Phytochemistry, Vol. 21, No. 2, pp. 455–456, 1982.
Printed in Great Britain.

0031-9422/82/020455-02$03.00/0
Pergamon Press Ltd.

p-HYDROXYBENZALDEHYDE AS A MAJOR CONSTITUENT OF THE EPICUTICULAR WAX OF SEEDLING *SORGHUM BICOLOR*

S. WOODHEAD, C. GALEFFI* and G. B. MARINI BETTOLO†

Centre for Overseas Pest Research, Wright's Lane, London W8 5SJ, U.K.; *Laboratorio di Chimica Biologica, Istituto Superiore di Sanita, Rome, Italy; † Centro per la Chimica dei Recettori e delle Molecole Biologimente Attive del CNR, Universita Cattolica del S. Cuore, via Pineta Sacchetti 644, Rome, Italy

(*Revised received* 3 *July* 1981)

Key Word Index—*Sorghum bicolor*; Gramineae; epicuticular wax; *p*-hydroxybenzaldehyde; locusts; feeding deterrent.

Abstract—*p*-Hydroxybenzaldehyde is present in concentrations up to 30% in the wax of *Sorghum bicolor* seedlings. It is highly deterrent to locusts, reducing their normal feeding by 90%.

Investigations into the antifeedant properties of seedlings of *Sorghum bicolor* (cv. 65D, from Botswana) to insects suggested that the epicuticular wax may be of importance [1, 2]. This led to analysis of the wax by TLC. Several components with R_f values corresponding to those expected for common wax chemicals were present [2, 3], and in addition, a polar constituent, clearly detectable in UV light, was found at concentrations amounting to 30% of the wax. This latter was highly deterrent to the test insect (*Locusta migratoria*), normal feeding being reduced by 90% when this chemical was offered to the insect in a choice test. In these tests, chemicals were applied to glass-fibre discs previously impregnated with sucrose at phagostimulatory concentrations and insects were individually given a choice between a control disc with sucrose only, and one with sucrose plus the test chemical. Full experimental details of insect methods are reported elsewhere [1].

Isolation and recrystallization of the chemical from hexane–$CHCl_3$ gave a compound with characteristics consistent with its identification as *p*-hydroxybenzaldehyde. Feeding tests with pure *p*-hydroxybenzaldehyde (Sigma) and third instar nymphs of *L. migratoria* in a choice situation showed that at concentrations in excess of 0.25% dry wt *p*-hydroxybenzaldehyde was a potent inhibitor of feeding by *L. migratoria* (Fig. 1). In surface extracts of young sorghum 65D (mean height 10 cm) *p*-hydroxybenzaldehyde was equivalent to 1.15 ± 0.28% of the plant dry weight. At such concentrations, feeding by *L. migratoria* was significantly reduced (Fig. 1), suggesting that this chemical is important in contributing to the deterrence of young sorghum to graminivorous insects [1].

Although phenolics have been previously identified as leaf wax components [3, 4], they are generally present as a small proportion of the wax, and are frequently methylated [5]. High concentrations have been reported in bud exudates and, for example, in the farina of *Primulas*. The major substance here is flavone itself, amounting to 75% of the secretion [6], with mono- and di-hydroxyflavones present to a lesser extent. The farina is secreted from glandular hairs [6]. Sorghum possesses trichomes, either predominantly of a 'prickle hair' type or of a glandular type, and some cultivars appear to have similar numbers of both (S. Woodhead, unpublished data). Sorghum 65D has almost exclusively glandular hairs and the origin of *p*-hydroxybenzaldehyde in secretions from such hairs is a possibility.

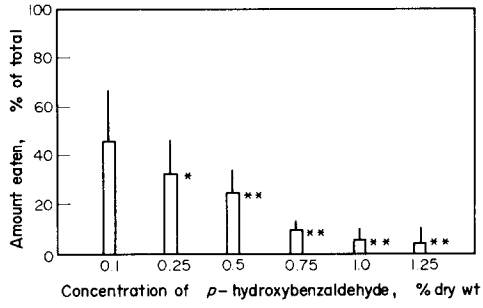

Fig. 1. Effect of *p*-hydroxybenzaldehyde on feeding by *Locusta migratoria*. Third instar nymphs were given *p*-hydroxybenzaldehyde at concentrations from 0.1 to 1.25% dry wt in a choice test [1]. The amount eaten in the test is expressed as a percentage of the total amount eaten, i.e. test disc plus control disc. Number of insects tested is 20 in each case. Results are expressed ±s.d. and are statistically significant at $P = 0.001$ (**), $P = 0.005$ (*), Sign test.

Previous reports on waxes of *Sorghum vulgare* [7] do not identify *p*-hydroxybenzaldehyde as a constituent. However, these studies were done on mature plants and we have evidence that the concentration of *p*-hydroxybenzaldehyde is dependent on the cultivar and in all cases decreases with increasing plant age [2]. All cultivars tested by us have *p*-hydroxybenzaldehyde in

the wax to some extent at all stages of growth up to flowering. The occurrence of free *p*-hydroxybenzaldehyde in sorghum epicuticular wax and its relationship to the cyanogenic glucoside dhurrin, also present in high concentration in seedling sorghum is of interest since *p*-hydroxybenzaldehyde is a hydrolysis product of dhurrin, and is discussed by us elsewhere [2].

EXPERIMENTAL

Seeds of *Sorghum bicolor* cv. 65D were obtained from the Botswana Ministry of Agriculture and grown in John Innes No. 2 compost and peat (1:1) in controlled environment rooms. Daylength was 14 hr and temp. was maintained at 30° ± 1° (day) and 25° ± 1° (night). Maximum illumination at plant level from banks of 65/80 W warm white fluorescent tubes was 7500 lx.

Epicuticular waxes were obtained from 7-day-old seedlings (height 10 cm) by immersion of the intact seedlings in distilled Analar chloroform for short periods, extraction being stopped prior to chlorophyll contamination. TLC was done on Si gel G plates (Merck), preactivated at 110° for 30 min, in *n*-hexane–Et_2O–HCO_2H (90:10:0.5). The most polar band (R_f 0.04) was scraped off, eluted with $CHCl_3$ and recrystallized from a mixture of *n*-hexane and $CHCl_3$. Yield was 30% of the total wax, crystals, mp 115°. UV spectrum in neutral solution gave λ_{max} 284 nm (log ϵ 4.20) and in alkaline solution λ_{max} 330 nm (log ϵ 4.44). Mass spectrometry (75 eV) gave *m/z* 122(83), 121(100), 93(41), 65(45), and NMR (in $CDCl_3$) indicates CHO, *s*, 9.90; CH (*ortho* to CHO), *d*, J = 8 Hz, 7.81; CH (*ortho* to OH), *d*, J = 8 Hz, 6.98; OH, *s*, 1.25.

REFERENCES

1. Woodhead, S. and Bernays, E. A. (1978) *Ent. Exp. Appl.* **24**, 123.
2. Woodhead, S. (1982) *Ent. Exp. Appl.* **31** (in press).
3. Martin, J. T. and Juniper, B. E. (1970) *The Cuticles of Plants*. Edward Arnold, London.
4. Richmond, D. V. and Martin, J. T. (1959) *Ann. Appl. Biol.* **47**, 583.
5. Harborne, J. B. (1980) in *Encyclopedia of Plant Physiology* (Bell, E. A. and Charlwood, B. V., eds.), Vol. 8, pp. 329–395. Springer, Berlin.
6. Blasdale, W. C. (1945) *J. Am. Chem. Soc.* **67**, 491.
7. Bianchi, G., Avato, P., Bertorelli, P. and Mariani, G. (1978) *Phytochemistry* **17**, 999.

Phytochemistry, Vol. 21, No. 2, pp. 456–457, 1982.
Printed in Great Britain.

0031-9422/82/020456-02$03.00/0

EPI-ILICIC ACID FROM *ALCANTARA EKMANIANA**

FERDINAND BOHLMANN, PAHUP SINGH, HAROLD ROBINSON† and ROBERT M. KING†

Institute for Organic Chemistry, Technical University of Berlin, D-1000 Berlin 12, West Germany; †Smithsonian Institution, Washington, DC 20560, U.S.A.

(*Received* 15 *June* 1981)

Key Word Index—*Alcantara ekmaniana*; Compositae; Vernonieae; sesquiterpenes; *epi*-ilicic acid.

Abstract—The aerial parts of *Alcantara ekmaniana* afforded, in addition to known compounds, *epi*-ilicic acid. The furanogermacranolides isolated indicate a close relationship between this genus and other members of the same tribe.

The genus *Alcantara* (Compositae, tribe Vernonieae) with one species has been investigated for flavones [1]. We now have studied aerial parts of *A. ekmaniana* (Philipson) H. Robins in more detail. In addition to stigmasterol, lupeol and its Δ-9,11- and Δ-12,13-isomers large amounts of costic (**2**) [2] and isocostic acid (**1**) [3] were isolated. Furthermore, as minor constituents ilicic acid (**4a**) [2], 1-hydroxycostic acid (**3**) [4] and the epimer **5a** of ilicic acid were isolated, which was transformed by addition of diazomethane to the methyl ester **5b** and the pyrazoline **10**. The ^{1}H NMR spectral data (Table 1) of **5b**, if compared with those of **4b**, showed that due to the axial hydroxy group in **5b** H-14 was shifted downfield, while the other signals were similar to those of **4b**. Therefore the only difference between **4b** and **5b** was the stereochemistry at C-4. The more polar parts afforded *p*-hydroxycinnamic acid and the furanoheliangolides **6** [5], **7** [5], **8** [6] and **9** [7], which have all been isolated from genera in the Vernonieae, most of them being placed in the subtribe Lychnophorinae [8] (*Eremanthus*, *Lychnophora*, *Piptolepis*, *Proteopsis*, *Vanillosmopis*). Only *Centratherum*, which may be related to the Lychnophorinae, and one *Vernonia*

*Part 391 in the series "Naturally Occurring Terpene Derivatives". For Part 390 see Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1982) *Phytochemistry* **21** (in press).