SYNTHESIS OF ^{14}C-LABELLED 3-(1-HYDROXY-2-PIPERIDINOETHYL)-5-PHENYLISOXAZOLE CITRATE (31252-S)

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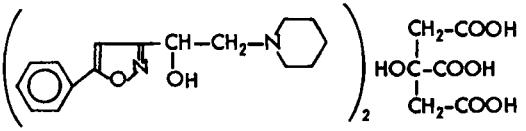
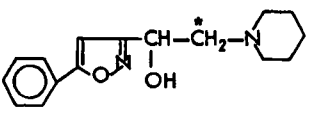
SUMMARY

3-(1-Hydroxy-2-piperidinoethyl)-5-phenylisoxazole citrate, 31252-S (I) has been labelled with carbon-14. The carbon-14 label was incorporated into the C-2 position of the side-chain to give (II) and into the C-4 position of the isoxazole ring of the molecule to give (III).

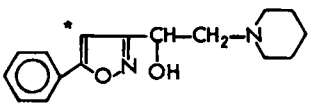
The carbon-14 labelled products (II) and (III) were obtained in 27% and 6.4% radiochemical yield based on methyl iodide-^{14}C, respectively.



(I)



(II)



(III)

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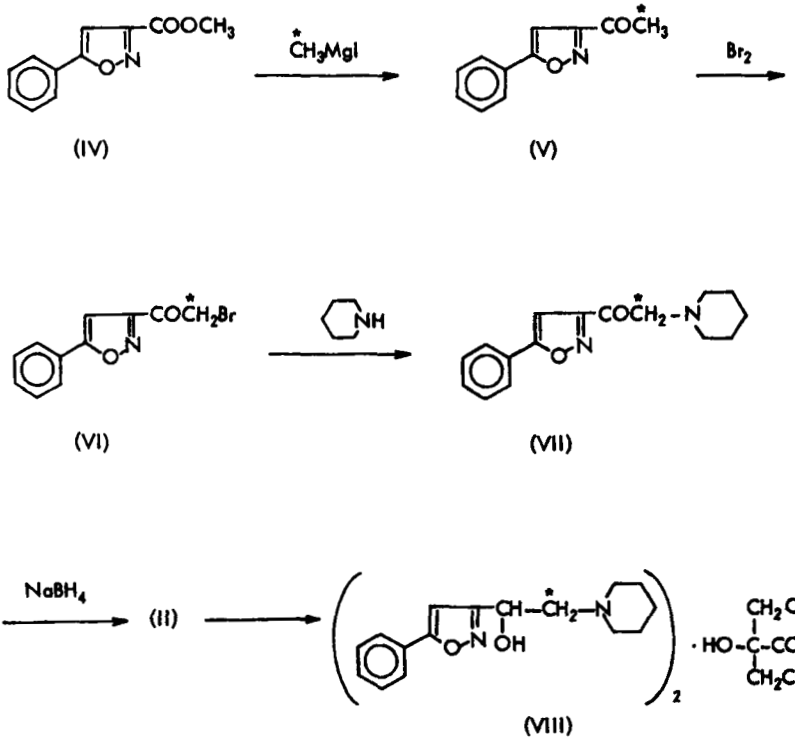
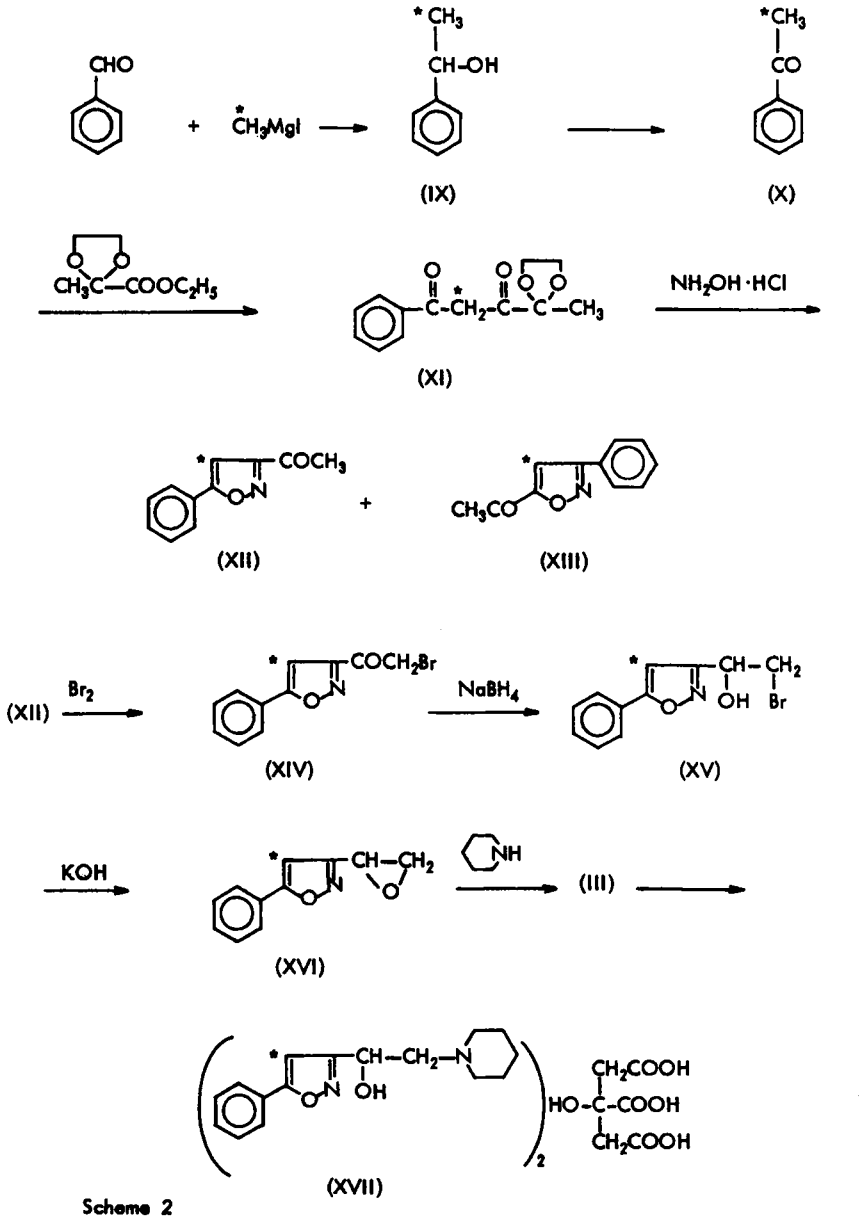
A number of 3-aminoalkylisoxazoles were synthesized in our laboratory by Kanō and Adachi [1]. Of these, 3-(1-hydroxy-2-piperidinoethyl)-5-phenylisoxazole exhibited powerful analgesic and antiinflammatory properties[2], and the citrate (31252-S) (I) was used practically for pharmaceutical preparations.

In order to conduct absorption and metabolism studies with 31252-S, the radioactive drug was required. Based on information already available on the metabolic fate of tritium-labelled 31252-S[3], it appeared that the carbon-14 label would be desirable. It was therefore carried out to label the carbon of the side-chain and of the isoxazole ring of the molecule with the carbon-14.

The side-chain labelled compound (II) was synthesized by the route indicated in Scheme



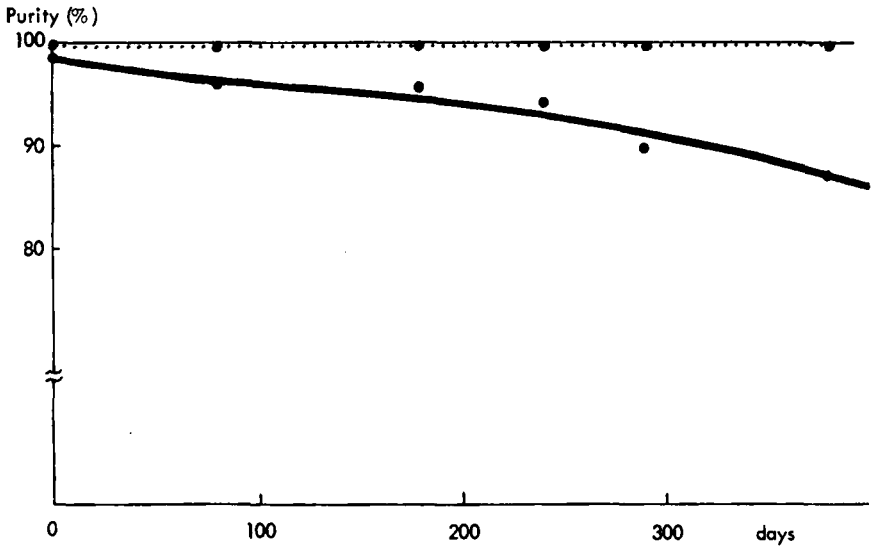
Scheme 1


CHO
+ *CH3MgI
*CH3
CH-OH
(IX)
*CH3
CO
(X)
CH3C—COOC2H5
NH2OH·HCl
(XI)
COCH3
CH3CO
(XII)
(XIII)
(XII) Br2
COCH2Br
(XIV)
NaBH4
CH—CH2
OH Br
(XV)
KOH
NH
(III)
(XVI)
CH—CH2—N
OH
CH2COOH
HO-C-COOH
CH2COOH
2
(XVII)

Scheme 2

I and the isoxazole ring labelled compound (III) by that in Scheme 2[4]. Overall radiochemical yields of (II) and (III) were 27% and 6.4% based on methyl iodide-^{14}C, respectively.

In order to investigate the radiochemical stability of the labelled compound, the radiochemical purity of labelled 31252-S (VIII) was examined over a one-year period. The dried solid sample (VIII) (12.0 mCi/mM) was stored in a refrigerator at 2-5° and its purity was determined every two or three months by radio-t.l.c. (solvent system : ethylacetate — methanol = 2 : 1). As shown in the Figure, its purity was 87.5% after one-year storage, although the purity of cold 31252-S (I)[6] was 99.6 - 100.4% under the same conditions.



Figure

Radiochemical purity of 3-(1-hydroxy-2-piperidinoethyl)-5-phenylisoxazole citrate-ethyl-^{14}C (VIII) during one-year storage. —— : (VIII) ····· : cold (I)

EXPERIMENTAL

Radioactivity determination was carried out with a ALOKA Model 653 liquid scintillation spectrometer (Japan) by the external standard method. Unless otherwise stated, silica gel plates (Merck 5724/0100) were used for radio-t.l.c. and scanned with a ALOKA Model JTC-201 TLC scanner.

3-Acetyl-5-phenylisoxazole-methyl-^{14}C (V)

The Grignard reagent was prepared from 1.83 g (12.9 mM, 600 mCi) of methyl iodide-^{14}C and 407.3 mg (16.75 mM) of magnesium in 32 ml of anhydrous ether-benzene (9 : 23). To the Grignard solution was added a solution of 2.223 g (22.2 mM) of triethylamine and 3.315 g (16.34 mM) of 3-carbomethoxy-5-phenylisoxazole (IV)[1] in 35 ml of anhydrous benzene with stirring at 5-10° for 30 min. The mixture was stirred for additional 2 hours at the same temperature, then decomposed by addition of a solution of ammonium chloride (5 g) in 20 ml of water, and acidified with 15% hydrochloric acid (35 ml). The aqueous layer was extracted with 30 ml of benzene. The combined benzene layer was washed with 6N-hydrochloric acid, water, and sodium hydrogen carbonate solution. The benzene solution was added to a solution of 10 ml of 15% potassium hydroxide and 30 ml of methanol and stirred for 3 hours at room temperature. The solution was washed with water, dried (Na_2SO_4), and evaporated to leave an yellow viscous oil (271 mCi, 45.1% yield based on methyl iodide-^{14}C), the radiochemical purity of which was shown to be about 90% by radio-t.l.c. (solvent system : benzene-ethyl acetate-n-hexane = 1 : 1 : 3). The residue was dissolved in n-hexane-benzene (1 : 1) and chromatographed on Florisil (40 g). Elution with benzene—n-hexane (1 : 1) gave 3-acetyl-5-phenyl-isoxazole-methyl-^{14}C (V), m.p. 100-102° (1.039 g; 257 mCi; specific activity, 46.3 mCi/mM) in 42.9% radiochemical yield.

3-Bromoacetyl-5-phenylisoxazole-methylene-^{14}C (VI)

To a solution of 1.039 g (5.5 mM, 257 mCi) of (V) in 12 ml of carbon tetrachloride was

added dropwise a solution of 900 mg (5.62 mM) of bromine in 3 ml of the same solvent with stirring at 35° and the mixture was allowed to stand overnight. Cold water (10 ml) was added to the mixture with stirring, and the precipitated crystalline product was collected by filtration to give (VI), m.p. 127-129° (1.27 g, 220 mCi) in 85.7% radiochemical yield. As its radiochemical purity was shown to be about 90% by radio-t.l.c., the product was used without further purification.

3-Piperidinoacetyl-5-phenylisoxazole-methylene-^{14}C (VII)

A solution of 1.27 g (220 mCi) of (VI) in 13 ml of acetone was added dropwise to a solution of 871.6 mg (10.24 mM) of piperidine in 2 ml of acetone with stirring vigorously, and the mixture was left for 30 min. at room temperature, during which time yellow precipitates were separated from the solution. The precipitated product (VII) was collected by filtration, washed with cold acetone, and used as the staring material in the next step.

3-(1-Hydroxy-2-piperidinoethyl)-5-phenylisoxazole-ethyl-^{14}C (II)

Sodium borohydride (594.3 mg, 15.7 mM) was added to a solution of the crude (VII) in 20 ml of methanol with stirring in an ice bath and the mixture was stirred for 45 min. at room temperature. To this mixture was added 13.5 ml of 10% hydrochloric acid, and this was filtered through a charcoal column. The filtrate was neutralized with 28% ammonium hydroxide and cooled in an ice bath to separate a precipitated crystalline product. The product was collected by filtration and washed with water to give (II) (1.546 g), which was recrystallised from methanol to give colourless prisms, m.p. 112-113° (912 mg, 161.5 mCi) in 73.5% yield based on (VI). The radiochemical purity was 98% by radio-t.l.c. (solvent system, ethyl acetate – methanol = 2 : 1).

3-(1-Hydroxy-2-piperidinoethyl)-5-phenylisoxazole citrate (31252-S)-ethyl-^{14}C (VIII).

Citric acid monohydrate (734 mg, 3.51 mM) was added to a solution of 912 mg (3.35

mM, 161.5 mCi) of (II) in 7 ml of methanol and heated under reflux for 30 min. The solution was left in a freezer overnight, and a precipitated product was collected and recrystallised from methanol to give (VIII), m.p. 143-145° (389 mg, 49.2 mCi; specific activity : 46.6 mCi/mM). The radiochemical purity was confirmed to be about 98% by dilution analysis and radio-t.l.c. (solvent systems, ethylacetate – methanol = 2 : 1 and chloroform – methanol – acetic acid = 45 : 5 : 1).

Acetophenone-2-^{14}C (X)

The Grignard reagent was prepared from 284 mg (1.17 mM, 5.0 mCi) of methyl iodide-^{14}C, dried by filtration through a magnesium perchlorate tube, and 48 mg (1.97 mM) of magnesium in 5 ml of absolute ether. Benzaldehyde (212 mg, 2.0 mM) was added dropwise to the Grignard solution with stirring at room temperature and heated under reflux for 1 hour. A solution of ammonium chloride in water was added to decompose the complex, and the mixture was extracted with ether (3 x 10 ml). The ether extract was washed with water, dried (Na_2SO_4), and evaporated to leave an oily residue (250 mg, 3.0 mCi) (IX containing cold benzaldehyde). The residue was oxidized with chromium trioxide-pyridine (prepared by 600 mg of CrO_3 and 3 ml of pyridine) in 20 ml of dichloromethane with stirring for 1 hour at room temperature. To this was added 300 mg of cold acetophenone as a carrier, and the mixture was poured into ice-water and the aqueous layer was extracted with ether. The combined extracts were washed with 2N-sulphuric acid, 2N-sodium carbonate, and water, dried (Na_2SO_4), and evaporated, leaving a residue. The residue was purified by distillation to give acetophenone-2-^{14}C (X) (450 mg, 2.5 mCi), b.p. 202°, to which cold acetophenone was added to bring the total weight up to 600 mg. Radiochemical yield was 50.0%.

1-Phenyl-4-ethylenedioxy-pentane-1,3-dione-2-^{14}C (XI)[5]

Acetophenone-2-^{14}C (X) (600 mg, 5.0 mM, 2.5 mCi) and ethyl pyruvate-ethylene ketal (800 mg, 5.0 mM) were added to a solution of sodium ethoxide (340 mg, 5.0 mM) in 8 ml of

ethanol and heated under reflux for 1 hour. The mixture was poured into ice-water and extracted with ether. The aqueous layer was adjusted to pH 6.0 by addition of acetic acid and extracted with ether (3 x 10 ml), and the extract was washed with water, dried (Na_2SO_4), and evaporated to leave a residue. The residue was crystallised from n-hexane to give (XI), m.p. 38-39.5° (610 mg, 1.3 mCi) in 52% yield.

3-Acetyl-5-phenylisoxazole-4-^{14}C (XII)

A solution of 610 mg (2.6 mM, 1.3 mCi) of (XI) and 268 mg (3.8 mM) of hydroxylamine hydrochloride in 20 ml of ethanol was heated under reflux for 2 hours, and the solvent was evaporated. The residue was dissolved in a solution of 6N-sulphuric acid (6 ml) and tetrahydrofuran (4 ml), heated under reflux for 1 hour, and extracted with ether. The extract was washed with water, dried (Na_2SO_4), and evaporated, leaving a residue. The residue was separated into 3-acetyl-5-phenylisoxazole-4-^{14}C (XII) (R_F 0.55) and 3-phenyl-5-acetylisoxazole-4-^{14}C (XIII) (R_F 0.45) by preparative t.l.c. on silica gel (solvent system, benzene — ethyl acetate = 2 : 1). The crude (XII) was recrystallised from light petroleum to give (XII), m.p. 98-99° (266 mg, 0.71 mCi) in 54.6% yield.

3-Bromoacetyl-5-phenylisoxazole-4-^{14}C (XIV)

To a solution of 266 mg (1.42 mM, 710 μCi) of (XII) in 7 ml of carbon tetrachloride was added a solution of 227 mg (1.42 mM) of bromine in 3 ml of carbon tetrachloride dropwise with stirring. The mixture was heated at 50° for 10 hours with stirring, and the precipitated crystalline product was collected and recrystallised from benzene - light petroleum to give (XIV), m.p. 129-130° (315 mg, 600 μCi) in 83.4% yield.

3-(1-Hydroxy-2-bromoethyl)-5-phenylisoxazole-4-^{14}C (XV)

Sodium borohydride (50 mg) was added to a solution of 315 mg (1.18 mM, 600 μCi) of (XIV) in 5 ml of methanol with stirring in an ice bath and the mixture was stirred for 1 hour at

room temperature. The mixture was extracted with ether (3 x 10 ml) and the extract was washed with water, dried (Na_2SO_4), and evaporated, leaving a residue. The oily residue was purified by preparative t.l.c. on silica gel (R_F 0.43, solvent system : benzene — ethyl acetate = 2 : 1) to give (XV) (220 mg, 420 μCi) in 69.7% yield.

3-(1,2-Epoxyethyl)-5-phenylisoxazole-4-^{14}C (XVI)

To a solution of 100 mg of potassium hydroxide in 4 ml of methanol was added 220 mg (0.82 mM, 420 μCi) of (XV), and the mixture was left for 3.5 hours at room temperature and extracted with ether (3 x 10 ml). The extract was washed with water, dried (Na_2SO_4), and evaporated, leaving a crystalline product. The product was recrystallised from cyclohexane to give (XVI), m.p. 79–80° (152 mg, 400 μCi) in 98.5% yield.

3-(1-Hydroxy-2-piperidinoethyl)-5-phenylisoxazole-4-^{14}C (III)

A solution of 152 mg (0.81 mM, 400 μCi) of (XVI) and 1.0 ml of piperidine in 10 ml of absolute bénzene was heated under reflux for 12 hours and washed with water, dried (anhydrous K_2CO_3), and evaporated to leave a residue. The residue was crystallised from methanol to give (III), m.p. 112–113° (180 mg, 320 μCi) in 80% yield. This compound was radiochemically pure by radio-t.l.c. (silica gel plate KGF-Merck, solvent system : ethyl acetate — methanol = 2 : 1).

3-(1-Hydroxy-2-piperidinoethyl)-5-phenylisoxazole citrate (31252-S)-4-^{14}C (XVII)

A solution of 68 mg (0.324 mM) of citric acid monohydrate in 0.5 ml of ethanol was added to a solution of 168 mg (0.62 mM, 300 μCi) of (III) in 7 ml of absolute benzene, heated at 60° with stirring for 30 min., and left in a freezer for 2 days. The precipitated crystalline product was collected and recrystallised from ethanol–acetone to give (XVII), m.p. 143–145° (215 mg, 286 μCi ; specific activity : 490 μCi/mM) in 95.0% yield.

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