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LEUCINE-DERIVED CYANOGENIC GLUCOSIDES IN THE ROSACEAE–SPIRAEOIDEAE

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Key Word Index—*Sorbaria arborea*; Rosaceae; cyanogenic glucosides; heterodendrin; cardiospermin-5-(4-hydroxy)-*E*-cinnamate.

Abstract—In addition to cardiospermin-5-(4-hydroxy)benzoate previously isolated from *Sorbaria arborea*, two further leucine-derived cyanogenic glucosides have been isolated from the same source. These are heterodendrin, already known from Sapindaceae and Mimosaceae, and a new compound, cardiospermin-5-(4-hydroxy)-*trans*-cinnamate.

INTRODUCTION

Cyanogenic compounds commonly found in the Rosaceae are prunasin (*R*-mandelonitrile-β-D-glucopyranoside) and amygdalin (*R*-mandelonitrile-β-D-gentiobioside), the latter occurring only in fruits and seeds whereas the former is located in the vegetative parts including the stems [1–3]. However, several observations indicate that cyanogenic substances other than those derived from phenylalanine may exist [4]. Recently cardiospermin-5-(4-hydroxy)benzoate has been isolated from *Sorbaria arborea* [5], which, biogenetically, is derived from leucine. During these investigations it was observed that there were other cyanogenic compounds in concentrations lower than those of cardiospermin-5-(4-hydroxy)benzoate and two of these are reported in this paper.

RESULTS AND DISCUSSION

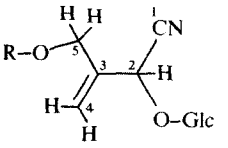
Extracts of leaves of *S. arborea* were chromatographed on polyvinylpyrrolidone [6] resulting in three cyanogenic fractions (I, II, III). Fraction III contained the main glucoside, cardiospermin-5-(4-hydroxy)benzoate. Fraction I was, at best, 2% of the total glucoside while the yield of fraction II was very poor and was not further investigated. However, HPLC of fraction III indicated small amounts of cyanogens other than the main glucoside, especially in the later eluates. Fraction I and the later eluates of fraction III were further purified on a silica gel column. After separation by preparative chromatography, fraction I resulted in nearly 6 mg of pure compound **1**. Fraction III was separated by HPLC on a RP-18 phase resulting in about 8 mg of compound **2**.

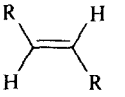
Hydrolysis of **1** by β-glucosidase resulted in HCN, (Feigl–Anger test [7]), glucose (TLC, GLC as sorbitol acetate) and a carbonyl compound, which was identified as its 2,4-dinitrophenylhydrazone on TLC as isobutyraldehyde. A comparison of the GLC on OV-225 showed coincidence with heterodendrin (2-β-D-glucopyranosyloxy-3-methyl-2*S*-butyronitrile) [8, 9]. The ^{1}H NMR spectrum in DMSO-d_6 was identical with that reported in reference [10] for heterodendrin and thus indicated that **1** is heterodendrin.

Compound **2** showed very similar behaviour in TLC to cardiospermin-5-(4-hydroxy)benzoate, but produced a violet colour with the anisaldehyde/H_2SO_4 spray reagent whereas the main glucoside appeared as a green spot. Hydrolysis by 1 N HCl yielded HCN, glucose and 4-hydroxycinnamic acid; no carbonyl compound was detectable. Hydrolysis by carboxyl esterase from porcine liver resulted in 4-hydroxycinnamic acid and cardiospermin (2-β-D-glucopyranosyloxy-3-hydroxymethyl-butyronitril-3-ene), both of which were identified by TLC and by GLC as their TMSi ethers. The ^{1}H NMR data are listed in Table 1. The new compound showed great similarity to cardiospermin-5-(4-hydroxy)benzoate with additional proton resonances occurring at δ 6.35 ppm (1 H, doublet, J = 16 Hz) and 7.63 ppm (1 H, doublet, J = 16 Hz), which indicates a *trans* (= *E*) cinnamoyl moiety. Like the main glucoside, esterification occurs at C-5–OH of cardiospermin as indicated by the *ca* 0.6 ppm downfield shift of the C-5-protons compared to cardiospermin. From these data, the new compound is a cardiospermin ester, cardiospermin-5-(4-hydroxy)*trans*-cinnamate.

Since heterodendrin found in the same plant has an *S*-configuration at C-2, it seems clear from biosynthetic arguments that the new ester also possesses an *S*-configuration at C-2. For this class of cyanogenic glucosides, leucine is assumed to be the biogenetic precursor, as indicated by incorporation experiments [11]. With regard to the biosynthetic sequence, heterodendrin is the cyanogenic glucoside most closely related to leucine. Since we have evidence of more cyanogenic products in very low concentration in this plant, further glucosidic intermediates may be obtainable. Besides the main glucoside, cardiospermin-5-(4-hydroxy)benzoate, the new ester, is *ca* 3% of the total cyanogen. This may arise as a byproduct of a non-specific *p*-hydroxybenzoate transferase in *S. arborea*. Nevertheless, this again demonstrates the ease of esterification of the 5-hydroxyl group of cardiospermin, as

Table 1. ^{1}H NMR spectra of two cyanogenic glucosides of *Sorbaria* compared to cardiospermin



Compound	R	H_2	2 × H_4	2 × H_5	*p*-OH-phenyl		Glc-H_1
Cardiospermin (MeOH-d_4)	H	5.45 (*s*)	5.54 (*s*) 5.51 (*s*)	4.23 (*s*) (broad)	—	—	4.5 (*d*), J = 7.5 Hz
Cardiospermin-5-(4-OH) benzoate (DMSO-d_6)	4-OH-benzoyl	5.55 (*s*)	5.65 (*s*) (broad)	4.9 (*s*) (broad)	6.85 (*m*) 7.9 (*m*)	—	4.65 (*d*), J = 7 Hz
Cardiospermin-5-(4-OH) cinnamate (DMSO-d_6)	4-OH-cinnamoyl	5.52 (*s*)	5.63 (*s*) 5.66 (*s*)	4.82 (*s*) (broad)	6.85 (*m*) 7.5 (*m*)	6.35 (*d*), J = 16 Hz 7.63 (*d*), J = 16 Hz	4.58 (*d*), J = 7.5 Hz

was also shown for cardiospermin-5-sulfate occurring in *Cardiospermum grandiflorum* [12].

EXPERIMENTAL

Isolation. Leaves of *Sorbaria arborea* grown in the Botanical Garden of the University of Freiburg were collected in September 1978 and freeze-dried. Dry matter (100 g) was extracted by petrol and subsequently by MeOH. The MeOH fraction was evapd to dryness, suspended in a small amount of H_2O and chromatographed on a polyvinylpyrrolidone column (2.5 × 68 cm, Polyclar AT, Serva, Heidelberg) with 0.5% HOAc. Fraction I was eluted within 204–425 ml, fraction II within 510–612 ml and fraction III within 1222–3655 ml. Fraction I was then chromatographed on Si gel (2 × 58 cm) with EtOAc–$CHCl_3$–MeOH (9:1:0.3) and a cyanogenic fraction was collected within 450–665 ml. This fraction was further purified by prep. TLC on Si gel using EtOAc–MeOH–HOAc (10:2:0.2) (detection: anisaldehyde–H_2SO_4) resulting in **1**. Fraction III was chromatographed on Si gel (4 × 64 cm) with EtOAc–MeOH–HOAc (99:1:0.1). Cyanogenesis was detected in 800–3850 ml, but 2700–3850 ml was used for further isolation. This fraction was purified by HPLC on Lichrosorb RP-18, 10 μm, 0.8 × 25 cm with AcCN–HOAc–H_2O (25:0.1:75) 2 ml/min, UV detection at 270 nm (R_t of cardiospermin-5-(4-OH)benzoate 9 min, R_t of **2** 14 min).

GLC systems. For sugars as hexitol acetates see ref. [5]. TMS-heterodendrin: OV-225, 3% on Chromosorb AW DMCS 80–100 mesh, 2 mm i.d. × 3 m, steel, N_2 (30 ml/min), 175–220°, 1°/min, FID, R_t of TMS-heterodendrin: 16.5 min. Products of hydrolysis of compound **2**: OV-17, 3% on Chromosorb AW DMCS 80–100, 2 mm i.d. × 1 m, glass, N_2 (20 ml/min), 100–250°, 4°/min, FID; R_t of TMS-*p*-hydroxybenzoic acid: 9.3 min, TMS-*p*-hydroxycinnamic acid: 18.4 min, TMS-cardiospermin: 27.4 min. TMSi ethers were prepared as described in ref. [13].

Hydrolysis with β-glucosidase (Serva, Heidelberg) from almonds using the Conway cell [14]: 24 hr at 37°, pH 6; with carboxyl esterase type II (Sigma), 2 hr in Tris buffer, pH 8, at room temp.

TLC systems. Si gel–EtOAc–MeOH–H_2O–HOAc, 84:13:2:1 (cardiospermin, 4-OH-benzoic acid, 4-OH-cinnamic acid); detection by UV 254 and anisaldehyde–H_2SO_4. Si gel–petrol–EtOAc–$CHCl_3$ 80:3:17 (isobutyraldehyde-2,4-dinitrophenylhydrazone); detection by UV 254. Si gel–AcCN–CS_2–H_2O, 85:5:10 (glucose), detection: anisaldehyde–H_2SO_4 [15].

^{1}H *NMR spectra.* Varian XL 100, FT-spectra, δ-scale, TMS standard.

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