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TETRAPHYLLIN B AND *EPI*-TETRAPHYLLIN B FROM *ADENIA GLAUCA* SCHINZ

K. C. SPENCER and D. S. SEIGLER, Department of Botany, University of Illinois, Urbana, Illinois 61801, USA

ABSTRACT

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Tetraphyllin B and *epi*-tetraphyllin B have been isolated from the South African plant *Adenia glauca* Schinz in an approximate ratio of 5:1.

The structures were established by ^{1}H-NMR.

INTRODUCTION

Adenia glauca is a climbing, tuberous, perennial shrub of the Passi-floraceae found growing in rocky areas of the dry bushveld of southern Africa.

The plant has been said to be poisonous to cattle (De Wilde, 1971), but children have been reported to eat the fruit without ill effects in Africa (Liebenberg, 1939; Watt, 1962).

The leaves of the plant base have been reported to be cyanogenic (Steyn, 1929; Watt, 1962), and the tuber non-cyanogenic and non-poisonous (Steyn, 1941; Steyn, 1949; Watt, 1962).

We found the tuber, leaves, stem and bark of this plant to be cyanogenic.

Tetraphyllin B and *epi*-tetraphyllin B have been isolated in a 1:1 ratio from *Adenia volkensii* Harms (Gondwe, Seigler & Dunn, 1978), and tetraphyllin B alone has been isolated from *Adenia digitata* Engl. (Spencer & Seigler, 1982).

In reporting the isolation of tetraphyllin B and *epi*-tetraphyllin B in a 5:1 ratio from *Adenia glauca*, we show that various relative amounts of different cyclopentene cyanogens can be produced by plants within the same genus.

MATERIALS AND METHODS

Isolation of the glycoside

A fresh tuber (24,0 g fw) of *Adenia glauca* Schinz, purchased from Abbey Garden, Carpenteria, California, was ground in a blender and added to 250 mℓ of boiling 80% methanol. The resulting suspension was filtered and the residue washed with 80% methanol. The extract was concentrated on a rotary evaporator at 40°C to yield a yellow syrup (100 mℓ).

Purification of the extract

The above concentrate was extracted exhaustively with $CHC1_3$, the aqueous phase being retained and chromatographed on paper (Whatman 3 MM, 23×47 cm) with

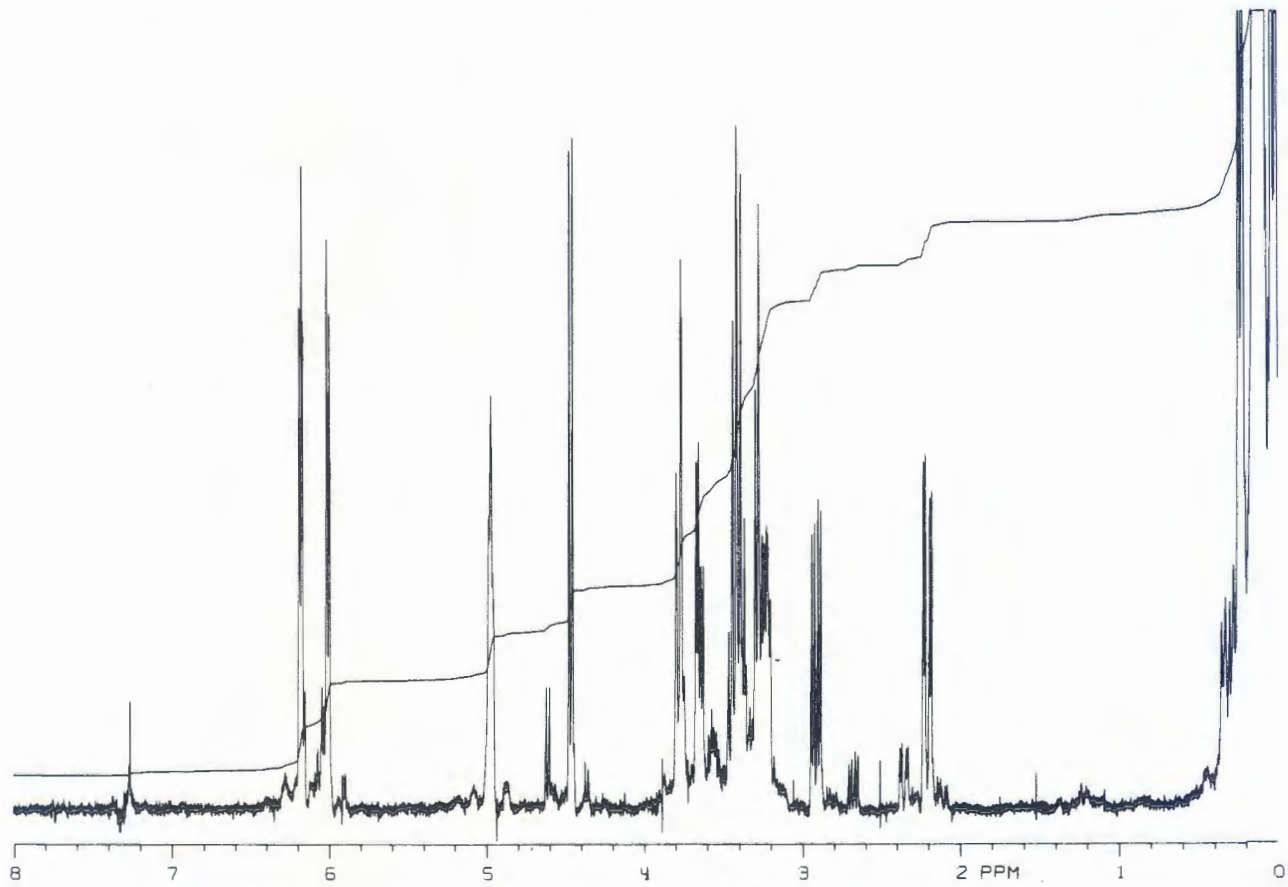

FIG. 1 ^{1}H-NMR spectrum of the TMS ethers of tetraphyllin B and *epi*-tetraphyllin B from *Adenia glauca*.

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$Me_2CO.H_2O$ (5:1). The cyanogen was detected by cutting a strip 1 cm wide from the centre of the chromatogram and then cutting 1 cm^2 sections from this strip, placing them in vials and testing them for HCN as follows:

A few drops of enzyme preparation in pH 6,8 phosphate buffer (see below) were added to the vials and HCN, released as a result of enzymatic hydrolysis, was detected with Feigl-Anger paper (Tantisewie, Ruijgrok & Hegnauer, 1969; Feigl & Anger, 1966). The cyanogen (Rf 0,7) was eluted in H_2O and concentrated under vacuum. This concentrate was rechromatographed on paper with $MeCOEt.Me_2CO.H_2O$ (15:5:3). The cyanogen (Rf 0,4) was eluted and concentrated to yield a crystalline white solid (23,4 mg, overall yield 0,1%).

Enzyme preparation

Leaves of *Passiflora foetida* L. (100 g) were ground in a blender with Me_2CO. The suspension was filtered and rinsed with Me_2CO. Solid material retained in the filter was dried under vacuum, resuspended in pH 6,8 phosphate buffer (500 mℓ), stirred in an ice-bath for 1 h and then filtered. The filtrate was dialyzed against pH 6,8 buffer for 12 h. The product was concentrated under vacuum to a final volume of 50 mℓ and its hydrolytic activity confirmed by testing fresh tubers of *Adenia* by the Feigl-Anger method.

Preparation of derivative

The TMS ether was prepared by dissolving 23 mg of dry sample in 0,5 mℓ of warm pyridine, adding 0,5 mℓ of 1,1,1,3,3,3-hexamethyl-disilazane and 0,5 mℓ of chlorotrimethylsilane. The mixture was warmed for 10 min and dried under N_2. The product was taken up in $CC1_4$, filtered and dried under vacuum for 24 h to remove traces of pyridine.

Spectral determination

The ^{1}H-NMR spectrum was determined on a Nicolet NT-360 (360 MHz) instrument as the TMS ether in $CDC1_3$.

Results and Discussion

The ^{1}H-NMR of the TMS ether of the unknown was identical with that of the TMS ethers of tetraphyllin B and *epi*-Tetraphyllin B previously reported (Gondwe *et al.*, 1978; Seigler, 1975).

Peak height ratios indicated that there was 5 × as much tetraphyllin B as *epi*-tetraphyllin B in the sample.

Adenia glauca must be regarded as a highly toxic plant, as we have shown that all its vegetative parts contain large amounts of the cyanogenic glucosides tetraphyllin B and *epi*-tetraphyllin B.

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