THE PREPARATION OF 3-ACETAMIDO-6-METHYL-8-N-PROPYL -S-[3- ^{14}C] TRIAZOLO-[4, 3-a] PYRAZINE [I.C.I. 58,301]

D.F. White and J. Burns
Imperial Chemical Industries Limited, Pharmaceuticals Division,
Alderley Park, Macclesfield, Cheshire, SK10 4TG, England.
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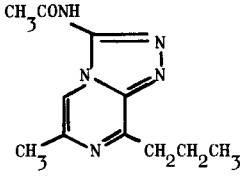
SUMMARY

The preparation of 3-acetamido-6-methyl-8-n-propyl-s-[3-^{14}C] triazolo-[4,3-a] pyrazine [I.C.I. 58,301] from sodium [^{14}C] cyanide in three stages is described. The overall radiochemical yield of product, at a specific activity of 3.6 μCi/mg, was 26.0%.

INTRODUCTION

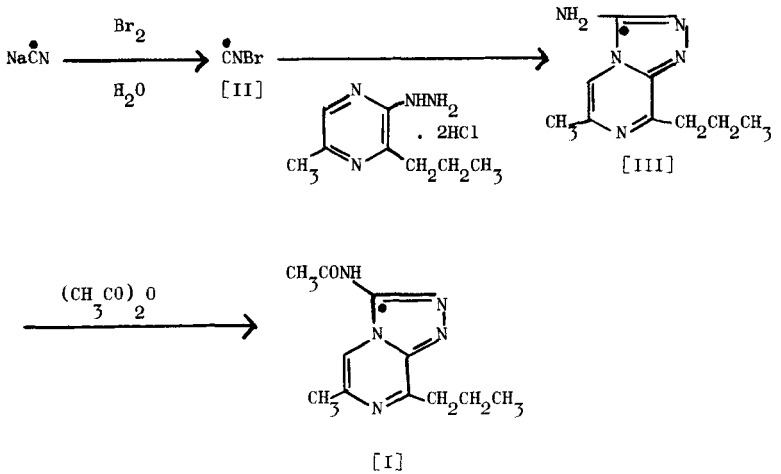
In the course of investigations into the action of various compounds as possible drugs for the treatment of asthma and related conditions, it was necessary to prepare a [^{14}C]-labelled form of 3-acetamido-6-methyl-8-n-propyl-s-triazolo-[4,3-a] pyrazine [I] (I.C.I. 58,301) [1] for a study of its metabolism in man, [2] and for metabolic and distributive studies including whole body autoradiography in animals. [3] The blood levels and spirometry of I.C.I. 58,301 in asthmatic patients, [4] its antibronchoconstrictor activity [5] and anti-anaphylactic effects [6] in various animal species have also been reported.



I.C.I. 58,301

[I]

The [^{14}C] material was prepared by the route indicated in the scheme below:



I.C.I. 58,301

SCHEME

MATERIALS

Sulphur free toluene (May and Baker Ltd.) was used without further purification. The 2,5-diphenyloxazole (PPO) and 1,4-bis(4-methyl-5-phenyloxazole) benzene (DMPOPOP) were purchased from Packard Instrument Ltd., Wembley; naphthalene (scintillation grade) was obtained from Thorn Electronics Ltd., Tolworth. Dioxan was purified by the method of Vogel. (7) Colloidal silica (Aerosil) was obtained from Buch, Beach, Segner Bayley Ltd. The sodium [^{14}C] cyanide was purchased from the Radiochemical Centre, Amersham.

All samples were counted on a Packard Tri-Carb Liquid Scintillation spectrometer Model 314; the sample containers were standard 20 ml glass screw-cap vials of low potassium content (Packard Instrument Ltd., Wembley). The photographic film used for autoradiography was 'Ilfex' X-ray film (Ilford Ltd., Essex, England). Merck Silica G.F. was obtained from Andermanns Ltd. All solvents used were either redistilled or of analytical reagent quality.

The solvent systems used for chromatography were as follows:-

(A) ethanol-cyclohexane (60 : 40.)

(B) toluene-ethyl acetate-ammonia (0.880) - ethanol (60 : 20 : 10 : 40.)

(C) chloroform - methanol (90 : 10.)

EXPERIMENTAL

^{14}C-cyanogen bromide [II]

Sodium [^{14}C] cyanide (2.72 mg) with a specific activity of 56.2 mCi/m mole was diluted with inactive sodium cyanide (149.28 mg) and dissolved in water (1.5 ml). The solution was added over 10 minutes to a mixture of bromine (445 mg) and water (0.1 ml) stirred in an ice bath. During the addition the internal temperature was kept below 10°C. The reaction mixture was stirred at 0 - 5°C for 2 hours.

3-amino-6-methyl-8-n-propyl-s-[3-^{14}C] triazolo-[4,3-a] pyrazine [III]

Sodium hydrogen carbonate (970 mg) was added to a stirred solution of 2-hydrazino-5-methyl-3-n-propylpyrazine dihydrochloride (540 mg) in water (3.8 ml). The solution was cooled in an ice bath, iso-butanol (3.5 ml) was added, and the reactants stirred at 0 - 10°C for 30 minutes. The mixture was added dropwise to the crude preparation of [^{14}C]-cyanogen bromide and stirred at 0 - 10°C for 1 hour. The whole reaction product was then evaporated to dryness under reduced pressure from a water bath at 25°C.

The residue was extracted with boiling iso-butanol (5 X 10.0 ml), and the combined extracts evaporated to dryness under reduced pressure at 40°C, to give a brown solid.

The solid was examined by thin layer chromatography (T.L.C.) using a Merck Silica G.F. plate developed with solvent system (A). The plate was examined under UV 366 nm and autoradiographed for 16 hr. Comparison of the UV and autoradiographic patterns showed that all the impurities were labelled.

A column (2.5 cm diameter) containing Merck Silica GF [20 g] was prepared and eluted with solvent system (A). The crude product was dissolved in the mobile phase (4.0 ml), and applied to the column; 50 X 2.0 ml fractions were collected, and 5 µl aliquots of alternate fractions were spotted on a Merck Silica GF plate and the plate developed with solvent system (A). The plate was run for 10 cm, dried, examined under UV 366 nm, and autoradiographed for 16 hr. The appropriate fractions containing one spot material with identical Rf to that of the pure reference compound were combined and evaporated to dryness under reduced pressure from a water bath at 30°C. Traces of silica were removed by the centrifugation of methanol extracts. Removal of the solvent under reduced pressure (bath at 30°C) gave a cream-coloured solid (420 mg).

This material was chromatographed on a Merck Silica GF plate developed with solvent system (A). The plate was examined under UV 366 nm and autoradiographed for 16 hr. An identical chromatographic pattern was shown by both means of detection. The combined fractions, on chromatographic examination, contained one radiochemically pure component.

3-acetamido-6-methyl-8-n-propyl-s-[3-^{14}C] triazolo-[4,3-a] pyrazine (I.C.I. 58,301) [I]

A mixture of 3-amino-6-methyl-8-n-propyl-s-[3-^{14}C] triazolo-[4,3-a] pyrazine (420 mg) and acetic anhydride (1.5 ml) was heated at 95 - 100°C for 1 hr. The mixture was cooled to room temperature, methanol (3.0 ml) was added, and the suspension stirred at room temperature for 30 min. The product was filtered off, washed with methanol and dried at 30 - 40°C to give a white powder (281 mg). The product was examined by T.L.C. on a Merck Silica GF plate developed with solvent system (B), examined under UV 366 nm, and autoradiographed for 16 hr. Comparison of UV and autoradiographic patterns showed that the one detectable impurity was labelled.

The product was recrystallised from n-butanol and dried at 35 - 40°C under reduced pressure to give I.C.I. 58,301 as a white powder (200 mg) (Found C, 56.8; H, 6.3; N, 30.0. $C_{11}H_{15}ON_5$ requires C, 56.7; H, 6.4; N, 30.0), representing an overall chemical yield of 30.4%.

The product was examined by T.L.C. in solvent systems (B) and (C) on Merck Silica GF plates and the plates autoradiographed for 16 hr. The autoradiographs were used to "map" the plates which were then segmented. Scintillation counting of the segmented plates indicated a minimum radiochemical purity of 99.6%. The specific activity was 3.6 μCi/mg (839 μCi/m mole) which represented an overall radiochemical yield of 26.0%.

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