

**SYNTHESIS IN THE FIELD OF PODOPHYLLOTOXIN AND RELATED ANALOGUES.
PART IV: SYNTHESIS OF β -APOPICROPODOPHYLLIN ANALOGUES WITH EXPANDED
LACTONE RING**

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ABSTRACT

β -Apopicropodophyllin (2) was converted to cyanoacid (3) by treating with sodium cyanide in ethanol. The resulting acid (3) was hydrolysed to give dicarboxylic acid (4) and this was further dehydrated to anhydride (5). Sodium amalgam reduction of 5 gave β -apopicropodophyllin homolactone (6).

INTRODUCTION

β -APOPICROPODOPHYLLIN (2), the dehydration product of podophyllotoxin (1) is a much stronger antimitotic agent than (1)^{1, 2}. Since at present no information is available on the effect of ring size of lactone part of (2) on antimitotic activity, it was considered worthwhile to synthesize β -apopicropodophyllin derivative with expanded lactone ring and to study the antimitotic activity of the product.

MATERIALS AND METHODS

Cyanide anion is an effective nucleophile that opens lactone ring to form new carbon to carbon bond. When a lactone is treated with sodium cyanide, the product is a cyanoacid³. This was supported by the fact that the reaction of α -pyrones with a nucleophile such as cyanide afforded the corresponding cyanoacid derivatives⁴.

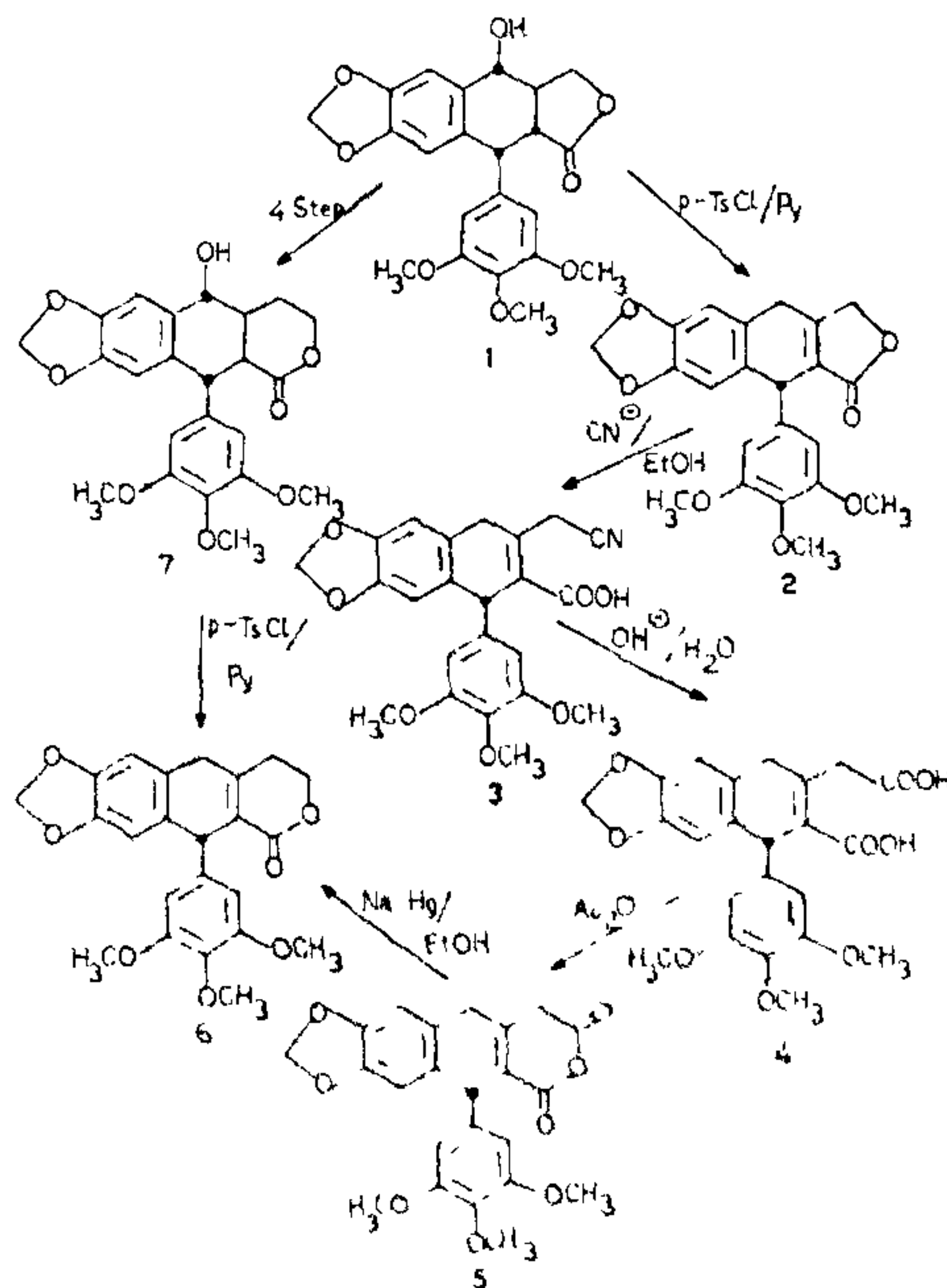
We had earlier used successfully¹, sodium cyanide in ethanol at reflux temperature to open the lactone ring in (1). By the same way, the dehydro α -pyrone ring system (α , β -unsaturated lactone) in (2) was opened by cyanide into cyanoacid which was further hydrolyzed to dicarboxylic acid. The anhydride obtained from dicarboxylic acid by dehydration was reduced to (6) by sodium amalgam reduction as shown in scheme. Structural characterisation is based on IR, NMR and mass spectra.

In order to prove that during reduction of anhydride to lactone, carbon-to-carbon double bond of lactone moiety was intact in the homolactone (6), β -apopicropodophyllin homolactone (6) was prepared starting from picropodophyllin homolactone (7) by dehydration and simultaneous isomerization of the double bond as in the preparation of β -apopicropodophyllin (2) from podophyllotoxin (1)⁵. The products obtained from both the routes were identified by thin

layer chromatography in five different solvent systems.

Experimental

Spectra were recorded on Perkin-Elmer 399-6B spectrophotometer in nujol ($\gamma_{\max} \text{cm}^{-1}$), PMR spectra in CDCl_3 on a varian 60 MHz instrument using TMS as internal standard (chemical shift in δ ppm) and mass spectra in Hitachi RMU-61 spectrometer and important fragments are given with the relative intensities (in the bracket). Purity of the compounds was checked by TLC.



Cyanoacid 3

In a typical reaction, a mixture of **2** (0.51 g, 1.3 mmol) and cyanide (0.83 g, 12 mmol) was refluxed for 30 hr on a water bath. Alcohol was removed by distillation under reduced pressure. The resulting solid mass suspended in a little water was acidified with 2N sulphuric acid and extracted into chloroform followed by water washing. The organic phase after drying (Na_2SO_4) was evacuated at *ca* 75 °C to a thick residue and the product (TLC: single spot in five different solvent system) precipitated as an amorphous white powder by the addition of hexane to the concentrated chloroform solution. Yield = 0.4 g (84%), neutralization equivalent 421 (theoretical 423); IR: 3440 (OH of carboxyl), 2150 ($-\text{C}\equiv\text{N}$), 1750 (CO of carboxyl) with shoulder at 1720, 1580 (aromatic $\text{C}=\text{C}$) cm^{-1} .

Dicarboxylic acid 4

Solution of **3** (0.35 g, 0.83 mmol) in 10% sodium hydroxide (15 ml) was refluxed for 3 hr. The reaction mixture was cooled to room temperature and then filtered. The product precipitated as an amorphous pale powder by addition of 2N sulphuric acid. The solid collected by filtration was thoroughly washed with water, yield = 0.25 g (68%), m.p. 90–92 °C; neutralization equivalent 223 (Theoretical 221); IR: 3400 (broad, OH of carboxyl), 1750 (CO of carboxyl), 1580 (aromatic $\text{C}=\text{C}$) cm^{-1} .

Anhydride 5

Solution of **4** (0.24 g, 0.55 mmol) in acetic anhydride (2 ml) was refluxed for 2 hr. The cooled reaction mixture was neutralized with saturated sodium bicarbonate solution and finally extracted into chloroform followed by water wash. The organic phase after drying (Na_2SO_4) was concentrated to a small volume (~2 ml) and the product precipitated as an amorphous pale brown powder by the addition of hexane to the concentrated solution; yield = 0.20 g (83%), m.p. 125–28 °C; IR: 1770 and 1735 (anhydride carbonyl), 1580 (aromatic $\text{C}=\text{C}$) cm^{-1} .

 β -Apopicropodophyllin homolactone (6)

Solution of **5** (0.11 g, 0.26 mmol) in ethanol (20 ml) was treated with 2% sodium amalgam (0.2 g, 0.004 g atom) and the mixture shaken to ensure thorough mixing and was kept at room temperature. After 18 hr, reaction mixture was filtered and the filtrate was concentrated to a small volume by distillation under

reduced pressure and the residue after dissolving in chloroform was successively washed with 2N hydrochloric acid, 5% sodium bicarbonate and finally with water. The organic phase after drying (Na_2SO_4) was concentrated to a small volume (~2 ml) and the product precipitated as an amorphous pale yellow powder by the addition of hexane to the concentrated solution, yield = 0.08 g (80%), m.p. 118–20 °C; IR: 1760 (CO of lactone), 1680 (conjugated double bond), 1590 (aromatic $\text{C}=\text{C}$) cm^{-1} ; PMR (CDCl_3): δ 2.0–2.6 (b, 2H, C_b-H), 3.4–3.7 (b, 2H, $-\text{CH}_2-\text{O}$), 3.85 (s, 6H, OCH_3), 3.9 (s, 3H, OCH_3), 4.2–4.6 (bm, 3H, PhCH_2- , $\text{Ph}-\text{CH}-$), 6.1 (s, 2H, $\text{O}-\text{CH}_2-\text{O}$), 6.30 (s, 1H, C_2-H or C_6-H), 6.40 (s, 1H, C_6-H or C_2-H), 6.80 (s, 1H, C_8-H aromatic), 6.95 (s, 1H, C_5-H); MS: m/z 410 (M^+ ; 10%), 398 (30), 394 (100), 379 (45), 351 (22), 168 (40). Anal: Found C, 67.10; H, 5.48% (Calcd for $\text{C}_{23}\text{H}_{22}\text{O}_7$, C, 67.32; H, 5.37%).

Conversion of picropodophyllin homolactone (7) to (6)

A mixture of **7** (0.05 g, 0.12 mmol) and *p*-toluene-sulphonyl chloride (0.07 g, 0.36 mmol) in dry pyridine (3 ml) was refluxed for 10 hr. The reaction mixture containing copious crystals of pyridine hydrochloride was evacuated at room temperature to remove most of the pyridine. The resulting slurry poured into chloroform (10 ml) was successively washed with 2N sulphuric acid (3 × 5 ml), 5% sodium bicarbonate solution (2 × 5 ml) and finally with water (3 × 10 ml). The organic phase after drying (Na_2SO_4) was completely evacuated at *ca* 75 °C to a thick residue. The residue was dissolved in chloroform and hexane was added dropwise until no more pale yellow precipitate formed on further addition.

The main solid fraction and an authentic sample of **6** synthesized from **2** were indistinguishable by TLC in five different solvent systems.

BIO-ASSAY

The above compound was tested for antimitotic activity by onion root tip method⁶ and was found to be less active ($\text{ID}_{50} = 6.5 \times 10^{-6}$ M) compared to the parent compound **2** ($\text{ID}_{50} = 2.5 \times 10^{-6}$ M).

ACKNOWLEDGEMENT

One of the authors (KMLR) is thankful to the CSIR, New Delhi for financial support.

29 June 1984; Revised 2 November 1984

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ANNOUNCEMENT

SUMMER SCHOOL IN THEORETICAL HIGH ENERGY PHYSICS 1-20 APRIL 1985, INDIAN INSTITUTE OF SCIENCE, BANGALORE.

The Science and Engineering Research Council of the Department of Science and Technology, Government of India is sponsoring a 5 year Series of Summer/Winter Schools in Theoretical High Energy Physics for the benefit of Ph.D. students as well as post doctoral scholars from all over India. The first School of the series is being held at the Centre for Theoretical Studies, Indian Institute of Science, Bangalore during 1-20 April 1985.

There will be three major courses on (i) Phenomenology of quarks and leptons, (ii) Introduction to Path Integral methods, (iii) Gauge Models of Fundamental Interactions, and three minor courses on (i) Physics of W and Z (ii) Anomalies, (iii) Composite models.

In this school, participation is limited to 25 persons, for whom round trip train travel as well as boarding and lodging will be provided. Applications from others able to find their own funds for travel, boarding and lodging, and who are also able to arrange their own accommodation, are also welcome and will be considered.

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