Proacacipetalin and Acacipetalin

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Summary The long-known cyanogenic glucoside acacipetalin, confirmed to be 2-(β-D-glucopyranosyloxy)-3-methylbut-2-enenitrile, can be produced from a natural β-D-glucopyranoside of 2-hydroxy-3-methylbut-3-enenitrile, which is distinguished from its epimer and named proacacipetalin.

THE crystalline cyanogenic glucoside acacipetalin[1,2] was obtained from Natal camelthorn, *Acacia sieberiana* DC. var. *woodii* (Burtt Davy) Keay and Brenan (*A. lasiopetala* in the sense of Steyn and Rimington, not Oliv.), and trassiebos, *A. hebeclada* DC. (*stolonifera* Burch.),† accounting for *ca.* 20% of the cyanide content. Extraction and evaporation of extracts were conducted in the presence of calcium carbonate, which is basic enough to epimerize mandelonitrile glycosides.[3] The most effective isolation procedure[2] also included treatment with basic lead acetate and ammonia. Hydrolysis[2] of acacipetalin with β-glucosidase or dilute acid gave, besides glucose and hydrogen cyanide, 2-methylpropanoic acid unaccompanied by aldehyde or ketone in acid and in the enzymatic reaction volatile acid plus acetone in 20% yield as the only carbonyl compound. Rimington accordingly formulated[2] acacipetalin as 2-(β-D-glucopyranosyloxy)-3-methylbut-2-enenitrile (I).

$Me_2C{=}C(OC_6H_{11}O_5)CN$ (I) $H_2C{=}C(Me)CH(OC_6H_{11}O_5)CN$ (II)

$Me_2CHCH(OC_6H_{11}O_5)CN$ (III)

($C_6H_{11}O_5$ = β-D-glucopyranosyl)

In a recent reinvestigation[4] of *A. sieberiana* var. *woodii* two cyanogenic glucosides were found, the major component being assigned the structure 2-(β-D-glucopyranosyloxy)-3-methylbut-3-enenitrile (II) and the minor component being possibly[5] the dihydro-derivative (III). The principal glucoside was not isolated in crystalline form but was characterized by the ^{1}H n.m.r. spectrum of its per-*O*-trimethylsilyl derivative and enzymatic hydrolysis to 2-methylprop-2-enal. Rimington's work was treated inconsistently and his acacipetalin was assumed to be not (I) but (II), either by itself[4,6] or admixed with (III).[5]

Through the kindness of Professor Rimington we have secured original specimens of acacipetalin, m.p. 177—179 °C (sintering from 172 °C), and its tetra-acetate,[2] m.p. 101—103 °C, and can confirm that these are pure compounds having the structures attributed to them in 1935 (Tables 1 and 2). Chromatography on silica gel of extracts of leaves and pods of *A. sieberiana* var. *woodii* and leaves of *A. hebeclada*† afforded syrupy concentrates of the main cyanogenic constituent, a single diastereoisomer of (II), incorrectly designated as acacipetalin in the literature,[4–7] and which we name proacacipetalin. Proacacipetalin was stable to pyridine at room temperature during 5 days and readily

TABLE 1. U.v. absorption spectra

Compound	Solvent	λ_{max}/nm (ε)
Acacipetalin	H_2O	220 (10,000)
Tetra-acetylacacipetalin	MeCN	218 (11,000)
Proacacipetalin	H_2O	None above 200 nm
Tetra-acetylproacacipetalin	MeCN	Shoulder at 210—220 (450); ε 1,800 at 200 nm

TABLE 2. Chemical shifts (δ) of aglucone hydrogens of (I) and (II) and derivatives

Compound	Solvent	Me	Olefinic	α-Hydrogen
Acacipetalin[a,b]	CD_3OD	1·89,[c] 1·95	—	—
Tetra-acetylacacipetalin	$CDCl_3$	1·82, 1·95	—	—
Trimethylsilylacacipetalin	CCl_4	1·72, 1·85	—	—
Proacacipetalin	D_2O	1·88	5.30, 5·42	5·42
	CD_3OD	1·90	5·17, 5.40	5.40
Tetra-acetylproacacipetalin	$CDCl_3$	1·85	Not assigned	N.a.
Trimethylsilylproacacipetalin	CCl_4	1·79	5·03, 5·30	4·89
Epiproacacipetalin	D_2O	1·88	5.30, 5·42	5.30
	CD_3OD	1·90	5·23, 5·38	5·28
Trimethylsilylepiproacacipetalin	CCl_4	1·79	5·05, 5·14	4·77

[a] Anomeric hydrogen, 4·65 (J 7·35 Hz). [b] ^{13}C N.m.r. [sample from (II); CD_3OD]: methyl carbons, δ 18·2 (17·9 for the monodeuterio-compound,‡ J_{CD} 19 Hz) and 20·75; other aglucone carbons, 115·5, 124·4, and 140·7; glucose carbons, 62·75, 71·5, 75·0, 78·25, 78·7, and 104·1 p.p.m. [c] Shifted to 1·87 for the monodeuterio compound‡ (^{1}H spectrum, 270 MHz; $|J_{H,D}|$ 2·1 Hz).

† We thank Dr. P. J. Robbertse (Department of General Botany, University of Pretoria) for confirming the identities of Steyn and Rimington's voucher specimens (ref. 1) as well as supplying material of these species. *Cf.* E. Palmer and N. Pitman, 'Trees of South Africa,' Balkema, Amsterdam, 1961, pp. 112, 148, 163; J. P. M. Brenan, in 'Flora Zambesiaca,' vol. 3, pt. 1, ed. J. P. M. Brenan, Crown Agents for Oversea Governments and Administrations, London, 1970, pp. 106—110. The synonym in chemical literature (ref. 7) is wrong.

‡ The acacipetalin formed in this reaction contained no more than one deuterium (ref. 8), all of which was in the more shielded methyl group (Table 2).

gave a crystalline tetra-acetate, m.p. 112—113·5 °C, $[\alpha]_D^{22}$ −37° (*c* 0·5 in ethanol), lacking conjugation (Table 1; the acetate was also devoid of i.r. absorption between 1900 and 2800 cm^{-1}, whereas acacipetalin and its acetate had sharp bands at 2230 and 2210 cm^{-1} respectively). Treatment with aqueous base abstracted the α-proton of proacacipetalin, the resulting anion being reprotonated more rapidly at the α- than the γ-position.[8] Thus in 0·7% triethylamine during 15 min at room temperature proacacipetalin was converted into a mixture of starting material and approximately an equal amount of the other diastereoisomer of (II), epiproacacipetalin, with only a little (I), whereas in 15% triethylamine at 15—20 °C during 3 h rearrangement to (I), m.p. 176—177 °C, was complete. The product after 3 h in 0·01 M ammonia[5] at room temperature was a mixture of all three compounds. When proacacipetalin was heated with calcium carbonate under reflux in 96% ethanol for 24 h most of it was converted into (I). The per-*O*-trimethylsilyl derivatives of the three isomers were separable by gas chromatography on poly(1,4-butanediol succinate) [3%, glass column 2·1 m × 4 mm, nitrogen 40 ml/min, 175 °C; retention times for (I) 15·6, proacacipetalin 16·9, epiproacacipetalin 19·8 min; on OV-25 phenyl methyl silicone the derivatives of (I) and epiproacacipetalin emerged together after trimethylsilylproacacipetalin].

In ^1H n.m.r. spectra signals from anomeric hydrogens (all doublets, *J ca.* 7 Hz) of the trimethylsilyl ethers of (I), proacacipetalin, and epiproacacipetalin appeared at δ 4·56, 4·33, and 4·03 respectively. Treatment of proacacipetalin with triethylamine in deuterium oxide‡ gave a diastereoisomeric mixture fully deuteriated at the α-position, and from the ^1H and ^2H n.m.r. spectra of this material and its trimethylsilylation product combined with the ^1H spectra of free and trimethylsilylated proacacipetalin the chemical shifts of the aglucone hydrogens were assigned by classes (Table 2). The chemical shift of the α-hydrogen of trimethylsilylproacacipetalin is less than the shifts of the olefinic hydrogens, not greater as proposed.[4,6]§ The chemical shift difference of 0·12 p.p.m. between the α-hydrogens of proacacipetalin and its epimer (free or trimethylsilylated) is similar to that[6,9] (0·16—0·19 p.p.m.) for epimeric pairs of mandelonitrile glycosides. For these latter pairs also, the order in which trimethylsilyl ethers are eluted on g.l.c. is the same as that in which n.m.r. signals of the anomeric or α-hydrogens appear during a sweep to higher field.[6,9,10] In these properties proacacipetalin is analogous to sambunigrin, dhurrin, and zierin of the (*S*)-mandelonitrile series, and epiproacacipetalin to prunasin, taxiphyllin, and holocalin of the (*R*)-series.

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§ The original assignment of chemical shifts (D. S. Seigler, C. Eggerding and C. Butterfield, *Phytochemistry*, 1974, **13**, 2330) for the corresponding hydrogens of the per-*O*-trimethylsilyl derivative of cardiospermin (II, CH_2OH replaces Me) appears more likely to be correct than the revised version (ref. 6).

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