SYNTHESIS OF ^{14}C-ANTHRALIN

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SUMMARY

1,8-dihydroxy-[10-^{14}C]9-anthrone (anthralin) was synthesized in good yield starting from readily available 3-methoxy-2-(2-methoxybenzyl)-benzoic acid (3).

^{14}C was introduced via carboxylation of the Grignard derived from 3-bromo-2(2-methoxybenzyl) anisole. This, in turn, was obtained from (3) by a Curtius and Sandmeyer reaction sequence.

Key words: carbon 14, anthralin

INTRODUCTION

Anthralin (1,8,dihydroxy-9-anthrone) (Dithranol) has been extensively used for more than 60 years in the topical treatment of psoriasis. Yet, very little is known concerning its metabolism and its mode of action at the molecular level.

For drug metabolism and skin penetration studies, we required anthra-

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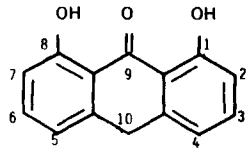

Fig. 1 : Numbering of anthralin.

lin labelled with a radioisotope in stable position(s). Because of the known propensity of tritium atoms in phenols to exchange in aqueous media, the commercially available ^{3}H-labelled anthralin did not suit our purposes. We therefore undertook the preparation of ^{14}C-labelled anthralin. *) **)

DISCUSSION

Anthralin is a notoriously unstable species[1], and it was therefore important to design a synthesis leading to a suitable stable precursor which could be stored as such, and which could be converted conveniently and efficiently to anthralin when required.

More obviously we required a synthetic route in which the label was introduced as late as possible.

The scheme outlined in Fig. 2 meets both these requirements, and in addition, has the further advantage of introducing the ^{14}C via the relatively inexpensive and readily available $^{14}CO_2$. Furthermore, it is in principle capable of ready extension to related anthrone derivatives.

The basis of the scheme is the conversion of the inactive acid (3) to its ^{14}C-labelled analogue (7) via the key bromo-derivative (6).

In both (6) and (7), the diphenylmethane oxidation level is critical. The alternative route via the corresponding benzophenone was ruled out on the

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*) For carbon numbering of anthralin, see Fig. 1.

**) While this manuscript was in preparation, Prof. Wiegrebe (Universität Regensburg) informed us that he had independently developed other routes potentially usable for ^{14}C-9 anthralin synthesis. (10)

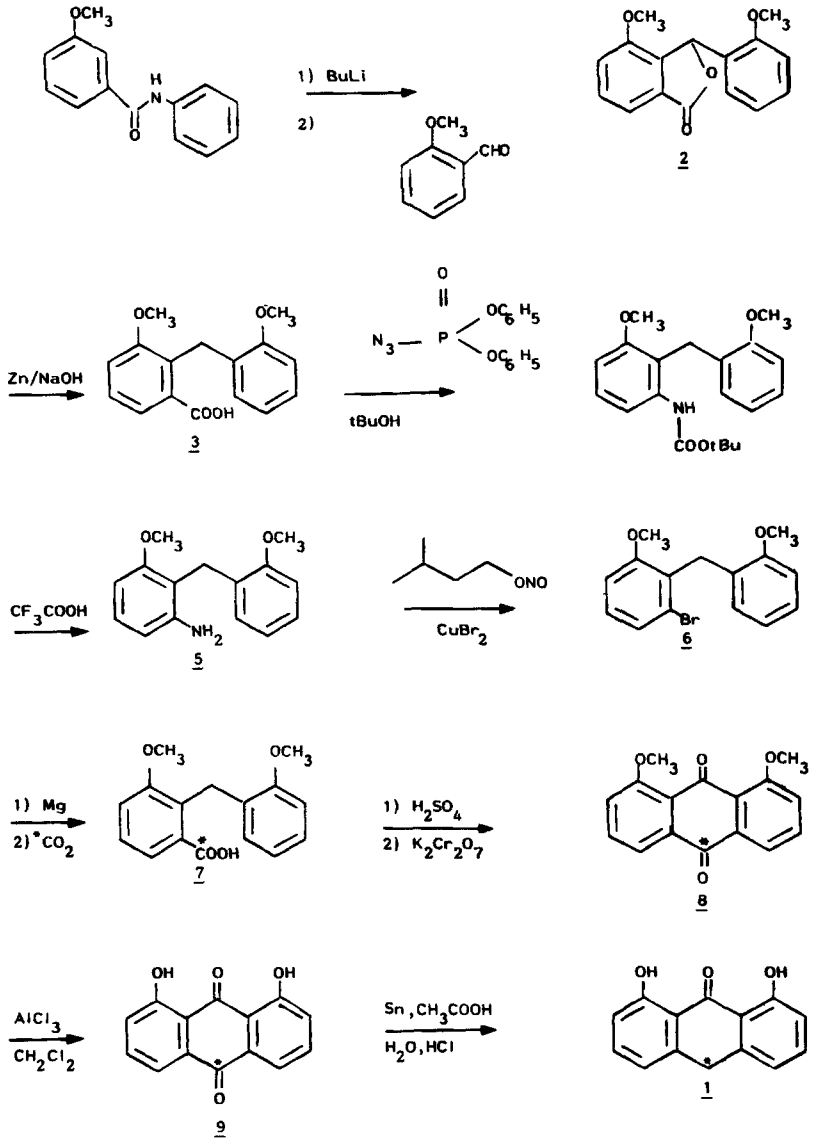

Fig. 2 : Scheme for the synthesis of [10-^{14}C] anthralin

grounds that: a) protection of the carbonyl group during Grignard formation would have been necessary, and b) the benzophenone analogue of (7) would be anticipated to cyclise less readily than (7) itself, and furthermore might conceivably be subject to Hayashi rearrangement[2] during cyclisation.

Cyclisation of (7) leads to the stable 1,8-dimethoxy-[10-^{14}C]anthraquinone (8) which can be stored indefinitely at low temperature and which can

be readily converted to [10-^{14}C]anthralin as required.

Numerous examples of anthrone and anthraquinone syntheses using the phthalide route have been reported[3]. In our case, condensation of the dianion of N-phenyl-m-anisamide with o-methoxy benzaldehyde, according to the method of Baldwin and Bair[3a] afforded a good yield of the phthalide (2). Treatment of (2) with zinc and aqueous potassium hydroxide[4] gave the benzyl benzoic acid (3). In our hands, and using a variety of conditions, this reduction gave only approximately 50% of the desired acid along with unreduced starting material.

Conversion of the acid (3) to the bromo compound (6) proved not to be straightforward. Direct Hunsdiecker reaction is known not to work well with aromatic acids bearing electron rich substituents[5], and we envisaged a Curtius/Sandmeyer sequence to effect the desired transformation. We found that the modified Curtius reaction using diphenylphosphorazidate[6] was best suited to our needs, affording a 60% yield of the carbamate (4). Cleavage of the t-butyl group in (4) is followed by spontaneous decarboxylation and the amine (5) is obtained in high yield. Carefully controlled reaction of (4) with cupric bromide and isopentylnitrite gave the desired bromo compound (6) in 30% yield (Sandmeyer reaction using HBr and $NaNO_2$ led only to mixtures of unidentified material) along with equivalent amounts of di- and tribrominated material (as evidenced by mass spectrometry) and as could be expected[4].

Conversion of the bromoderivative (6) to the chemically stable ^{14}C-labelled quinone (8) required only two steps: carboxylation of the Grignard reagent derived from (6) using $^{14}CO_2$ and cyclisation/oxidation[8,9] of the resultant ^{14}C-labelled acid (7), gave the quinone (8) in 36% yield.

[10-^{14}C]anthralin was readily prepared as required from (8) in ca. 80% yield[3d] by demethylation with aluminium chloride in dichloromethane to give (9), followed by reduction using tin and hydrochloric acid. Chromatography gave pure [10-^{14}C]anthralin with a specific activity of 57 mCi/mmol.

EXPERIMENTAL

Radioactivity was measured using bremsstrahlung counting (Berthold LB 2040). Radiochemical purity was determined by radio-chromatogram scanning (Berthold Automatic TLC linear analyser LB 2821). Thin layer chromatography (TLC) was performed on Merck 60F250 silica sheets, and High Performance Liquid Chromatography (HPLC) on a DuPont (8800) using either normal (Zorbax SIL) or reversed phase (Zorbax ODS) column. NMR spectra were determined on a JEOL FX90Q spectrometer and mass spectra on a Ribermag (R10-10C). Melting points are uncorrected, and analyses were performed by the Service de Microanalyse, C.N.R.S., Lyon.

$^{14}CO_2$ was generated from $Ba^{14}CO_3$, specific activity 57.2 mCi/mmol, (Commissariat à l'Energie Atomique, Saclay, France).

4-methoxy-3-(2-methoxyphenyl)phthalide (2)

In a nitrogen atmosphere, N-phenyl-3-anisamide (22.70 g, 0.1 mole) was dissolved in THF (250 ml). TMEDA (36 ml) was added and the solution was cooled to -78°C. n-Butyl lithium (138 ml, 1.6 M in hexane, 0.22 mole) was then added while maintaining the temperature below -70°C. The reaction mixture was then allowed to warm to room temperature (5 hours). The beige suspension was cooled again to -70°C and syphoned under nitrogen pressure into a solution of 2-methoxy-benzaldehyde (16.30 g, 0.120 mole) in THF (200 ml), cooled to -70°C. The reaction mixture was allowed to warm to room temperature overnight and aqueous hydrochloric acid (230 ml, 4N) was added with caution. After stirring for 1 hr, the mixture was concentrated to ca. 500 ml (aspirator) and extracted with dichloromethane (3 x 100 ml). The extract was dried ($MgSO_4$), evaporated and the solid residue was crystallised from diisopropyl ether affording (2) (20.9 g (77%)), sufficiently pure for the next stage.

One recrystallisation from diisopropylether afforded analytically pure (2):

IR: ($CHCl_3$) ν max (cm^{-1}) 1750 (CO)

NMR: ($CDCl_3$) δ 3.73 (3H,s,OCH_3), 3.84 (3H,S,OCH_3), 6.8-7.5 (8H, complex, ArH); MP 170-173°C (from CH_2Cl_2, diisopropylether)

Found: C, 70.86; H, 5.38 %. Calculated for $C_{16}H_{14}O_4$: C,71.1; H, 5.2 %.

3-methoxy-2-(2-methoxybenzyl) benzoic acid (3)

A mixture of the phthalide (2) (10 g, 37.0 mM), powdered zinc (60 g, 0.89 mole), copper sulfate (1 g) in aqueous KOH (10%, 500 ml) and pyridine (50 ml) was refluxed for 72 hours.

The insoluble material was filtered off and the colourless filtrate was acidified to pH 1 with 12 N aqueous HCl. The crystalline precipitate thus obtained was partitioned between ammonia (2 N, 200 ml), and methylene chloride (200 ml). The aqueous layer was washed twice with CH_2Cl_2, and acidified again to pH 1. The precipitate was washed with water, dried under high vacuum over P_2O_5 to afford (3) (5.20g, 52%). Found to be pure by HPLC (Zorbax ODS, using methanol as eluent).

The organic layer was evaporated to give 3.0 g of phthalide(2).

IR: ($CHCl_3$) ν max (cm^{-1}) 3300-2500 (OH), 1680-1670 (CO)

MP: 203°C from CH_2Cl_2, diisopropylether)

Found: C,70.43; H,5.78 %. Calculated for $C_{16}H_{16}O_4$: C,70.57; H,5.92 %.

t-Butyl-[N-3-methoxy-2-(2-methoxybenzyl)phenyl] carbamate (4)

A solution of acid (3) (2.72 g, 10 mM), diphenyl phosphorazidate (2.75 g, 10 mM) and triethylamine (1.40 ml, 10 mM) in t-butyl alcohol (30 ml) was refluxed under N_2 for 3 hours.

Evaporation of the volatile materials gave an oil which was applied to the top of a short (20 x 5 cm) column of silica gel (Merck 60, 70-230 mesh) and eluted (CH_2Cl_2 50, hexane 50). 50 ml fractions were collected and monitored by HPLC (column Zorbax ODS using methanol as eluent). The fractions containing material with k' = 3.8 were combined.

Evaporation of the solvents gave a crystalline residue which was recrystallised from hexane to afford pure (4) (2.04 g, 59.5%).

IR ($CHCl_3$) ν max (cm^{-1}) 3390 (NH), 1715 (CO)

NMR ($CDCl_3$) δ 1.53 (9H, s, tBu), 3.86 (3H, s, OCH_3), 3.96 (5H, broad s, OCH_3 and CH_2), 6.65-7.2 (7H, complex, ArH), 7.51 (1H, broad s, NH); MP 95°C.

Found: C, 70.41; H, 7.33; N, 4.03 %. Calculated for $C_{20}H_{25}NO_4$: C, 69.95; H, 7.3; N, 4.1 %.

3-methoxy-2-(2-methoxybenzyl)aniline (5)

The carbamate (4) (3.4g, 10 mM) was dissolved and stirred in cold (0°C) trifluoracetic acid (13 ml). After 5 min at 0°C, the bath was removed and the flask was allowed to warm to 20°C (20 min). The mixture was then partitioned between water (50 ml) and ether (100 ml). The ethereal solution was washed with NaOH (2 x 50 ml) and water until neutral, dried ($MgSO_4$) and the solution evaporated to afford a light brown oil which crystallised on standing: 2.40 g : 99%. One recrystallisation from diisopropylether afforded analytically pure (5).

IR ($CHCl_3$) ν max (cm^{-1}) 3480, 3380 (NH_2)

NMR ($CDCl_3$) δ 3.67 (2H, broad s, NH_2), 3.79 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.93 (2H, s, CH_2), 6.3-7.2 (7H, complex, ArH), MP 121-122°C.

Found: C, 73.91; H, 6.98; N, 5.77 %. Calculated for $C_{15}H_{17}NO_2$: C, 74.05; H, 7.05; N, 5.76 %.

3-Bromo-2 (2-methoxybenzyl) anisole (6)

To the aniline (5) (1.215 g, 5 mM) dissolved in cold (0°C) acetonitrile (3 ml) was added copper II bromide (6 mM) and then dropwise, isopentylnitrite (0.875 g, 7.47 mM) in acetronitrile (1 ml). The reaction mixture was allowed to warm slowly to room temperature (overnight), after which time it was poured into water, extracted (CH_2Cl_2), and purified by HPLC (Column Zorbax Sil E.V.), using a mixture of heptane 90% and CH_2Cl_2 10% as eluent. k' = 0.86.

Mass: m/e 308,306 (M^+)

NMR: ($CDCl_3$) δ 3.73 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.16 (2H, s, CH_2) 6.54-7.29 (7H, complex, ArH), MP 104°C (from CH_2Cl_2-diisopropylether).

Found: C, 58.35; H, 4.77; O, 10.59 %. Calculated for $C_{15}H_{17}BrO_2$: C, 58.6; H, 4.9; O, 10.4 %.

The dibrominated material formed in this reaction had k' = 1.04. MP: 104°C (from dichloromethane and diisopropylether).

3-methoxy-2-(2-methoxybenzyl) [carbonyl-^{14}C]benzoic acid

In a 50 ml, round-bottomed flask connected to a manifold flushed with N_2 were placed the bromoamisole (6) (337 mg, 1.110 mM), magnesium (28 mg 1.12 mM) and dry THF (5 ml). The reaction was initiated by heating (hair dryer) and heated at 40°C for 1 hr. The flask was then cooled to -78°C and the apparatus evacuated. Dry $^{14}CO_2$ was generated from $Ba^{14}CO_3$ (1mM, 57.2mCi/mmol) and concentrated sulfuric acid. The gas was first trapped in a flask containing P_2O_5 and cooled in liquid N_2. The liquid N_2 bath was removed and the gas transferred into the Grignard solution under vacuum. The reaction mixture was allowed to warm to room temperature over 18 hours. Saturated aqueous NH_4Cl (2 ml) was added. The pH was adjusted to 12 with 2N

NaOH. The mixture was partitioned between water (10 ml) and CH_2Cl_2 (10 ml). The organic layer was discarded. The aqueous layer was acidified to pH 1 with 12 N hydrochloric acid and extracted with ethyl acetate (50 ml). The organic layer was dried ($MgSO_4$) and the solvents evaporated to afford (7) (217 mg, 80% (radiochemical yield), 45.6 mCi) homogenous on silica gel, using as eluent a mixture of ether/hexane (6/4) containing acetic acid (1%): RF = 0.45.

1,8-Dimethoxy-[10-^{14}C]anthraquinone (8)

The benzoic acid (7) (166 mg, 34.8 mCi) was stirred for 1 hr with 96% sulfuric acid (0.27 ml). The flask was cooled to 0°C and water (0.9 ml) and potassium bichromate (0.26 g in 1.2 ml of water) were added, followed by acetic acid (4.5 ml).

The reaction mixture was refluxed for 75 min, cooled to room temperature, poured onto 5 ml of ice and adjusted to pH 12 using concentrated NH_4OH. The solution was extracted with CH_2Cl_2 (3 x 20 ml) and the organic layer washed with water, dried ($MgSO_4$) and the solvents evaporated to afford (8) (70 mg, 42 %, 14.8mCi) (chemical yield) ; homogeneous by thin layer chromatography using a mixture of ethyl acetate 5 and chloroform 5 as eluent, (RF: 0.6) and by HPLC (column Zorbax ODS, eluent : MeOH 8, water 2) k' = 1.5 and identical in chromatographic behaviour to authentic 1,8-dimethoxyanthraquinone (J. Maignan, L'Oréal Research Laboratories, Aulnay-sous-Bois, France).

The specific activity determined using mass spectrometry by measuring the ratio

$$\frac{M^+ \, ^{14}C \ (8)}{M^+ \, ^{14}C \ (8) + M^+ \text{ cold } (8)}$$

was found to be 56.9 mCi/mmol.

1,8-Dihydroxy-[10-^{14}C]anthraquinone (9)

Aluminium chloride (200 mg, 1.5 mM) was added to a solution of the quinone (8) (46 mg, 10.2 mCi) in dichloromethane (4 ml). The mixture was

stirred for 16 hours, poured into 3N HCl (20 ml) and extracted with dichloromethane (20 ml). After standard work-up, evaporation of the solvents afforded (9) (total activity 9.7 mCi, 95%), homogenenous by thin layer chromatography on silica gel using a mixture of benzene and ethyl acetate (6:4) as eluent (RF: 0.65).

1,8-Dihydroxy-[10-^{14}C]anthrone (anthralin) (1)

To (8) (2.4 mCi) were added successively a mixture of 12 N HCl (0.33 ml) and acetic acid (0.66 ml) and powdered tin (110 mg). The mixture was refluxed for 1 hour and the solvents evaporated (aspiration). The residue was taken up in $CHCl_3$ (500 µl) and isooctane(3 ml) was added. Chromatography (HPLC column: Dupont Zorbax SIL), using a mixture of diisopropyl ether 5, isooctane 5 containing acetic acid 1 °/oo afforded 1: 2.25 mCi, 94%. Single spot on TLC (silica gel, eluent: toluene 97, acetic acid 3. (RF: 0.7)

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