

DHURRIN IN *SORGHASTRUM NUTANS*\*

H. J. GORZ, F. A. HASKINS, RICHARD DAM and K. P. VOGEL

AR, SEA, U.S. Department of Agriculture and University of Nebraska, Lincoln, NB 68583, U.S.A.

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The isolation of dhurrin, the cyanogenic glycoside of *Sorghum*, was reported by Dunstan and Henry in 1902 [1]. This compound, now known to be (*S*)-*p*-hydroxymandelonitrile  $\beta$ -D-glucopyranoside [2, 3], was the first cyanogenic glycoside isolated from a representative of the Gramineae [4]. The occurrence of other cyanogenic glycosides in certain grasses is now recognized [4, 5], but according to Fat [4], *Sorghum* remains the only genus in which the presence of dhurrin has been firmly established. In this report, evidence is presented indicating that dhurrin also occurs in seedlings of the warm season perennial prairie grass, indiagrass, *Sorghastrum nutans* (L.) Nash.

## EXPERIMENTAL

Indiagrass seedlings were grown under fluorescent lights at 27°, in a mixture of vermiculite and perlite subirrigated with Hoagland's Solution No. 1. When seedlings were 12–14 days old, plumules were excised and were immersed immediately in 95% EtOH (ca 10 ml/g fr. tissue). After 20 min of heating at 80–85° with occasional stirring, the green extract was decanted. The tissue was rinsed  $\times 3$  with small vols. of 95% EtOH without heating, and rinsings were added to the extract. The combined solution was evapd to dryness at ca 80° with an air stream passing over the surface of the soln. The residue was taken up in H<sub>2</sub>O (ca 2.5 ml/g fr. tissue) and filtered. Aliquots of the amber filtrate were chromatographed by ascent on Whatman 3MM filter paper in *n*-BuOH–95% EtOH–H<sub>2</sub>O, 40:11:19 [6]. Putative dhurrin was detected as an absorbing band,  $R_f$  ca 0.70, on chromatograms exposed to NH<sub>3</sub> and examined under 254 nm UV light. The absorbing band was eluted with 95% EtOH, and the eluate was evapd to ca 2.5 ml/g fr. tissue. As described below, this conc eluate (A) was subjected to PC and to alkaline hydrolysis. Also, the putative dhurrin was acetylated, and the NMR spectrum and mp of the resulting pentaacetate were determined.

The following eight solvent systems, most of which are described by Seigler [6], were used in the chromatographic comparisons: (a) 2-butanone–acetone–H<sub>2</sub>O, 15:5:3; (b) *n*-BuOH–Py–H<sub>2</sub>O, 6:4:3; (c) *n*-BuOH–H<sub>2</sub>O, 50:9; (d) *n*-PrOH–H<sub>2</sub>O, 7:3; (e) *n*-BuOH–95% EtOH–H<sub>2</sub>O, 40:11:19; (f) *iso*-

PrOH–H<sub>2</sub>O, 7:3; (g) *n*-BuOH–HOAc–H<sub>2</sub>O, 12:3:5; and (h) 5% HOAc. Ascending chromatography on Whatman No. 1 paper in these eight solvents resulted in the following  $R_f$  ( $\times 100$ ) values for dhurrin, a sample of which was kindly supplied by Dr. E. E. Conn (Univ. California, Davis): (a) 79, (b) 88, (c) 59, (d) 81, (e) 68, (f) 83, (g) 69, and (h) 91. Corresponding  $R_f \times 100$  values for the absorbing spot from A in the various solvents were within two units of the values for the co-chromatographed dhurrin standard.

An aliquot of A was hydrolysed in 0.1 N NaOH at room temp. The absorption spectrum was scanned between 220 and 400 nm immediately after A was added to base and again 3 hr later. The typical dhurrin spectrum [3], with a strong absorption maximum at 255 nm, was observed in the initial scan. After 3 hr in base, the 255 nm peak had disappeared, and modest and strong maxima were evident at 238 and 330 nm, respectively. These maxima are characteristic of *p*-hydroxybenzaldehyde in alkaline soln.

Both dhurrin and its *R*-epimer, taxiphyllin, yield *p*-hydroxybenzaldehyde upon hydrolysis. Thus, the spectrum of an alkaline hydrolysate of A does not exclude the possibility that indiagrass might contain taxiphyllin rather than dhurrin. Taxiphyllin has been found in Gramineae of the bamboo genera, *Bambusa* and *Dendrocalamus* [7, 8], as well as in the gymnosperm, *Taxus* [2]. The pentaacetates of dhurrin and taxiphyllin may be distinguished by their NMR spectra and their mps [2]. A portion of A was taken to near dryness, drying being completed over P<sub>2</sub>O<sub>5</sub>, after which the dry residue was treated with Ac<sub>2</sub>O/Py [2]. The NMR spectrum of this acetate was identical to the spectrum published for dhurrin pentaacetate, and was readily distinguishable from the spectrum of taxiphyllin pentaacetate [2]. The mp (uncorr.) was 131.5–132°; values reported for the pentaacetates of dhurrin and taxiphyllin are 132–132.5 and 144–144.8°, respectively [2]. Hence it may be concluded that young seedlings of indiagrass contain dhurrin.

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