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ACACIPETALIN FROM SIX SPECIES OF *ACACIA* OF MEXICO AND TEXAS

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Abstract—Acacipetalin is the principal cyanogenic glycoside in *Acacia chiapensis, A. cochliacantha, A. hindsii, A. macracantha, A. schaffneri* var. *schaffneri* (all from Mexico) and *A. schaffneri* var. *bravoensis* (Texas).

INTRODUCTION

Bentham recognized six series within the genus *Acacia*. Three are principally Australian, two are widely distributed in Asia, Africa and the Americas, and the sixth is exclusively American [1]. In a recent revision of the genus, Vassal combined the three Australian series into one subgenus but otherwise accepted Bentham's basic groups. He did however, reorganize taxa within the proposed subgenera [2], which are more or less equivalent to Bentham's series. He further divided his proposed subgenus *Acacia* section *Acacia* into two subsections, the *Uniseriae* and the *Pluriseriae*.

Cyanogenesis has long been recognized among various *Acacia* species. Numerous reports of positive cyanide tests have appeared in the literature, especially by Australian and South African workers between 1920 and 1940, but few compounds were actually isolated and characterized. Two South African species, *Acacia sieberiana* var. *woodii* (≡*A. stolonifera*) and *Acacia hebeclada* (≡*A. lasiopetala*) were shown by Rimington to contain acacipetalin [3]. A revised structure for this compound has been published [4] and a related compound dihydroacacipetalin has been reported [5]. The presence of acacipetalin has recently been reported in *Acacia giraffae* [6] and an American species *Acacia constricta* [7]. All are members of Bentham's series Gummiferae (Vassal's subgenus *Acacia*). In an effort to investigate other members of this genus we have now isolated and characterized the cyanogens of six additional species of *Acacia*.

RESULTS AND DISCUSSION

In initiating this study on the distribution and identity of the cyanogen in plants of Bentham's *Gummiferae* and *Vulgares* (Vassal's subgenera *Acacia* and *Aculeiferum*) we examined the North American *Acacia* collection in the herbarium at the University of California at Berkeley. A total of 202 specimens representing 99 species were examined and, of these, 73 specimens of the following 14 species were cyanogenic: *A. acatlensis, A. californica, A. chiapensis, A. cochliacantha* (≡*A. cymbispina*); *A. collinsii, A. constricta*; *A. farnesiana, A. globulifera, A. hindsii*; *A. macracantha, A. milleriana*; *A. pringlei*; *A. schaffneri* var. *schaffneri*; *A. schaffneri* var. *bravoensis* (≡*A. tortuosa* in part). Only in the case of *A. constricta* has the cyanogenic glycoside been identified and shown to be acacipetalin [7].

A positive test for HCN, as described in the Experimental, on a dried herbarium specimen strongly indicates that a cyanogenic glucoside was present in the living plant. It should be understood, however, that a negative test is inconclusive since any cyanogenic glucoside present in fresh material may have been destroyed during either the preparation of the herbarium specimen or its subsequent storage. It is of interest that a majority of the herbarium specimens that gave positive tests for HCN did so in the absence of added linamarase and almond emulsin indicating that the dried plant material still contained enzymes capable of hydrolyzing the cyanogenic material on addition of buffer. In fact, a specimen of *A. farnesiana* collected in 1898 gave a positive test in the absence of added enzymes. The addition of enzyme to the specimen released twice as much HCN indicating that the amount of β-glucosidase remaining in the herbarium sample was limiting in our analytical procedure.

Subsequently the following species were examined in the field in Texas and Mexico and found to be strongly cyanogenic: *A. chiapensis, A. cochliacantha*; *A. hindsii, A. macracantha*; *A. schaffneri* var. *schaffneri*; and *A. schaffneri* var. *bravoensis*. Collections of these were made and the cyanogen isolated and characterized from each; it proved to be acacipetalin in all cases.

Although herbarium specimens of *A. collinsii* were cyanogenic, we did not encounter plants in the field that were cyanogenic. The following species that were examined in the field also were not cyanogenic: *A. berlandieri*; *A. gregii*; *A. neovernicosa*; *A. rigidula*; *A. pennatula*; *A. smallii*; *A. angustissima*; *A. cornigera*; and *A. sphaerocephala*. Specimens of *A. farnesiana* were routinely observed to be cyanogenic; the identification of the cyanogens in this species is presently under investigation.

Vassal [2] subdivided the subgenus *Acacia* section *Acacia* into two subsections, *Pluriseriae* and *Uniseriae*, based largely on fruit morphology. We have found acacipetalin in several species of the *Uniseriae* (see above) but to date the only member of the *Pluriseriae* which contains this compound is the widely distributed African species *Acacia giraffae*. *Acacia farnesiana* (*sensu lato*), a widely distributed tropical member of the *Pluriseriae*, is probably a complex of closely related

species or microspecies. Several of these taxa are cyanogenic [8, 9], but acacipetalin is not one of the cyanogenic compounds present.

The relationship of myrmecophyly and cyanogenesis has been investigated [8, 10]. Janzen concluded that those species with ants normally do not duplicate their defense systems and thus do not make compounds such as cyanogenic glycosides in quantity. One exception is *Acacia chiapensis* which possesses both types of defense systems. He concludes that in this case *Acacia chiapensis* is a marginal host for obligate *Acacia* ants and in many features of growth and habit, resembles non-ant *Acacias* [8]. Specimens of the myrmecophilous *A. hindsii*, which were grown from seeds collected in Guatemala, were devoid of cyanide when examined by Rehr *et al.* [8]. However, in the current study we found *Acacia hindsii* specimens in Oaxaca and Jalisco to be strongly cyanogenic. Subsequent reexamination of the materials used by Rehr *et al.* did reveal a very low level of cyanide. This species of *Acacia* is inhabited by an obligate *Acacia* ant and thus appears to be another exception to Janzen's previous observations. Myrmecophyly and production of cyanide occur in species from both the *Pluriseriae* and *Uniseriae* and thus cloud the validity of this taxonomic distinction.

EXPERIMENTAL

Materials. Young leaves and shoots (*ca* 1 kg) were collected from plants of *Acacia hindsii* Benth. (D. Seigler and G. Holstein, DS-9575, Barra de Navidad, Jalisco, Mexico); *Acacia chiapensis* Safford (D. Seigler and G. Holstein, DS-9810, 46.7 km north of Matias Romero, Oaxaca, Mexico); *Acacia cochliacantha* Humb. and Bonpl. ex Willd (≡*A. cymbispina* Sprague and Riley) (D. Seigler and G. Holstein, DS-9686, 35.4 km northwest of Izucar de Matamoros, Puebla, Mexico); *Acacia macracantha* Humb. and Bonpl. (D. Seigler and G. Holstein, DS-9579, 107.8 km southeast of Barra de Navidad, Colima, Mexico); *Acacia schaffneri* (S. Wats.) var. *schaffneri* F. J. Herman (D. Seigler and G. Holstein, DS-9435, 30 km northwest of San Luis Potosi, San Luis Potosi, Mexico); and *Acacia schaffneri* (S. Wats.) F. J. Herman var. *bravoensis* Isely (D. Seigler, S. Saupe and H. Welt, DS-10006, 40.2 km south of Catarina, Webb County, Texas, USA). A voucher specimen of each collection has been deposited in the University of Illinois Herbarium. Material for chemical investigation was air dried and ground.

Procedures. Leaf material (10–50 mg) from herbarium samples was examined by the picric acid paper method [11] in two ways. Half of each sample was placed in a vial, a few drops of 0.1 M phosphate buffer, pH 6.8, were added and the picrate test carried out. The other half of each sample was placed in a vial and a few drops of 0.1 M phosphate buffer, pH 6.8, containing a mixture of linamarase [12] and almond emulsin (Sigma) were added before the picrate test was performed. In this manner, each sample was tested for its ability to produce HCN in the absence or presence of added enzymes capable of hydrolyzing cyanogenic glucosides with aliphatic and aromatic aglycones [6]. Extracts of the plant material collected in the field were prepared and worked up as in ref. [4] except that a Sephadex G-10 column (1.5 cm × 80 cm) was substituted for the Si gel column. The residue from the $CHCl_3$ extraction, taken up in H_2O, was placed on the column and eluted with H_2O. A flow rate of 1 ml/min was used; 7 ml fractions were collected and tested for cyanogenic glycosides as previously described [4]. The major glycoside was 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 TMS derivative for NMR [13] and GLC [4, 5] analysis.

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