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2-AMINO-4-ACETYLAMINOBUTYRIC ACID, 2,4-DIAMINOBUTYRIC ACID AND 2-AMINO-6N-OXALYLUREIDOPROPIONIC ACID (OXALYLALBIZZIINE) IN SEEDS OF *ACACIA ANGUSTISSIMA*

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Key Word Index—*Acacia angustissima*; Leguminosae; non-protein amino acids; oxalylalbizziine (2-amino-6N-oxalylureidopropionic acid); 2-amino-4-acetylaminobutyric acid; 2,4-diaminobutyric acid.

Abstract—A new amino acid previously detected in 17 species of *Acacia* has been isolated from seeds of *Acacia angustissima* and identified as oxalylalbizziine. These seeds also contain more than 6% dry weight of 2-amino-4-acetylaminobutyric acid, which has not been reported previously in a legume, and lower concentrations of 2,4-diaminobutyric acid.

INTRODUCTION

The legume genus *Acacia* can be divided into four distinct subgroups based on the specific 'patterns' of non-protein amino acids which occur in the seeds [1]. More than two hundred species of *Acacia* examined can be assigned unequivocally to one of these four groups. *Acacia angustissima* (Mill.) Kunze (syn. *A. filicina* Willd.), a member of the section *Filicinae* (series *Filicinae* Benth.) has recently been introduced commercially into Queensland, Australia from Bolivia [Pedley, L., personal communication] and unlike all other *Acacia* species we have examined, its seed non-protein amino acid 'pattern' does not correlate with any of the four known 'patterns' within the genus.

Three non-protein amino acids were detected in these seeds; 2-amino-4-acetylaminobutyric acid, an amino acid originally isolated from the latex of *Euphorbia pulcherrima* [2] but not previously found in a leguminous species accounted for approximately 6% of the dry seed weight, 2,4-diaminobutyric acid for approximately 0.6% of the dry seed weight, and an 'unknown' amino acid for approximately 1.0% of the dry seed weight. This 'unknown' has previously been reported to occur in seeds of all *Acacia* species placed in the third chemically defined sub-group of Evans *et al.* [1] and was referred to by these authors as 'amino acid k, an unidentified derivative of 2,3-diaminopropionic acid'. We have now isolated and identified this compound as the novel amino acid, 2-amino-6N-oxalylureidopropionic acid (oxalylalbizziine, **1**).

RESULTS AND DISCUSSION

The 'unknown' was detected in seed extracts of *A. angustissima* as a very acidic ninhydrin-positive compound when the extracts were subjected to HV electrophoresis on paper. The compound gave a positive test for oxalate with potassium permanganate, and albizziine and 2,3-diaminopropionic acid were identified (by co-chromatography and co-electrophoresis) as products of acid hydrolysis (6 N HCl, 100°, 17 hr). Under the same conditions, free albizziine was partially hydrolysed to 2,3-diaminopropionic acid. When the 'unknown' was subjected to acid hydrolysis under milder conditions (0.1 N HCl, 20°, 10 min) the only ninhydrin-reacting compounds detected in the hydrolysate were albizziine and traces of unchanged 'unknown'. From these results it was concluded that the 'unknown' was an oxalylated derivative of albizziine. The presence of a free 2-amino group was shown by chelation with cupric ions [3], indicating that the oxalyl group was attached to a ureido nitrogen.

The structure of oxalylalbizziine was determined conclusively by a single crystal X-ray diffraction analysis. A computer generated, perspective drawing of the final X-ray model is given in Fig. 1. The X-ray study defined only the relative configuration of oxalylalbizziine and the enantiomer shown, which has the L (*S*)-configuration at C-2, is an arbitrary choice. The site of attachment of the

$$HO_2C.CO.NH.CO.NH.CH_2.CH(NH_2)CO_2H$$

1

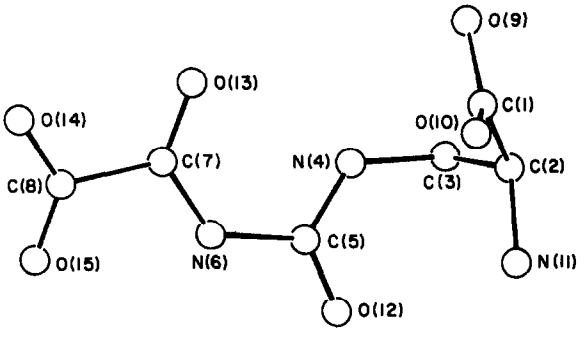

Fig. 1. A computer generated perspective drawing of oxalylalbizziine. Hydrogens are omitted for clarity and no absolute configuration is implied.

Table 1. Species of *Acacia* from the third chemically defined subgroup which contain oxalylalbizziine in their seeds

A. ataxacantha DC.
A. catechu (L.) Willd.
A. mellifera (Vahl) Benth. ssp. *detinens* (Burch) Brenan
A. erubescens (Welw. ex) Oliv.
A. ferruginea DC.
A. galpinii Burtt-Davy
A. goetzei Harms ssp. *goetzei*
A. hamulosa Benth.
A. modesta Wall.
A. nigrescens Oliv.
A. polyacantha Willd. ssp. *campylacantha* ([Hochst. ex] *A. Rich.*) *Brenan*
A. rovumae Oliv.
A. senegal (L.) Willd.
A. venosa (Hochst. ex) Benth.
A. welwitschii Oliv. ssp. *delagoensis* (Harms) Ross et Brenan
A. coulteri Gray ex Benth.
A. confusa Merr.

oxalyl group is the terminal nitrogen, N-6, of albizziine rather than N-4. As is commonly observed, oxalylalbizziine crystallized as a zwitterion but, based on bond distances and hydrogen location, the terminal carboxyl group is ionized. The α-carboxyl group appears to be rather standard with a C–1=O–10 distance of 1.200(8) Å and a C–1=(O–9)H distance of 1.313(7) Å. The terminal carboxyl group has C-8–O-14 and C-8–O-15 distances of 1.248(8) and 1.221(7) Å respectively. One molecule of water forms part of the asymmetric unit and is involved in two of the four hydrogen bonds. There are hydrogen bonds between water and O-15 with a distance of 2.760 Å; water and N-11, 2.971 Å; O-14 and N-11, 2.794 Å; and O-14 and O-9, 2.510 Å. The three latter hydrogen bonds are along the *x*, *z* and *y* screw axes respectively. Bond distances and angles agree well with generally accepted values. Archival X-ray data have been deposited with the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, U.K. and are available from them. A complete literature citation should be given when ordering.

Oxalylalbizziine (previously designated compound k) is present in 17 species of *Acacia*, in addition to *A. angustissima*, in the third chemical group of Evans *et al.* [1]; these species are listed in Table 1. This compound provides the only chemical link between species in this group and *A. angustissima* which does not contain any of the other amino acids considered to be chemical 'markers' for the group. 2,4-Diaminobutyric acid occurs as 0.6% of dry seed weight in *A. angustissima* and, although this compound is present in some species in the third chemically defined subgenus, it is not considered to be a reliable 'marker' amino acid for this group. The occurrence of 2-amino-4-acetylaminobutyric acid as 6% of the dry seed weight of *A. angustissima* is of particular interest as this compound has only been detected previously in a species of *Euphorbia* [2].

Seeds from 20 individual plants of *A. angustissima* were analysed separately and it was found that the concentrations of the three non-protein amino acids in the seeds varied very little from plant to plant.

EXPERIMENTAL

Isolation of oxalylalbizziine. Seeds of *A. angustissima* (400 g) were ground to a fine powder and extracted with continuous shaking in 70% ethanol at room temp. for 72 hr. After filtration the residue was re-extracted twice in the same way. The combined filtrates were concd under red. pres. and applied to a column of Amberlite IR-120, H^+ form (60 × 3 cm). The column was washed thoroughly with water and the oxalylalbizziine displaced with 0.2 N pyridine (400 ml). The pyridine fractions were evaporated to dryness under red. pres. and the acidic amino acid recrystallized twice from EtOH. Found: C, 31.12; H, 4.7.; N, 17.74%. $C_6H_9N_3O_6 \cdot H_2O$ requires C, 30.3; H, 4.6; N, 17.72%. MS showed abundant ions at *m/z* 237 and 146 [M − CO·COOH − H_2O].

Paper chromatography. The ascending method on Whatman No. 1 paper was used. Papers were developed with ninhydrin (0.2% in 95% aq. Me_2CO). R_f glu in BuOH–HOAc–H_2O (12:3:5) 0.07; R_f glu in PhOH–H_2O (4:1, w/v) in the presence of NH_3 0.08. These values were identical to those of synthetic oxalylalbizziine (prepared by the addition of oxalyl chloride to a soln of albizziine in HOAc).

Single crystal X-ray diffraction analysis. Suitable crystals of oxalylalbizziine **1** were grown by slow evaporation of an aq. soln. Preliminary X-ray photographs showed orthorhombic symmetry and accurate cell constants, determined by a least-squares fit of fifteen diffractometer measured 2θ-values, were $a = 5.222(1)$, $b = 13.014(2)$ and $c = 13.907(3)$ Å. Systematic extinctions, the presence of chirality and crystal density (1.67 g/cm^3) were uniquely accommodated by space group $P2_12_12_1$ with one molecule of $C_6H_9N_3O_6 \cdot H_2O$ forming the asymmetric unit. All unique diffraction maxima with $2\theta \leqslant 114°$ were collected on a computer-controlled four-circle diffractometer using graphite monochromated CuK$^-\alpha$ radiation (1.54178 Å) and variable speed, 1°ω-scans. Of the 778 reflections surveyed in this fashion, 750 (95%) were judged observed after correction for Lorentz, polarization and background effects.

A phasing model was achieved by a multisolution, weighted tangent formula approach [4]. A weighted E-synthesis of the most probable solution showed all fifteen nonhydrogen atoms of the molecule. The water of crystallization was located on a

subsequent F_0-synthesis. After block-diagonal least-squares refinement, all but one of the hydrogens were located on a ΔF-synthesis. This hydrogen, H-2, was included at a calculated position using an assumed geometry. Full matrix least-squares refinement with anisotropic nonhydrogen atoms and isotropic (fixed) hydrogens converged to a standard crystallographic residual of 0.074 for the observed reflections.

Fractional coordinates, thermal parameters, bond distances and bond angles have been deposited with the Cambridge Crystallographic Data Centre. All crystallographic calculations were made on a PRIME 400 computer operated by the Materials Science Center and the Department of Chemistry, Cornell University. The principal programs used were REDUCE and UNIQUE, data reductions programs, Leonowicz, M.E., Cornell University, 1978; BLS78A, anisotropic block-diagonal least squares refinement, Hirotsu, K. and Arnold, E., Cornell University, 1980; XRAY76, the X-ray system of Crystallographic Programs, edited by Stewart, J. M., University of Maryland, Technical Report TR-445, March, 1976; ORTEP, crystallographic illustration program, Johnson, C.K., Oak Ridge, ORNL-3794; BOND. molecular metrics program, Hirotsu, K., Cornell University, 1978; MULTAN-78, 'A System of Computer Prograams for the Automatic Solution of Crystal Structures from X-ray Diffraction Data,' University of York, England. Principal author P. Main. For literature description of MULTAN see refs [4, 5].

HV paper electrophoresis. Extracts and purified amino acids were subjected to electrophoresis on Whatman 3 mm paper (0.70 V/cm for 30 min) in buffer solns of pH 1.9, 3.6 and 6.5 [6].

Isolations of 2-amino-4-acetylaminobutyric acid. After the removal of oxalylalbizziine, the remaining amino acids were displaced from the ion exchange column with 1 N pyridine (175 ml). The pyridine soln was evaporated to dryness under red. pres., the residue was redissolved in water, and passed through a column (60 × 3 cm) of Amberlite IR-45 in the acetate form. The neutral amino acids did not bind to the column. The effluent was concd under red. pres. and 2-amino-4-acetylaminobutyric acid (2.6 g) crystallized from the concd soln. Found: C, 44.20; H, 7.81; N, 17.35. $C_6H_{12}N_2O_3$ requires: C, 45.00; H, 7.50; N, 17.50%. Identification was confirmed by co-chromatography and co-electrophoresis with authentic 2-amino-4-acetylaminobutyric acid prepared by the method of Leclerc and Benoiton [7]. The IR spectra of the isolated and the synthetic compounds were identical.

Quantitative determination. The approximate concns of the three non-protein amino acids in the seed extracts were determined with the aid of an amino acid analyser by comparing the peak areas given by the amino acids in the extracts with those given by standard solns of the pure compounds.

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