THE PREPARATION AND PURIFICATION OF ETHYL 6-ETHYL-4-HYDROXY [3-^{14}C]CINNOL-3-YL CARBOXYLATE (ICI 75,186)

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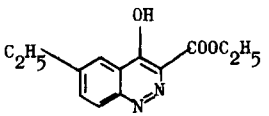
SUMMARY

The preparation of ethyl 6-ethyl-4-hydroxy[3-^{14}C]cinnol-3-yl carboxylate (ICI 75,186) from diethyl[2-^{14}C]malonate in five stages is described, together with the purification procedures involved. The overall radiochemical yield, at a specific activity of 3.3 μCi/mg, was 10%.

INTRODUCTION

In the course of investigations into the actions of various anti-asthmatic agents it was necessary to prepare a [^{14}C]-labelled form of ethyl 6-ethyl-4-hydroxy-cinnol-3-yl carboxylate (I), hereinafter referred to as ICI 75,186, for metabolic studies. The results of these metabolic studies will be reported elsewhere.[1]



ICI 75,186
(I)

The preparation of substituted 4-hydroxy-cinnol-3-yl carboxylic acids has been reported by Barber et al.[2], and the preparation of the esters, including ICI 75,186, by Gilman[3]. The method of choice for this synthesis of the labelled form of ICI 75,186 was essentially that of Gilman.

DISCUSSION

p-Ethyl aniline (1 m mole) was diazotised and coupled onto diethyl[2-^{14}C]malonate to give the hydrazone (II) which was hydrolysed with sodium hydroxide in aqueous ethanol. The resulting di-acid (III) was converted to the di-acid chloride (IV) by heating with thionyl chloride in toluene.

Various methods of cyclisation were explored in preliminary attempts to prepare the cinnoline (V), including:

(a) reaction of the di-ester (II) with phosphorus oxychloride,
(b) reaction of the di-acid (III) with polyphosphoric acid,
(c) reaction of the di-acid (III) with polyphosphoric ester[4],
(d) reaction of the di-ester (II) with polyphosphoric ester,
(e) reaction of the di-acid chloride (IV) with titanium tetrachloride in chlorobenzene.

Of these routes the last gave the best yield. It was also found that cyclisation would only take place when the acid chloride was pure. Thus, when the reaction mixture giving (IV) was evaporated to dryness, impure (IV) resulted and this would not undergo cyclisation. When, however, the reaction mixture was kept fairly concentrated, cooled to 0° and diluted with light petroleum (b.p. 40 - 60°), then (IV) crystallised out in a much purer form with only a slight loss in yield. This material was of sufficiently high purity to allow cyclisation to occur. Therefore, as shown in Fig. 1, the di-acid chloride (IV) was converted into the cinnol-3-yl acid (V) with titanium tetrachloride in chlorobenzene.

A second preparation of the acid (V) was carried out on the same scale. For convenience the two products were combined and esterified by passing dry hydrogen chloride gas through a boiling solution of (V) in absolute alcohol.

The crude ICI 75,186 (I) was purified by preparative-scale thin-layer

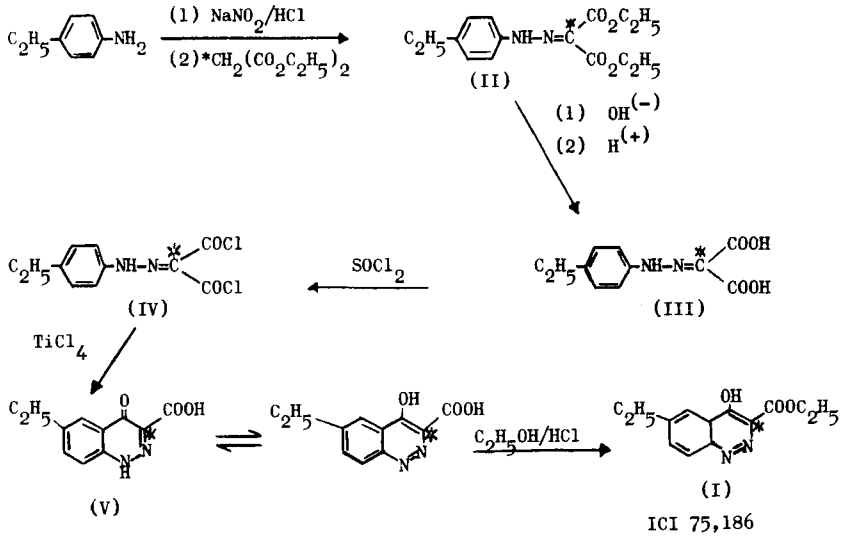


Figure 1

chromatography (p.t.l.c.) using Silica Gel GF as the adsorbent and mixtures of chloroform and absolute alcohol as eluents. In these laboratories the work up of p.t.l.c. separations usually involves removal of the band containing the required product, extraction from the silica with methanol, evaporation to dryness and finally removal of traces of silica by redissolving the product in a minimum volume of absolute alcohol followed by centrifugation. However, in this case, two properties of ICI 75,186 came to light which mitigated against this procedure - (a) the relative lability of the ester grouping, and (b) the tendency for the compound to undergo a morphological change resulting in a form which was much less soluble in ethanol. These factors necessitated two alterations to the normal procedure. Firstly, methanol could not be used at any stage as this would have resulted in transesterification, generating some of the methyl ester, as indeed it had done during a previous non-labelled synthesis. Secondly, the change in crystalline form made it extremely difficult to remove the last traces of silica and resulted in a relatively low recovery of the product, thus reducing the overall yield of ICI 75,186.

MATERIALS

Sulphur free toluene (May and Baker Ltd.) and absolute alcohol (B.P. Ltd.) were used without further purification. Chlorobenzene (B.D.H. Ltd.) was redistilled and stored until required over Molecular Sieve Type 4A (B.D.H. Ltd.). All other solvents used were of general laboratory grade. Titanium tetrachloride (B.D.H. Ltd.) was redistilled prior to use. Merck Silica Gel GF was obtained from Andermanns Ltd. and 2-(4'-t-butylphenyl)-5-(4"-biphenylyl)-1,3,4-oxadiazole (Butyl-PBD) from Koch-Light Laboratories Ltd.

Diethyl[2-^{14}C]malonate was purchased from the Radiochemical Centre, Amersham.

All samples were counted on a Packard Tri Carb Liquid Scintillation Spectrometer Model 3320 in standard 20 ml glass screw cap vials of low potassium content (Packard Instruments Ltd.). The photographic film used for autoradiography was Kodak "Kodirex" X-ray film.

The solvent systems used for chromatography were as follows:

A	chloroform-absolute alcohol	(85 : 15)
B	chloroform-absolute alcohol	(97 : 3)
C	light petroleum (b.p. 60-80)-acetone	(60 : 40)
D	chloroform-absolute alcohol-formic acid	(85 : 15 : 1)
E	toluene-ethyl acetate-absolute alcohol-ammonia (density 0.880)	(60 : 20 : 35 : 10)

EXPERIMENTAL

Diethyl[2-^{14}C]mesoxalate 4'-ethylphenyl hydrazone (II)

p-Ethyl aniline (121 mg; 1 m mole) was stirred at room temperature for 1 hour in a mixture of hydrochloric acid (0.25 ml of 36%) and water (0.15 ml) in order to form the hydrochloride. To the resultant suspension, stirred and cooled to -5 to -10°, a solution of sodium nitrite (73 mg; 1.05 m mole) in water (0.4 ml) was added over 45 min. The solution of the diazonium salt was stirred for a further 30 min at -5 to -10°. Meanwhile, diethyl [2-^{14}C] malonate (1.0 mCi) of specific activity 16.7 m Ci/m mole was iso-

topically diluted with inactive diethyl malonate (total weight 161 mg; 1 m mole) and added to a suspension of anhydrous sodium acetate (188 mg) in ethanol (0.6 ml) and water (0.1 ml). The diazonium chloride solution was added to this stirred suspension over 1 hour, the reagents and reaction mixture being kept at -5 to 0°. The mixture was then stirred at 0° for 1½ hours, allowed to warm to room temperature, stirred for a further 4 hours and the product extracted with toluene (5 X 10.0 ml). After being washed with water (2 X 5.0 ml) the extracts were dried over anhydrous magnesium sulphate and evaporated to dryness under reduced pressure to give (II) (238 mg; 82%) as a red oil.

[2-^{14}C]Mesoxalic acid 4'-ethylphenyl hydrazone (III)

The diethyl ester (II) (238 mg) was hydrolysed by stirring with aqueous sodium hydroxide (0.3 ml of 48%), water (2.0 ml) and ethanol (0.3 ml) for 16 hours at room temperature. The reaction mixture was washed with toluene (2 X 4.0 ml) and ether (2 X 4.0 ml), acidified with concentrated hydrochloric acid (36%) and the copious yellow precipitate which formed extracted into ether (4 X 10.0 ml). The extracts were washed with water (2 X 5.0 ml), dried over anhydrous magnesium sulphate and evaporated to dryness under reduced pressure. Drying for 16 hours under vacuum at room temperature gave (III) (155 mg; 81%) as a pale yellow powder.

[2-^{14}C]Mesoxalyl chloride 4'-ethylphenylhydrazone (IV)

The acid (III) (155 mg) was suspended in toluene (0.45 ml) and thionyl chloride (0.2 ml) added. The resultant deep-red solution was stirred at room temperature for 30 min, then at 70-75° (under reflux) for 1 hour. On cooling to 0° followed by the addition of light petroleum (b.p. 40-60°) (1.0 ml), the product crystallised out. These crystals were centrifuged from the mother liquors, washed with light petroleum (2 X 2.0 ml) and dried under vacuum at room temperature to give (IV) (135 mg; 75%) as lemon-yellow needles.

6-Ethyl-4-hydroxy[3-^{14}C]cinnol-3-yl carboxylic acid (V)

A solution of titanium tetrachloride (0.1 ml) in chlorobenzene (0.7 ml) was added to a stirred suspension of (IV) (135 mg) in chlorobenzene (0.8 ml) and the temperature of the mixture raised slowly to 95-100°. The dark red solution was stirred for 6 hours at this temperature then allowed

to cool with stirring for 10 hours. Dilute hydrochloric acid (5.0 ml of 2M) was added to decompose the titanium complex and the resulting emulsion stirred at room temperature for 4 hours. The aqueous layer was separated by centrifugation and discarded. The chlorobenzene solution and precipitated solids were extracted with dilute sodium hydroxide solution (20 X 3.0 ml of 1M) and water (3 X 3.0 ml). The combined extracts were washed with toluene (2 X 10.0 ml) and ether (3 X 10.0 ml) and acidified with concentrated hydrochloric acid (36%). After centrifugation, the precipitate was washed with water (4 X 5.0 ml) and dried at 45° under vacuum to give (V) (87.5 mg; 81%) as a cream-yellow powder.

The acidic solution, which retained a yellow colouration, was extracted with chloroform (3 X 5.0 ml), the extracts dried over anhydrous magnesium sulphate and evaporated to dryness under reduced pressure giving a further quantity (12 mg) of (V), total yield: 99.5 mg (92%).

This material was combined with that from a parallel run (78.5 mg) carried out on the same scale.

Ethyl 6-ethyl-4-hydroxy[3-^{14}C]cinnol-3-yl carboxylate, ICI 75,186 (I)

The acid (V) (178 mg) was suspended in absolute ethanol (5.0 ml) and the solvent heated to the boil. Dry hydrogen chloride gas was passed through the refluxing suspension. After 45 min all the acid had dissolved. Gas infusion was continued, under reflux, for a further 5¼ hours. The reaction mixture was cooled and the solvent removed under reduced pressure to give crude ICI 75,186 (168 mg) as a pale yellow sticky glass.

PURIFICATION OF ICI 75,186

The crude material was purified by preparative-scale thin-layer chromatography on twelve 20 X 40 cm glass plates coated with Silica Gel GF to a thickness of 0.5 mm using solvent system A as eluent. The band on each plate corresponding in Rf to a reference sample was removed and the product extracted from the silica by stirring with absolute alcohol (120 ml) for 16 hours. This suspension was filtered and the silica washed with absolute alcohol (4 X 100 ml). The ethanolic extracts and washings were evaporated to dryness under reduced pressure and the solid redissolved in a small volume of absolute alcohol. This

solution was centrifuged from a small amount of insoluble material and the solvent evaporated under a stream of dry nitrogen gas. The white solid was dried at room temperature under vacuum for 16 hours to give purified ICI 75,186 (120 mg; 63%).

The product was chromatographed on Silica GF plates, eluting with solvent systems B and C, and examined both under ultra violet light (254 nm) and by autoradiography (16 hours). Only one spot could be detected on each plate by either visualisation technique, thus showing the product to be relatively pure although still containing some ethanol-insoluble material. In an attempt to remove this, the solid was extracted into ethanol and ethyl methyl ketone. Unfortunately, t.l.c. showed that this procedure partially decomposed the product which thus had to be further purified by p.t.l.c. on six plates using solvent system A. The band on each plate corresponding in Rf to a reference sample was removed and extracted in the manner described for the first purification. After concentration to small volume (6 ml) the extracts were centrifuged to remove some insoluble material and the supernatant liquor removed. On evaporation under a stream of dry nitrogen gas and drying under vacuum the product was obtained as a colourless glass. The glass was triturated with light petroleum (b.p. 60 - 80°) and dried, giving the pure ICI 75,186 (69 mg) as a pale cream powder, representing an overall chemical yield of 12.5%

The product was examined on t.l.c., eluting with solvent systems B, D and E and the radiochemical purity determined by segmentation and liquid scintillation counting in a toluene-Butyl-PBD (6%) phosphor. This indicated a minimum radiochemical purity of 99%. Mass spectrometry and nuclear magnetic resonance spectroscopy showed no detectable impurities. The specific activity was 3.3 μCi/mg (802 μCi/m mole) which represented an overall radiochemical yield of 10%.

REFERENCES

1. Fromson J. M., Bramah S. E. and Pearson S. - in preparation.

2. Barber H. J., Washbourn K., Wragg W. R. and Lunt E. - J. Chem. Soc., 2828 (1961).

3. Gilman D. J. - U.K. Patent 1306839 (June 13, 1973).

4. Schramm G., Grötsch H. and Pollmann W. - Angew. Chem. Int. Ed., 1, 1 (1962).