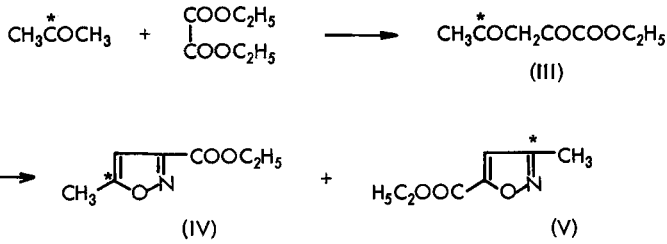
SYNTHESIS OF SOME ^{14}C-LABELLED ISOXAZOLE DERIVATIVES

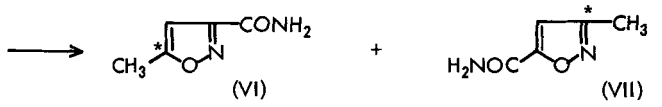
H. Minato, T. Nagasaki, and Y. Katsuyama
Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka, 553 Japan.
Received on March 25, 1975.

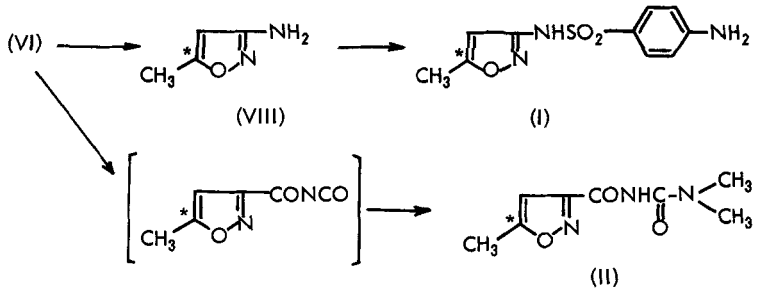
SUMMARY

Isoxazole derivatives, 3-sulfanilamido-5-methylisoxazole (I) and 3,3-dimethyl-1-(5-methyl-3-isoxazolylcarbonyl)-urea (II), have been labelled with carbon-14. The carbon-14 label was incorporated into the C-5 of the isoxazole ring to give (I) and (II) in 22.3% and 33.0% radiochemical yield based on acetone-^{14}C, respectively.

In the course of studies on chemistry and utilization of isoxazole derivatives in our laboratory, Kanō et al.[1] and Fujimoto et al.[2] had synthesised 3-sulfanilamido-5-methyl-isoxazole (sulfamethoxazole) (I) as a sulfa drug and 3,3-dimethyl-1-(5-methyl-3-isoxazolyl-carbonyl)-urea (II) as a diabetic agent, respectively. For pharmacological and action mechanism studies, it appeared to be desirable that the isoxazole ring of these agents is labelled with ^{14}C. Although the ^{14}C-labelling at C-4 of the isoxazole ring have been reported in the previous paper,[3] this communication describes the incorporation of ^{14}C into C-5 of the isoxazole ring of the molecule. The ^{14}C-labelled compounds were synthesised by the route demonstrated in the synthetic scheme.







Experimental

Ethyl acetopyruvate-4-^{14}C (III)

A mixture of 3 mCi of acetone-2-^{14}C (116 mg, 2 mM), 116 mg (2 mM) of carrier cold acetone [0.95 ml of a solution of acetone (123 mg) in ethanol (1 ml)], and 876 mg (6 mM) of ethyl oxalate [1.58 ml of a solution of ethyl oxalate (554 mg) in ethanol (1 ml)] was added dropwise to a solution of 138 mg (6 mM) of sodium in 4.6 ml of ethanol with stirring and stirred for 2 hours at room temperature. The precipitated sodium salt was collected and washed with ethanol (1 ml) and then ether (0.5 ml) to give 480 mg of a colourless powder of sodium salt of (III). This was dissolved in 10 ml of 10% H_2SO_4 and extracted with ether (10 ml x 3). The extract was washed with water, dried (Na_2SO_4), and evaporated to leave 1.94 mCi (421 mg) of (III) as a colourless mobile oil in 64.8% radiochemical yield.

3-Carboamino-5-methylisoxazole-5-^{14}C (VI)

A solution of 1.94 mCi of (III) (421 mg, 2.66 mM) and 240 mg (2.92 mM) of hydroxylamine-sulphate in 5 ml of ethanol was heated under reflux with stirring for 4 hours and then evaporated in vacuo to leave 630 mg of a residue (IV + V). To the residue was added 3 ml of conc. NH_4OH, and the mixture was stirred for 1.5 hours at room temperature.

The precipitates were collected by filtration and washed with water to give 144 mg of crystals (VI + VII). The filtrate and the washings were combined and extracted with chloroform-methanol (5 : 1) (10 ml x 3). The extract was washed with water, dried (Na_2SO_4), and evaporated to leave 85 mg of crystals (VI + VII).

The mixture (229 mg) of (VI) and (VII) was separated by preparative t.l.c. (silica gel KGF 254 plate, 20 cm x 20 cm x 750 μm; solvent system : benzene-ethyl acetate = 1 : 1) and extracted with chloroform-methanol (5 : 1) to give 1.23 mCi (213 mg) of (VI) as colourless plates, m.p. 172.5° (from water), R_F 0.36 in 63% yield (R_F value of (VII) is 0.17).

3,3-Dimethyl-1-(5-methyl-3-isoxazolylcarbonyl)-urea-^{14}C (II)

A solution of 239 mg (1.9 mM) of oxalyl chloride in 1.6 ml of absolute benzene was added to a solution of 1.23 mCi of (VI) (213 mg, 1.7 mM) in 3 ml of absolute benzene and heated gradually to 83° (bath temperature) for 20 min. The mixture was heated under reflux for 1.5 hours and then concentrated the volume up to a half at 83-85° (bath temperature) in vacuo. To this solution was added a solution of 242 mg (3.3 mM) of dimethylamine in 1 ml of absolute benzene with stirring at room temperature, and the mixture was stirred for 30 min. under sealed condition and evaporated to leave a residue. Ten % hydrochlotic acid was added to the residue to acidify with ice-cooling and the mixture was extracted with dichloromethane (10 ml x 3). The extract was washed with sodium chloride solution, dried (Na_2SO_4), and evaporated to leave 340 mg of residue. The residue was recrystallised from benzene-ether to give 184 mg of (II) as colourless prisms, m.p. 87-88° . The mother liquor of crystallisation was evaporated to leave 133 mg of a residue, which was purify by preparative t.l.c. (silica gel KGF 254 plate, 20 cm x 20 cm x 750 μm; solvent system : benzene-ethyl acetate = 1 : 1) to give 84 mg of (II), m.p. 87-88° , R_F 0.29. These products were combined and confirmed to be pure 3,3-dimethyl-1-(5-methyl-3-isoxazolcarbonyl)-urea-^{14}C (II) (268 mg, 985 μCi; specific activity, 0.73 mCi/mM) by t.l.c.-autoradiogram and t.l.c.-radioactinogram [X-ray film, silica gel KGF plate, solvent system = benzene-ethyl-acetate (1 : 1)] . Yield: 80.5% and 33.0% based on acetone.

3-Amino-5-methylisoxazole-5-^{14}C (VIII)

The acid amide (VI) (3.18 mCi; 428 mg, 3.4 mM) was added to a mixture of 260 mg (3.48 mM) of sodium hypochloride [1.91 ml of a solution of sodium hypochloride (136 mg) in water (1 ml)] and 139 mg (3.48 mM) of sodium hydroxide [2.16 ml of a solution of sodium hydroxide (64.4 mg) in water (1 ml)] with stirring in an ice-bath and stirred for 3 hours with ice-cooling. The mixture was heated for 10 min. in an oil-bath (bath temperature, 120°), and then 0.274 ml of a solution of sodium hydroxide (340 mg) in water (1 ml) was added. The mixture was heated under reflux for 1 hour and extracted with ether (6 ml x 3). The extract was washed with water, dried (Na_2SO_4), and evaporated to leave 266 mg of a crystalline residue (VIII), m.p. 65-66° (from benzene).

3-Sulfanilamido-5-methylisoxazole-^{14}C (I)

p-Acetylaminobenzenesuphonyl chloride (696 mg, 2.98 mM) was added to a solution of 266 mg (2.71 mM) of the above-mentioned amine (VIII) in 1.5 ml of pyridine in an ice-bath and stirred for 30 min. at room temperature. To this mixture was added 4 ml of water and 2 ml of 15% sodium hydroxide, and the mixture was heated at 80° to evaporate pyridine in vacuo. To the residue was added 4.5 ml of water and 2.5 ml of 15% sodium hydroxide, and the mixture was left for 45 min. at room temperature and then stirred for 30 min. in an oil-bath (bath temperature, 95-100°). The reaction mixture was adjusted to pH 4.5 by addition of 1.5 ml of 15% hydrochloric acid and 0.7 ml of 40% acetic acid. Soon after, crystals were separated from the solution and filtered. The collected crystalline product was washed with 2.5 ml of water and dried to give 629 mg of crude (I). The product was dissolved in benzene and chromatographed on 15 g of silica gel (Merck No. 60), and recrystallised from 80% methanol to give 3-sulfanilamide-5-methylisoxazole-^{14}C (I) as colourless plates, m.p. 169-170° (472 mg, 1.74 mCi; specific activity, 0.93 mCi/mM), which was confirmed to be pure compound by t.l.c.-autoradiogram and t.l.c.-radioactinogram [X-ray film, silica gel KGF plate, solvent system = benzene-ethylacetate (1 : 1)]. Yield: 54.7% based on the acid amide (VI).

References

1) Kanō, H and Ogata, K., Ann. Rept. Shionogi Res. Lab., 7 (1), 1 (1957); 8, 3 (1958); Nishimura, H., Nakajima, K., Okamoto, S., Shimaoka, N., and Sasaki, K., ibid., 8, 5 (1958); Minesita, T., Yamamoto, K., Takahashi, S., Hirota, R., Ishigami, T., Sekihara, S., and Muraoka, Y., ibid., 8, 17 (1958).

2) Fujimoto, M., Sumimoto, S., and Tochino, Y., Japanese Patent 686317 (47-37428); Sumimoto, S., Tochino, Y., and Fujimoto, M., ibid, 691581 (47-44735); Sumimoto, S., Fujimoto, M., and Tochino, Y., ibid., 691582 (47-44736).

3) Minato, H., Nagasaki, T., Yokoshima, T., Suga, K., and Yamaguchi, M., J. Label. Compounds, 10, (1974) in press.