SYNTHESIS OF ETHYL 4-(3,4,5-TRIMETHOXYCINNAMOYL)-[2,5-^{14}C] PIPERAZINYL ACETATE AND ETHYL 4-(3,4,5-TRIMETHOXY[β-^{14}C] CINNAMOYL)PIPERAZINYL ACETATE.

G. Hardy, I.P. Sword and D.E. Hathway.

Department of Metabolic Studies, Huntingdon Research Centre, Huntingdon PE18 6ES, England.

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SUMMARY

This paper describes the synthesis (i) of ethyl 4-(3,4,5-trimethoxycinnamoyl)-[2,5-^{14}C]piperazinyl acetate via [2,5-^{14}C]piperazine, and ethyl [2,5-^{14}C] piperazinyl acetate, and (ii) of ethyl 4-(3,4,5-trimethoxy [β-^{14}C]cinnamoyl)piperazinyl acetate via 3,4,5-trimethoxybromobenze and 3,4,5-trimethoxybenz [^{14}C]aldehyde.

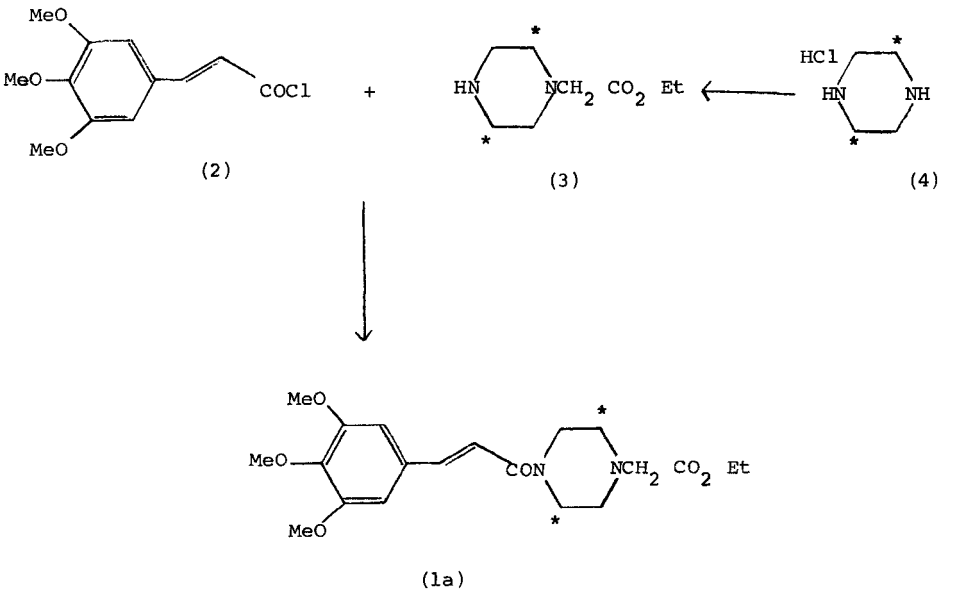
The unequivocal preparation of 3,4,5-trimethoxybromobenzene is described, and other methods for the preparation of 3,4,5-trimethoxybenz [^{14}C]aldehyde are examined.

The synthesis (Fauran et al., 1969) and pharmacological properties (Huguet et al., 1969; Pourrias et al., 1971a; Pourrias et al., 1971b) of a new, potent coronary dilator, ethyl 4-(3,4,5-trimethoxycinnamoyl)piperazinyl acetate, have recently been described. For the investigation of its metabolism (Chasseaud et al., 1972) in mammals two isotopically labelled

forms of the drug were required, viz. ethyl 4-(3,4,5-trimethoxycinnamoyl)-[2,5-^{14}C]piperazinyl acetate and ethyl 4-(3,4,5-trimethoxy [β-^{14}C]cinnamoyl)piperazinyl acetate, the syntheses of which are the subject of the present paper.

Schemes 1 and 2 exemplify the reaction sequences which were developed for the syntheses of ethyl 4-(3,4,5-trimethoxycinnamoyl)-[2,5-^{14}C]piperazinyl acetate and ethyl 4-(3,4,5-trimethoxy [β-^{14}C]cinnamoyl)piperazinyl acetate respectively.

Scheme 1

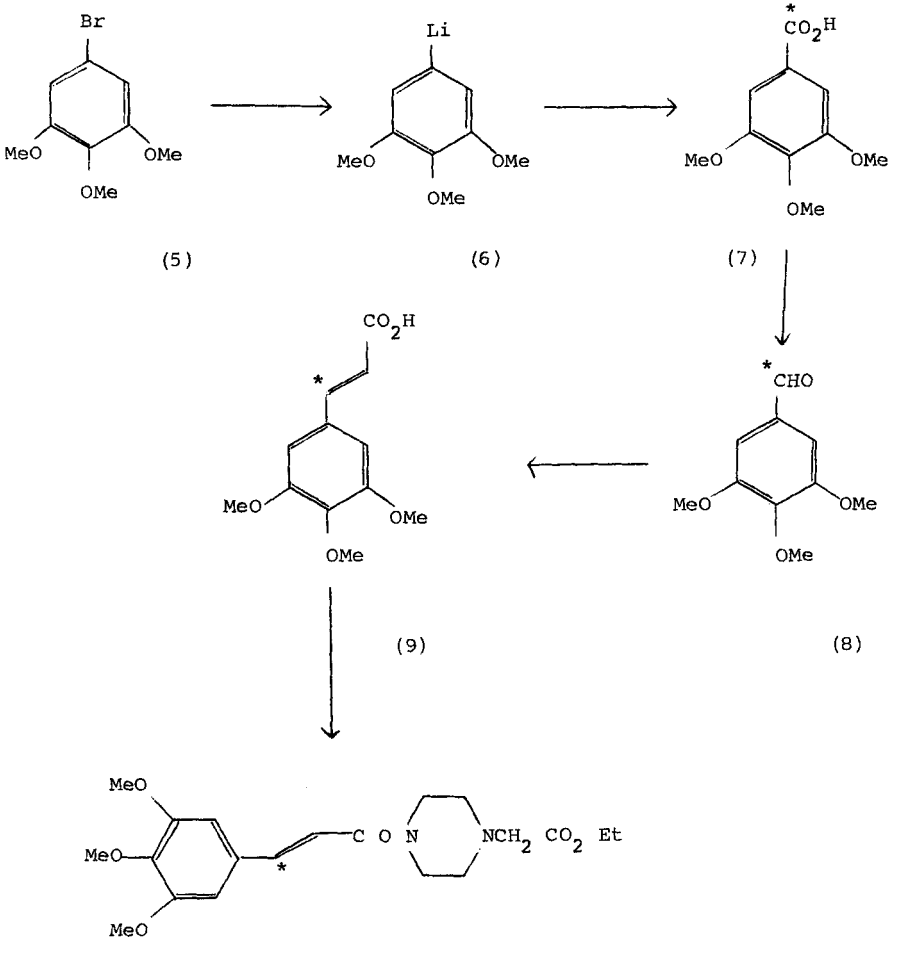


Scheme 1. Monoalkylation of piperazine hydrochloride with ethyl chloroacetate proceeds in poor yield with respect to piperazine, owing to formation of piperazine dihydrochloride, but improved results can be obtained through the recovery of the dihydrochloride and its conversion into monohydrochloride by reaction with piperazine hexahydrate. When this procedure was applied to commercially available /2,5-^{14}C/piperazine dihydrochloride (compound 4), good yields of ethyl/2,5-^{14}C/piperazinyl acetate (compound 3) were obtained. Condensation of that product with 3,4,5-trimethoxycinnamoyl chloride (compound 2) in the presence of anhydrous potassium carbonate gave ethyl 4-(3,4,5-trimethoxycinnamoyl)-/2,5-^{14}C/piperazinyl acetate (compound 1a), which was purified as the maleate salt.

Scheme 2. Ethyl 4-(3,4,5-trimethoxy /β-^{14}C/cinnamoyl)piperazinyl acetate was eventually prepared by 6-stage synthesis from 3,4,5-trimethoxybromobenzene (compound 5), but this starting material was difficult to obtain.

Thus in our hands, reaction of 3,4,5-trimethoxybenzoic acid under Hunsdiecker conditions afforded a single, halogen-free product with ester-like properties (cf. Dandiya et al., 1962), and also, debromination of 2,6-dimethoxy-3,4,5-tribromophenol with Zn dust yielded, in fact, a ternary mixture which analysed by gas chromatography and n.m.r. spectroscopy, not 4-bromo-2,6-dimethoxyphenol exclusively (cf. Kohn et al., 1947). Over a range of reaction conditions involving different batches of Zn, 3-bromo-2,6-dimethoxyphenol (70%, theoretical) preponderated, with 4-bromo-2,6-dimethoxyphenol (16%) and 2,6-dimethoxyphenol (14%) as minor products. Only by methylation of this mixture, followed by careful fractional crystallization of the low-melting product, were workable quantities of 3,4,5-trimethoxybromobenzene obtained.

Scheme 2



Whence, carbonation of the bromobenzene derivative was effected, essentially as described by Calvin (1949), to afford 3,4,5-trimethoxybenzoic[^{14}C]acid (compound 7), which was converted into the corresponding aldehyde (compound 8) by Rosenmund reduction

of the acid chloride. Condensation of the aldehyde with malonic acid yielded 3,4,5-trimethoxy /β-^{14}C/cinnamic acid (compound 9), from which ethyl 4-(3,4,5-trimethoxy /β-^{14}C/cinnamoyl)piperazinyl acetate (compound 1b) was prepared by the method described for the other isotopically labelled form of the drug (see Scheme 1).

Alternative syntheses of 3,4,5-trimethoxybenz /^{14}C/aldehyde (compound 8) were also investigated, but the cost of isotopic starting materials precluded synthesis of syring /^{14}C/ aldehyde with a 33% theoretical yield by Duff reaction of 2,6-dimethoxyphenol with hexamethylenetetramine (Allen et al., 1963), or by oxidation of the benzylidene derivative formed by Claisen rearrangement of the allylic ether corresponding to 2,6-dimethoxyphenol (Pearl, 1948). Attempted Vilsmeier-Haack formylation of 2,6-dimethoxyphenol under various reaction conditions gave starting material, or under neutral work-up conditions, a compound, which showed carbonyl absorption in the i.r., and which was easily hydrolysed to starting material. The product, presumably the O-formyl derivative, was not further investigated.

Experimental

Melting points (m.p.) were determined on a Kofler microscope hot-stage. Gas chromatography (g.c.) was carried out on a Perkin-Elmer F-11 instrument with flame ionisation detection using a 6ft column (inside diameter 1/8in) of 5% OV-1 on J.J's diatomite 'CQ' 80-100 mesh at 145°C. Nuclear magnetic resonance (n.m.r.) spectra were recorded in deuteriochloroform on a Varian HA-100 instrument using tetramethylsilane as internal reference; infra-red (i.r.) spectra as Nujol mulls on a Unicam SP200G spectrophotometer. ^{14}C activities were measured by liquid scintillation methods using a Nuclear Chicago Mark 1 counter. Merck t.l.c. plates (250μ, F_{254}) were used to monitor purity; preparative plates were coated with Merck Kieselgel G 1 mm thick. Radiochemical purity was monitored using Kodak X-ray film.

2,6-Dimethoxy-3,4,5-tribromophenol

Bromine (12.8g; 80mmole) was gradually added with stirring to pyrogallol-1,3-dimethyl ether (3.1g; 20mmole) contained in a wide-necked flask. When the addition was complete excess bromine was removed in vacuo leaving a pink residue which was recrystallized from benzene, to give colourless prisms (5.74g) m.p. 136-137° (Kohn & Grün, 1925, give m.p. 134°).

3,4,5-Trimethoxybromobenzene

2,6-Dimethoxy-3,4,5-tribromophenol (4.8g; 12.4mmole) was dissolved in warm glacial acetic acid (10 ml) and water (10 ml), then zinc dust (4.8g) was added to the solution in two portions. The mixture was heated at reflux for 45 minutes, cooled, filtered, poured into water (20 ml) and neutralised with a saturated solution of sodium hydrogen carbonate. Ether extraction afforded the crude monobromophenol (2.0g) which was immediately taken into 2N sodium hydroxide solution (10 ml) and treated with dimethylsulphate (1.0 ml). After heating at reflux for 2 hours, the mixture was cooled, diluted with water (10 ml) and extracted with ether (3 x 10 ml The combined ethereal extracts were washed with dilute sulphuric acid and water, then dried and evaporated to leave a waxy residue (1.25g). Fractional crystallisation of the product from 60/80 light petroleum followed by recrystallisation from n-pentane afforded colourless prisms (200mg) m.p. 75-76° (Kohn & Steiner, 1947 give m.p. 78°) n.m.r. ($CDCl_3$) τ, 3.31 singlet (2H) (aromatic protons) 6.18 & 6.02 singlets (9H) (3 x OCH_3).

3,4,5-Trimethoxybenzoic [^{14}C] acid

A solution of 3,4,5-trimethoxybromobenzene (120mg; 0.485mmole) in anhydrous ether (5 ml) was flushed with nitrogen then chilled to -60° with a dryice-isopropanol mixture whilst a 10% solution of n-butyl lithium in n-hexane (0.35 ml; 0.55mmole) was added. The mixture was allowed to warm to room temperature for two minutes

with stirring, then frozen in liquid nitrogen and the system evacuated to 0.2mm. Radioactive carbon dioxide was liberated by addition of concentrated sulphuric acid to barium carbonate-^{14}C (43mg; 0.218mmole; 60.7mCi/mmole) frozen out on to the reaction mixture, then reacted at -60° for 7 minutes. The solution was acidified with 2N hydrochloric acid and extracted with ether (4 x 10 ml). Combination of the ethereal extracts and re-extraction with 5% sodium hydrogen carbonate solution, followed by re-acidification yielded the crude carboxylic acid which was extracted into ether (4 x 10 ml) and washed with water. Evaporation yielded a colourless solid (21mg) m.p. 173-174° (from water).

3,4,5-Trimethoxybenz /^{14}C/ aldehyde

3,4,5-Trimethoxybenzoyl /^{14}C/ chloride (115mg; 0.5mmole) prepared from the acid (106mg) and thionyl chloride (0.3 ml) in benzene (5 ml), was dissolved in dry xylene (3 ml) containing 1 drop of quinoline-sulphur catalyst poison and reduced with hydrogen gas over 10% palladium on barium sulphate catalyst (50mg). The evolution of hydrogen chloride during the reaction was monitored by absorption into water and titration with 0.1N sodium hydroxide solution.

After 3 hours the reaction was stopped, the contents of the flask filtered free from solid material and solvent evaporated *in vacuo* to afford the aldehyde, pale yellow needles from aqueous ethanol (98mg) m.p. 74-75° (Huang, Tarbell & Arnstein, 1948 give m.p. 74-75°).

3,4,5-Trimethoxy /β-^{14}C/cinnamic acid

A mixture of 3,4,5-trimethoxybenz /^{14}C/aldehyde (98mg; 0.5mmole) and malonic acid (65mg; 0.625mmole) in dry pyridine (0.5ml) containing piperidine (1 drop) was heated at 95° for 2½ hours then at reflux for a further ½ hour. On cooling the resultant solution

was treated with 3N hydrochloric acid (3 ml) and the cinnamic acid which deposited was collected and recrystallized from ethanol, colourless needles (65mg) m.p. 123-124°, identical in all respects with an authentic sample.

Ethyl [2,5-^{14}C] piperazinyl acetate

To a mixture of piperazine hexahydrate (97mg; 0.5mmole) and piperazine dihydrochloride (75mg; 0.47mmole) comprising cold dihydrochloride (52mg; 0.32mmole) and piperazine [2,5-^{14}C]-dihydrochloride (23mg; 0.15mmole; 33.4mCi/mmole; CEA France) in ethanol (2 ml) was added ethyl chloroacetate (62mg; 0.51mmole) in ethanol (0.1 ml). The mixture was refluxed 2 hours then cooled, supernatant decanted, and the residue washed with cold ethanol (1 ml) and washings combined with supernatant. The residue (piperazine [2,5-^{14}C] dihydrochloride) was re-cycled and supernatant from both runs combined, evaporated *in vacuo* to a syrup then basified with excess aqueous potassium carbonate. Chloroform extraction (2 x 20 ml) afforded the product (164mg).

Ethyl 4-(3,4,5-trimethoxycinnamoyl)-[2,5-^{14}C]piperazinyl acetate maleate salt

3,4,5-Trimethoxycinnamoylchloride (246mg; 0.97mmole) in ethyl acetate (2 ml) was added over 5 minutes to a cold mixture of sodium hydrogen carbonate (120mg; 1.43mmole) and ethyl [2,5-^{14}C]piperazinyl acetate (164mg; 0.95mmole) in ethyl acetate (3 ml). The mixture was heated at reflux for one hour, thoroughly washed with hot water (60°; 2 x 5 ml), aqueous washings re-extracted with ethyl acetate (2 x 20 ml) and the combined organic phases dried and evaporated. The crude amide thus obtained (320mg) was warmed with maleic acid (95mg) in ethyl acetate (5 ml) for 5 minutes, then filtered hot through glass fibre paper on a medium porosity sinter. Evaporation of solvent *in vacuo* afforded the maleate salt (406mg).

This salt dissolved in a 1:1 mixture of ethyl acetate : ethanol (10 ml) was submitted to chromatography on preparative plates (ethanol as eluent). Authentic material was co-chromatographed as marker and the relevant band identified under u.v. light.

Removal of the band and recovery of the product in boiling 1:1 ethanol:ethyl acetate gave a total of 148mg of pure maleate, identical in all respects with authentic material (i.r., t.l.c. in 3 solvent systems). Autoradiography showed the derivative to be radiochemically pure. The specific activity was 7.422μCi/mg = 3.77mCi/mmole.

Ethyl 4-(3,4,5-trimethoxy /β-^{14}C/cinnamoyl)piperazinyl acetate maleate salt

This was prepared from 3,4,5-trimethoxy /β-^{14}C/ cinnamoyl chloride (69mg; 0.265mmole) and ethyl piperazinyl acetate (46.5mg; 0.270mmole), and was purified as the maleate salt exactly as described above. It was identical in all respects with authentic material. The specific activity was 14.87μCi/mg, 7.55mCi/mmole.

All of the other intermediates used in these experiments were prepared by the reactions described for the labelled compounds.

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