SYNTHESIS OF 2,6-DIMETHOXY [U-^{14}C]PHENOL.

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SUMMARY

The preparation of 2,6-dimethoxy [U-^{14}C]phenol was carried out in five steps from [U-^{14}C]phenol. [U-^{14}C]Phenol was converted to 2-[U-^{14}C]phenoxy-5-nitrobenzophenone which was sequentially hydroxylated with hydrogen peroxide-acetic acid to give the di-ortho hydroxylated phenolic intermediate, 2-(2",6"-dihydroxy [U-^{14}C]phenoxy)-5-nitrobenzophenone. Methylation of this intermediate and subsequent scission of the aryl ether link with piperidine gave the required di-ortho substituted phenol. An alternative sequence was investigated for the conversion of 2-(2"-hydroxyphenoxy)-5-nitrobenzophenone to 2-(2",6"-dimethoxyphenoxy)-5-nitrobenzophenone but it gave a poorer overall yield with an unstable intermediate. Characterization data are included.

2,6-Dimethoxyphenol is a constituent part of the anaesthetic M & B 16,573 and is released from the anaesthetic in vivo. Preliminary metabolic investigations have shown that 2,6-dimethoxyphenol is toxic in certain species, particularly the cat (1). A complete study of the metabolism and mode of excretion of this phenol necessitated a defined synthesis of ^{14}C-labelled 2,6-dimethoxyphenol in order that its toxic effects could be studied. Few published methods exist for the synthesis of di-ortho substituted phenols. One method has been outlined by Loudon and Scott (2) and the overall yield of 2,6-dimethoxyphenol obtained by these workers was described by them as good. However, even after examination of all the optimum conditions for each stage it was never

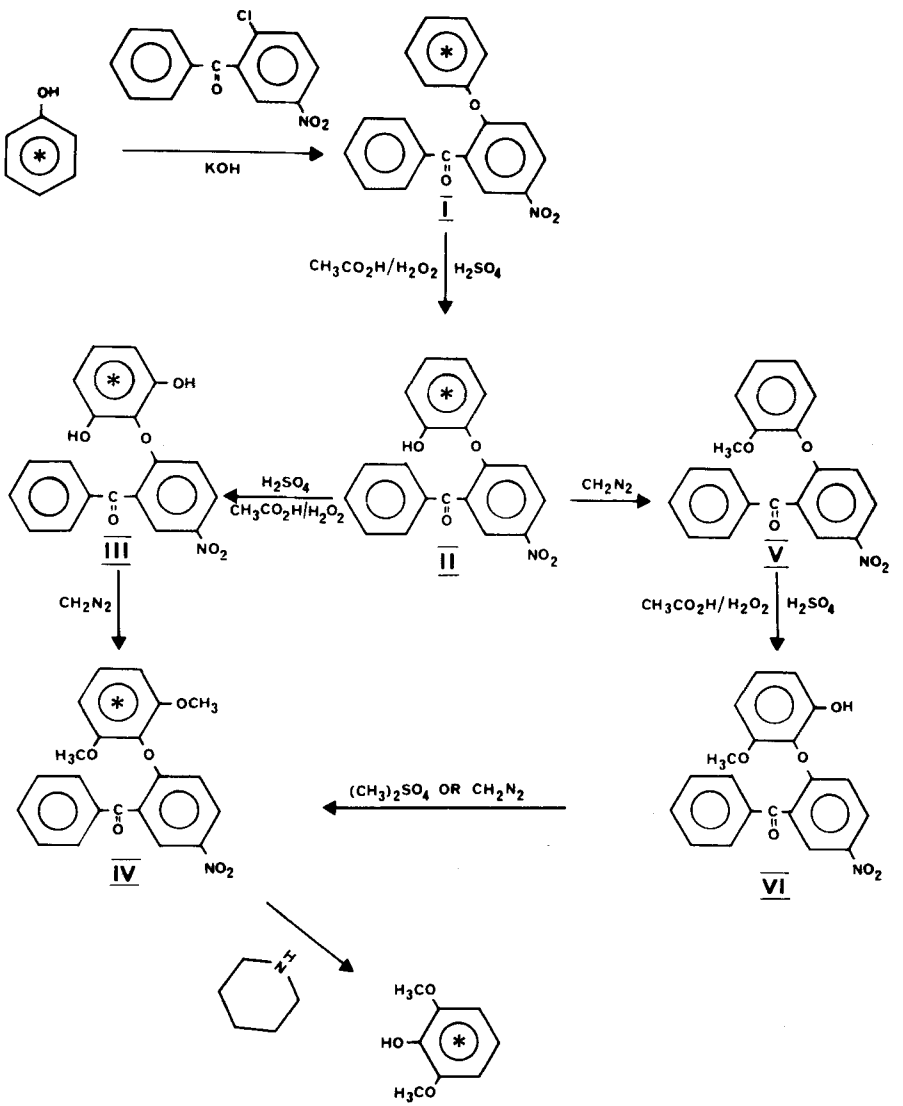

Fig. 1. SYNTHESIS OF ^{14}C-LABELLED 2,6-DIMETHOXY PHENOL

possible to obtain an overall yield of more than 5 - 10%. The present publication reports a full procedure for the synthesis of 2,6-dimethoxy [U-^{14}C]phenol using [U-^{14}C]phenol as the radiochemical precursor (Fig. 1).

The initial reaction consisted of coupling [U-^{14}C]phenol with 2-chloro-5-nitrobenzophenone to give a compound of the aryloxynitrobenzophenone type (I). This intermediate was formed in good yield and predisposed the phenol ring to ortho-substitution. Hydroxylation was effected by dissolving the compound in conc. H_2SO_4, diluting the solution with acetic acid and adding hydrogen peroxide. The hydroxylation appears to proceed through the intermediate but unisolated xanthylium sulphate to give the ortho-substituted phenolic ring, compound II, in good yield.

The introduction of a second ortho-substituent could be achieved by either one of two ways. The procedure adopted in the radiochemical synthesis required a further treatment with sulphuric acid followed by acetic acid and hydrogen peroxide to give the di-ortho substituted intermediate (III) directly and again in good yield. Methylation of the phenolic hydroxyl groups with diazomethane gave 2-(2",6"-dimethoxy [U-^{14}C]phenoxy)-5-nitrobenzophenone (IV), also in good yield. The ether link was split with piperidine and yielded the required 2,6-dimethoxy [U-^{14}C]phenol. Each step in the sequence was effected in at least 50% yield giving an overall yield for the di-ortho substituted phenol of 5 - 10% based on [U-^{14}C] phenol.

The alternative sequence from compound II involved three steps prior to splitting the ether link with piperidine. Here methylation of the mono ortho-substituted intermediate precedes the introduction of the second ortho-substituent which is then methylated separately.

The isolated intermediates from both sequences were purified by repeated crystallization and characterized by infra-red and nuclear magnetic resonance spectroscopy. In addition, prepared 2,6-dimethoxy [U-^{14}C]phenol was further characterized by ultra-violet spectroscopy and was shown to be homogeneous by

thin-layer chromatography.

MATERIALS AND METHODS

2-Chloro-5-nitrobenzophenone was purchased from the Aldrich Chemical Co., Milwaukee, Wisconsin, U.S.A., and was recrystallized from ethanol containing a small quantity of activated charcoal. Diazomethane (alcohol free) was prepared by the method of De Boer (3). 2,6-Dimethoxyphenol was supplied by Eastman Organic Chemicals Ltd., Rochester, New York, U.S.A. [U-^{14}C]Phenol (specific activity 337μCi/mg) was purchased from the Radiochemical Centre, Amersham, Bucks, U.K.

Infra-red spectra were measured on a Perkin-Elmer 257 spectrophotometer using the KBr disc method, the ultra-violet spectra were obtained on a Unicam SP 800A spectrophotometer using butan-1-ol as solvent and n.m.r. spectra were recorded on a Perkin-Elmer 100 MHz spectrophotometer using $CDCl_3$ as solvent. Thin-layer chromatography was performed on Silica Gel G using xylene-chloroform (1:3,v/v) as solvent.

EXPERIMENTAL

Preparation of 2-[U-^{14}C]phenoxy-5-nitrobenzophenone (I)

[U-^{14}C]Phenol (300μCi, 337μCi/mg) was mixed with phenol (2.23g, 2.0 mmoles) and fused with finely powdered KOH (1.2 mmole). 2-Chloro-5-nitrobenzophenone (5.24g, 1 mmol) was added and the reaction was allowed to proceed for 15 min; sufficient heat was applied to provide a homogeneous mixture at its boiling point. On cooling, the mixture was pulverized and washed with 10% (w/v) NaOH solution (3 x 10ml). After filtration the residue was washed with distilled water (500 ml) to neutral pH. The solid product was crystallized twice from aqueous acetic acid, 90% (v/v), dried and decolourized with activated charcoal in boiling benzene. Recrystallization was accomplished by dilution of the resultant filtered benzene solution (40 ml) with an equal volume of light petroleum (b.p.60 - 80°C); a final recrystallization was obtained from benzene-light petroleum (b.p.60 - 80°C) (2:3, v/v).

A yield of 60% was obtained for this stage, the product being white and crystalline with melting point 141 - 142° C.

The infra-red and n.m.r. spectra of the product were consistent with the patterns expected of authentic 2-[U-^{14}C]phenoxy-5-nitrobenzophenone. From the infra-red spectrum the following important absorptions were observed: 1275 - 1250cm^{-1} and 1075cm^{-1} (aromatic ether), 1665cm^{-1} (aromatic ketone), 840cm^{-1} (2 adjacent protons on an aromatic ring) and 800cm^{-1} (one adjacent proton on an aromatic ring). The n.m.r. spectrum of 2-[U-^{14}C]phenoxy-5-nitrobenzophenone reveals a sharp signal assigned to the proton between the carbonyl and nitro group of the benzophenone at 1.7τ, all the remaining protons were observed as a multiplet around 2.5τ.

Preparation of 2-(2"-hydroxy [U-^{14}C]phenoxy)-5-nitrobenzophenone (II)

2-[U-^{14}C]Phenoxy-5-nitrobenzophenone (3.6g, 1.15 mmole) was dissolved by shaking and warming in conc. H_2SO_4 (8ml). After 30 min, acetic acid (64ml) was added and the mixture treated with hydrogen peroxide (1.8ml of 30%, w/v) dropwise with shaking. After 20 min the mixture was poured onto crushed ice. The precipitate was filtered, washed with water, dried and crystallized from methanol. The resulting solid (m.p. 152-153°C) was obtained in a yield of 70%.

The infra-red spectrum showed a new absorption band at 3350 - 3400cm^{-1} indicating the presence of a phenolic hydroxyl group; the remainder of the spectrum was virtually identical with 2-[U-^{14}C]phenoxy-5-nitrobenzophenone. The n.m.r. spectrum confirmed the introduction of the phenolic hydroxyl group and the integral trace revealed a ratio of 1 hydroxyl proton to approximately 14 aromatic protons. The n.m.r. spectrum also confirmed that the phenolic hydroxyl group had been introduced ortho to the ester linkage.

The conversion of 2-(2"-hydroxy [U-^{14}C]phenoxy)-5-nitrobenzophenone to 2-(2",6"-dimethoxy [U-^{14}C]phenoxy)-5-nitrobenzophenone may be achieved by one of two pathways as shown in Fig. 1. In preliminary investigations using non-

labelled intermediates the favoured pathway was found to be:

Preparation of 2-(2",6"-dihydroxy [U-^{14}C]phenoxy)-5-nitrobenzophenone (III)

To 2-(2"-hydroxy [U-^{14}C]phenoxy)-5-nitrobenzophenone (2.45 g, 0.7 mmol) was added conc. H_2SO_4 (2.5 ml). The mixture was warmed to give a brick red coloured solution to which, on cooling, was added acetic acid (40 ml), followed by hydrogen peroxide (2.5 ml of 30%, w/v). After shaking for 10 min the colour changed to amber and the mixture was poured onto crushed ice. The precipitated 2-(2",6"-dihydroxy [U-^{14}C]phenoxy)-5-nitrobenzophenone was washed, dried *in vacuo* over P_2O_5 and crystallized from methanol. The bright yellow crystals gave a melting point of 180°C; yield 55%. The infra-red spectrum showed an intensification and broadening of the hydroxyl absorption band and the presence of three adjacent protons on a phenolic nucleus. In addition the infra-red spectrum confirmed the di-*ortho* substitution of the phenolic ring; a characteristic series of absorption bands was observed in the 2000 - 1660cm^{-1} region associated with the out-of-plane bending of the isolated protons (4). The integral trace on the n.m.r. spectrum gave a ratio of 2 hydroxyl protons to 10 aromatic protons which is consistent with the structure proposed. The hydroxyl proton peak (3.1τ) was a single peak confirming that di-*ortho* substitution had taken place giving a symmetrical substitution of the phenyl nucleus.

Preparation of 2-(2",6"-dimethoxy [U-^{14}C]phenoxy)-5-nitrobenzophenone (IV)

Methylation of the dihydroxy compound with excess diazomethane resulted in the formation of the yellow crystalline dimethyl ether of m.p. 170°C, (literature value 170°C). The addition of alcohol-free diazomethane to 2-(2",6"-dihydroxy [U-^{14}C]phenoxy)-5-nitrobenzophenone gave an initial effervescence and the methylation was allowed to proceed for 2 days at 0°C. During this time the majority of the dimethyl ether crystallized out of solution; the remainder was obtained by removal of solvent (total yield 50%).

The infra-red spectrum showed the loss of all the bands associated with

the hydroxyl absorption at 3400 - 3350cm^{-1} and the appearance at 2850cm^{-1} of an aromatic methoxyl absorption. The n.m.r. spectrum indicated that the substituted methoxyl groups were both in the ortho position as shown by the single peak at 6.3τ. The ratio of methyl protons to aromatic protons was 2.5:6.

The alternative pathway for the preparation of 2-(2",6"-dimethoxyphenoxy)-5-nitrobenzophenone was as follows; typical reaction conditions are as described:

Preparation of 2-(2"-methoxyphenoxy)-5-nitrobenzophenone (V) Methylation of 2-(2"-hydroxyphenoxy)-5-nitrobenzophenone (1.6g) was accomplished with excess ethereal diazomethane (alcohol-free) over 2 days at 0°C. This methylation yielded the methyl ether as a white crystalline solid which was recrystallized from ethanol to give a solid with m.p.124°C in 45% yield. The infra-red spectrum showed loss of phenolic hydroxyl absorption at 3350 - 3400 cm^{-1} and the appearance of the aromatic methoxyl absorption at 2850cm^{-1}. The n.m.r. spectrum showed the methoxyl protons at 6.3τ, the ratio of methoxyl protons to aromatic protons was 3:13.5.

Preparation of 2-(2"-methoxy 6"-hydroxyphenoxy)-5-nitrobenzophenone (VI)

2-(2"-Methoxyphenoxy)-5-nitrobenzophenone (0.5g) was dissolved, with warming, in 0.5ml conc. H_2SO_4. After cooling, acetic acid (8ml) was added and then hydrogen peroxide (0.5ml of 30%, w/v). This was left until the brick red colouration had faded to amber (approx. 20 min), then poured onto crushed ice, filtered, washed and dried. The dry cream non-crystalline product (yield 35% on average) was kept in the dark because of its instability to ultra-violet radiation.

The infra-red spectrum showed all the salient absorptions of 2-phenoxy-5-nitrobenzophenone (I), with the expected addition of hydroxyl and methoxyl absorption bands at 3350 - 3400cm^{-1} and 2850cm^{-1} respectively. The n.m.r. spectrum of this compound showed a ratio of aromatic to methoxyl to hydroxyl

protons of 10:2.2:1.2. The methoxyl protons were seen at 6.2 τ, the hydroxyl at 4.7 τ while the aromatic protons were seen as a multiplet around 2.5 τ.

Methylation of 2-(2"-methoxy 6"-hydroxyphenoxy)-5-nitrobenzophenone could be achieved either with ethereal diazomethane (alcohol free) at 0°C for 2 days, as described above for the ^{14}C-labelled preparation or with alkaline dimethyl sulphate. The use of alkaline dimethyl sulphate required that the compound for methylation be dissolved in anhydrous acetone and treated with sufficient dimethyl sulphate and potassium carbonate (10% excess) to methylate the phenolic group over 5h under reflux conditions. The cooled reaction mixture was centrifuged, the acetone evaporated at room temperature and the residue recrystallized twice from ethanol and dried over P_2O_5 in vacuo, to give 2-(2",6"-dimethoxyphenoxy)-5-nitrobenzophenone (IV) (average yield 50%). The infra-red and n.m.r. spectra were identical with those obtained with this intermediate in the radiochemical synthesis.

Preparation of 2,6-dimethoxy [U-^{14}C]phenol

Dry 2-(2",6"-dimethoxy [U-^{14}C]phenoxy)-5-nitrobenzophenone (1.6g) was heated under reflux with excess piperidine, (5ml/g of material), for 20 min. The initial yellow colour of the solution changed to dark brown upon refluxing. After cooling, the solution was diluted with an equal volume of benzene, washed twice with 2M-H_2SO_4 and the aqueous phase discarded. The benzene layer was then washed with water to neutral pH and extracted with 2M - NaOH (2x10ml). The alkaline extract was acidified to pH2 and further extracted with chloroform (2 x 10ml). The solvent was evaporated and a crystalline residue obtained on removal of final traces of solvent in vacuo. The crystalline residue was purified by sublimation between 60°C and 70°C in vacuo (1mm Hg) to give a white crystalline solid which was recrystallized from benzene-petroleum spirit (b.p.60-80°C), (1:3, v/v) to give a product of m.p.56°C (literature value 56°C).

The infra-red spectrum (Fig. 2b) was identical with that obtained using an authentic sample of 2,6-dimethoxyphenol. The absorption band due to the

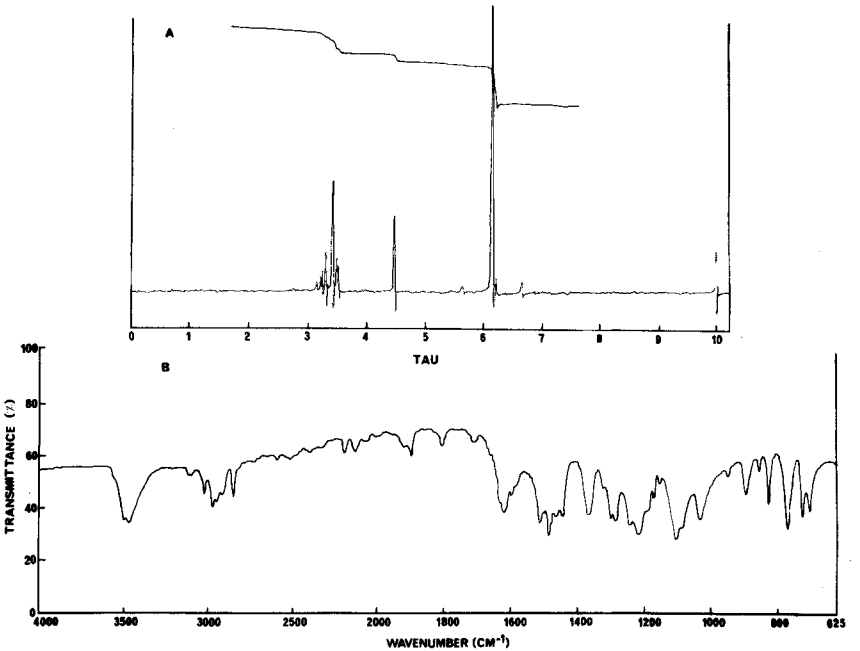

Fig.2 CHARACTERIZATION OF 2,6-DIMETHOXY [U-^{14}C] PHENOL
(a) N.M.R. SPECTRUM
(b) INFRA-RED SPECTRUM

phenolic hydroxyl group is seen at 3500cm^{-1}, the methoxyl groups at 2850cm^{-1}. The three adjacent protons present in 2,6-dimethoxyphenol give an absorption at 770cm^{-1} and an absorption due to the out-of-plane bending pattern of the di-ortho substituted aromatic ring was observed at 2000 - 1650cm^{-1} (4). The n.m.r. spectrum of 2,6-dimethoxy [U-^{14}C]phenol (Fig. a) shows a symmetrical molecule with respect to the methoxyl protons 6.1τ. The phenolic hydroxyl appears at 4.5τ. The para-proton of the ring is coupled to both the equivalent meta-protons and so this proton will be seen as a triplet and both meta protons as singlets. However, the resolution of the spectrum is not sufficiently clear to see this and the three aromatic protons appear as a multiplet at 3.4τ. From the integral trace the following proton ratios can be seen,

methoxyl 6: hydroxyl 1: aromatic 3; this is the expected ratio for 2,6-dimethoxyphenol. The ultra-violet spectrum was identical with that of authentic 2,6-dimethoxyphenol and showed an absorption maximum at 268nm corresponding to the phenolic hydroxyl absorption, ε 8.5 x 10^3. Thin-layer chromatography of 2,6-dimethoxy [U-^{14}C]phenol showed a single radio-active component with an R_F of 0.45.

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