Phytochemistry, 1977, Vol. 16, pp. 1599–1600. Pergamon Press. Printed in England.

SHORT REPORTS

CYANOGENESIS OF *DENDROCALAMUS*: TAXIPHYLLIN

ULRICH SCHWARZMAIER
Institute of Organic Chemistry, University Kiel, D-2300 Kiel, W. Germany

(*Revised received* 4 *April* 1977)

Key Word Index—*Dendrocalamus giganteus*; *D. hamiltonii*; Gramineae; bamboo; phytosterols; 4-hydroxybenzaldehyde; cyanogenesis; taxiphyllin; cyanoside metabolism; amino acids; L-asparagine.

Abstract—Taxiphyllin (1), L-asparagine, 4-hydroxybenzaldehyde and β-sitosterol are identified as the main extractives of the shoots of *Dendrocalamus giganteus* and *D. hamiltonii*. An analysis of the free amino acids is presented and the administration of taxiphyllin-[1-^{14}C] to the inner leaves of a bamboo shoot yielding labelled asparagine described.

INTRODUCTION

Continuing our studies on the cyanogenesis of Bambusoideae [1], we investigated the Asian species *Dendrocalamus giganteus* Munro and *D. hamiltonii* Nees et Arnott. The shoots are strongly cyanogenetic (90–100 mg HCN/100 g fr. wt); after proper cooking, however, they are safely edible. In this paper, we report on the characterization and the metabolism of the cyanogen.

RESULTS AND DISCUSSION

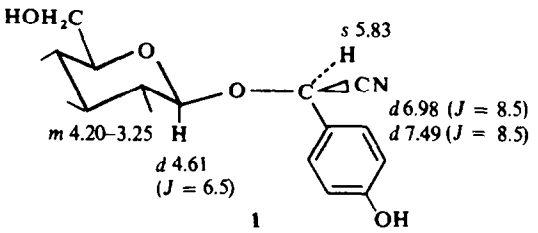
The extracts of the shoots as well as the $CHCl_3$ fraction MeOH fraction and the crystalline polar remainder thereof were obtained as described in [1]. The $CHCl_3$ fraction containing the lipids consisted mainly of 4-hydroxybenzaldehyde and a mixed phytosterol. The GLC analysis of the latter is given in Table 1.

The MeOH fraction on column chromatography gave 4-hydroxy-D(R)-mandelonitrile-β-D-glucopyranoside (taxiphyllin) (1) [2]. The structure was confirmed by enzymatic and thermal [1] liberation of 4-hydroxybenzaldehyde, HCN and glucose, the red colour with 2,3,5-triphenyltetrazolium chloride/conc NH_3 [3], the blue colour with hydroxylamine/$FeCl_3$ [4] and the PMR spectrum of the free cyanoside [5]. The occurrence of dhurrin (4-hydroxy-L(S)-mandelonitrile-β-D-glucopyranoside) known from the grass *Sorghum vulgare* Pers. [6] could be eliminated by PMR [5] (see Experimental) and TLC [4]. The mechanism of the thermal degradation of 1 responsible for the edibility of bamboo shoots has already been described in [1]. The isolation of 1 from *Dendrocalamus* and *Bambusa* species represents the first report of its occurrence in monocotyledons.

Table 1. GLC analysis of phytosterols*.

No	Compound	R_t (sec)	% of fraction *D. giganteus*	% of fraction *D. hamiltonii*
1	Campesterol	553	17.7	25.1
2	Stigmasterol	589	9.9	9.1
3	β-Sitosterol	679	72.4	65.8

*As TMSi ether derivative [1].



1

The crystalline polar remainder consisted mainly of L-asparagine. Further free amino acids detected by comparative autoanalysis are listed in Table 2. Of these,

Table 2. Free L-amino acids in the mother liquor of L-asparagine from *D. hamiltonii*

Compound	R_t (min)	Concentration (nmol/mg residue)
O-Phosphoserine	10	7
O-Phosphoethanolamine	19	275
Aspartic acid	47	71
Threonine	73	17
Serine	78	122
Asparagine	92	564
Glutamic acid	100	5
Glutamine	107	1
Proline	130	tr
Glycine	132	2
Alanine	137	4
Citrulline	144	1
α-Aminobutyric acid	148	tr
Valine	159	3
Isoleucine	194	2
Leucine	205	1
Tyrosine	229	4
Phenylalanine	248	8
β-Aminoisobutyric acid	265	tr
γ-Aminobutyric acid	284	tr
Ethanolamine	290	8
Allo-hydroxylysine	317	tr
Ornithine	337	tr
Lysine	350	5
Histidine	365	5
Arginine	429	68

tyrosine is significant as a biochemical precursor of (1) [7]. β-Cyano-L-alanine [7], not being contained in the applied test mixture, was identified as a trace by TLC.

The findings suggest that taxiphyllin (1) may serve as an intermediate [7] in the biosynthesis of L-asparagine in fast growing arboreous bamboos. In a preliminary experiment confirming this assumption, taxiphyllin-[1-^{14}C] synthesized after [8] was fed [9] to inner leaves of a shoot of *D. hamiltonii*. After 20 hr of administration and metabolism, the plant material was worked up. The asparagine and the unaltered taxiphyllin were separated by TLC, purified by PC and located on the chromatograms by radiogram scanning and visualization with ninhydrin and emulsin/picrate, respectively. The conversion of ^{14}C to asparagine was estimated autoradiographically [10] and found to be 5–8%. Acid hydrolysis of the amino acid yielded labelled aspartic acid. The results confirm the conclusions drawn from previous cyanide feeding experiments [7].

EXPERIMENTAL

General conditions were as those in ref. [1]. Plates precoated with Si gel F_{254} were used for TLC, paper S & S 2043 b Mgl for PC. The feeding experiment and the work-up were carried out as in ref. [9]. A measurement of the sp. act. could not be carried out. The degree of conversion was estimated by means of comparative autoradiography [10].

Plant material. Dendrocalamus giganteus (2 shoots, 850 g) and *D. hamiltonii* (2 shoots, 590 g) were harvested in the Botanical garden of Munich and Hambourg, respectively, in August 1976. The voucher specimens are deposited there. Because of the great chemical similarities, the results of both species are presented together, the contents of *D. hamiltonii* being given in parentheses.

Extractives. 4-Hydroxybenzaldehyde, mp 116° (EtOH–hexane), 0.039% (0.031%). Phytosterol, mp 135–137° (MeOH), $[\alpha]_D^{20}$ − 36.7° ($CHCl_3$), 0.025% (0.030%), mainly β-sitosterol (see Table 1). Taxiphyllin (1), identical with the cyanoside from *Bambusa* spp. [1] and *Taxus baccata* [1], PMR (2 N DCl/DSS, δ ppm) 5.83 (*s*, methine H), 4.61 (*d*, J = 6.5 Hz, anom. H); (dhurrin from *Sorghum vulgare* Pers. for comparison, 5.99 (*s*) and 4.81 (*d*, J = 7.2 Hz), respectively); (1)-pentaacetate, Ac_2O–C_5H_5N, mp 144° (EtOH–hexane), $[\alpha]_D^{20}$ − 22.2° (EtOH), PMR ($CDCl_3$–TMS, δ ppm) 5.54 (*s*, methine H). L-Asparagine, mp 232° (H_2O), $[\alpha]_D^{20}$ − 5.4° (H_2O), 0.64%, (0.59%). Further free amino acids detected in the mother liquor by autoanalysis are given in Table 2. β-Cyano-L-alanine was detected in traces by co-TLC with an authentic sample [1] on Si gel with *n*-BuOH–HOAc–H_2O (4:1:1) as eluent, ninhydrin: blue, R_f 0.33.

Feeding experiment. Taxiphyllin-[1-^{14}C] 100 mg crude DL-4-acetoxy-mandelonitrile-[1-^{14}C], obtained from K^{14}CN (Amersham Buchler) in the usual manner, 100 mg acetobromoglucose, 100 mg $Hg(CN)_2$, and 20 mg $CaSO_4$ were stirred for 12 hr at 40°. After filtration, the mixture was deacetylated by standing (48 hr, 20°) with 2 ml MeOH–conc H_2SO_4 (30:1) and purified by PLC on Si gel ($CHCl_3$–MeOH, 3:1). The obtained epimeric glycosides were re-acetylated with Ac_2O–C_5H_5N, and the acetates separated by PLC on Si gel ($CHCl_3$–Et_2O, 6:1). The most polar fraction on deacetylation and PLC in the above manner yielded optically pure taxiphyllin (10.4 mg, sp. act. 280 μCi/mmol).

Feeding technique. A pale inner leaf (1.5 g) of a shoot of *D. hamiltonii* in a small beaker containing taxiphyllin-[1-^{14}C] (5.0 μmol) in 1 ml 2% EtOH was placed in the dark in a gentle stream of air to increase the transpiration. As the feeding soln was consumed, further solvent was added. After 20 hr, the plant material was ground in 10 ml 90% EtOH containing 3% HOAc in a mortar using sand as an abrasive, transferred to a small column and eluted exhaustively with further solvent. The extract concd at room temp. was chromatographed by TLC on Si gel using *n*-BuOH–HOAc–H_2O (4:1:1) as eluent. The radioactive zones (taxiphyllin R_f 0.77, asparagine 0.21) were scraped off and extracted with 50% MeOH. The evapd concentrates were re-chromatographed on paper eluting with PhOH–H_2O (asparagine R_f 0.41) or *n*-BuOH–C_5H_5N–C_6H_6–H_2O 5:3:2.4:1) (taxiphyllin R_f 0.75), resp. The developed sheets were cut into strips, and asparagine and taxiphyllin located by scanning and by spraying with ninhydrin or emulsin/picrate (sandwich technique [1]), resp. In another expt, the paper zones were eluted with 50% MeOH and aliquots of the purified compounds and the original feeding soln co-chromatographed on Si gel using the above mentioned solvent. The developed and dried plates were autoradiographed. Furthermore, a small portion of the labelled asparagine was hydrolyzed by boiling 15 hr with 2N HCl, yielding labelled aspartic acid (TLC: R_f 0.32, PC: 0.08).

Acknowledgements—The author is indebted to Dr. E. Waldvogel, Kiel, Mr. J. Bogner, Munich, and Mr. A. Zechmeister, Hambourg, for the supply of plant material and to Dr. R. Tutschek and Mrs. S. Thönes, Kiel, for the amino acid analyses.

REFERENCES

1. Schwarzmaier, U. (1976) *Chem. Ber.* **109**, 3379; and literature cited therein.
2. Towers, G. H. N., McInnes, A. G. and Neish, A. C. (1964) *Tetrahedron* **20**, 71.
3. Schwarzmaier, U. (1975) *J. Chromatog.* **114**, 235.
4. Schwarzmaier, U. (1975) *J. Chromatog.* **111**, 430.
5. Schwarzmaier, U. (1976) *Chem. Ber.* **109**, 3250.
6. Mao, C.-H., Blocher, J. P., Anderson, L. and Smith, D. C. (1965) *Phytochemistry* **4**, 297.
7. Conn, E. E. and Butler, G. W. (1969) in *Perspectives in Phytochemistry* (Harborne, J. B. and Swain, T. eds.) p. 47. Academic Press, London; Schütte, H. R. (1973) *Fortschritte der Botanik*, Vol. 35, p. 103. Springer-Verlag, Berlin; and literature cited therein.
8. Schwarzmaier, U., unpublished results, reported on the 8th IUPAC Symp. Chem. Nat. Prod., New Dehli, India (1972), Abstract Book, p. 380.
9. Rosen, M. A., Farnden, K. J. F. and Conn, E. E. (1975) *J. Biol. Chem.* **250**, 8302.
10. Mangold, H. K. (1967) in *Dünnschicht-Chromatographie* (Stahl, E., ed.) 2nd ed., p. 157. Springer-Verlag, Berlin.