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

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SUMMARY

The synthesis of the title compound (5) is described. Protection of 4-O-(4,6-ethylidene-α-D-glucopyranosyl)-4'-demethyl-4-epipodophyllotoxin (1) with benzylchloroformate in the presence of pyridine gave 4-O-(4,6-ethylidene-α-D-glucopyranosyl)-4'-carbobenzyloxy-4-epipodophyllotoxin (2). Treatment of (2) with acetic acid-water produced 4-O-(α-D-glucopyranosyl)-4'-carbobenzyloxy-4-epipodophyllotoxin (3). Reaction of [^{14}C]acetaldehyde diethylacetal with (3) introduced the label in the 4',6'-ethylidene position (4). Hydrogenation using 30% palladium on carbon yielded the title compound (5) in an overall yield of 6%.

KEY WORDS:
4-O-(4,6-di[^{14}C]ethylidene-α-D-glucopyranosyl)-4'-demethyl-4-epipodophylltoxin, 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 4-O-(4,6-ethylidene-α-D-glucopyranosyl)-4'-demethyl-4-epipodophyllotoxin (VP-16).

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During the development of VP-16 as an anti-tumor agent, pharmacokinetic and drug disposition studies were essential to understand its absorption, tissue distribution, metabolism and elimination in various animal models used in the investigation of safety and efficacy.

4-O-(4,6-ethylidene-α-D-glucopyranosyl)-4'-demethyl-3'-O[^{14}C]-methyl-4-epipodophyllotoxin was originally synthesized.[6] It was found that this material underwent complete metabolism in the rat in less than 1 hr liberating [^{14}C] carbon dioxide. Consequently, a metabolically more stable position of the radiolabel had to be investigated.[7]

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

EXPERIMENTAL

[^{14}C] acetaldehyde diethylacetal was purchased from ICI Research 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 instrumentation. Nuclear magnetic resonance spectra were measured on a Bruker 360. Weighings were carried out on a Sartorius 200 balance and a Mettler Microanalytical M5AS balance.

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

To a solution of VP-16 (1) (2.5 g, 4.25 mmol) in methylene chloride (50 ml) was added pyridine (1.36 ml, 17 mmol) and benzyl chloroformate (5 ml, 17 mmol). The colorless solution was stirred at room temperature for 2 hr. The reaction mixture was washed with water (2 x 25 ml), dried, filtered and concentrated to a white foam. Purification by Silica gel chromatography using an eluent of 5% methanol in methylene chloride (Rf=0.2) yielded desired product (2) as a white foam (1.0 g, yield=33%).

High Pressure Liquid Chromatography was carried out on Waters Delta Prep. 3000 (analytical), Model 481 detector, Model 740 recorder. Eluent-50% acetonitrile in water. Flow Rate-2 ml/min. Detector-

Ultraviolet at 254 nm. Column-Nova-Pak C18 3.9 mm x 150 mm (Waters). Retention Time-7.5 min.

4-O-(α-D-glucopyranosyl)-4'-carbobenzyloxy-4 epipodophyllotoxin (3).

A solution of 2 (2 g, 2.76 mmol) in acetic acid-water (3:1, 8 ml) was heated at 70° for 17 hr. The reaction mixture was concentrated to dryness and purified by Silica gel flash chromatography using an eluent of 10% methanol in methylene chloride. The appropriate fractions (Rf=0.3) were concentrated to a white foam (3) (600 mg, yield=31%).

High Pressure Liquid Chromatography was carried out on Waters Delta Prep. 3000 (analytical), Model 481 detector, Model 740 recorder. Eluent-50% acetonitrile in water. Flow Rate-2 ml/min. Detector-Ultraviolet at 254 nm. Column-Nova-Pak C18 3.9 mm x 150 mm (Waters). Retention Time-2.06 min.

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

To a solution of 3 (347 mg, 0.5 mmol) in acetonitrile (4 ml) was added [^{14}C] acetaldehyde diethylacetal (47 mCi, 52 mCi/mmol) in acetonitrile (3 ml), non-labelled acetaldehyde diethylacetal (142 µl, 1 mmol) and p-toluenesulfonic acid (20 mg). This was stirred at room temperature for 30 min. The reaction mixture was concentrated to a white foam. Purification by Silica gel column-chromatography using 5% methanol in methylene chloride as an eluent (Rf=0.2) yielded 4 as a white foam (250 mg, yield=69%).

High Pressure Liquid Chromatography was carried out on Waters Delta Prep. 3000 (analytical), Model 481 detector, Model 740 recorder. Eluent-50% acetonitrile in water. Flow Rate-2 ml/min. Detector-Ultraviolet at 254 nm. Column-Nova-Pak C18 3.9 mm x 150 mm (Waters). Retention Time-7.5 min.

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

To a solution of compound 4 (250 mg, 0.35 mmol) in ethyl acetate (3 ml) and acetone (1.5 ml) was added 30% palladium on carbon (15 mg). This was hydrogenated in a Parr hydrogenator at 40 psi for 15 hr. An additional amount of ethyl acetate (5 ml) was added to dissolve the resulting white precipitate. The reaction-mixture was filtered through

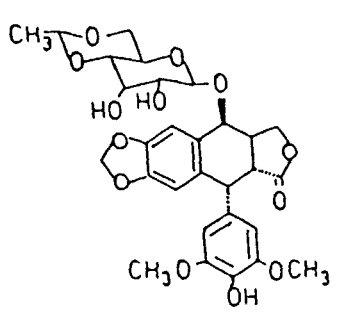
celite washed with methylene chloride (20 ml) and concentrated to a white foam. This was purified by column chromatography on Silica gel using 10% methanol in methylene chloride as an eluent. The fractions containing product (Rf=0.5) were concentrated in vacuo to a white crystalline solid (5) (155 mg, yield = 73%) having a radiochemical purity of 97.7% and a specific activity of 28 μCi/mg.

High Pressure Liquid Chromatography was carried out on Waters Delta Prep. 3000 (analytical), Model 481 detector, Model 740 recorder. Eluent-50% acetonitrile in water. Flow Rate-2 ml/min. Detector-Ultraviolet at 254 nm. Column-Nova-Pak C18 3.9 mm x 150 mm (Waters). Retention Time-1.9 min.

RESULTS AND DISCUSSIONS

Conditions of ester formation in the 4'-position of 4-O-(4,6-ethylidene-α-D-glucopyranosyl)-4'-demethyl-4-epipodophyllotoxin (VP-16) were optimized using excess benzylchloroformate and equal molar amounts of pyridine. This produced a mixture of tri, di, and monoesters. Column chromatography was used to separate and purify the desired monoester. Ring opening of the 4',6'-ethylidine was achieved by heating the monoesters in 3:1 acetic acid-water at 70°C for 17 hr. The reaction conditions were monitored by high pressure liquid chromatography. After 17 hr some starting material was present but no more product was forming. Decomposition was starting to occur. Ring closure of the ethylidine ring with [^{14}C] acetaldehyde diethylacetal catalyzed by p-toluenesulfonic acid progressed readily and purification was achieved by column chromatography. Deprotection of the 4' position proceeded under normal conditions. Purifiction by column chromatography yielded the desired product as a white crystalline solid having a radiochemical purity of 97.7% and a specific activity of 28 μCi/mg. All experimental conditions were optimized using non-radioactive materials.

SYNTHETIC PATHWAY



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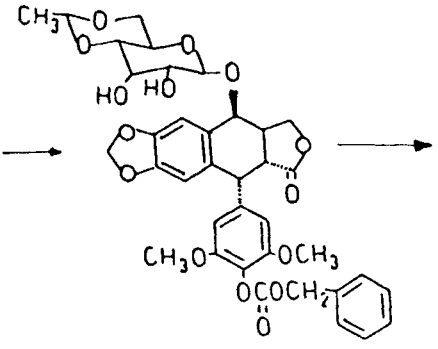

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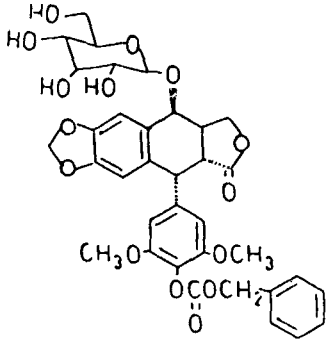

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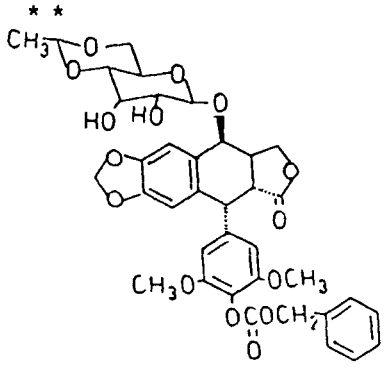

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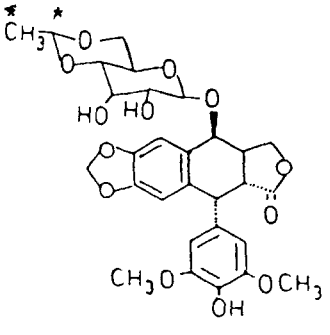

5

*Position of radiolabel

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7. Unreported results.