SYNTHESIS OF THE ^{14}C-LABELLED JUVENOID W 328

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SUMMARY

The labelled juvenoid 2-(4-(2-(ethoxycarbamatoethoxy)(benzene-U-^{14}C)benzyl) -1-cyclohexanone ethylene acetal (8) (W 328) was prepared starting from p-hydroxy(ring-U-^{14}C)benzoic acid (1) by a seven step synthesis in 11% overall yield. The reaction conditions previously reported for the "cold" synthesis of the W 328 (8) were modified to suit the small scale preparation.

Key Words: ^{14}C-labelled juvenoid W 328, p-hydroxy(ring-U-^{14}C)benzoic acid, ^{14}C-labelled pesticide

INTRODUCTION

The labelled juvenoid 2-(4-(2-(ethoxycarbamato) ethoxy)benzyl)-1-cyclohexanone ethylene acetal (8) (W 328) was required in order to study the mechanism of action of this potential pesticide on the flies Sarcophaga bullata. The compound 8 labelled in the urethane carbonyl position with ^{14}C, had already been prepared (1). On the basis of the preliminary results it was desirable to label the aromatic portion of the W 328 molecule either with ^{14}C or ^{3}H. The (benzyl-^{3}H)W 328 was prepared by CESG method (2). The results of the application of the ^{14}C or ^{3}H or doubly labelled W 328 on the flies are to be published in the journal Pesticide Science (6).

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For the synthesis of (benzene-U-^{14}C)W 328 (8), described in this paper, p-hydroxy-(ring-U-^{14}C)benzoic acid (1) was chosen as a starting material. It was synthesised from (ring-U-^{14}C)phenol according to the published procedure (3).

RESULTS AND DISCUSSION

The p-hydroxy(ring-U-^{14}C)benzoic acid (1) was esterified and etherified in one step to methyl-p-methoxy(ring-U-^{14}C)benzoate (2) by the action of diazomethane. The ester 2 was reduced by lithium aluminium hydride in ether to p-methoxy(ring-U-^{14}C)benzyl alcohol (3). From this point onwards the published synthesis of "cold" juvenoid was followed (4), with several improvements relating to the small scale work.

The conversion of the benzyl alcohol 3 to the corresponding p-methoxy(ring-U-^{14}C)benzyl chloride (4) was performed by using a 20% solution of hydrogen chloride in dioxane (instead of the action of concentrated hydrochloric acid/benzene mixture). The chloride 4 was used in the further reaction step directly after the evaporation of the excess reagent. Its reaction with the N-(1-cyclohexenyl) pyrrolidine in dioxane gave, after hydrolysis, 2-(4-methoxy (benzene-U-^{14}C) benzyl)-1-cyclohexanone (5). Deblocking of the phenol group of compound 5 by the action of hydrobromic acid in acetic acid gave 2-(4-hydroxy(benzene-U-^{14}C)benzyl)-1-cyclohexanone (6). The reaction of the phenol 6 with the ethyl 2-chloroethyl carbamate in the presence of ground sodium hydroxide in dimethyl sulfoxide, as described for the "cold" synthesis, sometimes unpredictably fails and therefore the improved arrangement, using sodium hydride as a base and ethyl 2-bromoethyl carbamate as a reagent in dimethyl formamide as a solvent, was used to prepare 2-(4-(2-(ethoxycarbamato)ethoxy) (benzene-U-^{14}C)benzyl)-1-cyclohexanone (7). The ketalisation of the ketone 7 with ethylene glycol gave eventually 2-(4-(2-(ethoxycarbamato)ethoxy-(benzene-U-^{14}C)benzyl)-1-cyclo-hexanone ethylene acetal (8).

In the "hot" experiment we started with 1973 MBq (53.3 mCi) of p-hydroxy(ring-U-^{14}C)benzoic acid (1), with the molar activity of 2294 MBq/mmol (62 mCi/mmol), and we obtained 214 MBq (5.8 mCi; 11%) of the target labelled juvenoid 8 with a radiochemical purity of 97% .

EXPERIMENTAL

Analytical TLC was performed on the Merck Kieselgel 60, UV 254 nm, 0.2 mm plates. Preparative TLC was performed on 2 mm 20 x 20 cm plate. The following solvent mixtures were used as mobile phase:

(A) n-heptane - ethyl acetate 4 : 1

(B) n-heptane - ethyl acetate 7 : 3

(C) diethyl ether - n-hexane 1 : 1 .

Radioactivity distribution was analyzed on the Berthold LB 2832 Linear Analyzer combined with multichannel analyzer Berthold-Silena and HP 97-S calculator.

Materials for flash-chromatography were from J.T. BAKER (Phillipsburg).

The radioactivity of the samples was assayed on a liquid scintillation counter Beckman LS 7800 with the correction on quenching (external standard).

The IR spectra were measured on the NICOLET 205 FT-IR Spectrometer using Diffuse Reflectance Optical unit SPECTRA-TECH. 10 µl of the 0.1% solution of the compound in tetrachloromethane were deposited on the KBr in a microcup. After 10 minutes of drying at the laboratory temperature the spectrum was measured. The Kubelka-Munk transformation was made prior to the evaluation.

Evaporations were made on a rotary evaporator HEIDOLPH VV-micro under the reduced pressure of water aspirator at a temperature of 30 - 40 °C.

The p-hydroxy(ring-U-^{14}C)benzoic acid (1) was prepared according to ref. 3 from the 4.74 GBq (128 mCi) of (ring-U-^{14}C)phenol. The yield of acid 1 was 2.37 GBq (64 mCi, 50%), the radiochemical purity according to TLC (benzene - dioxane - acetic acid 90 : 25 : 4) was 85% and the product was used without further purification.

Methyl p-methoxy(ring-U-^{14}C)benzoate (2)

To 2.3 GBq (62 mCi) of the p-hydroxy(ring-U-^{14}C)benzoic acid (1) in a 50 ml reaction flask, cooled to 0 °C, 25 ml of the diazomethane solution (prepared from 2.2 g of N-nitroso-N-methyl-N'-nitroguanidine (5)) was distilled. The flask was stoppered with a KOH filled drying tube, wrapped in aluminium foil and the mixture was left at the laboratory temperature for 48 hours. Radio-TLC (solvent A) revealed only the product 2 (Rf = 0.8) and the impurities originally present in the acid 1. The solution was evaporated to dryness and the benzoate 2 was directly used in the subsequent step.

p-Methoxy(ring-U-^{14}C)benzyl alcohole (3)

The benzoate 2 was dissolved in 7 ml of ether, the solution was filtered and taken to the syringe. This ether solution of 2 was than slowly added via the silicone rubber septum to the reaction flask containing stirred suspension of 110 mg of $LiAlH_4$ in 4 ml of ether under an Argon atmosphere. The

reaction flask was cooled in ethanol - carbon dioxide bath to -30 °C. On completion of the addition of the benzoate 2 the reaction mixture was brought to the laboratory temperature and stirred for another 2 hours. The reaction mixture was then cooled to -20 °C and the excess reagent was destroyed at first by moist ether and then 1.8 ml of 2M sulphuric acid was added. The ether layer was separated and the water phase was extracted with two 5 ml portions of ether. The united ether extracts were washed with water and then dried using Na_2SO_4. The radio-TLC (solvent A) showed that the 90% of the radioactivity was in the benzyl alcohol 3 (Rf = 0.2). The yield on acid 1 was 85% (1.96 GBq).

p-Methoxy(ring-U-^{14}C)benzyl chloride (4)

The ether solution of the benzyl alcohol 3 was concentrated to an oily consistency and the oily residue dissolved in 1.8 ml of 20% hydrogen chloride in dioxane and the solution stirred for 1.5 hours at the laboratory temperature. The solution was concentrated at 22 °C to several drops and 1.3 ml of dioxane was added. The chloride 4 was immediately used in the following step.

2-(4-Methoxy(benzene-U-^{14}C)benzyl)-1-cyclohexanone (5)

To the stirred solution of the chloride 4 in dioxane 0.52 ml of N-(1-cyclohexenyl) pyrrolidine was added, the flask stoppered and the reaction mixture heated to 115 - 118 °C with stirring for 2 hours. The reaction mixture was cooled to the laboratory temperature, 0.9 ml of water was added and the mixture heated to 100 °C for one hour. The reaction mixture was concentrated on the evaporator and the residue partitioned between water and ether. In the organic layer were recovered 1.88 GBq of the ketone 5 with a radiochemical purity of 90% (radio-TLC in solvent (A), Rf = 0.5). In view of the "cold" experiments, which showed, that the impurities contained in the ketone 5 have strongly negative effect on the purification in the next synthetic step, the crude ketone 5 was purified by flash-chromatography on silicagel, the mixture of n-heptane - ethyl acetate 10 : 1 was used as mobile phase; 1.56 GBq of pure ketone 5 was recovered.

2-(4-Hydroxy(benzene-U-^{14}C)benzyl)-1-cyclohexanone (6)

The solution of the ketone 6 was evaporated in a 25 ml pear shaped flask, 2 ml of acetic anhydride and 1.4 ml of 47% hydrobromic acid were added, the flask was flushed with Argon, stoppered, and the mixture was heated with stirring to 110 - 112 °C for one hour. The reaction mixture was diluted with 1.5 ml of water, 2 ml of ether was added and the mixture neutralised with solid $CaCO_3$

(altogether 200 mg were added in small portions, intensive foaming occurs after addition of the first portions). When all calcium carbonate was dissolved, the ethereal layer was taken off and the water phase was extracted with three 2 ml portions of ether. The united ethereal extracts were dried with $MgSO_4$ and the product was purified by flash-chromatography, mobile phase n-heptane - ethyl acetate 9 : 1; 12% of methylated ketone 5 was recovered and 1073 MBq (69%) of the demethylated ketone 6 was obtained.

2-(4-(2-(ethoxycarbamato)ethoxy)(benzene-U-^{14}C)benzyl)-1-cyclohexanone (7)

The solution of the demethylated ketone 6 in 1 ml of ethyl acetate was evaporated to a syrup in a 10 ml pear shaped flask. 45 Mg of the 46% suspension of NaH in mineral oil and 1 ml of amine free DMF were added. The flask was stoppered with a rubber septum with a thin hypodermic needle inserted for pressure relief. The mixture was stirred and heated in a 70 °C bath for 10 minutes. During this time all the NaH dissolves and the reaction mixture darkens. During the next 40 minutes the 200 µl of ethyl bromoethyl carbamate dissolved in 500 µl of DMF were gradually added using the syringe. The addition completed, the mixture was heated for another 30 minutes. After cooling 5 ml of water were added and the mixture was extracted with three 6 ml portions of ether. United ethereal extracts were dried with $MgSO_4$, concentrated and the residue was purified by flash-chromatography, mobile phase (A). The yield of the carbamate 7 was 413 MBq (38.5% on ketone 6).

2-(4-(2-(Ethoxycarbamato)ethoxy)(benzene-U-^{14}C)benzyl)-1-cyclohexanone ethylene acetal (8)

The solution of the carbamate 7 in toluene (266 MBq) was evaporated in a pear shaped flask to a syrup. Ethylene glycol (250 µl), p-toluenesulphonic acid monohydrate (about 5 mg) and benzene (10 ml) were added. The flask was equipped with an azeotropic distillation adapter and condenser. The reaction mixture was heated to boiling and stirred; water in the azeotropic adapter was trapped by molecular sieve Nalsit 4. After 90 minutes the reaction mixture was concentrated on a rotary evaporator to a volume of approx. 1 ml and the mixture was applied to a preparative TLC plate. The plate was eluted twice with the ether - n-hexane mixture 1:1. The band containing 8 (UV detection) was scratched off and the silicagel was extracted using 20 ml of ether. The ether solution was evaporated to dryness and the residue dissolved in 5 ml of toluene. The yield was 214 MBq (5.8 mCi), radiochemical purity was better than 97%.

IR (cm^{-1}) : 3350, 1720, 1520, 1250, 1165, 1100, 1075.

The identity of the product was confirmed by comparison of its IR spectrum with that of the authentic specimen.

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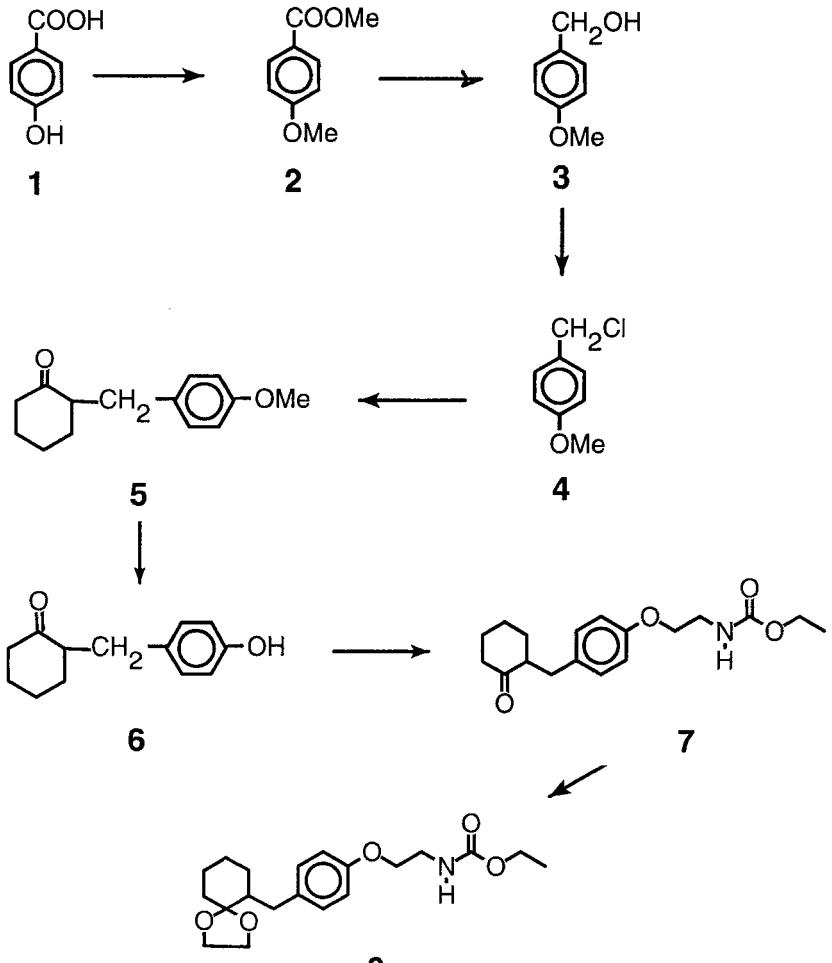
REACTION SCHEME



juvenoid W 328