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# A RE-ASSESSMENT OF *p*-HYDROXYBENZALDEHYDE IN *SORGHUM BICOLOR* SEEDLINGS

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**Key Word Index**—*Sorghum bicolor*, Gramineae, seedlings, *p*-hydroxybenzaldehyde, dhurrin

**Abstract**—Free *p*-hydroxybenzaldehyde is present in seedlings previously heated to inactivate hydrolytic enzymes and in chloroform extracts of the surface It is also present in epicuticular wax removed from plants by mechanical means

Woodhead *et al* recently reported the occurrence of *p*-hydroxybenzaldehyde (**1**) in the wax of seedlings of *Sorghum bicolor* cv 65D from Botswana [1], but Haskins and Gorz have subsequently suggested that it was only produced as a result of the enzymic hydrolysis of dhurrin (to hydrogen cyanide and **1**), and does not occur free in sorghum [2] This seemed possible since brief immersions of the tissues in chloroform were used to obtain the wax [3] and chloroform treatment (for 2 hr) has been used in the measurement of hydrogen cyanide potential in sorghum [4] Evidence is presented here in support of our original claim

*Chloroform extractions*

Sorghum (cv 65D) was grown as detailed previously [1] and seedlings 10 cm high were dipped into chloroform for periods of 15 sec, 1, 2, 5, 10 and 15 min, respectively The chloroform extracts were concentrated to dryness and taken up in 10 ml water Solutions were spectroscopically scanned from 200 to 400 nm in neutral solution, then immediately after dilution (1 10) with 0 1 M sodium hydroxide and finally after the basic solutions had stood at room temperature for 2 hr The solutions were also analysed for hydrogen cyanide [5] Seedlings that had been immersed in chloroform for 15 min were subsequently macerated and extracted in water (20 ml) at room temperature for 3 hr, filtered and the aqueous filtrate was diluted (1 10) with 0 1 M sodium hydroxide prior to recording the absorption spectrum

In neutral solution, chloroform immersions of up to 5 min showed no absorption maximum at 255 nm (characteristic of dhurrin) but all extracts showed one at 285 nm (characteristic of **1**) which increased in magnitude with the duration of the immersion [Table 1(a)] In 0 1 M sodium hydroxide, a maximum was observed at 330 nm in all extracts, again characteristic of **1** The absorbance at 330 nm did not increase on standing, as would have been expected if it was a result of the hydrolysis of dhurrin and no hydrogen cyanide was detected For immersions of more than 5 min, however, the neutral solution showed an additional maximum attributable to dhurrin, suggesting that immersions in chloroform of this length were disrupting the tissues, liberating dhurrin, and therefore possibly allowing its enzymic hydrolysis to **1** Extracts from macerated seedlings (after 15 min in chloroform) also showed maxima characteristic of dhurrin and **1** in water and in alkali In these extracts $A_{330}$ was much higher after 2 hr [Table 1(b)] and hydrogen cyanide was detected

In another experiment, fresh seedlings (10 cm high) were immersed in chloroform for 15 sec, followed by two immersions of 10 sec each, a procedure used for wax

Table 1 *p*-Hydroxybenzaldehyde in seedlings of sorghum 65D

| Treatment | *p*-Hydroxybenzaldehyde (% of plant dry wt) |
|---|---|
| (a) Fresh seedlings | |
| Immersion in $CHCl_3$ for | |
| 15 sec | 0 1–0 3 |
| 30 sec | 0 1–0 4 |
| 1 min | 0 1–0 5 |
| 2 min | 0 4–0 8 |
| 5 min | 0 8–1 1 |
| 10 min | 1 0–1 2 |
| 15 min | 1 1–1 2 |
| (b) 15 min immersion in $CHCl_3$ then seedlings macerated in water | 1 5–1 7 |
| (c) Immersion in $CHCl_3$ for 15 sec, then 2 × 10 sec | 0 7–0 8 |
| (d) Dried seedlings | |
| Immediate extraction with water | 0 5–0 7 |
| After 2 hr alkaline hydrolysis | 1 7–1 9 |

extraction from sorghum by other workers [6] TLC of this extract on silica gel in MeCOEt–EtOAc–$HCO_2H$–$H_2O$ (5 3 2 1) showed **1**, but no dhurrin on exposure of the plate to UV light In this extract, **1** was 24% of the wax and 0 8% of the plant dry wt [see Table 1(c)]

*p-Hydroxybenzaldehyde in mechanically collected samples*

*p*-Hydroxybenzaldehyde was positively identified in epicuticular wax samples removed from leaves and stem of older plants of cv 65D by purely mechanical means [3] and is also reported in similarly collected samples from other sorghum cultivars [7]

*p-Hydroxybenzaldehyde in oven-dried seedlings*

Seedlings were oven-dried at 75° for 2 5 hr to deactivate the hydrolytic enzymes that release **1** from dhurrin and subsequently macerated in water at room temperature for 3 hr [2] After filtering, samples were diluted 1 10 with 0 1 M sodium hydroxide and $A_{330}$ was read immediately and after standing at room temperature for 2 hr In both cases maxima were observed at 330 nm Table 1(d) shows the concentration of **1** in the dried seedlings and that liberated by alkaline hydrolysis of dhurrin

The presence of **1** in extracts of dried seedlings is good evidence for its occurrence in the free state in sorghum 65D Short immersions in chloroform (less than 5 min) yielded **1** but no dhurrin, and transmission electron microscopic analysis of sections of the seedlings' leaves treated in this way showed that the cell membranes were intact Extractions of 5 min and longer showed rupturing of the epidermal cell membranes, although some epicuticular wax was still present even after 10 min immersions Since dhurrin is located entirely in the vacuoles of epidermal cells [8], it will be accessible for hydrolysis as soon as these cells are disrupted, but before chlorophyll is observed in extracts since chloroplasts are not present in epidermal cells of sorghum In earlier analyses [1] the appearance of chlorophyll was taken as the criterion for the start of cellular disruption Estimates of the concentration of **1** will consequently have included some **1** from dhurrin hydrolysis and are therefore too high Current data from experiments described here using several different batches of seed of 65D indicate that the concentration ranges from 0 4 to 0 8% of plant dry wt and from 12 to 25% of the wax Previous estimates may have included **1** from hydrolysis of dhurrin in alkaline solution during analysis [3] if epidermal cells were ruptured by chloroform treatment, but not **1** from enzymic hydrolysis of dhurrin since mesophyll cells (where the hydrolytic enzymes are located [8]) were not ruptured Also, we may have been inaccurate in stating that **1** is solely a constituent of the epicuticular wax since there is the possibility that cuticular wax was also extracted [9]

The evidence presented in this paper clearly shows the presence of free **1** in the wax of seedlings of sorghum 65D We have, however, examined a number of cultivars of sorghum, largely from Indian sources, and have found only one other with an appreciable content of **1** in the wax extracts The quantities of **1** in the extracts do not always correlate with the quantities of hydrogen cyanide released by hydrolysis, as would be expected if release from dhurrin were the only source of **1** in the plant Concentration of free **1** decreases rapidly with increasing age of seedlings in all cultivars [3] and other workers have recently reported a similar age-related occurrence of free **1** in the wax of two sorghum cultivars [10] However, this is clearly a restricted phenomenon with many cultivars having little or no free **1** [2, 3, 6] There is active turnover of dhurrin in sorghum seedlings, with hydrogen cyanide incorporated into asparagine [11] and the consequent production of **1** Scant attention has been paid to the fate of this **1** and possibly it is secreted from the epidermal cells to the plant surface [12] where it can provide protection to the plant against herbivores [3]

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