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SHORT REPORTS

CYANOGENESIS IN *ACACIA FARNESIANA*

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Abstract—Linamarin and lotaustralin are the major cyanogens of *Acacia farnesiana*; a third unidentified cyanogen is also present. The amount of cyanide produced by plants within one population of the taxon varies from below the level of detection with picrate paper to approximately 5 μmol per g (1.4%) dried plant material.

INTRODUCTION

Among the mimosoid legumes, few species exhibit the systematic complexity of *Acacia farnesiana* (L.) Willd. This widespread assemblage of species or microspecies is probably native to the Americas [1–3] and has subsequently been introduced into other tropical and subtropical regions throughout the world. In the Americas, *Acacia farnesiana* (*sensu lato*) is found from the southern U.S. to Argentina. Plants are often irregular in form but are sometimes spreading and flat-topped [4, 5]. It is often distinguished only with difficulty from species such as *A. tortuosa* and *A. macracantha* [1, 6] and in Africa shows affinities toward *A. sieberiana* [1]. Several taxa have been segregated from the *Acacia farnesiana* species complex. *A. smallii* is widely distributed in the southern U.S. and northern Mexico [7]. *A. pinetorum*, another north American segregate, occurs in S. Florida [7]. *Acacia caven* is an extratropical South American taxon with close affinities to *A. farnesiana*. *Acacia farnesiana* (based on Mexican material which was not *A. smallii*) is a tetraploid (2n = 52). *A. caven* is a diploid (2n = 26). Apparently neither *A. smallii* or *A. pinetorum* have been examined cytologically [7].

Vassal [3] subdivided the subgenus *Acacia* section *Acacia* into two subsections *Pluriseriae* and *Uniseriae* based largely on fruit morphology. *Acacia farnesiana* and its relatives are members of the *Pluriserieae*. Only one cyanogenic member of the *Pluriserieae* has previously been investigated. *A. giraffae*, a common African savanna species, is known to contain proacacipetalin as the major cyanogen [8].

Acacia farnesiana has been reported to be both cyanogenic [9, 10] and acyanogenic [11]. Recent investigations have suggested that the cyanogens are linamarin and lotaustralin [8, 9, 12].

RESULTS AND DISCUSSION

Chemical identification

Purification and analysis by NMR spectroscopy of the TMSi ethers of cyanogenic fractions of *Acacia farnesiana* (from the University of California Arboretum, Davis) show that both linamarin and lotaustralin are present. Analysis of the TMSi ethers by GLC confirmed this observation. Evidence for the presence of a third, as yet uncharacterized, glycoside was obtained by PC. This glycoside occurs in only small amounts and is currently under further investigation.

Variation in cyanide production

Both cyanogenic and acyanogenic specimens of *Acacia farnesiana* (*sensu lato*) have previously been observed. The amount of cyanide is also known to vary in a single specimen sampled at different times of the year, and plant parts also differ in cyanophoric capacity (unpublished data). In an effort to assess the amount of variation within members of a single population, the leaves of shrubs of *Acacia farnesiana* were sampled. The population selected grows in Santa Rosa National Park, Guanacaste Province, Costa Rica. This is well outside the range of segregates such as *A. smallii*, *A. pinetorum* and *A. cavens*. The amount of cyanide varied widely (Table 1). Since the commonly used picrate (or Guignard) test cannot detect concentrations below *ca* 30–50 μg HCN per g of dried plant material (or *ca* 1.0–1.8 μmol HCN/g) [3], about one half of the specimens tested from the Guanacaste population would not have given a positive test if quantitative measurements had not been made.

In a number of field trials, *A. smallii*, *A. pinetorum* and *A. caven* were negative to the Guignard test with the exception of one sample of *A. smallii* (D. Seigler and G. Holstein, DS-9249, Coahuila, Mexico). To date cyanogens from this species have not been characterized.

EXPERIMENTAL

Plant materials. Each sample of leaves was stripped from a single large branch from a different shrub 1–2 m tall. Shrubs were tens of meters apart, and therefore each sample represents one individual. All the plants were growing in *ca* 10 ha of abandoned

Table 1. Cyanide analysis of dried samples of mature *Acacia* leaves from Santa Rosa National Park, Guanacaste Province, Costa Rica*

Plant	Grams tested	μmol HCN/g dry wt	Plant	Grams tested	μmol HCN/g dry wt
A	0.210	3.4	S	0.212	0.30
B	0.222	0.28	T	0.217	0.35
C	0.231	0.26	U	0.529	0.16
D	0.244	0.38	V	0.256	3.3
E	0.218	0.0	W	0.291	0.16
F	0.225	<0.1	X	0.240	2.9
G	0.227	4.3	Y	0.266	2.5
H	0.222	4.5	Z	0.247	0.23
I	0.254	0.26	AA	0.286	3.6
J	0.259	1.87	BB	0.394	0.0
K	0.247	2.5	CC	0.250	2.3
L	0.227	0.26	DD	0.291	0.25
M	0.297	0.0	EE	0.264	1.6
N	0.230	3.9	FF	0.231	1.6
O	2.218	1.4	GG	0.264	1.5
P	0.215	4.4	HH	0.232	3.1
Q	0.253	0.20	II	0.233	2.5
R	0.231	0.23	JJ	0.236	1.7

* 1 μmol cyanide per g is equivalent to 0.0027%.

pasture long ago cut out of deciduous forest in Santa Rosa National Park. All plants were large enough to be reproductive adults and all were growing in full sun. The leaves were mature at the time of picking (28 June, 1977) and had been produced in May (beginning of the rainy season). They were immediately air-dried in shade. Vouchers from this population are in the Herbarium of the Missouri Botanical Garden. Specimens of *A. farnesiana* from Mexico, *A. smallii* from Texas and Mexico, *A. pinetorum* from Florida and *A. caven* from Argentina were collected by D. Seigler *et al.* and voucher specimens of each collection have been deposited in the University of Illinois Herbarium (ILL).

Procedures. Leaf material from each specimen was examined by the picrate test [14]. Half of each sample was placed in each of two vials and the picrate paper test performed both with and without added linamarase and almond emulsin (Sigma) [15]. Extracts of plant material from *Acacia farnesiana*, a specimen in the arboretum at the University of California, Davis, were worked up as previously described [12, 16]. The glycosides were then purified by PC (×2) with 2-butanone Me_2CO–H_2O (15:5:3) and eluted to yield a purified sample which was converted to its TMSi derivative [17] for GLC [12, 16, 18] analysis. Quantitative analysis was performed on samples of dried plant material (*ca* 0.25 g) by the following procedure. The plant material was macerated and treated with linamarase in Pi buffer (pH 6.8) and 0.375 mg chloramphenicol to retard microbial activity. NaOH (0.5 ml, 1.0 N) was placed in the center well, the flask sealed and the sample placed on a shaker–incubator (35°, 18–24 hr). At that time the NaOH from the center well was removed and analysed for cyanide by the colorimeteric method of Epstein [19].

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