

¹⁴C-labelled 4-hydroxycoumarin derivatives.

I. Synthesis of 3-substituted derivatives.

A. B. SUSAN⁺, M. DINESCU and L. GAVAT⁺⁺

⁺ Institute for Atomic Physics, P. O. Box 35, Bucharest, Rumania

⁺⁺ Institute for Chemical Research, Spl. Independentei 202, Bucharest, Rumania

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SUMMARY

The synthesis and characterization of 3-R-substituted derivatives of 4-hydroxycoumarin-3-¹⁴C 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-3-¹⁴C with the following unsaturated ketones : benzylideneacetone, p-chlorobenzylideneacetone and p-nitrobenzylideneacetone.

INTRODUCTION

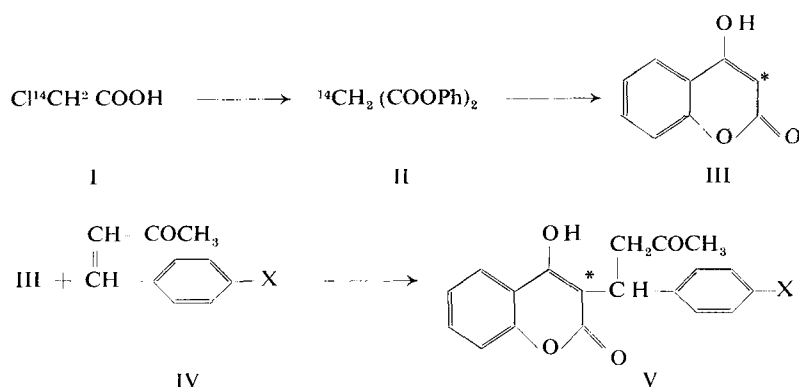
This paper describes the synthesis and characterization of three new labelled compounds prepared for use in raticide metabolism studies of certain 3-substituted 4-hydroxycoumarin derivatives possessing well-known blood anti-coagulant activity. These compounds are : 3-(α-acetonylbenzyl)-4-hydroxycoumarin-3-¹⁴C, (Va); (α-acetonyl-p-nitrobenzyl)-4-hydroxycoumarin-3-¹⁴C, (Vb), and (α-acetonyl-p-chlorobenzyl)-4-hydroxycoumarin-3-¹⁴C, (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 ¹⁴C-ring labelled derivatives appeared to be the Michael condensation ⁽¹⁷⁾ of 4-hydroxycoumarin (¹⁴C-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), 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 chemical purity was determined by an ascending 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-

* L. Gavai, M. Dinescu and A. B. Susan (paper to be published).

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-3-¹⁴C. 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-3-¹⁴C and yield based on diphenylmalonate-2-¹⁴C 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-3-¹⁴C (Vc)

A mixture of 71 mg (0.29 mmole) 4-hydroxycoumarin-3-¹⁴C (of an activity of 0.21 mCi), 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 5 ml 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 -acetonilybenzyl)-

* 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-3-¹⁴C (total activity 82.4 C), yield based on 4-hydroxycoumarin-3-¹⁴C 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-3-¹⁴C, 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-3-¹⁴C (Vb).

A mixture of 2.3 g (9.39 mmole) 4-hydroxycoumarin-3-¹⁴C (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-3-¹⁴C) 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-3-¹⁴C which disappeared after a second recrystallisation from boiling acetone,

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

The substance was prepared under identical conditions with the above mentioned compounds by the condensation of 4-hydroxycoumarin-3-¹⁴C with *p*-chlorobenzylideneacetone, yield 42 % based on 4-hydroxycoumarin-3-¹⁴C. 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-3-¹⁴C 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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REFERENCES

1. ANSCHUTZ, R. and BERTRAM, W. — *Ber. dtsch. Chem. Ges.*, **36** : 465 (1903).
2. ANSCHUTZ, R. — *Ann.*, **367** : 169 (1909).
3. HULTQUIST, M. E. — U. S. Pat. 2,449,038 (1948); *C. A.*, **43** : 693 (1949).
4. HULTQUIST, M. E. — U. S. Pat. 2,439,302 (1948); *C. A.*, **42** : 5051 (1948).
5. PAULY, H. and LOCKEMAN, K. — *Ber. dtsch. Chem. Ges.*, **48** : 28 (1915).
6. BOYD, J., ROBERTSON, A. and WHALLEY, W. B. — *J. Chem. Soc.*, 174 ((1948).
7. STAHMANN, M. A., WOLF, J. and LINK, K. P. — *J. Am. Chem. Soc.*, **65** : 2288 (1943).
8. MOLHO, D. — U. S. Pat. 3,032, 557 (1962); *C. A.*, **57** : 13730 (1962).
9. KAWASE, Y. and SAKASHITA, K. — *Bull. Soc. Chim. Japan*, **35** : 1869 (1962).
11. ZIEGLER, E. and JUNEK, H. — *Monatsh.*, **86** : 29 (1955).
12. ZIEGLER, E., JUNEK, H. and WILDTGRUBE, G. — *Monatsh.*, **87** : 386 (1956).
13. STARR, D. F. and DISANTO, C. C. — U. S. Pat. 2,752,360 (1956); *C. A.*, **51** : 1293 (1957).
14. KNOEVENAGEL, K. W. — U. S. Pat. 2,743,283 (1956); *C. A.*, **51** : 1292 (1957).
15. LEFEVRE, A. and MENTZER, C. — *Compt. rend.*, **255** : 2611 (1962).
16. IKAWA, M., STAHMANN, M. A. and LINK, K. P. — *J. Am. Chem. Soc.*, **66** : 902 (1944).
17. SUSAN, A. B. and DINESCU, M. — Unpublished.