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Synthesis of ^{14}C-labelled heterocyclic analogues of phenylalanine with pyrazole and furan rings

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SUMMARY

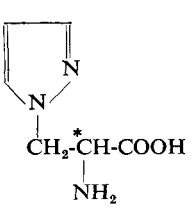
Diethyl acetamidomalonate-2-^{14}C was alkylated by 1-chloromethylpyrazole and the reaction intermediate hydrolyzed to give DL-3-(1'-pyrazolyl)-alanine-2-^{14}C. By the same method, but using 3-chloromethylpyrazole instead of the 1-isomer, DL-3-(3'-pyrazolyl)-alanine-2-^{14}C was prepared. Condensation of furfural with hydantoin-5-^{14}C gave 5-(2'-furylidene)-hydantoin-5-^{14}C, which was subsequently reduced to 5-(2'-furyl)-hydantoin-5-^{14}C and this compound was hydrolyzed to DL-3-(2'-furyl)-alanine-2-^{14}C. Methods for isolation and purification of the final products on microscale are described.

INTRODUCTION

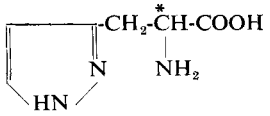
Analogues of phenylalanine are often used in studies of proteosynthesis problems. Some of them were also prepared in radioactive form by labelling with isotopic carbon, as for example DL-3-(4'-fluorophenyl)-alanine-3-^{14}C [1], threo-3-phenyl-DL-serine-1-^{14}C [2] and threo-3-phenyl-DL-serine-3-^{14}C [2, 3]. This work was directed to synthesis of further potential ^{14}C-labelled analogues of phenyl-alanine, bearing an unchanged α-alanine side chain, but with the benzene nucleus replaced by another aromatic system. From a number of such compounds, DL-3-(1'-pyrazolyl)-alanine (I), DL-3-(3'-pyrazolyl)-alanine (II) and DL-3-(2'-furyl)-alanine (III) were chosen; only the first of them was also found in living organisms [4].

As starting radioactive materials diethyl acetamidomalonate-2-^{14}C (for the synthesis of I and II) and glycine-2-^{14}C (for the preparation of III) were used. Thus, the radioactive carbon in all three products was located in position 2 of the aliphatic side chain. Labelling in the carboxyl group was

inconvenient owing to considerable danger of decarboxylation during biochemical experiments and great difficulties would be accompanied with labelling in other positions.



I



II

III

The synthesis of DL-3-(1′-pyrazolyl)-alanine-2-^{14}C (I) was based on a method used for preparation of the non-radioactive compound [5], but somewhat modified. The sodium derivative of diethyl acetamidomalonate-2-^{14}C was alkylated with 1-chloromethylpyrazole, liberated from its hydrochloride by the action of sodium ethoxide directly in the reaction mixture. The alkylated intermediate was hydrolyzed to the desired amino acid I, which was isolated in chromatographically pure form by column chromatography on cellulose. Unlike the original work [5], which used three molar equivalents of diethyl acetamidomalonate per one equiv. of 1-chloromethylpyrazole, the same yield of I was obtained, when only equimolar amounts of both components were allowed to react.

When the above mentioned alkylation was carried out with 3-chloromethylpyrazole instead of the 1-isomer, DL-3-(3′-pyrazolyl-)alanine-2-^{14}C (II) was formed. Also in this case it was shown, in contradiction to the literature [6], that no excess of acetamidomalonate was necessary. After hydrolysis, the pure product II was easily isolated as crystalline monohydrochloride.

DL-3-(2′-furyl)-alanine-2-^{14}C (III) was prepared by modification of a hydantoin synthesis, described originally by Deulofeu [7]. Glycine-2-^{14}C was converted into hydantoin-5-^{14}C (IV), *via* ethyl ureidoacetate-2-^{14}C (V) by the method of Bond [8]. Condensation of compound IV with furfural then gave 5-(2′-furylidene)-hydantoin-5-^{14}C (VI). It was found that this reaction proceeds better in the presence of N-ethylpiperidine instead of the recommended sodium acetate [7, 9]. Reduction of compound VI by means of sodium amalgam led to 5-(2′-furyl)-hydantoin-5-^{14}C (VII), which was hydrolyzed by barium hydroxide, without isolation. The DL-3-(2′-furyl)-alanine-2-^{14}C (III) so formed was finally purified on cellulose column, a chromatographically pure product being obtained.

EXPERIMENTAL

Diethyl acetamidomalonate-2-^{14}C and glycine-2-^{14}C were products of the Radiochemical Centre, Amersham, England. Radioactivity measurements were carried out on Mark I liquid scintillation counter (Nuclear Chicago). Paper chromatography was carried out by descending technique on Whatman No. 1 paper in *n*-butanol — acetic acid — water (4 : 1 : 5). Radioactive chromatograms were measured on Frieseke and Hoepfner apparatus. Cellulose used for column operations was the Chromatographic Grade Cellulose Powder of J. H. Munktell, Sweden.

DL-3-*(1′-Pyrazolyl)-alanine*-2-^{14}*C (I)*. Clean sodium (78 mg; 3.3 mg-atom) was dissolved in absolute ethanol (8 ml) with exclusion of air moisture. To the solution was added diethyl acetamidomalonate-2-^{14}C (348 mg; 1.6 mmole), specific radioactivity 1.88 mCi/mmole (8.6 μCi/mg). After stirring for 30 min the solution was cooled to 0° C and treated with 1-chloromethylpyrazole hydrochloride (245 mg; 1.6 mmole) [6] in absolute ethanol (4 ml). The reaction mixture was then stirred for 30 min at 0° C and then allowed to stand for 24 hours at ordinary temperature; during this time the alkaline reaction disappeared. Ethanol was evaporated *in vacuo*, the remainder dissolved in glacial acetic acid (4 ml), concentrated hydrochloric acid added (6 ml) and the whole was gently refluxed for 6 hours. The solution was again evaporated to dryness *in vacuo* and the evaporation repeated twice more after dissolving in a small volume of water. The residue was taken into water (2 ml) and the solution applied to a Dowex 50 × 8 (H^+-form, 100-200 mesh) column (22 × 1 cm). Chloride ions were eluted with water and amino acids displaced from the column by 5 % ammonia. The ninhydrin-positive fractions were pooled and evaporated to dryness, together with powdered cellulose (1 g). The resulting dry powder was placed on the top of a cellulose column (30 g; 22 × 2 cm), prepared in a solvent mixture *n*-butanol-ethanol-water (4 : 1 : 1). The column was eluted with the same solvent mixture at a rate of 1.5 ml/10 min., fractions containing 1.5 ml each being collected. Fractions 70-100 contained chromatographically pure I; they were pooled and evaporated to dryness *in vacuo*. The yield of white crystalline DL-3-(1′-pyrazolyl)-alanine-2-^{14}C (I) was 74 mg (30 %); spec. activity 1.8 mCi/mmole (12.2. μCi/mg).

A non-radioactive preparation had R_f = 0.31 and melted at 238-248° C (dec.); literature values are : R_f = 0.48 (ascending arrangement) [10], m.p. = 245-7° C (dec.) [5].

DL-3-(3′-*Pyrazolyl)-alanine*-2-^{14}C (II). Sodium (94 mg; 4.1 mg-atom) was dissolved in absolute ethanol (5 ml) and the solution treated with diethyl acetamidomalonate-2-^{14}C (434 mg; 2 mmoles) with specific radioactivity 50 μCi/mmole (2.3 μCi/mg). The mixture was stirred for 30 min., cooled to 0° C and slowly treated with a solution of 3-chloromethylpyrazole

hydrochloride* (306 mg; 2 mmoles) [6] in absolute ethanol (3 ml). The whole was allowed to stand for 36 hours at room temperature, the neutral mixture was evaporated *in vacuo*, the remainder dissolved in 2N hydrochloric acid (10 ml) and continously extracted with ether for 5 hours to remove the unreacted acetamidomalonate. The aqueous layer was then carefully made alkaline with solid sodium carbonate and again extracted with ether for 3 hours. The ethereal extract was dried with magnesium sulphate and evaporated *in vacuo*, giving a colourless oily residue. This was refluxed for 5 hours with glacial acetic acid (3 ml) and concentrated hydrochloric acid (4 ml). The acid solution was then decolorized with charcoal, evaporated to dryness *in vacuo*, absolute ethanol (1 ml) added and the evaporation repeated. This operation yielded 360 mg (95 %) of the white, crystalline and somewhat hygroscopic monohydrochloride of DL-3-(3′-pyrazolyl)-alanine-2-^{14}C (II. HCl) possessing a specific radioactivity 49 μCi/mmole (2.6 μCi/mg).

A non-radioactive preparation of this compound, obtained by the same way, had $R_f = 0.28$ and melted at 229-230° C (dec.; from aqueous acetone). Analysis : found C, 37.39; H, 5.13. Calc. for $C_6H_9N_3O_2$, C, 37.61; H, 5.26 %.

Ethyl ureidoacetate-2-^{14}C (V). — Glycine-2-^{14}C(300 mg, 4 mmoles) of a specific radioactivity 0.5 mCi/mmole (6.67 μCi/mg) was suspended in absolute ethanol (3 ml). Dry hydrogen chloride was then passed over the surface of the stirred mixture until solution was complete; at the end of this period, the mixture was heated at 80° C and hydrogen chloride was passed for one hour more. The solution was evaporated to dryness *in vacuo*, yielding 554 mg (99 %) of ethyl aminoacetate-2-^{14}C hydrochloride. This was again dissolved in water (1.75 ml), finely powdered potassium cyanate (475 mg) was added and the whole was stirred for 15 min. at ambient temperature. The mixture was then cooled in ice for 3 hours (seeding advisable), the precipitate of coumpound V filtered with suction and dried in a dessicator over phosphorus pentoxide. Yield 533 mg (90 %, calculated on glycine-2-^{14}C).

Hydantoin-5-^{14}C *(IV)*. — Compound V (520 mg) was stirred with 25 % hydrochloric acid (2.4 ml) till complete solution took place. The solution was evaporated to dryness at 100° C and the remaining solid dried in a dessicator over phosphorus pentoxide and potassium hydroxide. The yield was 394 mg of crude product IV, containing inorganic salts.

5-(2′-*Furylidene*)-*hydantoin*-5-^{14}C *(VI)*. — The impure compound IV was suspended in glacial acetic acid (1.38 ml) and after 10 min. under constant stirring, redistilled furfural (0.38 ml) was added. Careful addition of N-ethylpiperidine (6 × 0.2 ml) caused evolution of heat and the mixture turned dark. The mixture was heated for 2 hours at 140-150° C, cooled and poured into ice-cold water (50 ml). The aqueous suspension was allowed

* CAUTION! 3-Chloromethylpyrazole hydrochloride causes irritation of the skin and mucous membranes, even in mere contact with vapours arising from the crude compound. To be handled in a good hood or better in a «Dry Box».

to stand at 0° C overnight, the product VI was filtered by suction, washed with water and dried in a dessicator. Yield 400 mg (56 %, calculated on glycine-2-^{14}C).

DL-3-(2′-*Furyl*)-*alanine*-2-^{14}C *(III)*. — Compound VI (400 mg) was dissolved in hot ethanol (36 ml) and cooled. Some black tars remained undissolved and were discarded. Reduction was carried out by 3 % sodium amalgam (18 g), which was added in the course of four hours in 6 successive portions under vigorous shaking. The p_H of the reaction mixture was held at 8-9 by 6 % sulphuric acid in 50 % ethanol; at the end of the reaction the mixture was exactly neutralized. The solution containing 5-(2′-furyl)-hydantoin-5-^{14}C (VII) was decanted from mercury, filtered from precipitated sodium sulphate and evaporated *in vacuo*. Water (30 ml) and barium hydroxide octahydrate (2 g) were added to the residue and the whole was refluxed for 20 hours under exclusion of carbon dioxide. Then, barium carbonate was precipitated by gaseous carbon dioxide introduced *over* the surface of the mixture (to prevent frothing), 10 % sulphuric acid (3 drops) was added, and the mixture was allowed to stand for 24 hours. The precipitate was filtered by suction, washed with water and the filtrate evaporated *in vacuo* at a temperature not exceeding 30° C. The solid which remained was again dissolved in water (3 ml), cellulose powder (1 g) added and the suspension again evaporated to dryness. A cellulose column (30 g; 22 × 2 cm) was prepared in 85% ethanol; the dry evaporated mixture of amino acids and cellulose was placed on the top of the column and elution started, using 85 % ethanol. Fractions containing about 3.5 ml each were collected in 10 min. intervals and their composition was tested chromatographically. Fractions 30-85 (192 ml together) containing chromatographically pure III were pooled and evaporated *in vacuo*, yielding 290 mg (47 %, calculated on glycine-2-^{14}C) of white powdered III; specific radioactivity 0.48 mCi/mmole (3.1 μCi/mg).

A non-labelled preparation of III made by the same way, had $R_f = 0.43$ and melted at 243-8° C (dec.). The literature reported m.p. 250-260° C [7]; 260° C [11].

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