^{14}C-labelled 4-hydroxycoumarin derivatives. I. Synthesis of 3-substituted derivatives.

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SUMMARY

*The synthesis and characterization of 3-R-substituted derivatives of 4-hydroxycoumarin-3-^{14}C is described, where R is (α-acetonylbenzyl), (α-acetonyl-*p*-nitrobenzyl), or (α-acetonyl-*p*-chlorobenzyl). The compounds were prepared by the quinoline-catalysed condensation of 4-hydroxycoumarin-3-^{14}C with the following unsaturated ketones : benzylideneacetone, p-chlorobenzylideneacetone and p-nitrobenzylideneacetone.*

INTRODUCTION

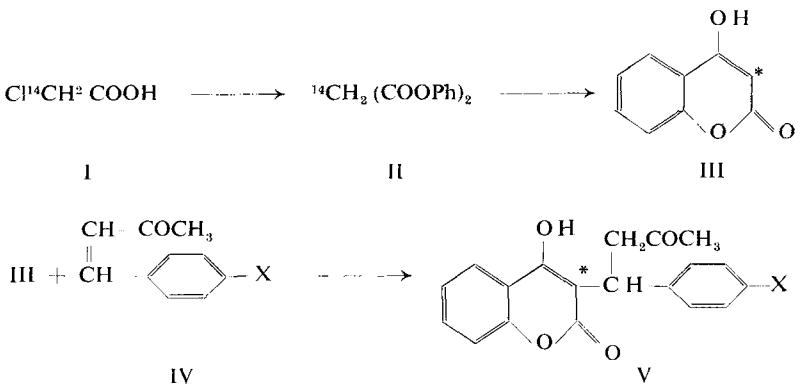
This paper describes the synthesis and characterization of three new labelled compounds prepared for use in raticide metabolism studies of certain 3-substituted 4-hydroxycoumarin derivatives possessing well-known blood anticoagulant activity. These compounds are : 3-(α-acetonylbenzyl)-4-hydroxycoumarin-3-^{14}C, (Va); (α-acetonyl-*p*-nitrobenzyl)-4-hydroxycoumarin-3-^{14}C, (Vb), and (α-acetonyl-*p*-chlorobenzyl)-4-hydroxycoumarin-3-^{14}C, (Vc) *.

Several synthetic routes to unlabelled 4-hydroxycoumarin and 3-R-substituted derivatives of 4-hydroxycoumarin have been described [1,16]. The most convenient method for the above mentioned ^{14}C-ring labelled derivatives appeared to be the Michael condensation [17] of 4-hydroxycoumarin (^{14}C-ring labelled) with the corresponding unsaturated ketones such as benzylideneacetone, *p*-chlorobenzylideneacetone and *p*-nitrobenzylideneacetone, in the presence of secondary amines (e.g, quinoline).

* Warfarin, Acenocoumarol and Coumachlor respectively are the registered trade names for the unlabelled compounds.

For the synthesis of the ring labelled intermediate, 4-hydroxycoumarin-3-^{14}C, the aluminium chloride promoted cyclization of diphenylmalonate was found to be the most suitable route. The starting malonic acid-2-^{14}C was obtained from 2-chloroacetic acid-2-^{14}C by an improved method [18]. The unsaturated ketones were obtained in the usual way, i.e. by condensation of acetone with benzaldehyde, *p*-nitrobenzaldehyde and *p*-chlorobenzaldehyde.

The reaction sequence used in the synthesis of the above mentioned 3-substituted 4-hydroxycoumarin -3-^{14}C derivatives is indicated below :



EXPERIMENTAL

The radioactivity was determined with a single channel scintillation counter NE 5503 (Nuclear Enterprises, England). The scintillator consisted of ethanol (150 ml), dioxane (300 ml), naphtalene (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 chemical purity was determined by an ascendent thin layer chromatographic method* in two 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 : silica gel buffered with 0.1 N boric acid. The radiochemical purity was determined by autoradiography of the TLC plates.

SYNTHESIS OF 4-HYDROXYCOUMARIN-3-^{14}C (III).

A homogeneous mixture 6.0 g (23.4 mmole, specific activity of 0.7 mC/mmole) diphenylmalonate-2-^{14}C and 6.2 g (46.5 mmole) of anhydrous alu-

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minium chloride * was gently heated until the temperature reached 150 °C and the mixture became fluid and then stirred for 20 minutes at 175-180 °C **. The reaction mixture was allowed to cool to 100 °C when 45 ml of a 7 % aqueous solution of hydrochloric acid was added stepwise *** in order to keep the temperature around 100 °C. The reaction mixture was then poured over 20 g of finely crushed ice leaving an oily layer which was allowed to stand for 2-3 hours, The solid product was filtered off, washed with water to pH 6.5-7, leaving crude crystalline 4-hydroxycoumarin-3-^{14}C. The solid was dissolved at 60 °C in 15 ml of 10% aqueous sodium hydroxide, chilled by adding 20 g finely crushed ice and acidified to pH 5.5 with a 10 % solution of sulphuric acid. The solution was stirred for 15 minutes with 1 g charcoal which was then removed and washed on the filter paper with three portions of 10 ml water at 60 °. The filtrate and washings were combined and acidified to pH 1-2 by adding about 3.5 ml of 50 % aqueous sulphuric acid. The resultant solid was filtered off, washed to pH 6-7 and dried on the boiling steam bath. Weight of pure 4-hydroxycoumarin-3-^{14}C and yield based on diphenylmalonate-2-^{14}C were 2.3 g and 60 % respectively. Melting points and the infrared spectrum of the product were identical with those of a standard sample of 4-hydroxycoumarin.

Synthesis of 3-(α-acetonylbenzyl)-4-hydroxycoumarin-3-^{14}C (Vc)

A mixture of 71 mg (0.29 mmole) 4-hydroxycoumarin-3-^{14}C (of an activity of 0.21 mC), diluted with 0.575 g (2.2 mmole) unlabelled 4-hydroxycoumarin, 0.57 g (3.9 mmole) benzylideneacetone, 80 mg (0.61 mmole) quinoline and 7 ml water was stirred for three hours at 85-90 °C. Then a solution of 0.725 g sodium carbonate in 2 ml water was added and stirred for 10 minutes at the same temperature. The mixture was then stirred for about 5 minutes with 0.55 g charcoal and filtered off. The charcoal residue was washed 5 times with 3 ml portions water, the filtrate and washings were diluted with about 50 ml water and then slowly acidified to pH 3 by adding about 5ml of 20% sulphuric acid. The crude product was filtered off, washed with about 75 ml water and recrystallised from 17 ml boiling acetone to which 0.4 g charcoal was added. To the hot acetone filtrate, 8 ml water were added and the solution was allowed to crystallise. The precipitated white crystals were filtered off and dried *in vacuo*, yielding 0.448 g pure 3-(α-acetonylbenzyl)-

* It is desirable to use a granulated aluminium chloride instead of a finely powdered product, otherwise the product solidifies and the stirring becomes inefficient.

** It is necessary to maintain the temperature and the reaction time within the recommended values in order to avoid the formation of polymers.

*** When adding the hydrochloric acid it is necessary to avoid the dropping of the temperature below 100 °C in which case the decomposition of the reaction complex is stopped and can be further performed only with difficulties, by gently heating of the reaction mixture.

4-hydroxycoumarin-3-^{14}C (total activity 82.4 C), yield based on 4-hydroxycoumarin-3-^{14}C was 39.2 %.

The infrared spectrum and the melting point of the product were identical with those of a standard sample. The TLC analysis in the above mentioned systems and the autoradiography showed a single strong active spot R_F value for the two systems 0.46 and 0.50 respectively), a weak spot belonging to the starting 4-hydroxycoumarin-3-^{14}C, besides other three nonradioactive weak spots (unidentified), one having lower R_F value as the above mentioned substance and two spots with greater R_F values.

SYNTHESIS OF 3-(α-ACETONYL-*p*-NITROBENZYL)-4-HYDROXYCOUMARIN-3-^{14}C (Vb).

A mixture of 2.3 g (9.39 mmole) 4-hydroxycoumarin-3-^{14}C (total activity 6.80 mC), 3 g (16,7 mmole) *p*-nitrobenzylideneacetone, 0.3 ml quinoline and 28 ml water was stirred for 3 hours at 85-90 °C and worked up in the same manner as shown above. The recrystallised pure product, 3.6 g (yield 72 % based on 4-hydroxycoumarin-3-^{14}C) showed identical melting point and infrared spectrum with a standard sample. The TLC analysis in the above mentioned systems and the autoradiography of the TLC plates showed a single strong active spots (R_F values in the two systems were 0.59 and 0.37 respectively) and a weak spot belonging to 4-hydroxycoumarin-3-^{14}C which disappeared after a second recrystallisation from boiling acetone,

SYNTHESIS OF 3-(α-ACETONYL-*p*-CHLOROBENZYL)-4-HYDROXYCOUMARIN-3-^{14}C(Vc)

The substance was prepared under identical conditions with the above mentioned compounds by the condensation of 4-hydroxycoumarin-3-^{14}C with *p*-chlorobenzylideneacetone, yield 42 % based on 4-hydroxycoumarin-3-^{14}C. The substance was characterized by melting point and infrared spectrum which were identical with those of a standard sample. The TLC analysis in the systems mentioned above and the autoradiography showed slight traces of radioactive 4-hydroxycoumarin-3-^{14}C and four nonradioactive impurities (unidentified), with R_F values lower than 4-hydroxycoumarin (two products) and two other ones with higher R_F values.

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