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DIHYDROACACIPETALIN—A NEW CYANOGENIC GLUCOSIDE FROM *ACACIA SIEBERIANA* VAR. *WOODII*

DAVID S. SEIGLER

Department of Botany, The University of Illinois, Urbana, Illinois, U.S.A.

and

CHARLES S. BUTTERFIELD, JOHN E. DUNN and ERIC E. CONN

Department of Biochemistry and Biophysics, University of California, Davis, California 95616, U.S.A.

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Abstract—The structure of a new cyanogenic glucoside, dihydroacacipetalin has been established, primarily on the basis of NMR and mass spectral data. This compound co-occurs with acacipetalin and is also derived from L-leucine in the plant *Acacia sieberiana* var. *woodii* (Leguminosae).

INTRODUCTION

Steyn and Rimington [1,2] isolated a cyanogenic compound from *Acacia hebeclada* and *A. sieberiana* var. *woodii* which they called acacipetalin. The structure of this compound, as isolated from *A. sieberiana* var. *woodii*, has recently been revised and the presence of a second compound reported [3] in that species. We now wish to clarify the nature of this second glycoside.

DISCUSSION AND RESULTS

The new glucoside, which we have called dihydroacacipetalin, occurs in the leaves and young stems of *Acacia sieberiana* var. *woodii*, a South African legume. In our materials it occurred in the ratio of 1:3 with acacipetalin, and it is likely that the compound isolated by Rimington and Steyn was a mixture of these two compounds, scarcely resolvable with techniques available to these workers. Mass spectra, NMR data and enzymatic hydrolysis indicate this compound to be the saturated analog of acacipetalin. In a similar manner to our examination of acacipetalin, we have compared the NMR spectrum of the TMS ether of this compound with that of the corresponding saturated lipid (see Table 1 and Ref. [4]). Methyl doublets (apparently three, centered at 1·15 δ) overlap the multiplet produced by the isopropyl hydrogen. The anomeric proton of glucose and the cyanohydrin methine proton overlap in a multiplet centered at ~4·2 δ. Other sugar protons occur at 3·0–3·6 δ as expected. The presence of methyl doublets (apparently three) and a multiplet (apparently three doublets) for the methine signals suggest hindered rotation about the single bond and the presence of rotamers. The NMR spectrum of synthetic isobutyraldehyde cyanohydrin has two doublets centered at 1·08 δ (J 6·5 Hz) which overlap a multiplet produced by the isopropyl protons and a doublet (J 5·5 Hz) at 4·28 δ.

Hydrogenation of the TMS ether of acacipetalin with Pd/C (5%) in light petroleum and subsequent examination of the NMR spectrum produces a doublet at 4·15 δ, a doublet at 4·05 δ and two doublets at 1·18 δ, with appropriate coupling constants, in addition to absorptions of unchanged acacipetalin. The field desorption mass spectrum [6] of dihydroacacipetalin had an (M + 1) ion at

Table 1. NMR data for dihydroacacipetalin and for the hydrogenated cyanolipid of *Ungnadia speciosa*

	Multiplicity		Chemical shift (δ)*	
	Glycoside	Lipid	Glycoside	Lipid
Methyl protons	3*d*?	2*d* (*J* 5·6 Hz)	1·15 } (7)	1·09
Isopropyl proton	*m*	*m*	† }	2·16
Cyanohydrin proton	3*d*?	*d* (*J* 5·6 Hz)	~ 4·3‡	5·2
Glucose protons	*m*		3·0–3·6 (~ 4)	
$-CH_2-$OTMS of glucose	*d* (*J* 3 Hz)		3·53 (~ 2)	
Anomeric proton	*d* (*J* 7 Hz)		~ 4·4‡	

* NMR spectra were measured on a 100 mc Varian HA-100 instrument in CCl_4 (dihydroacacipetalin) and $CDCl_3$ (hydrogenated cyanolipid). The presence of trace amounts of lipids overlap and partially obscure the methyl doublets and must be carefully avoided [5].

† The isopropyl proton is centered under and overlaps the two methyl doublets.

‡ Total of ~ two protons.

262 *m/e* (measured on a Varian MAT 731 mass spectrometer).

A mixture of acacipetalin and dihydroacacipetalin was hydrolyzed enzymatically with β-glucosidase (almond), subsequently distilled, and the 2,4-dinitrophenylhydrazone (2,4-DNP) prepared [3]. Examination of the NMR spectra of the precipitate revealed a doublet at 1·24 δ (*J* 6·5 Hz), identical to that of the doublet of authentic isobutyraldehyde 2,4-DNP.

The TMS ether of dihydroacacipetalin is readily separated by GLC from those of glucose and acacipetalin. Previous studies [7,8] have suggested that (S)-cyanogenic glucosides are eluted before the corresponding (R)-compounds. If a mixture of the two epimers of acacipetalin is prepared by allowing the compound to stand in the presence of 0·01 N NH_4OH, a new peak with a shorter retention time is observed, suggesting an (R)-configuration for the original acacipetalin. However, when dihydroacacipetalin is epimerized, the new peak has a greater retention time indicating an (S)-configuration for the dihydro compound. Comparison of NMR data [5] for linamarin and lotaustralin suggests that acacipetalin has an (R)-configuration, and as the anomeric protons in mixtures of acacipetalin and dihydroacacipetalin overlap, dihydroacacipetalin must have the same configuration.

EXPERIMENTAL

Plant material. Seeds of *Acacia sieberiana* var. *woodii* obtained from the Botanical Research Institute, Pretoria, S. Africa, were germinated and several young trees grown in a greenhouse. Voucher specimens of the plants are deposited in the University of Illinois herbarium.

Isolation and purification of glycosides. Dihydroacacipetalin was isolated from vegetative material in the manner previously described [3]. Ethanolic–aq. extracts were concentrated and mixtures obtained purified by PC.

GLC procedures. TMS ethers of the cyanogenic glycosides and glucose were prepared by treating dried samples with trimethylchlorosilane in C_5H_5N (Tri-Sil) for 30–60 min. Chromatography of the derivatives obtained was performed on a chromatograph equipped with an FID on a glass column (1·8 m × 2 mm i.d.) packed with 3% SP-2250 on Supelcoport 80/100 mesh. Carrier gas, He, 30 ml/min; temp. program 200° for 1 min, ΔT of 5°/min, final temp. 300°, held for 2 min. Injection temp., 260°; detector temp., 350°.

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