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SHORT COMMUNICATION

CYANOGENESIS IN SPECIES OF THE FERN GENERA *CYSTOPTERIS* AND *DAVALLIA*

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Abstract—The cyanogenic glucoside prunasin was identified in *Cystopteris fragilis* (Lam.) Bernh. and the cyanogenic glycoside vicianin was shown to be present in *Davallia bullata* Wall., *D. denticulata* (Burm.) Mett. and *D. fijiensis* Diels. The structure of vicianin was reinvestigated and its revised constants are presented.

CYANOGENESIS appears to be a relatively common feature of the Polypodiaceae.[1] For instance, four species of the genus *Cystopteris* have been reported to be cyanogenic[2, 3] and this property has also been verified in approximately eight species of the genus *Davallia*.[2, 4]

In a previous communication,[5] we reported the first isolation of a pure cyanogen from ferns, of prunasin from *Pteridium aquilinum* (L.) Kuhn, which recently has been confirmed by Bennett[6] working with *P. aquilinum* L. *var. esculentum* (Forst.f.). In the present communication we report the isolation and identification of prunasin (*O*-β-D-glucopyranosyl-D-mandelonitrile) from *Cystopteris fragilis* (Lam.) Bernh. Additionally, we have shown the cyanogenic glycoside vicianin to be present in *Davallia bullata* Wall., *D. denticulata* (Burm.) Mett. and *D. fijiensis* Diels.

The glycoside vicianin has hitherto been isolated only from the seeds of *Vicia angustifolia* (L.) Reichard.[7] Based on investigations by Bertrand and Weisweiller[8–11] and Helferich and Bredereck[12] the structure *O*-[6-*O*-(α-L-arabinopyranosyl)-β-D-glucopyranosyl]-D-mandelonitrile has been suggested for vicianin. The hexaacetyl derivative of the glycoside has been synthesized.[13]

Our investigations on the *Davallia* cyanogen have unambiguously proved its structure to be identical with that indicated for vicianin. Owing to some discrepancies between some

[1] R. HEGNAUER, *Chemotaxonomie der Pflanzen*, Vol. 1, p. 276, Birkhäuser Verlag, Basel (1962).
[2] M. GRESHOFF, *Kew Bull. Roy. Bot. Gardens* 397 (1909).
[3] A. NORDAL and A. JERMSTAD, *Pharm. Acta Helv.* **23**, 227 (1948).
[4] F. SMITH and C. T. WHITE, *Proc. Roy. Soc. Queensland* **30**, 84 (1918).
[5] H. KOFOD and R. EYJÓLFSSON, *Tetrahedron Letters* **12**, 1289 (1966).
[6] W. D. BENNETT, *Phytochemistry* **7**, 151 (1968).
[7] G. BERTRAND, *Compt. Rend.* **143**, 832 (1906).
[8] G. BERTRAND and G. WEISWEILLER, *Compt. Rend.* **147**, 252 (1908).
[9] G. BERTRAND and G. WEISWEILLER, *Compt. Rend.* **150**, 180 (1910).
[10] G. BERTRAND and G. WEISWEILLER, *Compt. Rend.* **151**, 325 (1910).
[11] G. BERTRAND and G. WEISWEILLER, *Compt. Rend.* **151**, 884 (1910).
[12] B. HELFERICH and H. BREDERECK, *Ann. Chem.* **465**, 166 (1926).
[13] D. N. CHAUDHURY and A. ROBERTSON, *J. Chem. Soc.* 2054 (1949).

of the constants reported for vicianin in the literature[7,9,13] and the values observed by us, we eventually isolated vicianin (as the fully acetylated derivative) from the seeds of *V. angustifolia* for direct comparison. Careful examination for other cyanogenic substances in the plants studied proved to be negative.

EXPERIMENTAL

Plant Material

The material of the *Davallia* species, consisting of the aerial parts, was collected in April 1967, in a greenhouse belonging to the Botanical Garden of Copenhagen, and it was extracted almost immediately after collection. The *Cystopteris fragilis* material (aerial parts) was obtained from wild plants growing in the lava of Hafnarfjardarhraun near Reykjavík, Iceland. The collection was made in July 1966, the specimen was transported by air at approx. 0° and extracted immediately when received.

Isolation of Vicianin

The fresh, green parts of *Davallia bullata* (18 g) were treated with boiling, abs. EtOH (250 ml) for 5 min. The ethanolic phase was filtered off and the residual material was dried, pulverized and extracted in a Soxhlet for 6 hr with MeOH (50 ml). The alcoholic extracts were combined and evaporated *in vacuo* at 30° yielding a thick syrup that was extracted repeatedly with warm H_2O. The aqueous extract (20 ml) was clarified by centrifugation and extracted continuously with hot EtOAc (50 ml) in a liquid–liquid extractor for 16 hr.

Concentration of the EtOAc extract gave a dark-coloured syrup (0·5 g) that was suspended in MeOH (2 ml) and mixed with silica gel (1 g). The mixture was dried at room temperature for 12 hr and transferred to a column of silica gel (40 g, containing 10% of H_2O). Irrigation of the column with a 5:95 mixture of MeOH–EtOAc at first, followed by a 1:9 mixture of MeOH–EtOAc and monitoring the fractions (10 ml) by TLC (silica gel G) using naphthoresorcinol–H_2SO_4 as localizing reagent, resulted in the isolation of an impure, cyanogenic syrup (80 mg). Rechromatography on a silica gel column using a 1:9 mixture of MeOH–EtOAc as solvent yielded a colourless solid (70 mg) that was crystallized to give 66 mg (0·4 per cent) of a pure, cyanogenic glycoside. It was identified as vicianin as follows:

Crystallized from Bz–MeOH as long, colourless needles it had m.p. 175–176° (corr.) (lit.,[7] m.p. approx. 160°, later[9] corrected to 147–148°). $[\alpha]_D^{20\cdot1}$ −20·0° (c, 0·5 in H_2O) (lit.,[7] $[\alpha]_D^{16-18}$ −20·7° (H_2O)). Emulsin or acid catalyzed hydrolysis of the glycoside indicated the release of HCN (picric acid test[14] or benzidine–cupric acetate test[15]). Glucose and arabinose were identified in the hydrolysates by co-chromatography (three solvents, PC); in Et_2O extracts of the hydrolysates, benzaldehyde was identified as the 2,4-DNPH derivative by co-chromatography (four solvents, TLC).

The glucose and arabinose residues in the glycoside were shown to possess the D- and L-forms respectively by treating a hydrolysate with phenylhydrazine, separating the osazones formed (column chromatography; silica gel; elution with a 2:8 mixture of MeOH–$CHCl_3$) and determining their specific rotations.[16] Treatment of the glycoside (15 mg) with conc. HCl, heating and extracting the product with Et_2O yielded *D*-mandelic acid (3 mg) (TLC, m.p., i.r., specific rotation). Methylation of the glycoside, essentially by the method of Kuhn *et al.*,[17] followed by hydrolysis, indicated the presence of 2,3,4-tri-*O*-Me-D-glucopyranose and 2,3,4-tri-*O*-Me-L-arabinopyranose (two solvents, PC).

Acetylation of the glycoside using the pyridine–Ac_2O method, gave a hexaacetate, m.p. 170–171° (corr.) (lit.,[13] 165°). $[\alpha]_D^{20\cdot1}$ −29·6° (*c*, 0·5 in $CHCl_3$). (Found: C, 54·56; H, 5·62; N, 1·88 per cent. Calc. for $C_{31}H_{37}O_{16}N$: C, 54·77; H, 5·49; N, 2·06 per cent.) The i.r. spectrum of the acetate was indistinguishable from that of vicianin hexaacetate isolated from *Vicia angustifolia*. Vicianin hexaacetate showed the NMR signals ($CDCl_3$) at δ 7·48 (S, 5 H), 5·60 (S, 1 H), 5·4–3·5 (M, 13 H) and 2·15, 2·06, 2·01 and 1·98 (partly resolved S, 18 H).

The fresh material of *Davallia denticulata* (35 g) and *D. fijiensis* (40 g) yielded 5·4 mg (0·15‰) and 21·1 mg (0·5‰) of pure vicianin respectively.

Isolation of Prunasin

The fresh leaves of *Cystopteris fragilis* (1230 g) yielded by a similar procedure 620 mg (0·5‰) of crystalline prunasin. Its identity was established by direct comparison with an authentic sample by mixed m.p., co-chromatography, specific rotation and i.r. analysis.

[14] M. MIRANDE, *Compt. Rend.* **149**, 140 (1909).
[15] F. FEIGL and V. GENTIL, *Microchim. Acta* **1**, 44 (1959).
[16] N. K. RICHTMYER, in *Methods in Carbohydrate Chemistry* (edited by R. L. WHISTLER and M. L. WOLFROM), Vol. 2, p. 129, Academic Press, London and New York (1963).
[17] R. KUHN, H. EGGE, R. BROSSMER, A. GAUHE, P. KLESSE, W. LOCHINGER, E. RÖHM, H. TRISCHMANN and D. TSCHAMPEL, *Angew. Chem.* **72**, 805 (1960).

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