SYNTHESIS OF 3-AMINO-1-METHYL-9H-[4-^{14}C]PYRIDO[3,4-b]INDOLE (3-AMINO[4-^{14}C]HARMAN)

Masao Tada, Kazunori Sato, and Atsushi Oikawa
Research Institute for Tuberculosis and Cancer, Tohoku University,
4-1 Seiryomachi, Sendai 980, Japan

SUMMARY

A seven-step synthesis of 3-amino-1-methyl-9H-[4-^{14}C]pyrido-[3,4-b]indole from DL-[methylene-^{14}C]tryptophan and acetaldehyde is described.

Key Words: 3-aminoharman, 3-amino-1-methyl-9H-[4-^{14}C]pyrido-[3,4-b]indole, DL-[methylene-^{14}C]tryptophan.

INTRODUCTION

9H-Pyrido[3,4-b]indole (norharman) and its 1-methyl derivative (harman) have been found to have synergistic or co-mutagenic effects in bacterial mutation.[1,2] The action of various amino 9H-pyrido[3,4-b]indoles on induction of sister-chromatid exchange in mammalian cells and their presence in L-tryptophan and protein pyrolysates have been reported.[3-6] In order to study biological actions further, we have prepared 3-amino-1-methyl-9H-pyrido-[3,4-b]indole labelled at C-4, a position that is unlikely to be lost during its metabolism. This paper describes the seven-step synthesis of 3-amino-1-methyl-9H-[4-^{14}C]pyrido[3,4-b]indole (1*) from DL-[methylene-^{14}C]tryptophan (2*).

0362-4803/87/060631-06$05.00

Received October 7, 1986
Revised October 31, 1986

RESULTS AND DISCUSSION

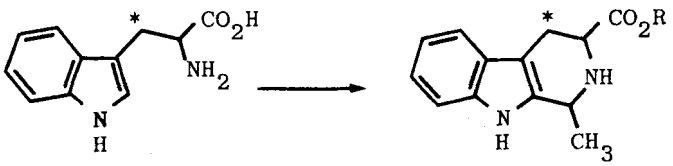
The method for the synthesis of unlabelled 3-amino-1-methyl-9H-pyrido[3,4-b]indole (1) has been described by Snyder et al.,[7,8] but is impractical for the preparation of the ^{14}C-labelled compound. Preparation of (1*) by a modified procedure minimized loss of the expensive radioactive starting material (2*).

DL-Tryptophan (2) was treated with acetaldehyde under acidic condition to afford 1-methyl-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]-indole-3-carboxylic acid (3) in an 88% yield. When the resulting condensation mixture was evaporated to dryness without filtration, the residue afforded crude (3) which was converted to the methyl ester (4) with methanol in the presence of sulfuric acid. The ester (4) was then dehydrogenated with sulfur to yield methyl 1-methyl-9H-pyrido[3,4-b]indole-3-carboxylate (5). Compound (5) was converted into azide (6) via the hydrazide (7) and the azide (6) was heated with n-pentanol in xylene to give the pentyl carbamate (8).[9]

Alkaline hydrolysis of (8) afforded 3-amino-1-methyl-9H-pyrido[3,4-b]indole (1) in an 18.1% overall yield from (2).[4,8] A Lobar column gave satisfactory results in purification of (1). The procedure was reproducible on a scale ranging from 1 g to 100 mg of (2).

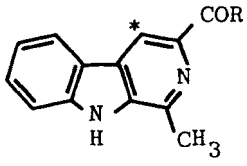
The radiolabelled synthesis carried out as above gave 3-amino-1-methyl-9H-[4-^{14}C]pyrido[3,4-b]indole (1*) with a specific activity 0.93 mCi/mmol. The radiochemical yield was 17.7% and radiochemical purity ca. 92%.

The detailed effect of (1) in the induction of sister-chromatid exchanges have been currently investigated and the results will be reported elsewhere.

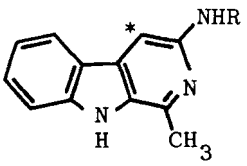


2

3: R=H
4: R=CH_3



5: R=OCH_3
7: R=$NHNH_2$
6: R=N_3



8: R=$CO_2C_5H_{11}$
1: R=H

* indicates ^{14}C-label.

EXPERIMENTAL

DL-[methylene-^{14}C]Tryptophan (54 mCi/mmol) was purchased from Amersham International plc, Buckinghamshire, England. Column and thin-layer chromatographs were run on a Merk Lobar LiChroprep RP 8 column (25 x 310 mm), and Merk pre-coated silica gel 60 F_{254} plates (5 x 20 cm) and pre-coated RP 8 plates (5 x 10 cm), respectively.

3-Amino-1-methyl-9H-[4-^{14}C]pyrido[3,4-b]indole (1*). DL-[methylene-^{14}C]Tryptophan (2*) (2 mg) was supplied in aqueous solution containing 2% ethanol which was evaporated to dryness under a reduced pressure. To the residue, DL-tryptopan (2) (100 mg) was added as a carrier. To a cold suspension of the mixture in 0.1 N sulfuric acid (0.4 ml) and water (1 ml), was added acetaldehyde (0.25 ml) . The mixture was stirred at room temperature for 24 hr, filtered, washed with cold water, and dried (P_2O_5) to give 99.2 mg (88%) of (3*).[7] To a suspension of (3*) (99 mg)

in methanol (3 ml), conc. sulfuric acid (0.15 ml) was added and the mixture was heated under reflux for 12 hr. After addition of ice-water, the resulting mixture was neutralized with sodium hydrogen carbonate and extracted with ethyl acetate (150 ml). The extract was washed with water and dried over magnesium sulfate to afford the ester (4*) (85 mg). A mixture of (4*) (85 mg) and sulfur (26 mg) in xylene (2 ml; dried with "Dry Soda") was heated at 160 °C for 6 hr and the solution evaporated to dryness under a reduced pressure. The residue was extracted with dichloromethane/methanol (5:1 v/v; 200ml) and the extract was evaporated to dryness to give crude (5*). A mixture (2 ml) of hydrazine hydrate, n-pentanol, ethanol, and water (11:25:7:1 by volume), was added to the crude (5*) and heated under reflux for 5 hr. The solution was evaporated under a reduced pressure to give the hydrazide (7*). Sodium nitrite (40 mg) in water (0.8 ml) was added to (7*) in conc. hydrochloric acid (0.1 ml) and water (4 ml) under cooling with ice-water. The resulting mixture was stirred at 0 °C for 30 min, neutralized with saturated sodium hydrogen carbonate solution and extracted with ethyl acetate/ethanol (9:1 v/v; 200 ml). The extract was washed with water and dried over magnesium sulfate to give the azide (6*). A mixture (6*) in xylene (1 ml; dried with "Dry Soda") and n-pentanol (0.1 ml; dried with molecular sieve 3A) was heated at 130 °C for 30 min, and then evaporated to dryness under a reduced pressure to afford crude n-pentyl carbamate (8*) (76.2 mg).[9] The crude (8*) was suspended in a mixture of potassium hydroxide (20 mg), n-butanol (0.2 ml), and water (0.02 ml), heated under reflux for 3 hr, and then poured into ice-water, and made neutral with sodium hydrogen carbonate. The resulting solution was then extracted with ethyl acetate/ethanol (9:1 v/v; 150 ml), washed with water, and dried over magnesium sulfate. After removal of the solvent, the residue was dissolved

in acetonitrile/methanol(85:15v/v, 2ml) and chromatographed over a Robar column to give 17.5 mg (18.1% based on 2*) of the amino compound (1*). Its retention time is 32 min. The chromatographic conditions were as follows: Eluent, flow rate, and detector are acetonitrile/methanol (85:15 v/v), 3.8 ml/min, and UV (254 nm), respectively. The product proved identical with an authentic sample.[8]

Radio-TLC of (1*) were carried out over a pre-coated silica gel 60 F_{254} plate and a pre-coated RP 8 plate to afford R_f 0.26 and 0.31, respectively. Their mobile phases were dichloromethane/methanol (4:1 v/v) and acetonitrile/methanol (85:15 v/v), respectively. The detection was made with a TLC scanner II (Berthold) and a UV lamp. The radioactivity of (1*) was obtained by measurement with a Beckman Model LS 1801 scintillation spectrometer. The radiochemical yield and purity and specific activity are 17.7%, ca. 92%, and 0.93 mCi/mmol (4.7 μCi/mg), respectively.

ACKNOWLEDGEMENT

The present work was partially supported by Grants-in-Aid for Cancer Research from the Ministry of Education, Science, and Culture, Japan.

REFERENCES

1. Matsumoto T., Yoshida D., and Mizusaki S.- Mutat. Res. 56: 85 (1977)
2. Nagao M., Yahagi T., and Sugimura T.- Biochem. Biophys. Res. Commun. 83: 373 (1978)
3. Tohda H., Tada M., Sugawara R., and Oikawa A.- Mutat. Res. 116: 137 (1983)
4. Tada M., Saeki H., and Oikawa A.- Bull. Chem. Soc. Japan 56: 1450 (1983)

5. Tada M., Saeki H., and Oikawa A.- Mutat. Res. 121: 81 (1983)

6. Tohda H., Sugawara R., Tazawa J., Tada M., and Oikawa A. - Mutat. Res. 129: 63 (1984)

7. Snyder H. R., Hansch C. H., Katz L., Parmerter S. M., and Spaeth E. C.- J. Amer. Chem. Soc. 70: 219 (1948)

8. Snyder H. R., Parmerter S. M., and Walker H. G.- J. Amer. Chem. Soc. 70: 237 (1948)

9. As easy removal of an excess of n-pentanol from the reactant mixture, n-pentanol instead of benzyl alcohol[8] was used in the rearrangement reaction. Compound (8) was obtained as an oily substance, chromatographically pure.