SYNTHESIS IN THE FIELD OF PODOPHYLLOTOXIN AND RELATED ANALOGUES.
PART IV: SYNTHESIS OF β-APOPICROPODOPHYLIN 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-APICROPODOPHYLIN (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 cyanoacid\(^3\). This was supported by the fact that the reaction of α-pyrones with a nucleophile such as cyanide afforded the corresponding cyanoacid derivatives\(^4\).

We had earlier used successfully\(^1\), sodium cyanide in ethanol at reflux temperature to open the lactone ring in (1). By the same way, the dehydro α-pyrene 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-B spectrophotometer in nujol (\(\gamma_{\text{max}} \text{ cm}^{-1}\)). 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.
Cyanoacil 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$_2$SO$_4$) was evaporated 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 (–C≡N), 1750 (CO of carboxyl) with shoulder at 1720, 1580 (aromatic C=C) cm$^{-1}$.

Dicarboxylic acid 4

Solution of 3 (0.35 g, 0.83 mmol) in 10% sodium hydride (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 225 (Theoretical 221); IR: 3400 (broad, OH of carboxyl), 1750 (CO of carboxyl), 1580 (aromatic C=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$_2$SO$_4$) 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 C=C) cm$^{-1}$.

$\beta$-Apocypodophyllin 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$_2$SO$_4$) 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 C=C) cm$^{-1}$; PMR (CDCl$_3$): δ 2.0–2.6 (b, 2H, C$_6$–H), 3.4–3.7 (b, 2H, –CH$_2$–O), 3.85 (s, 6H, OCH$_3$), 3.9 (s, 3H, OCH$_3$), 4.2–4.6 (bm, 3H, PhCH$_2$–), 5.6 (s, 1H, O–CH$_2$–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$_7$–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% (Caled for C$_{23}$H$_{22}$O$_7$, C, 67.32; H, 5.37%).

Conversion of pieropodophyllin 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 x 5 ml), 5% sodium bicarbonate solution (2 x 5 ml) and finally with water (3 x 10 ml). The organic phase after drying (Na$_2$SO$_4$) was completely evaporated 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 method and was found to be less active (ID$_{50}$ = 6.5 x 10$^{-6}$ M) compared to the parent compound 2 (ID$_{50}$ = 2.5 x 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

ANNOUNCEMENT

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For further information and application forms please write to: Dr N. Mukunda, Director, DST School on THEP, Centre for Theoretical Studies, Indian Institute of Science, Bangalore 560012. Last date for receipt of completed applications: 15 February 1985. Notice of acceptance will be despatched by: 25 February 1985.