Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXIV, No. 11

SYNTHESIS OF DL-[7-^{14}C]INDOSPICINE AND DL-2-AMINO[7-^{14}C]PIMELIC ACID

Elzbieta Wieczorkowska* and Mervyn P. Hegarty
Division of Tropical Crops and Pastures, CSIRO
Cunningham Laboratory, 306 Carmody Road, St. Lucia,
Qld. 4067, Australia.

*Chemistry Department, Royal Veterinary and
Agricultural University, 40 Thorvaldsensvej,
DK-1871 Frederiksberg C, Denmark.

SUMMARY

A new method of synthesis of DL-[7-^{14}C]indospicine and DL-2-amino-[7-^{14}C]pimelic acid and their nonradioactive equivalents is reported. The combined radiochemical yield of both compounds, starting from potassium [^{14}C]cyanide was 15.3%. Indospicine was isolated as the flavianate.

Key Words: Indospicine, 6-amidino-2-aminohexanoic acid, [7-^{14}C]-indospicine, 2-amino[7-^{14}C]pimelic acid, non-protein amino acids.

INTRODUCTION

Indospicine (6-amidino-2-aminohexanoic acid), a non-protein amino acid that occurs free in some species of the legume Indigofera, is a potent arginine antimetabolite and is hepatotoxic and teratogenic to mammals (1,2). Unfortunately no information is available on the biosynthesis of this unusual amidino amino acid, nor is it known if or how it contributes to the intermediary nitrogen metabolism of the producer plant. To gain further insight into the mechanism of action and metabolic fate of indospicine in biological systems, ^{14}C-labelled indospicine was synthesized.

*To whom correspondence should be addressed.

0362-4803/87/111273–08$05.00

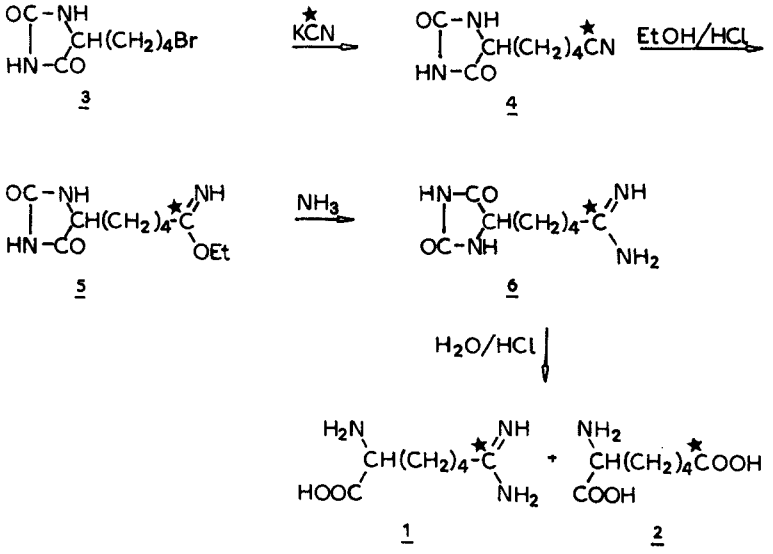
Received September 15, 1986
Revised October 13, 1986

Simultaneously ^{14}C-labelled 2-aminopimelic acid was obtained. This amino acid occurs in some ferns (3) and a ^{14}C-labelled isotopomer will be useful in metabolic studies.

RESULTS AND DISCUSSION

Published methods (4,5) for the synthesis of indospicine 1 are lengthy and cumbersome as they started with 6-hydroxynorleucine and involved a number of steps associated with the introduction and removal of protecting groups. We describe here a convenient four step synthesis (Scheme 1) of DL-indospicine 1 and DL-[7-^{14}C]-indospicine 1a. The synthesis also yielded DL-2-amino[7-^{14}C]pimelic acid 2a. An important feature of the method is protecting groups are not needed because the amino acid is formed in the last step by controlled acid hydrolysis of 6. Only partial loss of the amidine function occurs under these conditions.

Scheme 1



3 4 5 6 1 2

★ Indicates position of ^{14}C-label

5-(4-Cyanobutyl)hydantoin 4 was prepared from 5-(4-bromobutyl) hydantoin 3 (5-(4-tosyloxybutyl)hydantoin can also be used). The cyanide 4 was converted into the amidine 6 by the Pinner method (6) i.e. treatment of the cyanide 4 with ethanol and hydrogen chloride to give the imidate ester 5 followed by treatment of the crude 5 with ammonia.

The hydrolysis of 6 in hydrochloric acid was detailed investigated. The results are presented in Table 1.

Table 1

Molarity of HCl	Temp. ^{o}C	Time hr	Indospicine Yield %	2-aminopimelic acid Yield %
3	reflux	24	28.7	30.4
6	90	46	27.0	5.4
6	90	120	35.0	26.0
6	90	288	16.0	37.8
6	reflux	6	28.3	8.1
6	reflux	24	35.8	48.4
6	reflux	48	9.6	71.9
6	122-125 sealed tube	30	2-5	75-90

The indospicine 1 and 2-aminopimelic acid 2 were readily separated by ion-exchange chromatography and isolated as flavianate of 1 and free amino acid 2. [7-^{14}C]Indospicine 1a and 2-amino-[7-^{14}C]pimelic acid 2a was synthesized in the same way using [^{14}C]-KCN. The crude 5-(4-[^{14}C-cyano]butyl)hydantoin 4a was diluted with unlabelled material 4 and converted without purification into 6a. The radiochemical overall yield of 1a and 2a from [^{14}C]KCN was 15.3% (6.8% for 1a and 8.5% for 2a).

EXPERIMENTAL

General Chemical Methods

Quantitative amino acid analyses were carried out with a Beckman Multichrom B, amino acid analyser, on a 17.5 cm column of cation-exchange resin Type M 71. Thin-layer chromatograms were run on silica gel 60 layers (Merck), chloroform-ethanol 85:15 was used as solvent. The thin-layer sheets were exposed to chlorine gas and the colour developed by spraying with starch-potassium iodide reagent (7). Paper chromatograms were run on Whatman Chromatography Paper No. 1 in butanol-acetic acid-water (12:3:5).

^{1}H NMR spectra were used on a Jeol FX 90Q instrument, Animal Health Research Laboratory CSIRO, Parkville, Victoria. Chemical shifts are given in ppm downfield from TMS in deuterio-DMSO. Infrared spectra were recorded on a Perkin-Elmer 157G spectrophotometer. Microanalyses were performed by Microanalytical Service, Department of Chemistry, University of Queensland.

Melting points are uncorrected. Specific activities were determined by weighing and measurement of radioactivity by liquid scintillation counting on a Packard 4000-instrument. Counting efficiency was determined by use of an external standard. [^{14}C]KCN was supplied by Amersham International plc, England. 5-(4-Bromobutyl)hydantoin was prepared from dihydropyran by the method of Gaudry (8).

5-(4[^{14}C-amidino]butyl)hydantoin hydrochloride 6a

Crude 5-(4-[^{14}C-cyano]butyl)hydantoin 4a was prepared from potassium [^{14}C]cyanide (30.4 mg, 3 mCi) and 5-(4-bromobutyl)hydantoin 3 (117.5 mg, 0.5 mmol) in water (0.25 ml) and ethanol (1 ml) by refluxing for 5 hrs.

The solvent was removed under reduced pressure and to the residue acetone (10 ml) and 6 M HCl (0.05 ml) were added.The precipitate of potassium salts was removed by filtration and the un-

labelled cyanide 4 (429 mg, m.p. 98-99°C) was added to the solution. The acetone was evaporated to give pale yellow crystals from which traces of water were removed azeotropically with benzene. Dry chloroform (8 ml) and absolute ethanol (0.175 ml) were added, the reaction mixture cooled to -3° and saturated with hydrogen chloride. After standing at 0° for 18 hr hydrogen chloride and chloroform were removed, the residue dissolved at 0° in ethanol (10 ml) saturated with ammonia, and stirred at 0° for 3 hr. Yield of 5-(4-[^{14}C-amidino]butyl)hydantoin hydrochloride 6a as colourless crystals 504 mg, spec. act. 1.76 μCi/mg. Radiochemical yield 29.6%.

DL-[7-^{14}C]Indospicine 1a and DL-2-amino[7-^{14}C]pimelic acid 2a

6a (314 mg, 1.34 mmol, 552.6 μCi) was hydrolysed by gently refluxing with 6 M HCl (30 ml) for 24 hr. The reaction mixture was evaporated to dryness at 40°. The residue was taken up in water and applied to a column of Dowex 1 x 4 (200-400 mesh, acetate form) and then washed with water until the effluent gave a negative ninhydrin reaction. Analysis of water effluent (amino acid analyser, sodium citrate buffer, pH 4.69, 0.55 M) showed the presence of indospicine (retention time 81 min.). Yield 0.42 mmol, 31.3%. The DL-2-amino[7-^{14}C]pimelic acid was eluted from the column with 1 M acetic acid. Analysis of the acetic acid eluate (amino acid analyser, sodium citrate buffer, pH 3.30, 0.35 M) showed presence of 2a (retention time 45 min.). Yield 0.58 mmol, 43.3%.

Isolation of DL-[7-^{14}C]indospicine as flavianate

The water effluent was evaporated to dryness, taken up in (2 ml) and flavianic acid (500 mg) in boiling water (18 ml) was added. The solution was refluxed for 10 min, filtered and allowed to cool. Monoflavianate of 1a, precipitated as orange plates was isolated by filtration and washed successively with cold water and ethanol. Yield 153 mg. 0.31 mmol, spec. act. 0.833 μCi/mg. Radio-

chemical yield from potassium [^{14}C]cyanide 6.8%. The radiochemical purity was established by use of amino acid analyser and paper chromatography to be greater than 99%. The flavianate (19.0 mg, 15.8 μCi) was converted to the acetate by stirring the hot solution with excess of Dowex 1x4 (200-400 mesh, acetate form). The resin was filtered off and the filtrate was applied to the small column of the same resin. Analysis of the water effluent showed the presence of 6.73 mg of indospicine. Spec. act. 2.32 μCi/mg. Yield 99%.

Isolation of DL-2-amino[7-^{14}C]pimelic acid 1a

The acetic acid eluate was evaporated to dryness and the last traces of acetic acid were removed by repeated evaporation with water. After recrystallisation of the residue from aqueous ethanol 71 mg of pure 2a, spec. act. 2.25 μCi/mg was obtained. Radiochemical yield from 6a - 30.3%, overall radiochemical yield from potassium [^{14}C]cyanide - 8.5%. The radiochemical purity established by use of amino acid analyser and paper chromatography was 99%.

5-(4-Cyanobutyl)hydantoin 4

4 was prepared as described above from 5-(4-bromobutyl)hydantoin 3 (23.5 g, 0.1 mol), potassium cyanide (8 g, 0.12 mol), water (30 ml) and ethanol (80 ml), by refluxing for 5 hrs. The solvents were removed under reduced pressure. To the residue, methanol (100 ml) was added and potassium bromide collected by filtration. The filtrate was evaporated, the residue acidified with hydrochloric acid and continuously extracted with ethylacetate for 4 hr. The extract was washed with water, dried over magnesium sulfate and evaporated to dryness, to give yellow thick oil (11.6 g) which crystallized on standing in the refrigerator. Recrystallisation from acetone-toluene yielded 3.8 g (25%) of 4 m.p. 85-90°. Thin-layer chromatography showed a major spot Rf 0.61 and traces of impurities: Rf 0.29 (5-(4-hydroxybutyl)hydantoin); Rf 0.47 (unidentified); Rf 0.71 (5-(4-bromobutyl)hydantoin); Rf 0.83 (unidentified). Pure 4

was obtained by repeated recrystallisation from ethylacetate. Colourless crystals m.p. 101-2°. Rf 0.61. IR max, cm^{-1} 2280 (CN). ^{1}H NMR: 1.2-1.7 (6H); 2.38 (2H); 3.98 (H); 7.95 (H); 10.6 (H). Calcd. for $C_8H_{11}N_3O_2$: C, 53.02; H, 6.11; N, 23.19; Found: C, 52.73; H, 6.31; N, 22.96.

5-(4-Etoxycarboximidobutyl)hydantoin hydrochloride 5 and 5-(4-amidinobutyl)hydantoin hydrochloride 6

A flask containing 1.81 g (0.01 mol) of 4 in 30 ml of dry chloroform and 0.7 ml (0.012) mol of absolute ethanol was cooled to -3° while dry hydrogen chloride saturated the reaction mixture. The contents were allowed to stand at 0° for 18 hrs and excess of hydrogen chloride and chloroform were removed under reduced pressure to give pale yellow crystalline mass of 5. The latter was dissolved at 0° in 40 ml ethanol saturated with ammonia and stirred at 0° for three hours. The amidine hydrochloride 6 separated as colourless crystals, m.p. 162-165, yield 1.75 g (74.6%). After recrystallisation from water-ethanol (1.5:8.5) m.p. 171-2°. ^{1}H NMR: 1.2-1.8 (6H).2.32 (2H); 3.87 (H); 6.2 (b.- removed by shaking with D_2O). Calcd. for $C_8H_{15}N_4O_2Cl$: C, 40.94; H, 6.44; N, 23.87. Found: C, 41.47; H,6.74; N, 24.08.

DL-Indospicine 1 and DL-2-aminopimelic acid 2

The crude 6 (940 mg, 4.0 mmol) was gently refluxed with 6 M HCL (20 ml) for 24 hrs. The hydrolysate was evaporated to dryness. The residue was applied to a column of Dowex 1x4 (200-400 mesh, acetate form) as described above. Analysis of water effluent (amino acid analyser) showed the presence of 1.42 mmol of indospicine, subsequently isolated as flavianate. Yield 542 mg (1.11 mmol). The contents of indospicine 1.09 mmol (98% purity). Anal: Calcd. for $C_{17}H_{21}N_5O_{10}S$: C, 41.88; H, 4.34; N, 14.37. Found: C, 42.23; H, 4.58; N, 14.21. The monoflavianate can be quantitatively converted to the acetate as described above.

The synthetic DL-isomer and natural L-isomer had identical Rf values on paper chromatography and identical retention times in the amino acid analyser.

The 2-aminopimelic acid 2 was eluted from the column with 1 M acetic acid. Analysis of acetic acid eluate (amino acid analyser) showed presence of 2. Yield 1.64 mmol, 41%. After evaporation and recrystallisation of the residue from aqueous ethanol 179 mg of pure 2 was obtained. It was identical (m.p. and mixed m.p., IR spectrum, behaviour in amino acid analyser and on paper chromatography with commercial DL-2-aminopimelic acid.

ACKNOWLEDGEMENT

We thank Mr. Graham Simpson for skilful technical assistance. We are grateful to Professor, Dr. Peder Olesen Larsen and Dr. Jan Wieczorkowski for a critical revision of the manuscript.

Support from the Danish Natural Science Research Council (to E.W.) is acknowledged.

REFERENCES

1. Hegarty, M.P. and Pound, A.W. - Aust.J.Biol.Sci. 23, 831 (1970).
2. Hegarty, M.P. -In "Effects of Poisonous Plants on Livestock", R.F. Keeler, K. van Kampen and L.F. James (eds.). - Academic Press, New York, pp. 575-585 (1978).
3. Murakami, N. and Hatanaka, S.-I. - Phytochemistry 22, 2735 (1983).
4. Culvenor, C.C.J., Foster, M.C. and Hegarty, M.P. - Aust.J.Chem 24, 371 (1971).
5. Fujioka, T., Satoh, T., Tanizawa, K. and Kanaoka, Y. - J.Biochem. 87, 1229 (1980).
6. Pinner, A. - "Die Imidoather und ihre Derivate". Oppenheimer, Berlin, 1892.
7. Rydon, H.N. and Smith, P.W.G. - Nature 169, 922 (1952).
8. Gaudry, R. - Can. J. Res. (B). 26, 387 (1948).