SYNTHESIS OF ^{14}C-LABELED BENZOFURANO[2,3-b] BENZOPYRAN-6-ONE AND ITS 4-HYDROXYCOUMARIN DERIVATIVE

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SUMMARY

2-Isopropyl-8,9-dimethoxy-benzofurano[2,3-b]furo[2,3-h][1][11-^{14}C]benzopyran-6-one(**IVb**) and 3-(2-hydroxy-4,5-dimethoxyphenyl)-4-hydroxy-5'-isopropylfurano[2',3':7,8][2-^{14}C]coumarin(**Vb**) were prepared from isorotenone(**Ib**) for use in metabolic and other studies. Deoxybenzoin derivative(**IIb**), obtained from isorotenone, was reacted with ethyl[^{14}C]formate in the presence of sodium to give the corresponding [2-^{14}C]-2'-hydroxyisoflavone(**IIIb**), which was transformed into the ^{14}C-labeled benzofurano[2,3-b]furo[2,3-h]benzopyran-6-one compound (**IVb**) by the subsequent oxidative cyclization with selenium dioxide. Hydrolysis of compound **IVb** in alkaline media gave ^{14}C-labeled 4-hydroxycoumarin(**Vb**).

Keywords: ^{14}C-benzofurano[2,3-b]furo[2,3-h]benzopyran-6-one, ^{14}C-4-hydroxy coumarin, carbon-14

INTRODUCTION

Natural products having a ring system of benzofurano[2,3-b]benzopyran-6-one(coumarono-chromone) have become interesting new type among the isoflavonoids, and it has also been remarked that these compounds are naturally co-occurring substances with rotenone which has an interesting biological activity as an insecticide. Lisetin(1-3),identified as the first natural product of this type, and Milletin(4) were isolated from the roots of *Piscidea erythrina L.*(Jamaican Dogwood) and the seeds of *Millettia auriculata*, respectively,

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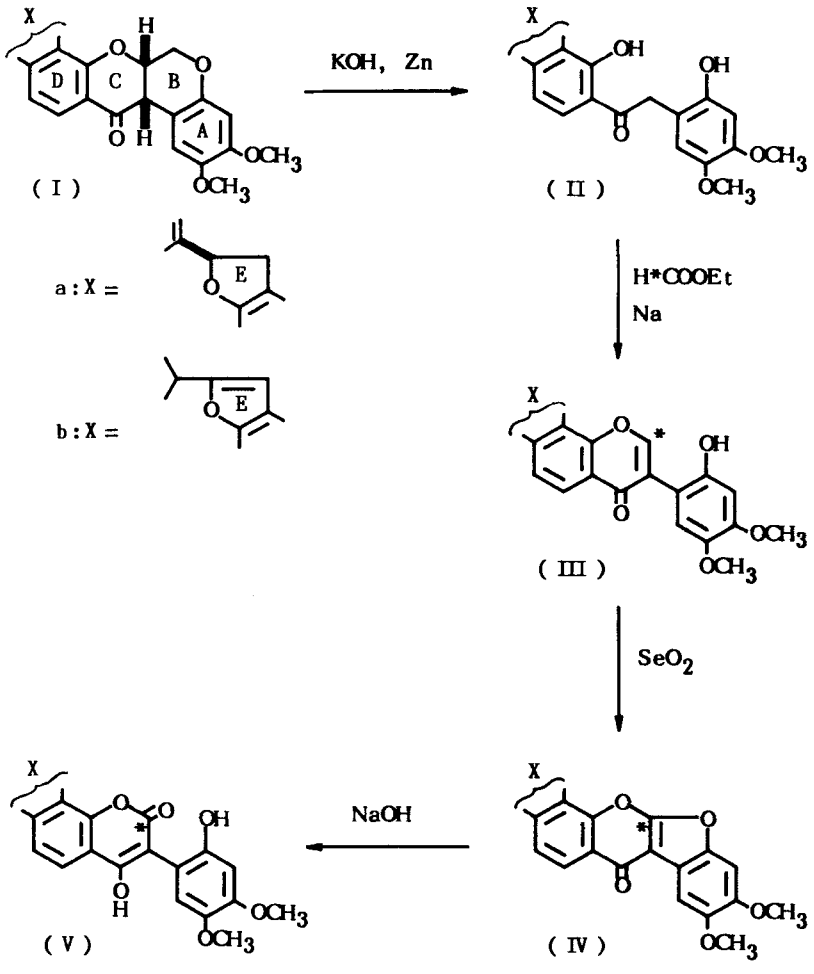
together with some rotenoids and isoflavonoids.

Previously, we reported that compound **IV** having coumarono-chromone structure is formed by the photooxygenation of dehydrorotenone(5,6) and prepared readily through 2'-hydroxy isoflavone **III** from rotenone(**Ia**)(7). This paper deals with the synthesis of ^{14}C-labeled benzofurano[2,3-b]benzopyran-6-one compound(**IVb**) and the corresponding 4-hydroxycoumarin derivative(**Vb**) for metabolic and other studies.

DISCUSSION

The synthetic procedure employed here(scheme 1) is a modification of the method described previously(7). Isorotenone(**Ib**),which has the biological activity as rotenone(**Ia**)(8), was heated under reflux in ethanolic alkaline solution in the presence of zinc dust to give the deoxybenzoin **IIb**, known as isoderritol(9). Condensation of **IIb** with ethyl[^{14}C]formate(10) in the presence of powdered sodium according to the procedure of Crombie *et al.*(11) gave 2'-hydroxyisoflavone **IIIb**. In this process, however, a ten-fold excess of ethyl formate are usually employed, resulting in dilution of specific activity of the product obtained. It was revealed in preliminary experiments that while the yield of the isoflavone **III** was fairly good, the radiochemical yield in this process was below 1%. In order to have high specific activity of compound **IIIb**, the total amounts of radiolabeled ethyl formate was allowed to react with **IIb** for 1 hr, and subsequently, additional ethyl formate in limited amounts was added to the reaction mixture at intervals of 1 hr. Crystallization of the product gave the [2-^{14}C]-isoflavone(**IIIb**) in 5.7% radiochemical yield.

Compound **IIIb** was readily converted to ^{14}C-labeled



Scheme 1

benzofurano[2,3-b]benzopyran-6-one(**IVb**) in good yield according to the procedure described previously(7). Meanwhile, an attempt to prepare radiolabeled compound **IVa** results in low yield owing to the sensitivity of the isopropenyl moiety in compound **IIIa** to selenium dioxide(7).

[2-^{14}C]-4-Hydroxycoumarin(**Vb**) was prepared by the hydrolysis of **IVb** with sodium hydroxide according to the method described previously(5,6). The preparation of 4-

hydroxycoumrins from deoxybenzoin was achieved usually by the reaction of deoxybenzoin with ethyl chloroformate. However, this process is not practical for the radiochemical synthesis, since labeled ethyl chloroformate is not readily available. Therefore, the above reaction is an applicable process for the synthesis of 4-hydroxycoumarins labeled with carbon-14 in position 2.

EXPERIMENTAL

Sodium[^{14}C]formate(4.0 mCi/mmol, 1 mCi) was purchased from New England Nuclear, Boston, Mass. Rotenone was purchased from Aldrich Chemical Co. Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Liquid scintillation counting was done with a Packard Tri-Carb 460 CD liquid scintillation counter equipped with automatic external standarization.

Isorotenone(Ib)

Rotenone(Ia,15g, 38 mmol) was dissolved in a mixture of acetic acid (180 g) and concentrated sulfuric acid (100 g). The mixture was stirred at 60°C for 1 hr. and then ice-cooled water (700 ml) was added to the resulting mixture. The precipitate obtained was collected by filtration, washed with water and crystallized from methanol to give 10 g(67%) of isorotenone, mp, 180°C (lit.(12),181-182.5°C).

2-Hydroxy-4,5-dimethoxybenzyl 4-hydroxy-2-isopropylbenzofuran-5-yl ketone(Isoderritol)(IIb)

Isorotenone (**Ib**,10 g, 25 mmol) was heated in ethanol(300 ml) and 30% ethanolic potassium hydroxide solution (100 ml) was added. Zinc powder (25 g) was added to the boiling solution, and the mixture was heated under reflux with

vigorous stirring for 2.5 hr. The zinc powder was filtered off and the filtrate was acidified with 2N-hydrochloric acid with ice cooling. The precipitate was collected by filtration and then dissolved in ether; the ether solution was extracted with 10% potassium hydroxide solution. The aqueous solution was acidified again and extracted with ether. The ether extract was washed with water and dried over anhydrous sodium sulfate. The ether was removed under reduced pressure and the residue was recrystallized from methanol to give 3.5 g(37 %) of isoderritol as yellow plates, mp.146-147°C(lit.(9),148°C).

7-(2-Hydroxy-4,5-dimethoxyphenyl)-2-isopropylfurano[2,3-h][2-^{14}C]benzopyran-6-one(**IIIb**)

Sodium (380 mg) was powdered under hot toluene in a 25 ml pear-shaped flask(13), and the solvent was changed to dry ether. The flask was cooled in an ice-cold water bath and the ether was evaporated by a stream of dry nitrogen. Isoderritol(**IIb**)(750 mg, 2.03 mmol) put into the flask, and then 910 mg(11.7 mmol,79.4 μCi/mmol) of ethyl[^{14}C]formate, prepared from sodium[^{14}C]formate(4.0 mCi/mmol) according to the method of Ropp(10), was added to the mixture. The reaction mixture was allowed to stand at 5°C for 1hr and then additional amounts (3 x 1 ml) of freshly distilled ethyl formate were added to the reaction mixture at intervals of 1hr. After the mixture was allowed to stand at room temperature overnight, the sodium remaining in the mixture was removed by the addition of ethyl formate(3 ml). The resulting mixture was poured into water and extracted with chloroform(3 x 10 ml). The chloroform solution was washed with 10 % sodium hydroxide (10 ml) and water(2 x 10 ml), successively, and then the extract was dried over anhydrous

magnesium sulfate and evaporated to dryness under reduced pressure, the yellow residue was recrystallized from methanol to give the radiolabeled isoflavone(**IIIb**) (530 mg,1.40 mmol, sp.act. 38 μCi/mmol,5.7% radiochemical yield), mp.181-182°C (lit.(12), 182-183°C).

2-Isopropyl-8,9-dimethoxybenzofurano[2,3-b]furo[2,3-h][1][11a-^{14}C]benzopyran-6-one (**IVb**)

Compound **IIIb**(870 mg,2.23 mmol,37 μCi) was dissolved in freshly distilled isopentyl alcohol(85 ml) by means of heating, and then selenium dioxide(2 g) was added to the solution. The reaction mixture was heated under reflux with stirring for 10 hr. The precipitated selenium was removed by filtration, the filtrate was evaporated to dryness, and the residue was extracted with chloroform. The extract was washed with water and dried over anhydrous magnesium sulfate. After removal of the solvent, the crude product was recrystallized from chloroform-methanol to give 610 mg (1.6 mmol,73% yield, sp.act.16.8 μCi/mmol) of pure **IVb** as white solid, mp.214-215°C(lit.(5), 214-215°C).

3-(2-Hydroxy-4,5-dimethoxyphenyl)-4-hydroxy-5'-isopropylfurano[2',3':7,8][2-^{14}C]coumarin(**Vb**)

Compound **IVb** (250 mg,0.70 mmol) was hydrolyzed by refluxing in a mixture of 10 % potassium hydroxide solution (20 ml) and methanol(50 ml) for 2 hr under N_2 atmosphere. After cooling, the solution was acidified with 2N-hydrochloric acid and extracted with ether. The extract was washed with water and dried over sodium sulfate. The filtrate was evaporated to dryness and the residue was recrystallized from ethanol to give 180 mg(0.45 mmol,sp.act. 16.8 μCi/mmol) of pure **Vb** as brown needles, 253-254° C(lit.(5),253-254°C).

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