v^{NH} - 3135 cm^{-1}

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