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PRUNASIN-6′-MALONATE, A CYANOGENIC GLUCOSIDE FROM *MERREMIA DISSECTA*

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Key Word Index—*Merremia dissecta*; Convolvulaceae; prunasin, malonylprunasin; cyanogenesis.

Abstract—The cyanogenic glucosides prunasin and 6′-*O*-malonylprunasin have been isolated from the leaves of *Merremia dissecta*. Malonylprunasin is the first example of a malonyl conjugate of the cyanogenic glycosides.

INTRODUCTION

Cyanogenesis is known from a few members of the Convolvulaceae including sweet potatoes (*Ipomoea batatas* Lam.) [1] but cyanogenic compounds have never been isolated from these plants. Hegnauer [2] has observed that *Merremia dissecta* (Jacq.) Hallier, aereal parts and seeds, produces detectable amounts of hydrocyanic acid when crushed and he has argued [2] that phenylalanine-derived cyanoglycosides may be its source. In the following the structure elucidation of the cyanogenic glucosides of the leaves of *M. dissecta* is presented.

RESULTS AND DISCUSSION

The methanolic extracts of young, newly opened leaves contained 127 μmol/g dm of enzymatically liberated HCN and the old fully developed leaves 37 μmol/g dm. The extracts showed two cyanogenic zones when chromatographed on silica gel plates. The cyanogenic compounds were isolated and purified using low pressure column chromatography and preparative TLC on silica gel. The less polar compound was identical in all respects with prunasin (TLC, GLC of the TMS–ether, ^{1}H NMR), (*R*)-2-β-D-glucopyranosyloxyphenylacetonitrile [3]. The more polar compound yielded prunasin and its epimer sambunigrin [4] upon alkaline hydrolysis and malonic acid as indicated by GC. The negative ion FAB mass spectrum showed a signal for the deprotonated molecular ion at *m/z* 380 and for the deprotonated dimer at *m/z* 761, indicating the unknown had M_r of 381. The 1D and 2D COSY ^{1}H NMR spectra in CD_3OD allowed ready identification of the aromatic and sugar moieties. The downfield shift of the H-6′A,B signals (4.55, 4.40 ppm) compared to those in prunasin (3.93, 3.76 ppm) indicated acylation at C-6′. In DMSO-d_6 the characteristic AB system of a malonic acid residue was observed at 3.43 ppm [5] which in CD_3OD was absent due to deuterium exchange of the labile methylene protons. The presence of the malonyl moiety was confirmed from the ^{13}C NMR spectrum and its position at C-6′ was indicated by the low field shift of C-6′ and upfield shift of C-5′ (β-effect) compared to prunasin [6] (Table 1).

Thus the new compound is the C-6′-malonyl ester of prunasin, (*R*)-2-(6-*O*-malonyl-β-D-glucopyranosyloxy) phenylacetonitrile. This is the first report on a malonyl ester of a cyanogenic glucoside. As in almost all cases of the malonyl esters of anthocyanins [7], flavonoids, isoflavonoids, betalains and ginsenosides [8], the malonyl moiety is linked via the C-6 of the glucose moiety; only recently isomeric malonylated benzyl-β-D-glucosides at the 2, 3, 4 and 6 position of the glucose moiety were isolated from the fruit pulp of *Carica papaya* [9]. A rough estimation of the prunasin:prunasin malonate ratio was obtained from the cyanide detection with the picrate-sandwich-method [10] of the crude methanolic extract: young leaves contain more than 95% of prunasin and less than 5% of its malonate; old leaves contain less than 90% and more than 10% respectively. The shift of the ratio with age favouring the malonate may be a hint that malonylation traps the glucosides for a vacuolar storage [8]; but in view of the decrease of the cyanoglucosides in older leaves it also supports the hypothesis that malonylation keeps a compound in an almost metabolically inactive space, the vacuole [8].

Reports on cyanogenic constituents of the Lamiidae are rare. *Eremophila maculata* F. Muell. (Myoporaceae) [11] and *Linaria striata* DC and *L. minor* Desf. (Scrophulariaceae) [12] were reported to contain prunasin; *Perilla frutescens* Britt. var. *acuta* Kudo contains prunasin and its 2′-*O*-glucoside [13]; however, *Borago officinalis* L. contains dhurrin, the 4-hydroxylated *S*-epimer of prunasin [14]. This report underlines the tendency of this taxonomic group of plants to use phenylalanine as a biogenetic precursor [15] for producing its cyanoglycosides.

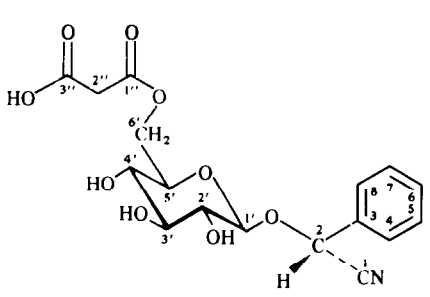

Table 1. ^1H and ^{13}C NMR data for prunasin-6′-malonate

	^{13}C NMR		^1H NMR	
	CD_3OD	DMSO-d_6	DMSO-d_6	
1	119.23	118.42		
2	69.04	67.25	H-2	5.88 (*s*)
3	134.92	133.67		
4	130.09	128.85	H-4	
5	128.95	127.29	H-5	
6	130.96	129.53	H-6	7.57–7.45 (*m*)
7	128.95	127.29	H-7	
8	130.09	128.85	H-8	
1′	102.54	101.71	H-1′	4.31 (*d*) 7.2
2′	74.70*	72.93*	H-2′	
3′	77.80	76.19	H-3′	3.15–3.07 (*m*)
4′	71.66	69.91	H-4′	
5′	75.53*	73.74*	H-5′	3.40 (*m*)
6′	65.47	64.13	H-6′A	4.33 (*dd*) (2.1; 11.7)
			H-6′B	4.20 (*dd*) (7.0; 11.8)
1″	168.75	166.84		
2″	n.o.	41.51	H-2″	3.43 (centre of AB system)
3″	168.75	167.94		

*Signals interchangeable.
n.o: Not observed.

EXPERIMENTAL

The plants were grown from seeds obtained from Prof. R. Hegnauer (Leiden), in the greenhouse and were identified after ref. [16]. A voucher is deposited in the herbarium of the Institute of Pharmaceutical Biology and Phytochemistry of the WWU Münster.

^1H and ^{13}C NMR spectra were recorded at ambient temp. The negative ion FAB mass spectrum was recorded on a Kratos MS 50 spectrometer with a Kratos FAB source; 3-nitrobenzyl alcohol was used as matrix.

Prunasin for comparison purposes was from the collection of A.N. β-Glucuronidase was obtained from Sigma (G 0876).

In several experiments 10–50 g lyophilized leaves were extracted with cold MeOH (Ultra turrax), the MeOH was concd, the residue shaken with H_2O, filtered or centrifuged and the clear solution lyophilized. The residue, dissolved in the mobile phase, was chromatographed on silica gel (150 g) in a 3.5 × 47 cm column using EtOAc–Me_2CO–H_2O (160:4:1) first; prunasin was eluted between 500 ml and 850 ml. The solvent was then replaced by EtOAc–Me_2CO–MeOH–H_2O (20:15:5:4) and prunasin malonate was eluted between 2400 ml and 2500 ml. Both cyanogenic fractions were further purified by prep. TLC on precoated silica gel plates (Merck) with EtOAc–Me_2CO–MeOH (4:3:1) (prunasin fraction) and EtOAc–Me_2CO–MeOH–H_2O (20:15:5:4) (prunasin malonate fraction). The cyanogenic zones were scraped off and the silica gel eluted with MeOH, taken to dryness, dissolved in H_2O, filtered and lyophilized. The residues were analysed.

Detection of cyanogenesis in solution was performed by using the Feigl–Anger test [17] with β-glucuronidase as an unspecific hydrolysing enzyme [10]. Cyanogenic zones on TLC were detected after spraying with β-glucuronidase using the sandwich technique according to [10]. Enzymatically liberated HCN was quantitatively estimated as described in [18]. Prunasin malonate was hydrolysed to give prunasin/sambunigrin and malonic acid in 0.5 M Na_2CO_3 at 40° for 40 min.

GLC of TMS-prunasin and its C-2-epimer TMS sambunigrin was performed on a DB-5 column (0.25 mm × 30 mm): 200–250°, 5°/min; flow 0.8 ml N_2; FID. R_t TMS-prunasin 9.55 min, R_t TMS-sambunigrin 9.90 min. The glucosides were derivatized to the TMS-ether using *N,N*-bis-trimethylsilyltrifluoroacetamide (BSTFA). Malonic acid was derivatized to its TMS-ester using MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide) and chromatographed by GLC on DB-5 (0.25 mm × 30 m), 50–160°, 5°/min; flow 1.5 ml N_2; R_t malonyl-TMS 15.5 min.

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