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Synthesis of high specific radioactivity [^{3}H]emodin

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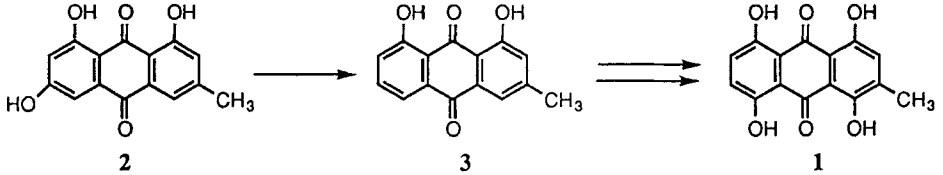
Summary.

Emodin, a precursor of chrysophanol in the biosynthesis of cynodontin by *Pyrenochaeta terrestris*, has been tritiated with a high specific radioactivity (12.5 Ci/mmol) by hydrogenolysis of bromomethyl emodin.

Introduction.

An important step of the biosynthesis of cynodontin **1** (1) by *Pyrenochaeta terrestris* is the transformation of emodin **2** into chrysophanol **3** (2) (Scheme 1). The mechanism of this **2** → **3** transformation is actually under extensive investigation (2, 3) in connection with the general problem of deoxygenation of metadiphenols derived from polyketides (4). This study requires a high specific radioactivity emodin owing to the low emodin deoxygenase activity present in *P. terrestris* extracts.



Scheme 1 : Biosynthesis of cynodontin **1**

First attempts to prepare [^{3}H]emodin using Wilzbach method (5) yielded low specific radioactivity [^{3}H]emodin (2, 6). Furthermore, two of the aromatic hydrogen atoms of emodin **2** being located on the metadiphenol part of the molecule are, as such, potentially exchangeable with the

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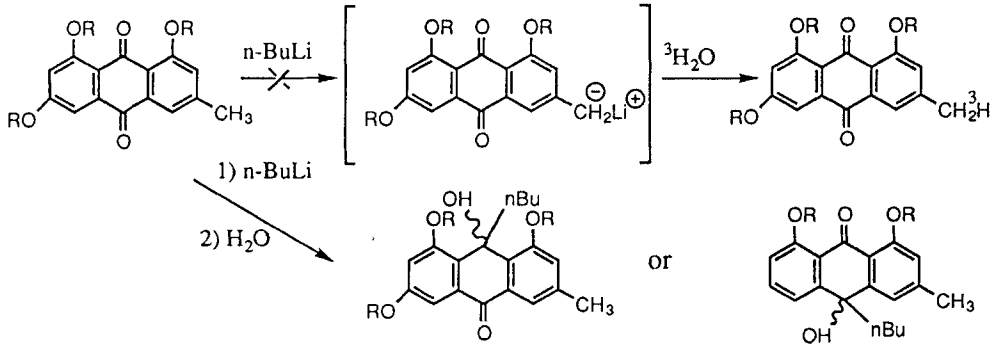
solvent through a reversible ketonization-enolization process (4c). Thus it was important to have a readily access to high specific radioactivity emodin with the label located on a non exchangeable position.

We report here the synthesis of 12.5 Ci/mmol [^{3}H]emodin bearing the label on the methyl group.

Results and discussion.

The most obvious way to label the benzylic position of emodin was a metallation-hydrolysis sequence which should have led almost directly to [^{3}H]emodin. After protection of the hydroxyl groups as acetates (R=CH_3CO), the treatment with n-butyllithium resulted almost quantitatively into the addition of a n-butyl radical on one of the carbonyl groups of the quinone with no trace of the expected metallation product (Scheme 2).



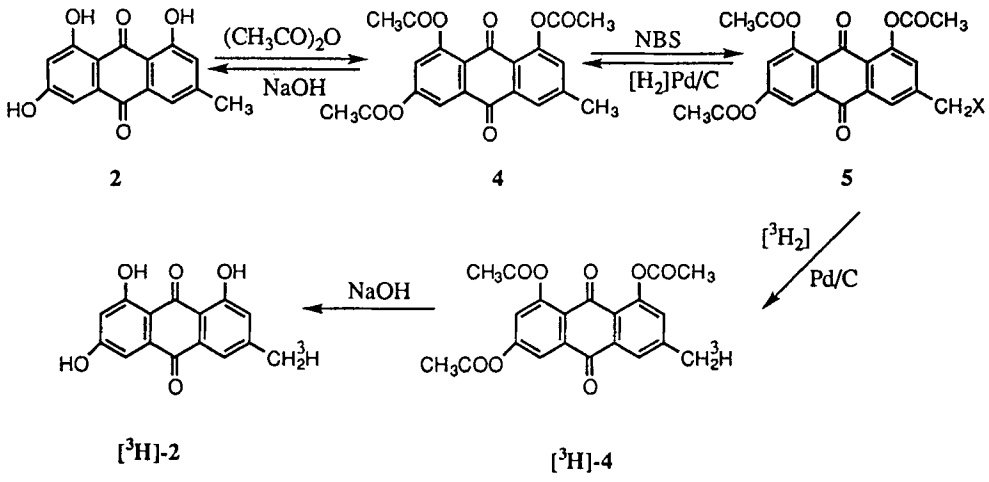
Scheme 2 : Action of n-BuLi on emodin

Another classical way for labeling almost quantitatively a benzylic position is the hydrogenolysis of a halomethyl derivative of emodin. The bromo substituted emodins have been described recently (7) but the experimental details were scarce and the product composition seemed to depend strongly on experimental conditions. We decided to reinvestigate the bromination of emodin.

Photochemical or radical induced bromination of emodin with N-bromosuccinimide in carbon tetrachloride failed, presumably because of the low solubility of emodin in that solvent. Peracetylation of emodin yielded emodin triacetate **4** that proved to be soluble enough for radical induced bromination.

Unexpectedly reaction of **4** with 1.1 eq. of N-bromosuccinimide in carbon tetrachloride in the presence of benzoyl peroxide yielded a mixture containing unreacted triacetate **4** (35 %) along with monobromomethyl triacetate **5** (55 %) and dibromomethyltriacetate (10 %). The dibromomethyl derivative could be eliminated by crystallization but the mixture of **4** and **5** could not be resolved easily in a preparative way. Use of a slight excess of triacetate **4** yielded reproducibly a mixture of **4** (20 %) and **5** (80 %).

Hydrogenolysis of this mixture in methanol with palladium on charcoal (10 %) afforded pure **4** that could be deacetylated smoothly into **2** upon treatment with sodium hydroxide (Scheme 3).



Scheme 3 : Synthesis of [^{3}H]emodin

The same sequence of reactions where the hydrogenolysis has been run with tritium gas (10 Ci) afforded pure [^{3}H]emodin whose specific radioactivity was 12.5 Ci/mmol as estimated by mass spectrometry with label located exclusively as expected on the methyl position of emodin **2** as checked by ^{3}H NMR spectroscopy.

Experimental.

^{1}H NMR and ^{13}C NMR spectra were recorded respectively at 200 MHz and 50 MHz on a Bruker AC200 spectrometer in $CDCl_3$. Chemical shifts δ are expressed in ppm relative to tetramethylsilane. ^{3}H NMR spectrum was recorded at 320 MHz on a Brucker AC300 spectrometer. Melting points were determined using a hot stage Köfler apparatus.

Chemical. Emodin and N-bromosuccinimide were from Janssen. 10 % Palladium on charcoal was from Fluka and trifluoroacetic acid was from Sigma. Tritium gas was from Radium Chemie AG (Switzerland). All other chemicals or solvents were of the highest purity available.

1,6,8-triacetoxy-3-methyl-9,10-anthraquinone **4**. Acetic anhydride (5 mL) was added dropwise to a suspension of emodin **2** (0.27 g, 1 mmol) in pyridin (5 mL). Upon solubilization the orange colour of the mixture vanished to yellow and the solution was stirred at room temperature for 1h30. Solvents were evaporated under vacuum and the green crude product was crystallized from a methylene chloride / ethanol mixture (1/1) yielding **4** (0.36 g, 90 %) as yellow green needles (F = 208°C).

^{1}H NMR δ : 2.34 (s, 3H, CH_3CO) ; 2.43 (s, 6H, CH_3CO) ; 2.49 (s, 3H, CH_3) ; 7.1 (bs, 1H, H arom) ; 7.22 (d, J = 2.5 Hz, 1H, H arom) ; 7.94 (d, J = 2.5 Hz, 1H, H arom) ; 8.00 (bs, 1H, H arom).

^{13}C NMR δ : 21.01 (CH_3CO_2) ; 21.61 (CH_3) ; 118.22 (*C*H) ; 122.95 ; 123.12 ; 123,31 (*C*H) ; 125.98 (*C*H) ; 130.84 (*C*H) ; 133.92 ; 135.53 ; 146.32 ; 150.13 ; 151.34 ; 154.54 ; 167.91 (CH_3CO) ; 169.00 (CH_3CO) ; 169.41 (CH_3CO) ; 179.60 (*C*O) ; 181.34 (*C*O).

Bromination of 4. N-bromosuccinimide (0.18 g, 1 mmol) and benzoyl peroxide (5 mg, 18.7 μmol) were added to a suspension of **4** (0.40 g, 1 mmol) in carbon tetrachloride (100 mL). After refluxing overnight, the mixture was cooled and filtrated over scintered glass. The filtrate was taken to dryness under vacuum. The yellow residue was crystallized from ethylacetate and yielded a mixture of **4** and 1,6,8-triacetoxy-3-bromomethyl-9,10-anthraquinone **5** (**4/5** = 20/80) that could not be separated by conventional methods.

Hydrogenolysis of 5. A mixture of **4** and **5** (**4/5** = 20/80, 10 mg) was hydrogenolyzed in dry methanol (2 mL) with palladium (Pd/C 10 %, 2 mg). After 4.5 h the suspension was filtered and the palladium washed with hot methanol. Concentration under vacuum yielded **4** that was used for the next step without purification.

Hydrolysis of 4. **4** (10 mg), 25 μmol) was heated at 80°C for two hours in sodium hydroxide (1 mol/L, 2 mL). After cooling, the pH was dropped to 4-5 with 10 % citric acid and the mixture was diluted with water and extacted with ethyl acetate. The organic layer was washed with water, brine and dried over magnesium sulfate. Elimination of solvent yielded pure **2** almost quantitatively.

^{1}H NMR (DMSO) δ : 2.42 (s, 3H, CH_3) ; 6.60 (d, J = 2.4 Hz, 1H, H arom) ; 7.13 (d, J = 2.4 Hz, 1H, H arom) ; 7.18 (d, J = 1 Hz, 1H, H arom) ; 7.50 (d, J = 1 Hz, 1H, H arom) ; 11.40 (s, 1H, OH) ; 12.02 (s, 1H, OH) ; 12.10 (s, 1H, OH).

^{13}C NMR (DMSO) δ : 22.34 (CH_3) ; 108.69 (C_2) ; 109.61 (C_4, C_{13}) ; 114.04 (C_{12}) ; 121.22 (C_5) ; 124.87(C_7) ; 133.48 (C_{11}) ; 135.77 (C_{14}) ; 148.98 (C_6) ; 162.20 (C_8) ; 165.26 (C_1) ; 166.43 (C_3) ; 181.98 (C_{10}) ; 190.39 (C_9). This spectrum is in good agreement with reference (8).

1,6,8-trihydroxy-3-[^{3}H]methyl-9,10-anthraquinone ([^{3}H]emodin). A mixture of **4** and **5** (**4/5**, 20/80, 7.2 mg) was dissolved in dry methanol (7 mL) in a 9 mL flask. After addition of Pd/C (10 %, 3.5 mg), tritium gas (10 Ci) was introduced in the flask using a Toeppler pump. The suspension was stirred at room temperature for 4 h. Following filtration (Millex 0.22 μ) the catalyst was rinsed with methanol (5 mL) and the yellow solution was taken to dryness under vacuum. Hydrolysis with sodium hydroxide (1 mL, 1 mol/L) was achieved at 80°C for 2 h. The pH of the solution was dropped to 4 with 10 % citric acid. [^{3}H]emodin was extracted with ethyl acetate (4 x 5 mL). The organic solution was dried on magnesium sulfate and exchangeable tritium was eliminated by evaporation of methanol (2 x 10 mL) yielding a total radioactivity of 236 mCi. Purification using HPLC (column Zorbax ODS 9.8 x 250 mm. Eluents : A, acetonitrile / 0.1 % trifluoroacetic acid. B, water / 0.1 % trifluoroacetic acid. Gradient 55 % A → 90 % A in 60 minutes, 1.5 mL/min) yielded [^{3}H]emodin (27 mCi). Radiochemical purity (> 99.9 %) was checked by HPLC

Retention time : 13.66 min) and by thin layer chromatography (RP18 $F_{254}S$ Merck, eluent methanol/water 80/20 R_F = 0.12 or Silicagel $60F_{254}$ Merck, eluent ethylacetate/cyclohexane 50/50 R_F = 0.85). Specific radioactivity of 12.5 Ci/mmol was determined by mass spectrometry (C.I., methane) M/Z = 271 (100 %) ; 273 (76.8 %) ; 275 (10.1 %). The location of the label on the methyl group was confirmed by ^{3}H NMR ($CDCl_3$) δ = 2.42 ppm.

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