the petrol extract was triturated with $CHCl_3$ and the solubles separated on Si gel with $CHCl_3$–MeOH–NH_3 [14]. Final purification was by HPLC on Micropak mCH 10 with MeCN. The amide was identified by UV spectra and retention times.

Biological assays. The compound (satd soln in H_2O, 157 mg/l.), was added at various concns to Petri dishes containing 5 snails (*Physa occidentalis* or *Lymnaea* spp: collected near Vancouver) and visual observations made of the responses. Similar tests were made with cercariae. Extracts of preserved roots of *H. longipes* were tested similarly, and against the common guppy (*Lebistes reticulatus*).

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DHURRIN, THE CYANOGENIC GLUCOSIDE OF *CERCOCARPUS LEDIFOLIUS*

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Key Word Index—*Cercocarpus ledifolius*; Rosaceae–Rosoideae; cyanogenic glucoside; dhurrin.

Abstract—Dhurrin (2-β-D-glucopyranosyloxy-2-(4-hydroxy)phenyl-2*S*-acetonitrile) was isolated as a cyanogenic compound from *Cercocarpus ledifolius* and identified by its hydrolysis products, chromatographic properties and ^1H NMR spectroscopy.

INTRODUCTION

Within the Rosaceae the phenylalanine-based cyanogenic glycosides amygdalin and prunasin are found mainly in the subfamilies Maloideae and Prunoideae [1] and hence have been considered to be the typical cyanogens of the family. However, other types of cyanogenic glycoside may occur in this family [2–4]. Recently the leucine-derived cyanogenic glucosides heterodendrin [5] and the 4-hydroxybenzoic acid ester [6] and 4-hydroxycinnamic acid ester of cardiospermin [5] have been detected in *Sorbaria* (Rosaceae–Spiraeoideae). We now describe the isolation and identification of dhurrin from *Cercocarpus ledifolius* Nutt. (Rosaceae–Rosoideae), a shrub or tree, growing in the western parts of the U.S.A. [7].

RESULTS AND DISCUSSION

Air-dried leaves and twigs of *C. ledifolius* were collected in the Sierra Nevada of northern California. The

Table 1. Comparison of the ^{1}H NMR data of the *Cercocarpus* glucoside with that for dhurrin[9] and taxiphyllin[10] all dissolved in acetone-d_6*

Substance	H-2 (cyanohydrin-H)	Glc H-1	Aromatic protons	Residual sugar protons
Dhurrin[9]	5.92, *s*	1H 4.68, *d*, 1H *J* = 7.5 Hz	7.41, *m*, 2H 6.92, *m*, 2H	3.1–3.9
Present glucoside	5.94, *s*	1H 4.69, *d*, 1H *J* = 7.5 Hz	7.40, *m*, 2H 6.90, *m*, 2H	3.2–3.9
Taxiphyllin[10]	5.84, *s*	1H 4.36, *d*, 1H *J* = 7.5 Hz	7.44, *m*, 2H 6.94, *m*, 2H	3.2–3.9

*Reference: TMS δ-scale.

methanolic extract of the material was purified using low pressure column systems on polyvinylpyrrolidone and Si gel[8]. The resulting colourless powder showed UV λ_{max}^{MeOH} at 230 nm ($\epsilon = 10^4$) and a shoulder at 274 nm. After addition of alkali (sodium hydroxide), a bathochromic shift was observed with peaks at 255 and 290 nm respectively, indicating a free phenolic hydroxyl group[8]. The substance showed strong cyanogenic activity on hydrolysis with dilute acids (CF_3COOH) or β-glucosidase from sweet almonds; further products of hydrolysis were glucose (TLC, GLC) and 4-hydroxybenzaldehyde (TLC). The cyanide–glucose ratio was close to 1 : 1. From these results a 4-hydroxylated mandelonitrileglucoside was expected[8]. GC on OV-225 showed minor peaks and a main peak that had the same retention time as TMSi-dhurrin, 2*S* configuration, which was quite separate from the 2*R* epimer, TMSi-taxiphyllin[8]. The ^{1}H NMR of the underivatized substance in acetone-d_6 is presented in Table 1. Data again are consistent with dhurrin[9] and exclude taxiphyllin[10] from the resonance position of the cyanohydrin proton and the anomeric glucose proton. Thus, the cyanogenic compound of *C. ledifolius* is 2-β-D-glucopyranosyloxy-2(4-hydroxy) phenyl-2*S*-acetonitrile (dhurrin).

Dhurrin is known from members of the Magnoliidae and the Liliopsida[11–13], from some Hamamelidales[14, 15], from Boraginaceae[16] and Proteaceae[17], but has hitherto never been found in the Rosaceae. The present data again show that the Rosaceae are heterogenous in their synthesis of cyanogens[4]. Thus, they are similar to other large plant groups, such as the Leguminosae [18] or Euphorbiaceae[19,20], in producing different types of cyanogenic glycosides. The biogenetic precursor of dhurrin in all plants tested so far is tyrosine[21]. Future investigations will show whether *Cercocarpus* also uses tyrosine for dhurrin synthesis and whether this character is useful for subfamily classification within the Rosaceae.

EXPERIMENTAL

Ca 100 g air-dried, pulverized plant material (leaves and twigs) of *C. ledifolius* were extracted with petrol and subsequently with MeOH. The MeOH extract was purified by chromatography on polyvinylpyrrolidone (2.5 × 50 cm; H_2O containing 0.5% HOAc) and the cyanogenic fractions (440–660 ml) were concd and chromatographed on Si gel (2.5 × 50 cm; EtOAc–t-BuOH–0.5% HOAc, 10:1:0.15). The resulting fractions (500–640 ml) were concd and lyophilized and the resulting colourless powder was used for further investigation.

Hydrolysis. Enzymatic. Ca 2 mg glucoside were incubated with 0.5 mg β-glucosidase (Serva, Heidelberg) in citrate buffer pH 6 for 4 hr at 38°. Cyanide was separated under N_2, trapped in 0.1 N NaOH and quantitatively estimated by the anthranilic acid–C_5H_5N method[22]. The residue was extracted with Et_2O and analysed for *p*-hydroxybenzaldehyde; in the remaining aq. phase glucose was detected and quantitatively estimated with the glucose oxidase method. *Acidic. Ca* 2 mg glucoside were incubated with 2 N trifluoroacetic acid for 1 hr at 95°.

GC systems. Cyanogenic glucosides. As their TMSi-derivatives; OV-225, 3% on Chromosorb AW DMCS 80–100 mesh; 180–240°, 1°/min; FID; R_t, TMSi-dhurrin: 49.2 min, R_t, TMSi-taxiphyllin 52.1 min. *Glucose.* After reduction to sorbitol as sorbitol penta-acetate on ECNSS-M[20].

TLC systems. Cyanogenic glucosides. SiO_2–EtOAc–MeOH–H_2O (8:1:1), detection: UV 254 nm, anisaldehyde–H_2SO_4; R_f dhurrin: 0.30; R_f prunasin: 0.43. *Glucose.* As described in Ref.[23]. *p-Hydroxybenzaldehyde.* SiO_2–toluene–MeOH (17 : 3), detection: UV, 254 nm, 2, 4-DNPH reagent, R_f, 0.53.

Cyanide. Quantitatively as described in Ref.[22]; qualitatively with Feigl–Anger paper[24] after hydrolysis with β-glucosidase.

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FATTY ACID COMPOSITION OF SALT STRESSED *ARACHIS HYPOGAEA* SEEDLINGS

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Key Word Index—*Arachis hypogaea*; Leguminosae; groundnut; seedling; fatty acid composition; salt treatments.

Abstract—Etiolated *Arachis hypogaea* seedlings grown in the presence of sodium chloride and sodium sulphate were analysed for their percent fatty acid composition. A higher proportion of oleic and linoleic acids resulting in a higher unsaturated–saturated fatty acid ratio was noticed in 6–10 day old embryonic axes of the salt treated seedlings.

INTRODUCTION

The fatty acid composition of seeds depends on the variety of seeds and on the conditions of growth [1, 2] and changes during their development [3, 4]. Several environmental factors also alter the level and composition of plant cell lipids. Several plant species adapt to extreme environmental conditions and lipid metabolism may become an important factor in the functioning of plant cell membranes under extreme conditions [5]. Changes in fatty acid composition were related to the resistance to various environmental stresses [6–10]. In the present experiment we studied the influence of single salt solutions on the fatty acid composition of groundnut seedlings during a 10 day period after germination.

RESULTS AND DISCUSSION

Palmitic, stearic, oleic and linoleic acids were the major fatty acids associated with the seedling metabolism of groundnut. The composition of fatty acids usually indicates the mode of utilization of the component fatty acids. If one acid is not metabolized relative to others, it apparently appears that the acid has been synthesized. Oleic acid was the major fatty acid in the groundnut cotyledons throughout the period of germination. Changes in the percentage of linoleic acid, with a higher amount on days 2–4 in the cotyledons possibly indicate its initial utilization in metabolism. Rapid changes in the percentage of individual fatty acids may be due to an interconversion of fatty acids usually associated with seed germination [11].

Salt treatments lowered the percent of palmitic acid and caused an increase in the percent of linoleic acid in the cotyledons (Table 1) and the embryonic axes (Table 2), particularly, during the later days of growth. A clear shift in the pattern of unsaturated fatty acids resulting in a high unsaturated–saturated fatty acid ratio was noticed in 6–10 day old embry-