Synthesis of C-9-^{14}C-1,8-dihydroxy-3-carboxyanthraquinone

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Summary

The synthesis of C-9-^{14}C-rhein is reported, $^{14}CO_2$ is used as a ^{14}C-source. After preparing ^{14}C-1,8-dimethoxy-3-methylanthraquinone by a condensation reaction, the product is demethylated and the 3-methyl group converted to the corresponding 3-carboxy group. The radio-active yield of the total synthesis, starting with 1 Ci $^{14}CO_2$ is 6,9% (6,9 mCi); 352 mg ^{14}rhein is produced with a specific activity of 55,7 mCi/mmol.

Key words: ^{14}C-rhein, ^{14}C-chrysophanol, C-9-^{14}C-1,8-dihydroxy-3-carboxyanthraquinone, C-9-^{14}C-1,8-dihydroxy-3-methylanthraquinone, ^{14}C-anthraquinone.

Introduction

Sennosides are widely used in the human constipation therapy and act as prodrugs which generate sennidines in the colon by bacterial hydrolysis; these sennidines are further transformed in situ to the active compounds rhein-9-anthrone and rhein. Although these laxatives have been taken by humans for centuries, little is known about the mechanism of action, toxicology and metabolism. In order

0362-4803/88/010023-11$05.50

Received January 1, 1987
Revised April 4, 1987

to study the metabolism of rhein in the rat we found it necessary to use a ^{14}C-labelled drug: prior attempts with "cold" rhein showed, after oral administration , a recovery of only 17 to 20% anthracene derivatives in urine and faeces after 72 h, the gap of some 80% being inexplicable (1).

This seems to be a constancy in the metabolism of anthraquinones: other authors dealed with the same low-recovery problem too (2, 3, 4).

So the use of radio-active anthraquinones is to be considered as a must in metabolism studies in order to find "non-anthraquinone" metabolites and/or important body sites of retention.

The idea to use tritiated compounds was dismissed because of the high acidic character of the anthraquinone aromatic ring protons (5, 6).

Recently three routes were described to synthetize C-9-^{14}C-dithranol using phtalic acid (by a Friedel-Crafts acylation), CH_3I (o-lithiation according to (7)) or CO_2 (o-lithiation according to (8)) as a ^{14}C-source (5).

Although $^{14}CO_2$ is a troublesome and hazardous substance to manipulate, the third pathway was choosen due to the relatively low price of the ^{14}C-source.

Experimental

General

Melting points were determined on an Electrothermal Melting Point Apparatus and are uncorrected. Thin Layer Chromatography (TLC) was carried out using commercially prepared 200 micron layer silica gel F_{254} plates (Merck). Preparative column chromatography was carried out with a glass column (diam. 40 mm), using for each run 50 g silicagel 60 for column chromatography (15-40 micron) (Sigma). Radio-TLC's were made by a Thin Layer Scanner II LB 2723

(Berthold). Radio-active samples were counted in Lumagel (Lumac) by a Berthold beta-counter BF 5003 A.

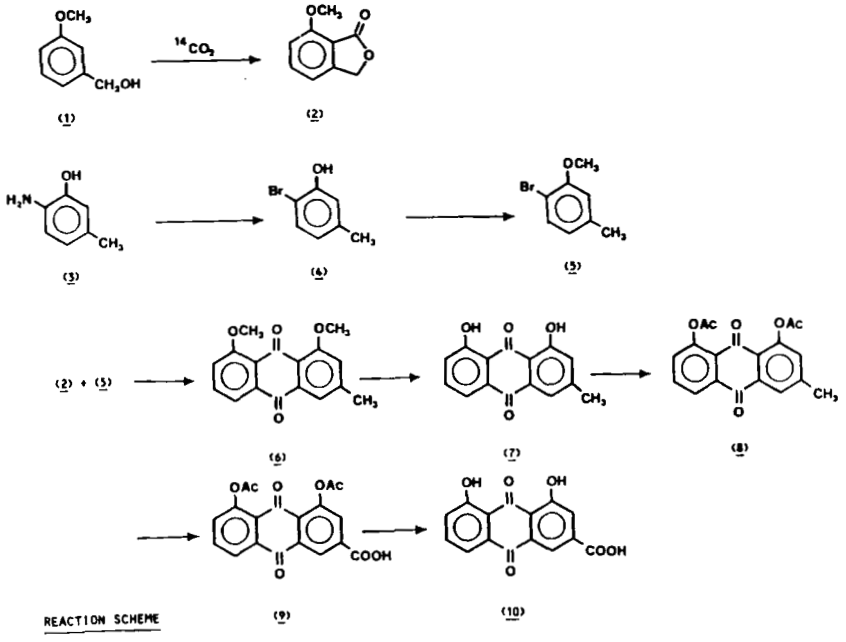
Unlabelled products, prepared by the same synthetic pathways, were analyzed by mass spectrometry and m.p. comparisons with the literature values (for synthetic strategy see Reaction Scheme).

Mass spectrometry was carried out on a HP 5995A Gas Chromatograph/Mass Spectrometer.

n-Hexane, THF and diisopropylamine were dried on $LiAlH_4$ or Na/Pb alloy and freshly distilled before use.

3-Methoxybenzylalcohol (Janssen Chimica) was purified by vacuum fractional distillation (93-94 C/0,8 mm Hg) and kept in a dry, inert atmosphere prior to use.

$^{14}CO_2$ was purchased from Amersham (1 Ci; 55,7 mCi/mmol; radioch. purity: > 95%).



^{14}C-7-methoxyphtalide (2)

15,2 g of a 1,6 M n-butyllithium solution in n-hexane (35,6 mmol) is added dropwise to a very rapidly stirred suspension of 3-methoxybenzylalcohol (1) (2,46 g; 17,8 mmol) in 36 mL dry n-hexane under dry and inert atmospheric conditions (Ar) at room temperature. The brick-red mixture is heated at 55-60°C for 14 hours and cooled to -78°C (dry ice/aceton). A separate tightly closed reaction vessel placed in liq. nitrogen is connected with a $^{14}CO_2$ containing break-seal ampoule (17,95 mmol), the content of the latter being transferred under vacuum and condensed as dry ice in the receiving vessel, before atmospheric pressure is re-established by an attached balloon filled with dry nitrogen gas.

The lithium-ortho-lithiobenzylalcoholate suspension in n-hexane is pumped over in one run through a cooled (-78°C) teflon tube (.125" i.d.) by means of a nitrogen gas stream into the $^{14}CO_2$-condensation reaction vessel, while the liq. nitrogen is rapidly replaced by an aceton/dry ice mixture. The yellow suspension is stirred for another 15 minutes, before being warmed up to room temperature, and is acidified (4 N HCl).

After one hour the non-incorporated $^{14}CO_2$ is expelled from the excipient with a vigorous "cold" CO_2 stream and trapped in a series of three wash bottles filled with 25% NH_3, before opening the reaction vessel.

The suspension is extracted with chloroform, the solvent removed under reduced pressure and the crude mixture purified in two runs by prep. column chromatography (first run: ethylacetate 50/n-hexane 50; second run: chloroform) and dried under vacuum over P_2O_5.

Yield: 819 mg (28%)

Radiochem. purity: > 99% (R_f: 0.31, solvent system:
ethylacetate 50/n-hexane 50)

m.p.: 103-105°C (lit. 107-109°C (9))

Mass spectrum m/e (rel. intensity): 29.0 (15.5), 39.1 (23.2), 50.1 (22.2),51.1 (29.0), 63.1 (23.5), 76.1 (19.4), 77.1 (48.2), 78.1 (24.3), 92.1 (20.7), 105.1 (27.4), 106.1 (19.1), 118.0 (100), 119.0 (18.0), 135.1 (66.5), 146.1 (39.2), 164.1 (26.6)

6-Bromo-3-methylanisole (5)

18 g of 6-amino-3-cresol (3) (146,1 mmol, Janssen Chimica), dissolved in 22,2 g concd. sulfuric acid and 110 mL water, is diazotated below 10°C with a solution of 10,2 g of $NaNO_2$ (147,8 mmol) in 210 mL water. Replacement of the diazonium group with bromine is effected by dropping the solution (kept at max. 10°C) into a suspension of 11,52 g of CuBr (80,4 mmol) and 12 mL of a 48% HBr solution in water, while a vigorous current of steam is passed through the reaction mixture (steam distillation). The oil in the distillate is extracted with ether. After removing the solvent under reduced pressure, methanol (100 mL) is added. At 5°C the mixture is methylated with a solution of diazomethane in ether (ca. 0,5 M, 400 mL). In these conditions 6-bromo-3-cresol (4) selectively reacts in the mixture of other phenols. The solution is extracted five times with 50 mL 0,1 N KOH, washed with water, dried over Na_2SO_4, and the solvent removed under reduced pressure. The oil is dried under vacuum over P_2O_5.

Yield: 15,9 g 6-bromo-3-methylanisole (59% over the two steps)

Chemical purity: > 98% (R_f: 0.66, solvent system: chloroform)

Mass spectrum m/e (rel. intensity): 39.0 (37.7), 50.0 (30.9), 51.0 (57.4), 52.0 (23.4), 63.0 (28.0), 65.0 (22.9), 77.1 (68.3), 78.1 (100), 91.1 (51.1), 121.0 (42.5), 156.9 (23.2), 158.9 (21.5), 200.0 (88.1), 202.0 (85.1)

^{14}C-1,8-dimethoxy-3-methylanthraquinone (6)

A solution of Li-diisopropylamide is prepared by adding at -78°C 7,04 g of a 1.6 M n-butyllithium solution in n-hexane (16,50 mmol) under dry and inert atmospheric conditions to a stirred solution of 1,67 g dry diisopropylamine (16,50 mmol) in 50 mL dry THF. A solution of ^{14}C-7-methoxyphtalide (2) (819 mg, 4,99 mmol) in dry THF is dropwise added to the reaction mixture and warmed up to -35°C. A solution of 2,11 g 6-bromo-3-methylanisole (5) (10,49 mmol) in dry THF is dropped into the carbanion solution, which is further allowed to warm up to room temperature. A stream of oxygen is then passed through for 15 minutes, and the solvent removed under reduced pressure, before the whole is acidified with a mixture of concd. hydrochloric acid and ice.

The suspension is extracted with ethylacetate, and the crude product purified by prep. column chromatography (n-hexane 30/ethylacetate 70).

Yield: 570 mg (40%)

Radiochem. purity: > 98% (R_f: 0.19, solvent system: n-hexane 30/ethylacetate 70)

m.p.: 197-198°C (lit. 195°C (10))

Mass spectrum m/e (rel. intensity): 63.1 (20.7), 76.1 (26.7), 139.1 (25.6), 151.1 (20.0), 152.1 (52.9), 153.1 (28.5), 165.1 (69.5), 265.1 (20.3), 267.1 (100), 282.2 (27.1)

The unlabelled compound proved to possess identical physical properties as 1,8-dimethoxy-3-methylanthraquinone, obtained by methylation of the two phenol groups of unlabelled, commercially available chrysophanol (7), by dimethylsulphate in an alkaline solution (method: as for lucidin (12)).

^{14}C-1,8-dihydroxyanthraquinone (^{14}C-chrysophanol) (7)

A stirred solution of 570 mg ^{14}C-1,8-dimethoxy-3-methylanthraquinone (6) (2,02 mmol) in 29 mL concd. phosphoric acid is heated for 35 minutes, under reflux, while a stream of nitrogen is passed through the reaction vessel. After cooling to 0-5°C and dilution with water, the whole is neutralized with a solution of NaOH in water to ca. pH 5 and extracted with chloroform. The solvent is removed under reduced pressure, and the crude product purified by prep. column chromatography (chloroform 60/n-hexane 40). The unreacted dimethyl and the partly reacted monomethyl derivatives are reprocessed with 10 mL concd. phosphoric acid.

Yield: 445 mg (87%)
Radiochem. purity: > 98% (R_f: 0.61, solvent system:
n-hexane 30/ethylacetate 70)
m.p.: 192-193°C (lit. 196°C (11))
Mass spectrum m/e (rel. intensity): 63.1 (20.1), 115.0 (23.8),
152.1 (25.7), 226.1 (21.7), 254.1 (100)

The unlabelled product proved to possess the same physical properties as commercially available chrysophanol.

^{14}C-1,8-diacetoxy-3-methylanthraquinone (8)

A mixture of 445 mg ^{14}C-chrysophanol (7) (1,75 mmol) and 450 mg Na-acetate (anh.) in 4,5 mL acetic anhydride is heated under reflux for 15 minutes. The suspension is quenched with water at 60°C and allowed to cool to room temperature, before being extracted with chloroform. The solvent is removed under reduced pressure and the crude product purified by prep. column chromatography (chloroform 70/hexane 30).

Yield: 497 mg (84%)

Radiochem. purity: > 99% (R_f: 0.13, solvent system:
n-hexane 30/chloroform 70)

m.p.: 207-209°C (lit. 207-208°C (11))

Mass spectrum m/e (rel. intensity): 43.1 (100), 115.0 (16.4), 139.1 (10.5), 152.1 (10.4), 254.1 (86.2), 255.1 (13.6), 296.1 (17.1)

The unlabelled product proved to possess the same physical properties as 1,8-diacetoxy-3-methylanthraquinone, synthetized according to Bellaart (11) starting with commercial chrysophanol.

^{14}C-1,8-dihydroxy-3-carboxyanthraquinone (^{14}C-rhein) (10)

A solution of 960 mg CrO_3 (9,6 mmol) in 2,4 mL acetic anhydride, diluted with 2,4 mL acetic anhydride is added by parts to a suspension of 497 mg ^{14}C-chrysophanoldiacetate (9) (1,47 mmol) in a 1:1 mixture of acetic anhydride/acetic acid (14 mL), the whole being heated for 6 hours at 55-60°C. The dark-green suspension is quenched wich water, extracted with ethylacetate and washed with water.

The solvent is removed under reduced pressure. The ^{14}C-1,8-diacetoxy-3-carboxyanthraquinone (9) is dissolved, avoiding the influence of light, in 200 mL 2,5% Na_2CO_3 and extracted several times with chloroform, before being boiled for 20 minutes, while a slight nitrogen stream is passed through the reaction vessel. Cooled to room temperature, the solution is slowly acidified and after most CO_2 has escaped reheated for 30 minutes at 100°C.

The precipitated ^{14}C-rhein (10) is collected by centrifugating and is washed with water and methanol.

Yield (two steps): 363 mg (87%)

Radiochem. purity: > 98% (R_f: 0.33, solvent system: ethylacetate 77/methanol 13/water 10)

Spec. activity: 54 mCi/mmol

m.p.: > 310°C (lit. 321-322°C (11))

Mass spectrum m/e (rel. intensity): 63.1 (19.2), 126.1 (20.1), 127.1 (17.0), 155.1 (18.7), 228.1 (15.1), 239.1 (17.0), 256.1 (16.6), 284.1 (100), 285.1 (16.9)

The unlabelled product proved to possess the same physical properties as rhein, synthetized from commercial aloin according to Bellaart (11).

Discussion

The idea to synthetize anthraquinone derivatives by condensation of the lithium salts of phtalides with arynes, generated in situ from bromobenzenes, goes back to Dodsworth (13). In the first step the phtalide derivative is synthetized by an electrophilic attack of carbon dioxide on the in situ produced Lithium-ortho-lithiobenzylalcoholate (8). In order to keep the high specific activity of $^{14}CO_2$ (55,7 mCi/mmol), we used equimolar quantities, which accounts for the yield of only 28% : prior "cold" attempts with a large excess of dry ice, which is recommended in this type of reaction to avoid formation of ketone derivatives (14), showed good yields of 45-55%.

The subsequent nucleophilic addition reaction of the 7-methoxyphtalide (2) to the aryne derivative proved, as Dodsworth (13) pointed out, to be regioselective: a TLC of the crude ethylacetate extract (solvent system: ethylacetate 70/n-hexane 30) shows besides an intense spot of (6) (R_f: 0.19) another faint yellow spot (R_f: 0.28), which is believed to be an isomeric form,

produced in a low yield, of the major product (6) (yield: 40 %) of this step. The oxidation of the 3-methyl group to the corresponding 3-carboxy group with CrO_3 at this stage gave very low yields (3-7%) of 1,8-dimethoxy-3-carboxyanthraquinone. The methoxy groups of (6) seemed to be responsible because the 1,8-diacetoxy derivative was readily oxidized in good yields (70-90%).

So we demethylated (6), according to a method described for 1,8-dimethoxy-3-carboxyanthraquinone (15) with concentrated phosphoric acid (yield 87%), acetylated the two phenol groups with a mixture of acetic anhydride and sodium acetate (yield 84%), before oxidizing with CrO_3 in a mixture of acetic acid and acetic anhydride (Bellaart (11)). The produced (9) is purified by extraction of unreacted (8) with chloroform from an alkaline solution and immediately hydrolyzed in the same medium. Care should be taken to avoid day light because of possible deterioration of rhein (10) in this reaction.

The radio-active yield of the total synthesis, starting with 1 Ci $^{14}CO_2$ is 6,9% (69 mCi).

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