

BENZYLISOQUINOLINE ALKALOIDS FROM *ANAMIRTA COCCULUS* AND *DIPLOCLISIA GLAUCESCENS*

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Abstract: Oxypalmatine and stepharine have been isolated in addition to (-)-8-oxotetrahydropalmatine in a comprehensive investigation of the non-quaternary alkaloidal fraction of *Anamirta cocculus*. Stepharine was isolated from *Diploclisia glaucescens*. All proton and carbon resonances in the three alkaloids have been assigned. It is reported that stepharine has spermicidal activity.

INTRODUCTION

Anamirta cocculus (L.) Wight et Arn., a woody climber of the family Menispermaceae, occurs in South-East Asia. The genus *Anamirta* belongs to the tribe Cosciniae. The other two genera *Arcangelisia* and *Coscinium* in the same tribe are noted for their content of protoberberine alkaloids. The sesquiterpene mixture picrotoxin is commercially isolated from berries of *A. cocculus* that are used as fish poisons.¹ Four quaternary alkaloids, berberine, palmatine, magnoflorine, columbamine and one tertiary alkaloid, (-)-8-oxotetrahydropalmatine have been isolated from the stem and root of the plant.²

Diploclisia glaucescens (Bl.) Diels (= *Cocculus macrocarpus* W. & A.) is also a liana of the family Menispermaceae, occurring in the southern parts of the Indian sub-continent and China. The genus *Diploclisia* belongs to the tribe Menispermae reputed to contain a wide variety of benzyloquinolines.¹ *D. glaucescens* is reported to be used in the treatment of biliousness and venereal diseases.³ A preliminary survey had indicated the presence of alkaloids in the plant.⁴

METHODS AND MATERIALS

Plant Material

A. cocculus was collected in July from the Uva Province. *D. glaucescens* was collected in May from the Central Province. Both plants were identified by comparison with herbarium samples available at the National Herbarium, Royal Botanic Gardens,

Peradeniya. Voucher specimens of both plants have been deposited in the Department of Botany, University of Peradeniya.

Apparatus

UV spectra were recorded on a Shimadzu UV-240 spectrophotometer (Tokyo, Japan). IR spectra were recorded on a JASCO IRA-1 spectrometer (Tokyo, Japan). Mass spectra were recorded on Varian MAT-112 (Cambridge, England) and 312 double focussing mass spectrometers connected to DEC PDP 11/34 computer systems. ^1H NMR spectra were recorded on a Bruker AM-400 NMR spectrometer at 400 MHz (Bremen, Germany). ^{13}C NMR spectra were recorded at 100 MHz on the same instrument. Optical rotations were taken on a Polartronic D polarimeter (Cambridge, England). CD was recorded on a JASCO J-600 spectrophotometer (Tokyo, Japan).

Extraction and Isolation

The dry, ground, mature stem of *A. cocculus* (40 kg) was sequentially extracted with hot light petroleum (40-60 $^{\circ}$) and hot methanol. Evaporation of the methanol gave a dark brown solid (3.5 kg) which was taken up in 2N HCl and the acidic extract washed with light petroleum (40-60 $^{\circ}$). Basification with 20% NH_4OH followed by extraction with chloroform gave the non-quaternary alkaloids (7.2 g) as a brown solid. Separation of the latter over silica gel (eluent methanol-chloroform), followed by preparative thin layer chromatography (eluent acetone-light petroleum) gave (-)-8-oxotetrahydropalmatine (316 mg), oxypalmatine (172 mg) and stepharine (300 mg).

The dry, ground, mature stem of *D. glaucescens* (2.5 kg) was extracted with *n*-hexane, and then with methanol. Evaporation of the methanol gave a dark-brown solid (260 g) which was dissolved in 2N HCl, washed with ether, the acidic extract basified with 20% NH_4OH and the bases extracted with dichloromethane. Evaporation of the solvent gave the non-quaternary alkaloids (10 g). Separation of the latter over silica gel (eluent methanol-chloroform) followed by preparative thin layer chromatography (eluent acetone-light petroleum) gave stepharine (180 mg). TLC of the total alkaloidal fraction showed the absence of (-)-8-oxotetrahydropalmatine and oxypalmatine.

RESULTS AND DISCUSSION

Processing of the stem of *A. cocculus* for non-quaternary alkaloids afforded (-)-8-oxotetrahydropalmatine, oxypalmatine and stepharine in yields of 0.0008%, 0.0005% and 0.0008% respectively. A similar procedure was used to isolate stepharine in 0.007% yield from the stem of *D. glaucescens*.

The identity of (-)-8-oxotetrahydropalmatine was confirmed by comparison of physical constants and spectral data with the reported values.² The negative optical rotation and the trough in the CD curve for the alkaloid at 240 nm gave evidence for a *trans*-quinolizidine ring system (H-14^α) in the alkaloid.⁵ The identity of oxypalmatine and stepharine was established by comparison of physical and spectral data^{5,6} as well as by direct comparison with authentic samples. Oxypalmatine is reported for the first time from the family Menispermaceae. The isolation of oxypalmatine from the bark of *Enantia polycarpa* (Annonaceae) constituted the first report of the isolation of the alkaloid from a natural source.⁷ Stepharine, first isolated from the roots of *Stephania glabra* is widespread in the Menispermaceae.⁸

The ¹³C NMR signals of all three alkaloids were edited by DEPT analysis. Homo COSY and hetero COSY spectra were used in the assignment of all the proton and carbon resonances in the three alkaloids.

Stepharine showed moderate spermicidal activity, causing 100% immobilization of spermatozoa of fresh human semen at a concentration of 10 mg/ml within 2 min when observed on a microscopic slide.

Details of physical constants and spectral data for the three alkaloids are available on request.

While both *A. cocculus* and *D. glaucescens* contain non-quaternary alkaloids, the latter has a higher content of them. The isolation of stepharine from both plants shows the incorporation of a single benzyltetrahydroisoquinoline precursor in both plants. The isolation of (-)-8-oxotetrahydropalmatine and oxypalmatine from *A. cocculus* shows the ability of the enzyme systems in this plant to incorporate an additional one-carbon precursor (berberine bridge).

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