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Synthesis of 4-0-(4,6-ethylidene-α-D-glucopyranosyl)-4'-demethyl-3'-0-[^{14}C]methyl-4-epipodophyllotoxin

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SUMMARY

The synthesis of the title compound (5) is described. Treatment of 4-0-(4,6-ethylidene-α-D-glucopyranosyl)-4'-demethyl-4-epipodophyllotoxin with sodium metaperiodate in acetonitrile formed the highly colored 4-0-(4,6-ethylidene-α-D-glucopyranosyl)-3',4'-dioxo-4-epipodophyllotoxin (1). Hydrogenation using 10% palladium on carbon produced the 3',4'-dihydroxy compound (2). Protection of the 4' hydroxy was achieved by benzylation (3). Treatment with excess [^{14}C]methyl iodide under basic conditions introduced the label on the 3' position (4). Deprotection yielded the title compound in an overall yield of 6%.

KEY WORDS:
4-0-(4,6-ethylidene-α-D-glucopyranosyl)-4'-demethyl-3'-0-[^{14}C]methyl-4-epipodophyllotoxin, leukemias, microtubule, antitumor.

INTRODUCTION

The mechanism by which podophyllotoxin blocks cell division is related to its inhibition of microtubule assembly in the mitotic apparatus.[1a,b] The clinical application of podophyllotoxin in the treatment of cancer[2,3] has been limited by severe toxic side effects during the administration of the drug.[4,5] In an attempt to discover less toxic analogues, a variety of podophyllotoxin derivatives have been prepared, one of which is VP-16. 4-0-(4,6-ethylidene-α-D-glucopyranosyl)-4'-demethyl-4-epipodophyllotoxin. VP-16 is effective in the treatment of a variety of leukemias and solid tumors.[6-9] This was shown not to be an inhibitor of microtubule assembly, suggesting that their antitumor properties were due to another mechanism of action.[10] The basic exploratory investigations on the disposition of VP-16

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in animals and man, (i.e. absorption, distribution, metabolism and excretion) were performed by tracer methodologies using the radiolabelled material.

This document describes the synthesis of 4-O-(4,6-ethylidene-α-D-glucopyranosyl)4'-demethyl-3'-O[^{14}C]methyl-4-epipodophyllotoxin.

EXPERIMENTAL

[^{14}C]Methyl Iodide was purchased from Amersham Corporation. All chemicals used in the synthesis were purchased commercially and used without any further purification. All other solvents were either redistilled or of analytical reagent quality. Thin layer chromatography plates used were Analtech silica gel GF, scored by 10 X 20 cm., 250 microns thickness. Radioactivity was measured by a Beckman LS9000 liquid scintillation counter. All the high pressure liquid chromatography was carried out on Waters Associates and Whatman instrumentation. Nuclear magnetic resonance was measured on a Bruker 360. Weighings were carried out on a Sartoris 200 balance and a Mettler Microanalytical M5AS balance.

4-O-(4,6-ethylidene-α-D-glucopyranosyl)-3',4'-dioxo-4-epipodophyllotoxin (1).

Etoposide (VP-16) (1 g) was suspended in acetonitrile (50 ml). To this was added a 0.5M solution of sodium metaperiodate (4.14 ml) causing an immediate deep red solution which was stirred at room temp for 4 hrs. The resulting solid was removed by filtration and the filtrate concentrated to a deep red semi-solid. Purification was achieved by chromatography on silica gel (524 mg). Yield = 53%.

Thin Layer Chromatography: 50% ethyl acetate, 50% methylene chloride, Analtech silica gel plates, visualization was with ultraviolet at 254 nm., Product Rf=0.17.

4-O-(4,6-ethylidene-α-D-glucopyranosyl)-3',4'-dihydroxy-4-epipodophyllotoxin (2).

Compound (1) (524 mg) was dissolved in methanol (30 ml) and to this was added 10% palladium on carbon (500 mg). This was shaken in an atmosphere of hydrogen at 20 psi for 5 hrs. The catalyst was removed by filtration through celite and the filtrate evaporated. The residue was purified by column chromatography on silica gel (25 g) using an eluent of 5% methanol in methylene chloride yielding a colorless solid (150 mg). Yield = 69%.

Thin Layer Chromatography: 5% methanol in methylene chloride, Analtech silica gel plates, visualization was with ultraviolet at 254 nm., Product - Rf=0.15.

4-O-(4,6-ethylidene-α-D-glucopyranosyl)-3'-hydroxy-4'-benzyloxy-4-epipodophyllotoxin (3).

To a solution of (2) (150 mg) in dry acetone (5 ml) was added potassium carbonate (145 mg) followed by benzyl bromide (895 mg). A nitrogen atmosphere was added and the reaction sealed and stirred at room temperature for 48 hrs. The reaction mixture was concentrated under reduced pressure and the resulting material purified by preparative high pressure liquid chromatography on silica gel. (Wt. = 96 mg.) Yield = 55%.

High Pressure Liquid Chromatography was carried out on Waters Associates instrumentation with the following parameters: Eluent-50% acetonitrile in water. Flow Rate-6 ml/min. Detector-Ultraviolet at 254 nm. Temperature-22.5°. Column-Whatman P-10 ODS 3 M9/50. Retention Time-11.9 min.

4-O(4,6-ethylidene-α-D-glucopyranosyl)-3'-O-[^{14}C]methyl-4'-benzyloxy-4-epipodophyllotoxin (4).

To a solution of (3) (96 mg) in dry acetone (2 ml) was added potassium carbonate (76 mg) followed by [^{14}C] methyl iodide (170 mCi, 59 mCi/mmol, 20 eq.) and the reaction was sealed and stirred at room temperature for 48 hrs. The reaction mixture was then concentrated under a stream of nitrogen, dissolved in acetone (10 ml) and the insoluble material removed by filtration. The filtrate was concentrated under reduced pressure and the resulting foam purified by preparative high pressure liquid chromatography. (Wt. = 60 mg.) Yield = 61%.

High Pressure Liquid Chromatography was carried out on Waters Associates instrumentation with the following parameters: Eluent-50% acetonitrile in water. Flow Rate-6 ml/min. Detector-Ultraviolet at 254 nm. Temperature-22.5°C. Column-Whatman P-10 ODS 3 M9/50. Retention Time-15.8 min.

4-O-(4,6-ethylidene-α-D-glucopyranosyl)-4'-demethyl-3'-O-[^{14}C]methyl-4-epipodophyllotoxin (5).

To a solution of (4) (60 mg) in methanol (20 ml) was added 10% Palladium on carbon (60 mg) and hydrogenated at 20 psi for 1 hr. at room temperature.

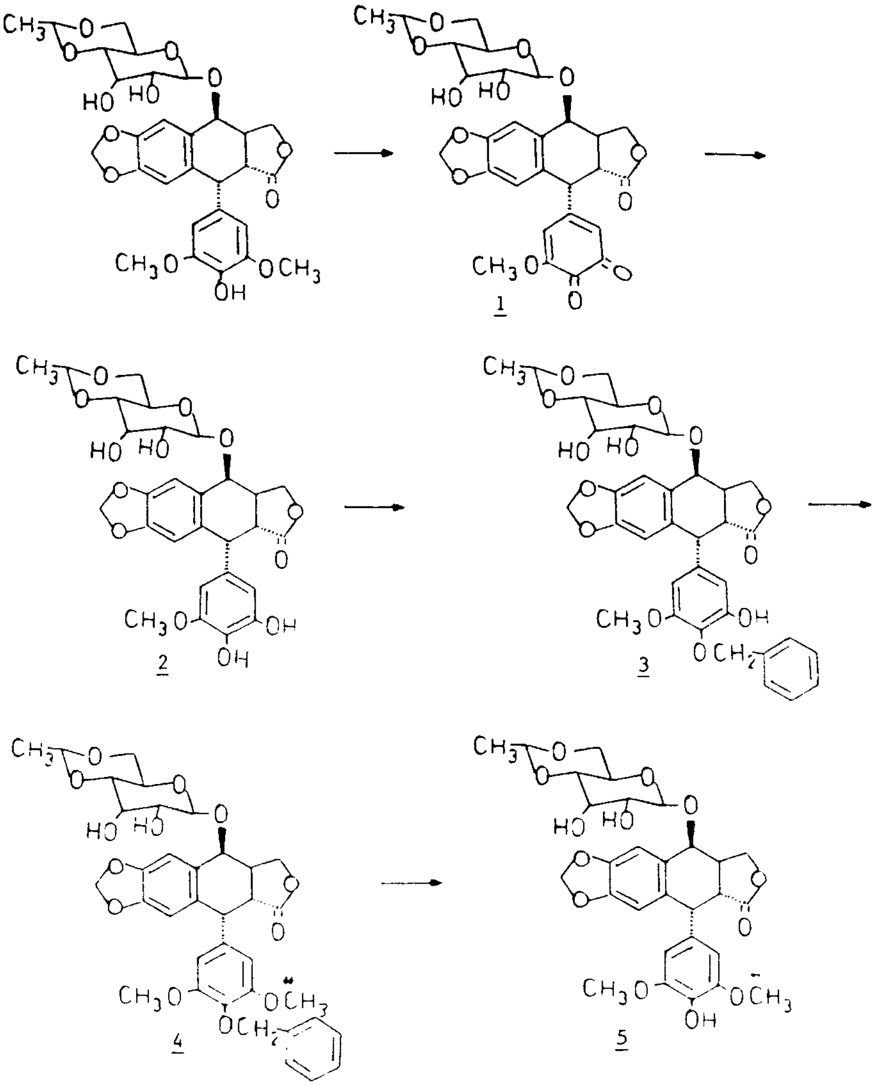
The catalyst was removed by filtration and the filtrate concentrated under reduced pressure to a light grey solid. This was purified by preparative high pressure liquid chromatography. (Wt. = 25 mg.) This was diluted with non-radioactive compound to produce a material having a specific activity of 8.3 μCi/mg and a radiochemical purity of 97%.

High Pressure Liquid Chromatography was carried out on Waters Associates instrumentation with the following parameters: Eluent-25% acetonitrile in water. Flow Rate-6 ml/min. Detector-Ultraviolet at 254 nm. Temperature-22.5°C. Column-Waters Phenyl column. Retention Time-12.1 min.

RESULTS AND DISCUSSIONS

Oxidation of etoposide (VP-16) occurred extremely rapidly under the mild conditions of sodium metaperiodate. A deep red color appeared indicating the existence of the chromatophore. Reduction to the dihydroxy compound proceeded readily under normal conditions. Both the diketo compound and the dihydroxy compound appeared to be stable. Protection of the 4' hydroxy was achieved by benzylation using one equivalent of benzyl

SYNTHETIC PATHWAY



* position of radiolabel

bromide under the basic conditions of potassium carbonate. The majority of the benzylation took place at the 4' position. Using molecular models, one sees that the the 4' hydroxy is slightly bent out of the plane of the aromatic system, thus allowing it to be benzylated. Stereochemical hindrance still existed in the reaction as demonstrated by the low yield.

Methylation of the 3' hydroxy was very specific. Methyl iodide appeared to be the only methylating agent that had any success. An 80% yield of methylated product was realized when a 40 fold excess of methyl iodide was used. At an excess of 20 fold, a yield of 60% was realized. This appeared to be a realistic yield. Anything less than this excess yielded only 5-11% product. Virtually all methylating agents that could be radiolabelled were tried without success. While methylation occurred, using these other agents, yields were extremely low.

Deprotection of the 4' position went readily and purification was achieved using high pressure liquid chromatography.

All experimental conditions were optimized using non-radiolabelled materials.

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