SYNTHESIS OF 3-[4-(1-ETHOXYCARBONYL-1-METHYLETHOXY)PHENYL]-5-(3-PYRIDYL)-1,2,4-[3-^{14}C]OXADIAZOLE (AT-308-^{14}C)

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Ethyl p-chlorophenoxyisobutyrate[1,2] is hypocholesterolemic in rats maintained on normal diets and the other hypocholesterolemic agents have also been introduced into clinical use. From the studies[3-5] of the relationship between structure and activity of 1,2,4-oxadiazole derivatives, 3-[4-(1-ethoxycarbonyl-1-methylethoxy)phenyl]-5-(3-pyridyl)-1,2,4-oxadiazole (AT-308)[6,7] was found to be one of the most potent hypocholesterolemic agent in both normocholesterolemic and hypercholesterolemic rats. The carbon-14 labelled compound of AT-308 was desired for the study of metabolic fate in animals, and the labelled position at 3 in 1,2,-4-oxadiazole ring was chosen from the economical point of view. According to the procedure of Harris[8] and Barber[9], p-hydroxybenzo-[^{14}C]nitrile (III) was synthesized from p-bromophenol and cuprous [^{14}C]cyanide (II) which prepared by reacting K^{14}CN with $CuSO_4$ in the presence of $NaHSO_3$ as shown in the reaction scheme. p-Hydroxy-[amidoxime-^{14}C]benzamidoxime (IV) obtained in 81% yield from III was reacted with nicotinoyl chloride to afford 3-(4-hydroxyphenyl)-5-(3-pyridyl)-1,2,4-[3-^{14}C]oxadiazole (V) in an excellent yield. Condensation of V with ethyl 2-bromoisobutyrate gave 3-[4-(1-ethoxycarbonyl-1-methylethoxy)phenyl]-5-(3-pyridyl)-1,2,4-[3-^{14}C]oxadiazole (VI) with a specific activity of 3.9

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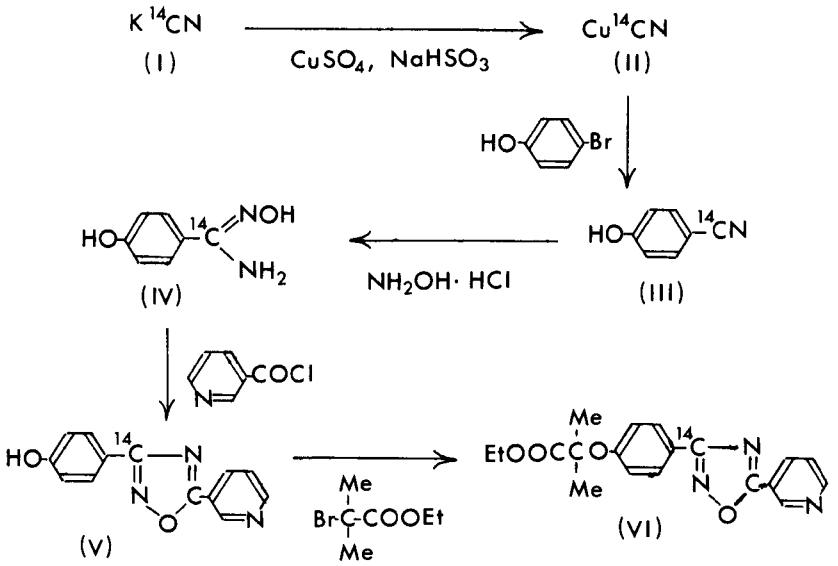
mCi/mmole in 13% radiochemical yield based on I. The radiochemical purity of VI was determined by both reverse isotope dilution and radiochromatographic methods, and found to be greater than 99%.



EXPERIMENTAL

Potassium [^{14}C]cyanide (I)

$K^{14}CN$ purchased from The Radiochemical Centre, Amersham, England, was diluted to 31 mCi/8 mmole with unlabelled KCN.

Cuprous [^{14}C]cyanide (II)

A solution of 1860 mg of $CuSO_4$ in 6 ml of water was kept at 60° in a water bath. Into this copper solution, 520 mg of $NaHSO_3$ in 1.5 ml of water was added with stirring over a 1-2 min period, followed immediately by a solution of 520 mg (31 mCi/8 mmole) of

$K^{14}CN$ in 1.5 ml of water. After 10 min, the hot mixture was filtered and the product was washed with boiling water, then with alcohol. It was dried over P_2O_5 in a vacuum desiccator for 24 h; yield, 610 mg of fine powder in 85%.

p-Hydroxybenzo-[^{14}C]nitrile (III)

A mixture of 610 mg of II and 990 mg of p-bromophenol in 1.6 ml of dimethyl formamide was heated at 150-160° for 4 h and then concentrated to dryness in vacuo. To the residue was added 11.6 ml of 6N HCl and stirring for 30 min. A solution of 2.3 mg of $FeCl_3 \cdot 6H_2O$ in 3 ml of water was added to the acidic solution of the residue, and further stirring for 30 min at 85°. After the resulting mixture was extracted with ether, the extract was washed with water, decolorized with animal charcoal, dried over anhydrous Na_2SO_4 and evaporated. The residue was dissolved in a small amount of CH_2Cl_2 and allowing to stand in a refrigerator. The crystalline product was filtered off, washed with benzene and dried to give 360 mg of III in 44% yield based on II.

p-Hydroxy-[amidoxime-^{14}C]benzamidoxime (IV)

Into a solution of 245 mg of Na_2CO_3 in 1.5 ml of water was dissolved 320 mg of hydroxylamine hydrochloride. The solution was added to 360 mg of III in 1 ml of ethanol and the mixture was heated at 65-70° for 5 h with stirring. After the reaction mixture was kept in a refrigerator over-night, the crystalline product was filtered off, washed with water and dried to give 372 mg of IV in 81% yield based on III.

3-(4-Hydroxyphenyl)-5-(3-pyridyl)-1,2,4-[3-^{14}C]oxadiazole (V)

A mixture of 372 mg of IV in 15 ml of pyridine and 530 mg of nicotinoyl chloride hydrochloride was stirred for 30 min at room temperature and then heated at 110° for 3 h. After removal of pyridine in vacuo, the residue was crystallized by the addition of 3 ml of water. The product was filtered, washed with cold water and dried to give 560 mg of V in 95.7% yield based on IV.

3-[4-(1-Ethoxycarbonyl-1-methylethoxy)phenyl]-5-)3-pyridyl)-1,2,4-[3-^{14}C]oxadiazole (VI)

A mixture of 840 mg of K_2CO_3, 140 mg of KI and 1.12 ml of ethyl 2-bromoisobutyrate was added to 560 mg of V in 8.4 ml of dimethyl formamide. The reaction mixture was heated at 70-80° for 15 h with stirring and then poured into 60 ml of water. The resulting mixture was extracted with ethyl acetate, and the extract was washed with water, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was chromatographed over 60 g of silica gel using benzene, followed by a mixture of acetone and benzene (1:19, v/v) as the eluents. Evaporation of the fraction of VI left a residue which was recrystallized from ethanol. VI with a specific activity of 3.9 mCi/mmole was obtained in 13% radiochemical yield based on I.

Thin-layer radiochromatography of VI

The thin-layer chromatograms (t.l.c) used were 200 mm plate precoated with silica gel and developed with the following three systems:

(a) silica gel-f (Tokyokasei, Ltd.). Developing solvent, AcOEt and benzene (1:1, v/v): Rf. 0.63.

(b) silica gel F-254/366 (Woelm). Developing solvent, AcOEt and benzene (1:1, v/v): Rf. 0.54.

(c) silica gel F-254/366 (Woelm). Developing solvent, $CHCl_3$ and AcOEt (1:1, v/v): Rf. 0.63.

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