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TWO CYANOGENIC GLUCOSIDES, TETRAPHYLLIN B AND *EPI*-TETRAPHYLLIN B, FROM *ADENIA VOLKENSII*

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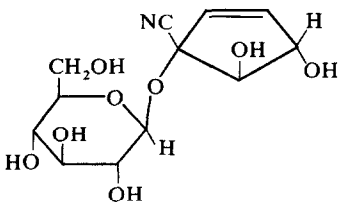
Key Word Index—*Adenia volkensii*; Passifloraceae; cyanogenic glycosides; tetraphyllin B; *epi*-tetraphyllin B.

Abstract—Tetraphyllin B and its previously unknown epimer have been isolated from the Kenyan plant *Adenia volkensii*. The structures were established by their NMR spectra and by GLC.

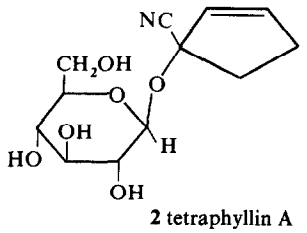
INTRODUCTION

Adenia volkensii, a perennial, tuberous shrub of the Passifloraceae, grows in certain dry areas of Kenya. The indigenous people of these areas have long known that the plant is poisonous and have used it to commit homicide. Fendall reported five cases of poisoning in Kenya after the victims had taken a cupful of a local gruel which had, allegedly, been deliberately contaminated with an extract of *A. volkensii* [1]. Much more common is the use of the tuber to kill hyenas, hence, the local Kamba name for the plant 'kiliambiti' or 'eater of hyenas'. The precise nature of the poisonous principle in the plant has remained unknown, although its cyanophoric nature was first reported almost thirty years ago by Hudson who also stated that the plant was potentially poisonous to livestock [2]. Maitai *et al.* [3] recently showed that all parts of the plant are cyanogenic with the tuberous root accounting for about 90% of the cyanogens. Gondwe has shown that even the flowers of this plant are cyanogenic [4]. Most members of the Passifloraceae are cyanogenic and often contain more than one cyanogenic compound [5]. It has been suggested that the cyanogen in *Adenia lobata*, *Passiflora adenopoda*, *P. allardii*, *P. caerulea* and *P. suberosa* is probably gynocardin [5]. The structure of this cyclopentanoid compound (**1**) was determined by Coburn and Long [6] after many years of investigation.

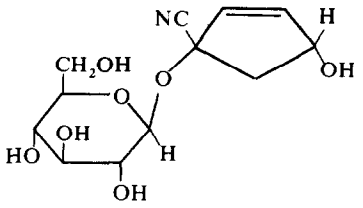
Paris *et al.* isolated another glycoside (barterin) from *Barteria fistulosa* (Passifloraceae), a medicinal plant from the Congo [7]. They also concluded that this compound contained a cyclopentene ring in the aglycone portion of the molecule. Russell and Reay isolated two new cyanogenic glycosides from the passifloraceous fruit *Tetrapathea tetrandra* (New Zealand passion fruit). These compounds, closely related in structure were named tetraphyllin A and B (**2** and **3**); tetraphyllin B is probably identical in structure to barterin. Another compound, deidaclin from *Deidamia clematoides*. has been reported to possess a similar structure to that of tetraphyllin A [9] but NMR spectra of the TMS ethers of the two compounds are not identical [10]. The findings of these workers suggested that the cyanogenic glycoside in *Adenia volkensii* would also be a cyclopentene derivative.



1 gynocardin



2 tetraphyllin A



3 tetraphyllin B

RESULTS AND DISCUSSION

Analysis of the unknown by hydrolysis with β-glucosidase and determination of captured HCN and glucose indicate the presence of a β-glucoside and a 1:1 ratio of HCN to glucose. These results are confirmed by analysis of the NMR spectra of TMS ethers prepared from the mixture. GLC analysis of the TMS ethers showed a prominent peak identical in retention time to tetraphyllin B under several sets of experimental

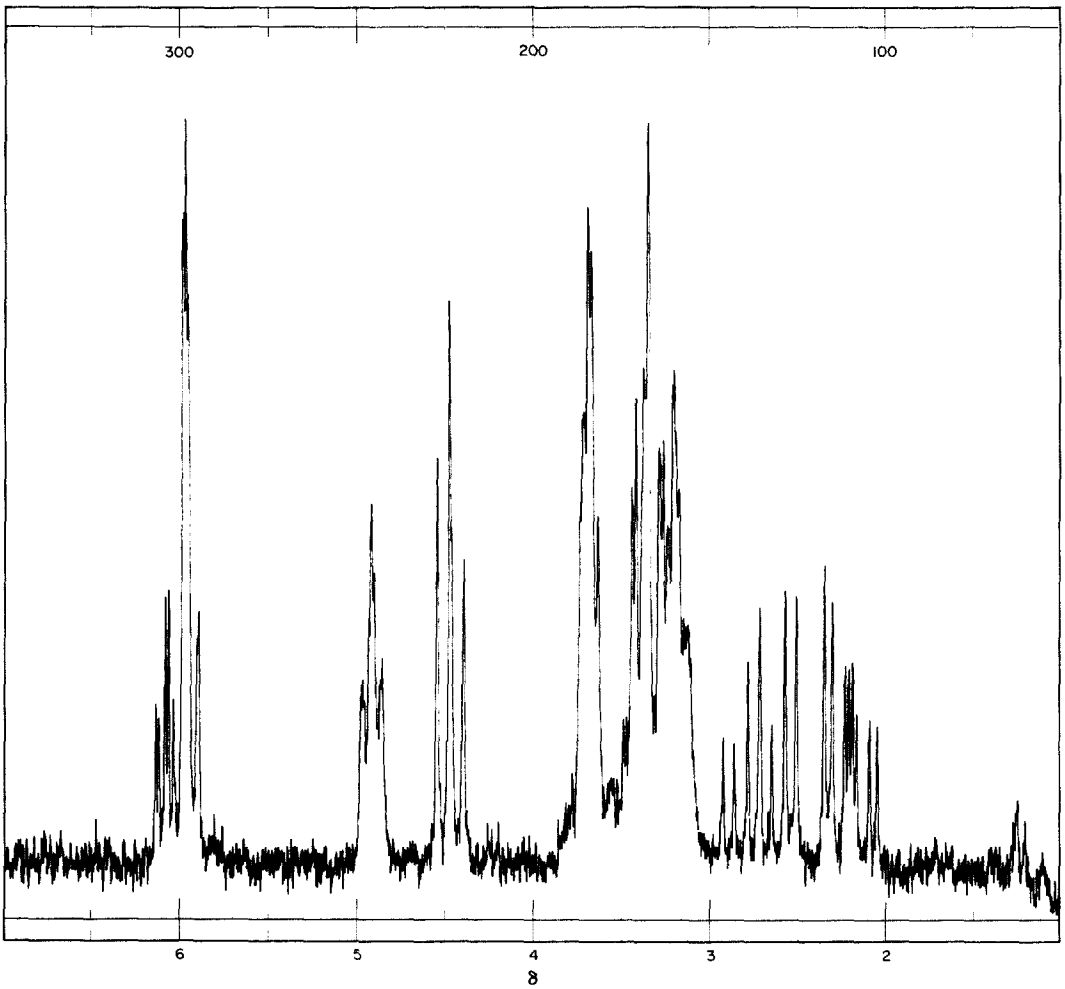


Fig. 1. NMR spectrum of the TMS ethers of tetraphyllin B and its epimer.

conditions. A portion of the original sample was hydrolyzed and TMS ethers prepared from the remaining material. The retention time of the major peak of the gas chromatographic pattern was unchanged but greatly reduced in proportion to other peaks observed.

Examination of the NMR spectrum revealed that all peaks characteristic of tetraphyllin B were present. Moreover, peaks corresponding to the other cyclopentene glycosides, tetraphyllin A and deidaclin were absent thereby excluding these compounds [10]. In addition to the peaks of tetraphyllin B, however, are several additional absorptions (Fig. 1) suggesting that our sample is a mixture of tetraphyllin B and at least one other compound.

Besides the two vinyl proton doublets centered at 6.12 and 5.92 δ respectively is a singlet at 5.95 δ. All vinyl protons show evidence of allylic coupling (< 1 cps). In addition to the anomeric proton absorption at 4.44 δ, is a second doublet at 4.54 δ. Overlap of these produces an apparent triplet. The coupling constant (7 Hz) and chemical shift of each is consistent with that of β-glycosides [12]. An 8 line pattern now complements the 8 line pattern seen in tetraphyllin B (Fig. 1). All other absorptions are identical in chemical shifts. Integral values of the vinyl protons, anomeric protons and the 15 (16) line pattern observed are in a 2:1:2 ratio. Those of sugar protons and the multiplet centered at 4.92 δ are in a 1:6 ratio. Irradiation of the triplet at 4.92 δ reduces the vinyl protons to a 4 line pattern with a sharp singlet (5.95 δ) superimposed (Fig. 2). The 15 line pattern is simplified to a clear 8 line pattern. This confirms Russell and Reay's suggestion that the proton at 4.92 δ is located between the vinyl and methylene protons [8].

Several factors suggest that tetraphyllin B and the co-occurring epimer differ at the chiral center bearing nitrile and oxygen and not at the allylic alcohol. These data suggest that our cyanogenic specimen is a mixture of the two epimers which differ at the chiral carbon atom bearing the nitrile groups and the glucoside moiety. One of these is the previously known tetraphyllin B and the other is the previously unreported epimer. The position of the absorption for the allylic proton at 4.92 δ does not differ in the two compounds. Further, the coupling constants of this proton and the two methylene protons are identical; the chemical shift of the 4 pairs of quartets corresponding to the methylene protons differs only slightly. The two quartets centered at 2.15 and 2.23 δ

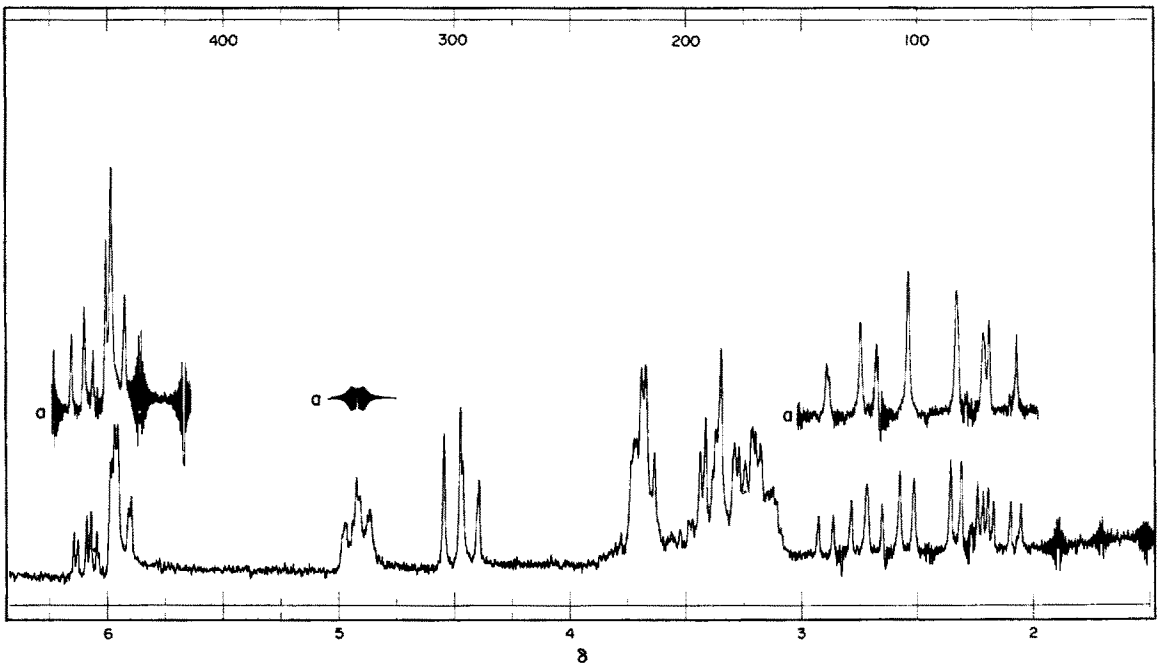


Fig. 2. Decoupled NMR spectrum of a mixture of tetraphyllin B and its epimer.

possess a coupling constant of 5 cps, identical to that for the two *trans*-coupled protons in the TMS ether of gynocardin, known to have only two *trans*-coupled protons [10]. The other two sets of quadruplets (2.83 and 2.62 δ) have a coupling constant of 7 cps which is probably the result of *cis* coupling. The relative positions of the methylene protons and the allylic proton do not appear to differ in the two compounds. The structure of tetraphyllin A (**2**) and deidaclin can only differ at the chiral center bearing nitrile and oxygen. Inversion of this center produces only slight changes in the chemical shifts of the adjacent methylene protons. A shift of the anomeric proton absorption also suggests that it is the chiral center which differs as inversion of the allylic center which is more distantly located, would not be predicted to produce significant effects. Only 1:1 mixtures of the two compounds are observed. This ratio was unchanged despite considerable care to avoid conditions which could lead to racemization. One sample was purified with MeCOEt–Me_2CO–H_2O, avoiding the presence of acid. It was identical in properties to those samples which were purified using *n*-BuOH–HOAc–H_2O. Further, Russell and Reay [8] did not observe the epimeric compound in their studies of tetraphyllin B. As the chiral center would be difficult to invert, the two epimers almost certainly co-occur in the plant *Adenia volkensii*.

EXPERIMENTAL

Isolation of the glycoside. Two fr. tubers of *Adenia volkensii* (400 g) were frozen (−27°), cut into small pieces and ground with a mortar and pestle. The tissue was quickly transferred to boiling EtOH (1 l., 80%) and boiled for 5 min to extract the compound and inactivate any hydrolytic enzymes present. The resulting suspension was filtered through cheese cloth and the residue washed with hot EtOH (100 ml). The extract was concd *in vacuo* at room temp. to yield a syrup (20 ml). The crude aq. extract had a pH of 5.5.

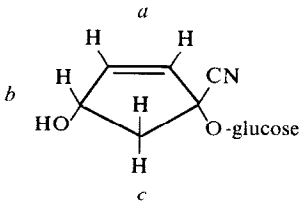
Purification of the extract. The conc extract was placed on a Sephadex column (G-10) and fractions (50 × 5 ml) collected with H_2O as eluant. A few drops of each fraction were transferred to a small vial buffered to pH 6.8 and a few drops of a mixture of linamarase and almond emulsin added. Any HCN released as a result of the action of these enzymes was detected with picrate paper [10]. The cyanogenic material (fractions 15 to 21) was concd to about 10 ml *in vacuo* and chromatographed on paper (Whatman 3 MM, 46 × 57 cm) in MeCOEt–Me_2CO–H_2O (15:5:3). After the cyanogen was detected on the PC as previously described [13], it was eluted with 80% EtOH. The eluate was then concd *in vacuo* to a yellow syrup (20 ml) and freeze-dried to yield a viscous yellow solid (2.2 gm). A portion of the solid was redissolved in 15% EtOH and purified by PC in *n*-BuOH–HOAc–H_2O (4:1:1). The eluate was concd under N_2 to yield a yellow product (200 mg).

Determination of HCN. To determine the total amount of HCN present, the product above was divided into two portions and each dissolved in about 5 ml H_2O. Each soln was placed in the main compartment of a conical flask that contained a center well. NaOH soln (1 ml, 0.5 M) was placed into the well to absorb any HCN released by hydrolysis. To the soln of cyanogen in one flask was added emulsin in phosphate buffer (2 ml, 1 mg emulsin/ml, pH 6.8) while H_2O (2 ml) was added to the other. The mouth of each flask was closed with a tight-fitting rubber cap and the flasks incubated at 30° for 24 hr. The HCN captured in the NaOH soln was then estimated by the method of ref. [11]. The flask with enzyme contained 10.6 μmol HCN whereas that without contained only 0.08 μmol.

Determination of sugars. The reaction mixtures then examined for sugar content by Benedict's test, the mixture that contained β-glucosidase enzymes was positive whereas the other gave no ppt. Quantitative determination of glucose by the glucose oxidase method [14] indicated the presence of 13.9 μmol with enzyme and 0.2 μmol without.

Table 1. The NMR spectral data for the TMS ethers of tetraphyllin B, *epi*-tetraphyllin B and the epimeric mixture from *Adenia volkensii*

	1a	2a	3a	1b	2b	3b	1c	2c	3c	1d	2d	3d	1e	2e	3e	1f	2f	3f
Chemical shift (δ)	6.12 5.92	5.95	6.12 5.92 +5.95	4.92	4.92	4.92	2.83 2.15*	2.62 2.23*	2.74 2.20‡	4.44 1	4.53	4.49	3.68 2	3.68	3.68	3.0-3.6	3.0-3.6	3.0-3.6
Integral value	2			1			2									4		
Multiplicity	2 *d*†	s	*2d* and sat 5.95	distorted *t*	distorted *t*	distorted *t*	ABX (8 pks)	ABX (8 pks)	2 (15 pks) evident	*d*	*d*	*t* (two overlapping *d*)	*d*	*d*	*d*	complex pattern	complex pattern	complex pattern
Coupling constant (cps)	6.0	—	6.0(*d*)	—	—	—	—	—	—	7.0	7.0	each *d*7.0	~3.0	~3.0	~3.0		—	—



1 Tetraphyllin B
2 *epi*-Tetraphyllin B.
3 The epimeric mixture

d Anomeric proton of sugar
e $-CH_2OTMS$ of sugar
f Other sugar protons.

* Two quartets centered at these δ values
† The doublet centered at 6.12 δ is finely split (> 1 cps) and is probably distant from allylic proton
‡ Two apparent octets centered at approximately these values (two peak overlap completely, antother two partially)

Spectral determinations. All NMR spectra were measured on a Varian HA-100 Spectrometer as the TMS derivatives in CCl_4. These derivatives were prepared as previously described [10].

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