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TETRAPHYLLIN B FROM *ADENIA DIGITATA*

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Key Word Index—*Adenia digitata*; Passifloraceae; tetraphyllin B; cyanogenic glycoside; ^{1}H NMR; ^{13}C NMR.

Abstract—Tetraphyllin B has been isolated in good yield from the South African plant *Adenia digitata*. Its structure was established by ^{1}H and ^{13}C NMR.

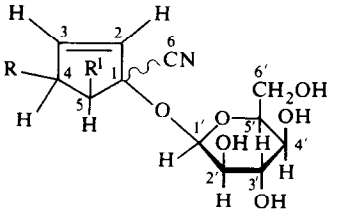
INTRODUCTION

Adenia digitata Engl. is a tuberous, perennial shrub found growing in dry South African localities. The fruit and tuber are reported to be extremely toxic to man; the plant has caused death upon ingestion under accidental, suicidal and homicidal circumstances [1–3]. Toxicity is due to a combination of a highly toxic protein, modeccin [1–4], and a cyanogenic glycoside [1–3]. We have found the roots, leaves, stem and bark of this plant to be cyanogenic. Tetraphyllin B (**1b**) has previously been isolated from *Barteria fistulosa* [5], *Turnera diffusa* [6], *Tetrapathaea tetrandra* [7], and, with an epimer, from *A. volkensii* [8]. In reporting the isolation of tetraphyllin B (**1b**) from *A. digitata*, we show that both an epimeric mixture of cyclopentene cyanogens and a single epimer may be produced by plants within the same genus.

RESULTS AND DISCUSSION

The NMR spectra of the unknown, its TMS ether, and its penta-acetate were identical with those of tetraphyllin B (**1b**) previously reported [6, 9]. The presence of a single glucose unit as the sugar moiety of the cyanogen was confirmed by the glucose oxidase method.

The ^{13}C NMR spectra of tetraphyllin B (**1b**) have not previously been published. Analysis of the proton noise decoupled ^{13}C NMR spectrum of the unknown in D_2O (Fig. 1) reveals the presence of 12 carbons. Their chemical shifts (Table 1) correspond closely with those previously reported for the related compounds deidaclin (**1a**) and gynocardin (**1c**) [10]. Analysis of the single frequency off-resonance proton decoupled spectrum of the unknown reveals that the peaks at δ 81.91 and 119.18 are not coupled to protons, and they are assigned to C-1 and C-6 respectively. Peaks at δ 47.09 and 61.38 are each coupled to two protons, and are assigned to C-5 and the methylene carbon of glucose, respectively. The peaks occurring in the olefinic region of the spectrum, at δ 143.09 and 132.41, are each coupled to one proton, and are assigned to C-2 and C-3, respectively. The other five sugar carbon peaks are assigned according to their identity in chemical shift with those of deidaclin and gynocardin. The chemical shift of δ 74.95 is then attributable to C-4. The last six carbons all appear as doublets in the SFORD spectrum. The presence of single peaks in the decoupled spectrum is indicative of the presence of only a single epimer of tetraphyllin B (**1b**) in our sample.



1a R = R′ = H Deidaclin

1b R = OH, R′ = H Tetraphyllin B

1c R = R′ = OH Gynocardin

EXPERIMENTAL

Isolation of the glycoside. A fresh tuber (1080 g fr. wt) of *A. digitata* Engl. obtained from the Botanical Research Institute, Pretoria, South Africa, was ground in a Waring blender and added to 2 l. boiling 80% MeOH. The resulting

Table 1. ^{13}C NMR spectral data for tetraphyllin B (**1b**) from *A. digitata* and for deidaclin (**1a**) and gynocardin (**1c**) [10] (15 MHz in D_2O)

Carbon	1a	1b	1c
1	84.98	82.91 *s*	85.65
2	143.14	143.09 *d*	140.91
3	128.73	132.41 *d*	128.39
4	31.43	74.95 *d*	78.59
5	37.67	47.09 *t*	87.36
6	120.92	120.18 *s*	116.67
1′	100.13	100.53 *d*	100.26
2′	73.78	73.69 *d*	73.69
3′	77.08	77.02 *d*	77.01
4′	70.34	70.23 *d*	70.61
5′	76.42	76.37 *d*	76.43
6′	61.47	61.38 *t*	61.70

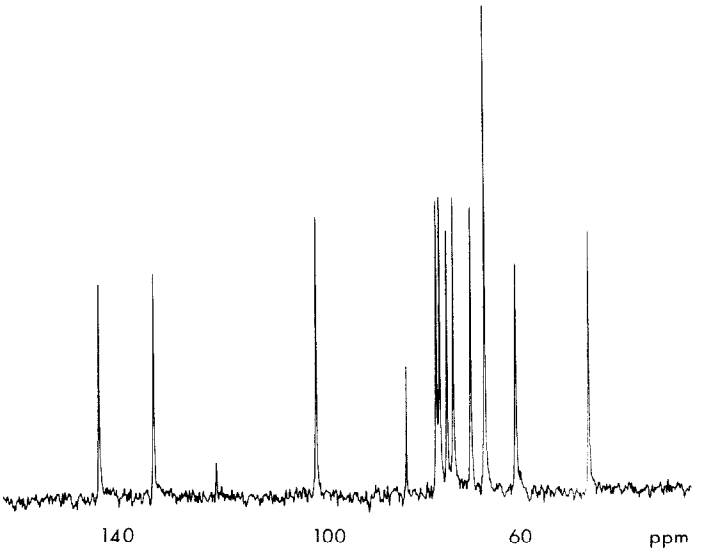


Fig. 1. The proton noise decoupled ^{13}C NMR spectrum of tetraphyllin B (**16**) in D_2O (ref. dioxane).

suspension was filtered and the residue washed with 80% MeOH (2 l). The extract was concd in a rotary evaporator at 40° to yield a yellow syrup (100 ml).

Purification of the extract. The concentrate was extracted exhaustively with $CHCl_3$, the aq. phase was retained and placed on a Sephadex G-10 column. Fractions were collected (20 × 20 ml) with H_2O as eluant. A few drops of each fraction were transferred to a vial, buffered to pH 6.8 and a few drops of enzyme preparation added (see below). HCN released as a result of enzymatic hydrolysis was detected with Feigl–Anger paper [11, 12].

The cyanogenic material (fractions 7–12) was concd to *ca* 20 ml and placed on a cellulose column (Whatman CF 1 powder–microcrystalline cellulose, 1 : 1). Fractions were collected (100 × 20 ml) with Me_2CO–H_2O (5 : 1) as eluant. The cyanogenic material (fractions 8–74) was concd to *ca* 10 ml and placed on a cellulose column and eluted with MeCOEt–Me_2CO–H_2O (15 : 5 : 3). The cyanogenic material (fractions 19–48/100) was concd to *ca* 1 ml and chromatographed on paper (Whatman 3MM, 23 × 57) in Me_2CO–H_2O (5 : 1). The cyanogen was detected by cutting a strip 1 cm wide from the centre of the chromatogram, cutting 1 cm^2 sections from this strip, placing them in vials and testing for HCN as above. The cyanogen (R_f 0.57) was eluted in H_2O and concd to yield a crystalline white solid (1.903 g, overall yield 0.18%).

Enzyme preparation. Leaves of *Passiflora foetida* L. (100 g) were ground in a blender with Me_2CO (500 ml). The suspension was then filtered and rinsed with Me_2CO. Solid material retained in the filter was dried under vacuum, resuspended in pH 6.8 Pi buffer (500 ml), stirred in an ice bath for 1 hr and then filtered. The filtrate was dialysed against pH 6.8 buffer for 12 hr. The product was concd under vacuum to a final vol. of 50 ml and its hydrolytic activity confirmed by testing fresh tuber of *Adenia* by the Feigl–Anger method.

Determination of HCN. To determine the total amount of HCN present in the original leaf material, 0.1 g dry wt was placed in 1.0 ml Pi buffer, pH 6.8, in the main compartment of a centre well flask. NaOH (0.5 ml, 0.1 M) was placed into the well to absorb any HCN released by hydrolysis. A few drops of enzyme preparation (see above) were added to the buffer and the flask was stoppered and incubated in a shaking water bath at 25° for 12 hr. The HCN captured in the NaOH soln was then estimated by the Lambert method [13].

Determination of sugar. Quantitative determination of glucose was carried out using the glucose oxidase method [14]. The cyanogen was incubated with the above enzyme preparation for 4 hr, then subjected to the glucose oxidase test. The results indicated the presence of 1 mol glucose per mol cyanogen (0.5 mg tetraphyllin B = 1.8 μmol; observed glucose activity = 1.8 μmol).

Preparation of derivatives. The TMS ether was prepared as previously described [9]. The penta-acetate derivative was prepared by dissolving 20 mg of unknown in 0.5 ml pyridine, and adding 0.5 ml Ac_2O. The mixture was warmed gently on a steam bath for 10 min, then concd under vacuum to dryness. The resulting solid was dissolved in $CHCl_3$ and subjected to prep. TLC on Si gel G (Brinkman) in $CHCl_3$–C_6H_6–MeOH (40 : 9 : 1). Representative plates were visualized with acid dichromate and charring at 200°. The penta-acetate (R_f 0.4) was desorbed in $CHCl_3$.

Spectral determination. ^1H NMR: 90 MHz in D_2O or $CDCl_3$ (TMS ether and penta-acetate); ^{13}C NMR: 15 MHz in D_2O.

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