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SHORT REPORTS

4,5-DIHYDROXYPIPECOLIC ACIDS IN THE SEED OF *JULBERNARDIA*, *ISOBERLINIA* AND *BRACHYSTEGIA*

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Key Word Index—*Julbernardia paniculata*; *Isoberlinia tomentosa*; *Cryptosepalum* sp; *Brachystegia* sp.; Leguminosae; Brachystegioideae; 2(S)-carboxy-4(R),5(R)-dihydroxypiperidine; non-protein amino acids.

Abstract—2(S)-carboxy-4(R),5(R)-dihydroxypiperidine has been isolated from seed of *Julbernardia paniculata*. The distribution of non-protein amino acids in the genera *Julbernardia*, *Isoberlinia*, *Brachystegia* and *Cryptosepalum* is also reported.

INTRODUCTION

L-Pipecolic acid (2(S)-carboxypiperidine) and *trans*-5-hydroxy-L-pipecolic acid (2(S), 5(S) configuration) are constituents of the non-protein amino acid pools of many legume seeds [1]. *Trans*-4-hydroxy-L-pipecolic acid, however, appears to have a more restricted distribution within this group [2,3]. Recently a class of compound has been isolated in which pipecolic acid is hydroxylated at both the 4 and 5 positions [4,5]. Two of the four possible stereoisomers have been isolated from *Calliandra* (Mimosaceae) [4,5] and *Derris* (Papilionaceae) [5]. We now wish to report the isolation of a third isomer from *Julbernardia* (Caesalpiniaceae) and the occurrence of this and the two previously reported isomers in the related genera *Brachystegia* and *Isoberlinia*.

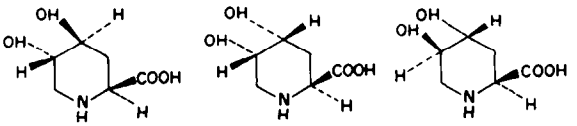
RESULTS AND DISCUSSION

Two-D PC of 70% ethanolic extracts of *Isoberlinia tomentosa* and *Julbernardia paniculata* seeds revealed unidentified compounds which gave a blue-grey colour with ninhydrin. These were tentatively identified as isomers of 4,5-dihydroxypipecolic acid (2-carboxy-4,5-dihydroxypiperidine) by co-chromatography with standard compounds (all 4 stereoisomers are resolved using solvent systems 1 and 2). Their identity was confirmed by comparison of the PMR spectra of the isolated compounds with those of authentic compounds. The four isomers had distinctive PMR spectra. The isomer present in *I. tomentosa*, 2(S)-carboxy-4(S),5(S)-dihydroxypiperidine (**1**), has previously been isolated from *Derris elliptica* which also contains its diastereoisomer, 2(S)-carboxy-4(R),5(S)-dihydroxypiperidine (**2**) [5]. The isomer present in *J. paniculata* was 2(S)-carboxy-4(R),5(R)-dihydroxypiperidine (**3**) which has not been previously reported.

Isoberlinia and *Julbernardia* are members of the Brachystegioideae subfamily of the Caesalpiniaceae [6]. This group is of great ecological and economic importance as the dominant forest trees over large areas of Central Africa. The non-protein amino acid composition of seeds of eight species of *Brachystegia* and of six other species of the Brachystegioideae was investigated by 2-D PC (Table 1). The non-protein amino acids were identified by co-chromatography with authentic compounds. Two major groups of non-protein amino acids were present: γ-substituted derivatives of glutamic acid and glutamine and pipecolic acid and its hydroxylated derivatives.

The two species of *Isoberlinia* were very similar in their non-protein amino acid composition, and were unusual in completely lacking any γ-substituted glutamate or glutamine. *Cryptosepalum*, however, was unusual in being rich in γ-substituted derivatives of glutamate and glutamine but containing only traces of pipecolic acid and no detectable amounts of hydroxylated pipecolic acids.

The genus *Brachystegia* is large, variable in morphology and difficult to classify [7–9]. For example, the type species *B. spiciformis* has a wide geographical range and includes many locally distinct morphological forms. Hybrids between several of the species are also common. Samples of seed of *Brachystegia* species, collected in different locations, were variable in non-protein amino acid composition, both between species and within the material identified as *B. spiciformis*. Isomers of 4,5-dihydroxypipecolic acid occurred in *B. boehmii* (**2**), *B. microphylla* (**2**) and one population of *B. spiciformis* (**3**).



1 2 3

The results suggest that the non-protein amino acid composition could be used as a taxonomic criterion, both within the genus *Brachystegia* and the Brachystegioideae as a whole.

EXPERIMENTAL

Chromatographic methods. Solvents for PC were: (1) 75% (w/w) PhOH in the presence of NH_3 vapour (2) *n*-BuOH–HOAc–H_2O, 90:10:29 (3) EtOH–20N NH_3–H_2O, 18:1:1 (4) EtOAc–Pyr–H_2O, 2:1:2, (upper phase) (5) *n*-BuOH–HCO_2H–H_2O, 15:3:2.

Table 1. The occurrence of certain non-protein amino acids in seeds of various species of the Brachystegioideae

Species	Country of origin	γ-Methylene glutamic acid	γ-Ethylidine glutamic acid	γ-Ethyl glutamic acid	γ-Methyl glutamic acid	γ-Methylene glutamine	Pipecolic acid	*Trans*-5-hydroxypipecolic acid	*Trans*-4-hydroxypipecolic acid	(1)	(2) 4,5-Dihydroxypipecolic acids	(3)	3-Hydroxyproline
Julbernardia paniculata (1)	Z	S	T	—	W	M	T	T	—	—	—	W	—
Julbernardia globiflora (1)	R	S	—	—	W	—	T	W	—	—	—	S	—
Isoberlinia tomentosa (2)	K	—	—	—	—	—	S	M	—	W	—	—	—
Isoberlinia angolensis (1)	Z	—	—	—	—	—	M	S	—	W	—	—	—
Cryptosepalum maraviense (2)	K	S*	—	T	W	M	T	—	—	—	—	—	—
Cryptosepalum exfoliatum (1)	Z	S*	—	W	W	M	T	—	—	—	—	—	—
Brachystegia spiciformis (1)	Z	W	T	—	W	M	T	T	—	—	—	M	—
„ „ (2)	K	W	—	—	T	—	S*	M	—	—	—	—	—
„ „ (1)	Z	—	—	—	—	—	S*	S	—	—	—	—	—
„ „ (2)	R	M	—	—	—	—	S*	W	—	—	—	—	—
Brachystegia manga (1)	Z	T	—	—	—	—	S	M	—	—	—	—	—
Brachystegia x *longifolia* (1)	K	W	—	—	—	—	S*	T	T	—	—	—	M
Brachystegia boehmii (1)	R	W	—	—	—	W	S*	M	W	—	W	—	—
Brachystegia glaucescens (2)	R	T	—	—	T	—	S*	M	—	—	—	—	—
Brachystegia utilis (1)	Z	T	—	—	—	—	M	T	—	—	—	—	—
Brachystegia microphylla (2)	Z	T	—	—	—	—	S*	W	W	—	T	—	—
Brachystegia allenii (1)	R	T	—	—	—	—	S*	W	W	—	—	—	—

Relative concentrations of amino acids: S: strong; M: moderate; W: weak; T: trace. Asterisk indicates that compound was the principal component of the free amino acid pool. Country of origin: Z: Zambia; R: Rhodesia; K: Katanga province of Zaire. Number in parenthesis indicates number of populations analysed.

Isolation of 4,5-dihydroxypipecolic acids. Seeds of *I. tomentosa* (50 g) and *J. paniculata* (30 g) were ground and extracted with 70% EtOH (10 ml/g). The extracts were applied to columns of Zeokarb 225 and the amino acid fraction eluted with ethanolic 7N NH_3. Dihydroxypipecolic acid was separated by preparative PC in solvents 1 and 5 followed by adsorption onto columns of Dowex 50 and elution with 0.2 N NH_3. A few mg of each compound were isolated. PMR spectra were recorded at 100 MHz in D_2O with TSS as a standard. **1** was converted to the hydrochloride because the standard was only available in this form. **1** and the standard gave identical spectra with multiplets in the regions δ 4.0–3.5 (4 H), 3.1–2.8 (1 H), 2.7–2.4 (1 H) and 2.1–1.6 (1 H). **3** as the free compound gave an identical spectrum to that of the standard compound with multiplets in the regions δ 4.1–3.75 (3 H), 3.5–3.1 (2 H) and 2.4–2.0 (2 H).

Amino acid distribution. Samples of ground seed were extracted with 70% EtOH and amino acid fractions separated using small Zeokarb 225 columns. The individual components were resolved on 2-D PC in solvents 1 and 2.

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PRUNASIN IN *HOLOCALYX BALANSAE*

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Key Word Index—*Holocalyx balansae*; Caesalpiniaceae; cyanogenic glucoside; prunasin; holocalin.

Die Samen des südamerikanischen Baumes *Holocalyx balansae* Mich. (Caesalpiniaceae) enthalten Holocalin (2-β-D-glucopyranosyloxy-2-(*m*-hydroxyphenyl)-2R-acetonitril) [1,2]. Das in [2] ebenfalls gefundene Zierin (2-β-D-glucopyranosyloxy-2-(*m*-hydroxyphenyl)-2S-acetonitril) ist offensichtlich ein Artefakt, für deren Bildung bei der Bearbeitung cyanogener Glykoside neben der Alkalikatalyse kürzlich weitere Gründe mitgeteilt wurden [3,4]. Zusätzliche Angaben über cyanogene Verbindungen in *H. balansae* fehlen. Als biogenetische Vorstufe für Holocalin und Zierin wird Phenylalanin diskutiert und *m*-Hydroxylierung im Verlauf der Biosynthese erwogen [5,6].

Aus den in [1] und [2] verwendeten Samen von *H. balansae* haben wir zwei Exemplare der Pflanze gezogen, die inzwischen *ca* 1m hoch sind. Die paarig gefiederten Blätter sind stark cyanogen; sie enthalten *ca* 0,85% mit β-Glucosidase freisetzbare Blausäure (bezogen auf Trockensubstanz). Die cyanogene Verbindung selbst ist gleichmäßig über das gesamte Blatt verteilt. GC-Untersuchungen methanolischer Extrakte des lyophilisierten Materials nach [2] zeigten, daß Holocalin nicht nachweisbar ist; dagegen war das Vorliegen von Prunasin (2-β-D-glucopyranosyloxy-2-phenyl-2R-acetonitril) wahrscheinlich. Die isolierte Verbindung ist dc, gc [2,7] sowie IR-spektroskopisch mit Prunasin identisch. Das Peracetat besitzt einen Fp (korr) von 135,5–137°, sein ^{1}H-NMR (60 MHz, in $CDCl_3$) ist bezüglich chemischer Verschiebung, Kopplungskonstanten und Integration identisch mit demjenigen von Prunasin-tetraacetat in [8]. Weitere cyanogene Glykoside, insbesondere Sambunigrin (2-β-D-glucopyranosyloxy-2-phenyl-2S-acetonitril) sind nicht nachweisbar. Prunasin liegt damit in den Blättern von *H. balansae* in hoher Konzentration von *ca.* 10% (bezogen auf Trockensubstanz) vor. Die Ordnung der Leguminosae (Fabales) zeichnet sich durch Cyanglykoside verschiedener Struktur aus (Linamarin, Lotaustralin, Acacipetalin, Dihydro-acacipetalin; Vicianin, Sambunigrin). Während dieser Arbeit wurde Prunasin aus der Mimosaceae *Acacia deanei* ssp. *paucijuga* isoliert und Mandelonitrilglykoside für weitere *Acacia* spec. wahrscheinlich gemacht [12]. Mit *Holocalyx balansae* wurde Prunasin erstmalig bei den Caesalpiniaceae nachgewiesen.

Prunasin und Holocalin sind bereits in *Sambucus nigra* L. (Caprifoliaceae) nebeneinander gefunden worden [9]. Obwohl andere Biosynthesewege hierdurch nicht auszuschließen sind, macht es der erneute Nachweis des gemeinsamen Vorkommens sehr wahrscheinlich, daß eine *m*-Hydroxylierung auf einer späten Stufe der Biogenese erfolgt und dabei Prunasin die direkte Vorstufe für Holocalin darstellt.

EXPERIMENTELLES

7 ausgewachsene Blätter wurden in flüssigem Stickstoff pulverisiert, gefriergetrocknet (3,2 g Trockensubstanz), 1 hr mit Petrol und anschließend 12 hr mit H_2O-gesättigtem EtOEt extrahiert. Die EtOEt-Phase wurde bis zur Hälfte eingeengt, 12 hr bei 4° belassen und der Niederschlag abzentrifugiert. Der Bodensatz wurde in H_2O gelöst, über wenig Kohle filtriert und das Filtrat lyophilisiert. Das erhaltene Präperate war dc (SiO_2; EtOAc–AcOH–MeOH–H_2O 12:3:3:2, Detektion: Feigl-Anger-Test, Anisaldehyd–H_2SO_4) und gc (2,7) rein und mit Prunasin R_f bezw. t_R-gleich. Das IR-Spektrum (KBr-Pressling) war identisch mit dem von Prunasin (aus Amygdalin nach [10] hergestellt). Zur Darstellung des Peracetates wurde 18 hr in 0,7 ml C_5H_5N und 0,7 ml Ac_2O acetyliert, der Ansatz getrocknet, mit 1 ml MeOH aufgenommen und mit 5 ml H_2O versetzt. Der Niederschlag wurde abzentrifugiert und aus MeOH–H_2O umkristallisiert: Fp(Korr) 135,5–137°, (Prunasintetraacetat 136–137° [11]).

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