

¹⁴C-labelled 4-hydroxycoumarin derivatives.

II*. Synthesis of 3-*n*-hexyl-4-hydroxycoumarin-10-¹⁴C

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SUMMARY

*The synthesis and characterization of 3-*n*-hexyl-4-hydroxycoumarin-10-¹⁴C is described. This new labelled substance was prepared by starting from phenol-1-¹⁴C which was transformed in a single step into the final product by a ZnCl₂ and POCl₃ catalysed condensation with *n*-hexyl-malonic acid.*

INTRODUCTION

The present paper describes the synthesis and characterisation of 3-*n*-hexyl-4-hydroxycoumarin-10-¹⁴C prepared for use in raticide metabolism studies. Owing to the interest of studying the metabolic behaviour of 4-hydroxycoumarin-3-substituted derivatives possessing blood anticoagulant activity and raticide action, the synthesis of the above mentioned substance labelled in the coumarine ring was performed.

There are several synthetic routes to unlabelled 3-*n*-hexyl-4-hydroxycoumarin such as the reaction of diphenylmalonate with malonic acid followed by the introduction of the *n*-hexyl substituent into the needed position of the coumarine ring, the AlCl₃-promoted intramolecular condensation⁽¹⁾ of *n*-hexyl-diphenyl malonate and the condensation⁽²⁾ of *n*-hexylmalonic acid with phenol in the presence of zinc chloride and phosphorus oxychloride.

For the preparation of 3-*n*-hexyl-4-hydroxycoumarin-10-¹⁴C the last mentioned synthetic route was found to be the most suitable as it needs only one radioactive step, i.e. the condensation of *n*-hexylmalonic acid with phenol-1-¹⁴C leading to 3-*n*-hexyl-4-hydroxycoumarin-10-¹⁴C. The radiochemical yield

* First part in this journal.

expected for this method (based on the starting phenol-1- ^{14}C) is about two times greater as for the intramolecular condensation of diphenyl *n*-hexylmalonate- ^{14}C labelled in the aromatic rings, where one half of the phenol- ^{14}C is lost on hydrolysis. Finally, the choice of the above mentioned synthetic route was conditioned by the higher radiochemical purity of the final reaction product as for the intramolecular condensation of diphenyl *n*-hexylmalonate, promoted by AlCl_3 .

EXPERIMENTAL

Melting points and boiling points were not corrected. The radioactivity measurements of the compounds labelled with ^{14}C were made by means of a single channel scintillation counter NE 5505 Nuclear Enterprises, England). The scintillator consisted of ethanol (150 ml), dioxane (300 ml), naphthalene (50 g) and 2,5-diphenyloxazole (P. P. O.) (5 g), made up to 1 l with toluene. The counting efficiency was determined for each sample by means of the Internal Standard method. The radiochemical purity was determined by the autoradiography of TLC plates for which an ascendent method in two systems was found *. The infrared spectra were recorded on a UR-10 (VEB Carl Zeiss, Jena) spectrometer in potassium bromide pellet.

n-Hexyl bromide.

A mixture of 120 g (1.17 mole) *n*-hexylalcohol, 180 g (1.17 mole) sodium bromide, 110 ml of 94 % sulphuric acid and 135 ml water was heated under reflux for two hours. The crude *n*-hexyl bromide was obtained by distillation of the reaction mixture followed by the separation of the organic layer which was washed with cold water, concentrated sulphuric acid and a 10 % aqueous solution of sodium carbonate and dried a few hours on calcium chloride. The pure product was then obtained by distillation at 156 °C/750 mm, yield 148 g (90 %), n_{D}^{20} 1.4478 (lit. ⁽³⁾ b.p. 155.5/748 mm and n_{D}^{20} 1.44778).

n-Hexyl malonic acid.

Into an ethanolic solution of sodium ethoxide prepared from 14 g sodium and 240 ml ethanol, 125 ml diethyl malonate and 100 ml *n*-hexyl bromide were added; the reaction mixture was heated on the steam bath about four hours when the reaction mixture became neutral. The major part of the ethanol was then removed by distillation and the remaining residue was treated 200 ml water. The oily organic layer was separated, washed with water, dried

* L. Gavai, M. Dinescu and A. B. Susan (paper in preparation).

on calcium chloride and purified by distillation, yielding pure diethyl *n*-hexylmalonate, b.p. 150-155 °C/20 mm, n_D^{20} 1.4290.

The diethyl *n*-hexylmalonate was hydrolysed to the corresponding acid by refluxing a mixture of 25 ml ester, 12.5 g potassium hydroxide, 42.5 ml ethanol, the residue was dissolved in water, the unsaponified ester was removed by extraction with ether, the aqueous solution was evaporated to dryness and acidified by adding a 10 % solution of hydrochloric acid. The resulting crystals were filtered off yielding pure *n*-hexyl malonic acid, m.p. 103-105 °C (after recrystallisation from benzene).

Synthesis of 3-n-hexyl-4-hydroxycoumarin-10-¹⁴C.

A mixture of 0.3847 g (4.09 mmole) phenol-1-¹⁴C (specific activity 2.2 mCi/mmole), 90 mg (0.95 mmole) unlabelled phenol, 0.94 g (5 mmole) *n*-hexyl malonic acid, 4.895 g (35.1 mmole) zinc chloride and 3.285 ml (35.1 mmole) phosphorus oxychloride was heated with stirring for 38 hours at 60-65 °C. After cooling to room temperature, the reaction mixture was treated with crushed ice and dissolved in a 10 % sodium carbonate solution. The crude crystalline ¹⁴C-coumarin derivative was obtained by precipitation with hydrochloric acid followed by filtration. Recrystallization from boiling acetone gave pure *n*-hexyl-4-hydroxycoumarin-10-¹⁴C of a specific activity of 2.15 mCi/mmole (yield based on phenol-1-¹⁴C was about 48 %). The infrared spectrum and the melting point (150-151 °C) were identical with those of a standard sample. The thin layer ascendent chromatography in the systems : A) petroleum ether/ethyl ether/chloroform/acetic acid (1/0.5/0.7/0.1), stationary phase : silica gel, and B) chloroform/methanol/toluene (10/2/3), stationary phase buffered with 0.1 N boric acid, showed a very high degree of purity.

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