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A NEW CYANOGENIC GLYCOSIDE FROM *HORDEUM VULGARE*

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Key Word Index—*Hordeum vulgare*; Poaceae; cyanogenic glycosides; 2-β-D-glucopyranosyl-oxy-3-methyl-(2*R*)-butyronitrile; (*R*)-*epi*-heterodendrin.

Abstract—From leaf extracts of 10-day-old seedlings of barley, a new cyanogenic glycoside has been isolated. This compound is 2-β-D-glucopyranosyl-oxy-3-methyl-(2*R*)-butyronitrile, the epimer of heterodendrin.

INTRODUCTION

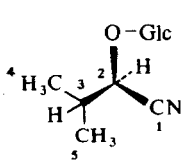
There exist several reports on cyanogenesis in cereals [4, 5 cf. also 2, 3, 26] but only in a few cases is the cyanogenic principle known. Thus dhurrin occurs in *Sorghum vulgare* [1] and *Triticum spelta* [27], and triglochinin in *Eleusine coracana* [6]. In this paper, we report the structure of the cyanogenic glycoside of *Hordeum vulgare*.

RESULTS AND DISCUSSION

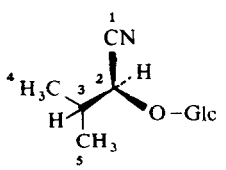
Five mg of the unknown cyanogenic glycoside were isolated from primary leaves of 10-day-old barley seedlings. The chromatographic data excluded an aromatic structure (no dark spot on TLC plates with fluorescence indicator, no reaction with TTH [22]) and ruled out the presence of triglochinin [6, 7]. The assay for double bonds [8, 9] also failed. Co-chromatography with heterodendrin isolated from *Heterodendron oleaefolium* [10] on Si gel in solvent 4 (see Experimental) resulted in two spots (hR_f-values: heterodendrin 38, barley-glycoside 30) whereas in all other solvents on Si gel and on cellulose there was no separation. The authentic heterodendrin, however, dissolved in EtOH and stored for several months, showed after chromatography on Si gel in solvent 4 a weak second spot corresponding to that of barley glycoside **1**. Similarly, **1** showed, after the same treatment, a weak second spot corresponding to that of **2**. Thus **1** and **2** seem to be partly interconvertible. In the GLC systems used, TMS ether of **1** showed a longer retention time than TMS-heterodendrin of 0.5 and 1 min, respectively.

The 2,4-DNPH of the aglycone of **1** showed an identical chromatographic behaviour to that of authentic isobutyraldehyde in solvents 8–13 on Si gel 60 and Si gel G. The ^{1}H NMR of the 2,4-DNPH of the aglycone was identical to that of authentic isobutyraldehyde 2,4-DNPH. The sugar moiety was identified as glucose by TLC comparison; it also yields a positive gluco-test [24]. The identity of the 2,4-DNPH derivative of the aglycone

Table 1. ^{1}H NMR data and structure of barley glycoside and heterodendrin



epi-Heterodendrin (**1**)



Heterodendrin (**2**)

Solvent	Substance	Methyl-H	C_2-H	Glc-H_1	C_3-H	Sugar
DMSO-d_6	**2**	0.95 (*d*) 3H 1.02 (*d*) 3H	4.71 (*d*) 1H *J* = 5.5	4.31 (*d*) 1H *J* = 7.5	~1.95 (*m*) 1H	~3–4
	1	0.94 (*d*) 3H 1.01 (*d*) 3H	4.53 (*d*) 1H *J* = 5.5	4.29 (*d*) 1H *J* = 7.5	~2.0 (*m*) 1H	~3–4
MeOH-d_4	**2**	1.17 (*d*) 3H 1.23 (*d*) 3H	~4.8*	4.62 (*d*) 1H *J* = 7.5	~2.1 (*m*) 1H	~3.2–4.2
	1	1.04 (*d*) 3H 1.10 (*d*) 3H	4.49 4.43 4.35 *J* = 5.5 2H (*t*)† *J* = 7.5		~2.1 (*m*) 1H	~3.2–4.1

δ scale, value in ppm. Coupling constants in Hz.
* On the shoulder of the CD_3OH-signal.
† This seems to be a pseudotriplet resulting from an overlapping of the two expected doublets.

of **1** with that of isobutyraldehyde shows that **1** is a glucoside of 2-hydroxy-3-methylbutyronitrile.

From the longer retention time in the GLC systems of TMS **1** compared with heterodendrin [10–12] which possesses the (*S*)-configuration [12], one may assume an isobutyraldehydecyanohydrin-glucoside with an (*R*)-configuration in the chiral centre of the aglycone. Thus the TMS-derivatives with (*S*)-configuration, in the case of mandelonitrile derivatives and proacacipetalin/epiproacacipetalin, were observed to be eluted earlier than derivatives with the (*R*)-configuration [13–15]. In a HPLC system using SiO_2 as a stationary phase, **1** was eluted 2.4 min after heterodendrin. This behaviour is in agreement with that of mandelonitrile glycosides with (*R*)-configuration [25] and reveals also an (*R*)-configuration at C_2 of **1**.

The ^{1}H NMR spectra of the new glucoside are closely related to those of heterodendrin [10] and are shown in Table 1. However, in DMSO-d_6 as well as in MeOH-d_4, the doublet of the anomeric sugar proton and the doublet of the cyanhydrin-H at C_2 are both shifted upfield in comparison to heterodendrin. Similar shifts of these protons are observed for (*R*)-mandelonitrile glycosides and epiproacacipetalin compared to the (*S*)-epimers [14–16] indicating also an (*R*)-configuration for **1**. The aglycone:glucose ratio is 1:1; the coupling constant of 7.5 Hz of the doublet of the anomeric glucose-H proves a β-configuration of the glycosidic linkage.

All the above data show that **1** is 2-β-D-glucopyranosyloxy-3-methyl-(2*R*)-butyronitrile (Table 1), or (*R*)-*epi*-heterodendrin. From its structure it may be a product of leucine metabolism. Cyanogenic glucosides derived from leucine have been isolated from Sapindaceae, Leguminosae and Rosaceae-Spiraeoideae (see [18]). This is the first report on a cyanogenic glycoside which seems to differ from the biogenetic scheme outlined for the Liliatae by Hegnauer [17, 18], who assumes tyrosine as the only precursor for cyanogenic glycosides in this taxonomic group.

EXPERIMENTAL

Plant material. Primary leaves of 10-day-old seedlings of *Hordeum vulgare* L., cv Dura, were grown on soil at greenhouse conditions. After harvesting, the leaves were dried at 60° and pulverized.

Isolation procedure. 180 g plant material were extracted several times with 10-fold hot 80% EtOH. The various fractions were combined and concd to a syrupy consistency. The syrup was eluted several times with hot (50°) demineralized H_2O. The cyanogenic glycoside was separated and purified according to the following scheme: CC (polyamide MN, SC6, H_2O); CC (Si gel 60 for CC, Merck, EtOAc with increasing MeOH concentrations up to 20%); PC (Whatman 3 MM, 90% aq. PrOH, elution: MeOH); PLC (2 mm layer, Si gel PF 254 – Merck, $CHCl_3$–MeOH (5:1), elution: MeOH), PLC (2 mm layer, Si gel PF 254 – Merck, EtOAc–MeOH–H_2O (93:5:2), elution: MeOH). Hydrolysis was done by the microdiffusion technique of Tantisewie [19].

Identification. Glycoside. TLC solvents: (S1) PrOH–$CHCl_3$–H_2O (17:2:1), (S2) $CHCl_3$–MeOH (3:1), (S3) MeCOEt–Me_2CO–H_2O (15:5:3), (S4) EtOAc–MeOH–H_2O (93:5:2), (S5) *n*-BuOH–EtOH–H_2O (7:2:2), (S6) EtOAc–Me_2CO–H_2O (4:5:1) (S7) *n*-BuOH–HOAc–H_2O (12:3:5). Adsorbents: Si gel 60 and Si gel F 254, (precoated plates, Merck S1, S2, S3, S4), Cellulose and Cellulose F 254 (precoated plates, Merck, S3, S5, S6, S7). Detection. Spot behaviour in UV (254 nm), sandwich-method with picrate paper [20], spraying with 0.1 M *p*-nitrobenzaldehyde and 0.1 M *o*-dinitrobenzene [21], anisaldehyde [8], TTH [22] and fluorescein–bromine [8, 9]. GLC: OV-1 3% on chromosorb (AW-DMCS 80–100 mesh), steel (3 m × $\frac{1}{8}$"), 175–200°, 1°/min, N_2 (25 ml), FID (10^{-10}/8–16); UCCW 10% on chromosorb (AW-DMCS 80–100 mesh), glass column (2.5 m × 2 mm), 210–250°, 1°/min, N_2 (30 ml), FID 10^{-10}/16, derivatization with HMDS and TMCS in C_5H_5N. HPLC: lichrosorb Si 60, 5 μm, 25 × 0.4 cm: EtOAc–MeOH 95:5, 1.5 ml/min, RI detector (System Knauer). ^{1}H NMR spectroscopy: FT spectra, 100 MHz, solvents—CD_3OD and DMSO-d_6. Acetate of hordeoside—prepared in C_5H_5N and acetanhydride; mp 144° (DSC-analysis, Heraeus TA 500 S).

Aglycone (2,4-DNPH derivative). TLC solvents: (S8) C_6H_6–C_6H_{10} (3:2), (S9) EtOAc–petrol $C_3H_7OC_3H_7$ (3:7:7), (S10) $CHCl_3$–C_6H_6 (1:1), (S11) EtOAc–petrol (1:4), (S12) C_6H_6–$CHCl_3$–petrol (1:1:1), (S13) EtOAc–petrol (1:2). Adsorbents: Si gel 60 (precoated plates, Merck S8–S12), Si gel G (S13). Detection: colour in daylight and absorption in UV (254 nm), spraying with K_3 [Fe(CN)$_6$] [23]. ^{1}H NMR spectroscopy: FT spectra, 100 MHz, solvent $CDCl_3$.

Sugar moiety. Glucotest according to Boehringer [24]. TLC solvents: (S14) EtOAc–C_5H_5N–H_2O–HOAc–propionic acid (10:10:2:1:1), (S15) BuOH–C_5H_5N–HOAc–EtOAc–H_2O (10:2:2:5:4), (S16) EtOAc–C_5H_5N–HOAc–H_2O (36:36:7:21), (S17) $CHCl_3$–MeOH–H_2O (16:9:2), (S18) EtOAc–MeOH–HOAc–H_2O (12:3:3:2). Adsorbents: Cellulose (precoated plates, Merck, S14, 15, 16), Si gel 60 (precoated plates, Merck, S17, 18).

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