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RING CLEAVAGE OF PHENOLS IN HIGHER PLANTS: BIOSYNTHESIS OF TRIGLOCHININ

JERZY W. JAROSZEWSKI and MARTIN G. ETTLINGER

Chemical Laboratory II, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark

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Key Word Index—*Triglochin maritima*; Juncaginaceae; *Thalictrum aquilegiifolium*; Ranunculaceae; biosynthesis; phenolics; cyanogenic glucosides; triglochinin.

The ability of higher plants to catabolize phenolic substances has recently become well documented, but the early products of ring cleavage have mostly remained uncharacterized [1, 2]. Thus whereas the oxidation of 1,2-diphenols to muconic acids by intradiol ring cleavage is a familiar process in microorganisms [3], few instances have been reported where muconic acids are formed by plants or plant enzymes [4, 5]. One example of apparent occurrence of intradiol aromatic ring cleavage in flowering plants is the biosynthesis of the widely distributed cyanogenic glucoside triglochinin [6].

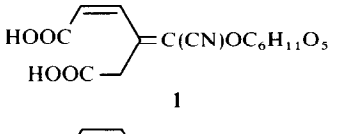
Triglochinin (**1**) is expected to be formed by isomerisation of either of the two epimeric protriglochinins (**2**) [7], which in turn may arise from **3** by intradiol ring fission [6]. A preliminary biosynthetic study of **1** in the monocotyledon *Triglochin maritima* indicated its origin from tyrosine [6], the side chain of the amino acid being presumably modified by the general pathway known for other cyanogenic glycosides [8]. Experiments with the dicotyledon *Thalictrum aquilegiifolium*, the major cyanogenic glucoside of which was formulated in effect as a monomethyl ester of **1** [9], also broadly supported a pathway from tyrosine through **4** and **5** to **1** [10].

In this paper we report further results of feeding experiments with *Triglochin maritima* and *Thalictrum aquilegiifolium*. The plants were in early flower and contained **1** as the only evident cyanogenic constituent [11, 12]. After feeding with (*RS*)-tyrosine-[2-^{14}C] and **4**, **5** or **6** labelled with ^{14}C in the cyano group, **1** was isolated by CC on polyamide and ion-exchange chromatography [13], followed by repeated prep. PC. Since the label from the substrates was expected to be incorporated into the cyano group of **1**, the glucoside was hydrolyzed enzymatically and the specific activity of the hydrogen cyanide evolved determined.

The results (Tables 1 and 2) demonstrate that all the precursors employed may be utilized in the biosyntheses of **1** in both plants. In general, the label was incorporated with high specificity except when the per cent incorporation was low. Of all substrates, **4** was most effectively converted into triglochinin. The cyanohydrins **5** and **6**, which might decompose partially in the plant tissues with lower apparent incorporation as a consequence, were each utilized to a lesser degree [lower in the monocotyledon (Table 1) than the dicotyledon (Table 2)]. Whether **6** is indeed involved in the biosyntheses (arising from **4** by way of **5** or 3,4-dihydroxybenzyl cyanide and being glucosylated to give **3**) or the observed conversion of **6** to **1** is due to lack of specifificity of the glucosyl transferase [14] involved (i.e., if under natural conditions glucosylation of the cyanohydrin group takes place in **5** prior to ring hydroxylation) cannot be judged at present. In cases where a glucoside of **5** co-occurs with **1** the configurations at the α-carbon atoms of the former and of cyanohydrin intermediates in biosynthesis of **1** are likely at all events to be the same. Thus **2** and **3** in *Triglochin maritima* would posses the (*R*) configuration [12] while in at least some Ranunculaceae [11] they will be the (*S*) forms. Our experiments demonstrate for the first time conversion of a 1,2-diphenol (**6**) to triglochinin, strongly supporting the involvement of **3**.

EXPERIMENTAL

Materials. Triglochin maritima L. (Juncaginaceae) was transplanted to pots from Nivaa Bay, Zealand, Denmark.



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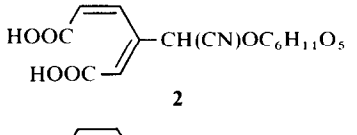

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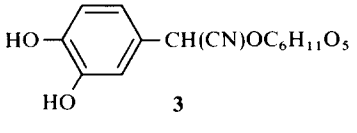

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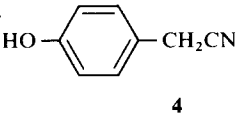

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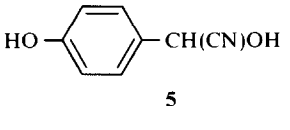

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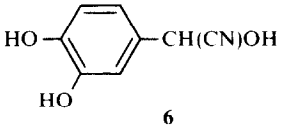

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Table 1. Incorporation of radioactivity into **1** and derived HCN in *Triglochin maritima*

Substrate	Taken up, μCi	Triglochinin isolated, nCi	% of triglochinin activity isolated as HCN	HCN isolated, mg	% radioactivity incorporated into HCN	Dilution
tyrosine	9.63	4.91	55	0.76	0.028	5.1×10^5
tyrosine	11.47	17.4	82	1.27	0.12	1.6×10^5
4	8.00	166	94	0.87	2.0	5.5×10^2
4	11.12	203	98	0.35	1.8	1.7×10^2
5	4.69	1.79	26	0.26	0.010	3.1×10^4
5	3.20	1.58	29	0.25	0.014	3.0×10^4
6	1.89	2.45	74	0.38	0.10	1.4×10^4
6	1.92	2.83	100	0.90	0.15	2.1×10^4

Table 2. Incorporation of radioactivity into **1** and derived HCN in *Thalictrum aquilegiifolium*

Substrate	Taken up, μCi	Triglochinin isolated, nCi	% of triglochinin activity isolated as HCN	HCN isolated, mg	% radioactivity incorporated into HCN	Dilution
tyrosine	12.5	68.1	88	3.47	0.48	1.0×10^5
tyrosine	12.5	50.9	61	4.15	0.25	2.4×10^5
4	5.02	191	87	2.76	3.3	1.6×10^3
4	7.18	976	100	3.32	13.6	3.4×10^2
5	4.04	12.2	95	0.95	0.29	4.5×10^3
5	4.37	165	100	1.04	3.8	3.5×10^2
6	1.83	33.3	100	1.21	1.8	2.4×10^3
6	1.82	39.3	100	0.84	2.2	1.4×10^3

Thalictrum aquilegiifolium L. (Ranunculaceae) was obtained from the Botanical Garden, University of Copenhagen. (*RS*)-tyrosine-[2-^{14}C] (48.8 Ci/mol) was purchased from the Radiochemical Centre, Amersham, England. 4-Hydroxybenzyl cyanide-[^{14}C] (2.66 Ci/mol) was prepared as described elsewhere [15]. (*RS*)-α-4-Dihydroxybenzyl cyanide-[^{14}C] (1.49 Ci/mol) and (*RS*)-α,3,4-trihydroxybenzyl cyanide-[^{14}C] (1.82 Ci/mol) were prepared from the corresponding aldehydes and cyanide-[^{14}C] by the bisulphite method [16, 17].

Methods. Radioactivity was determined by liquid scintillation counting and detected on chromatograms by two-dimensional scanning or autoradiography. The hydrolysis of **1** was carried out with almond emulsin in Conway microdiffusion cells during 48 hr at pH 5.5, HCN being trapped in 0.1 M NaOH [18]. Cyanide was determined by the Epstein method as modified by Jørgensen [19].

Feeding. Radioactive compounds were administered by immersing cut ends of branches, leaves or inflorescences (5–25 g) in aqueous solutions of the labelled compounds. The plants were given 20 hr of illumination during a 26-hr metabolic period.

Isolation of triglochinin. The plant material was dropped into boiling 80% EtOH, boiled for 10 min, chilled, homogenized and filtered. The filtrate was evaporated in vacuum, lyophilized, dissolved in several ml of water and applied to a polycaprolactam column (1.5 × 6 cm), which was eluted with 100 ml of water. The eluate was evaporated to a small volume and applied to an Amberlite IR-45 column (1.5 × 10 cm) previously washed with 2 M NaOAc and then H_2O. The column was rinsed with 200 ml H_2O and 100 ml of 1 M HOAc, and **1** was eluted with 150 ml of 5 M HCOOH. The eluate was evaporated and lyophilized, the residue dissolved in a small amount of MeOH and applied to a sheet of Whatman 3 paper (46 × 57 cm), and the chromatogram developed (descending) during 8–12 hr with EtOAc–HOAc–H_2O (16:3:2). Areas containing **1** located under UV light) were cut out and extracted with MeOH and the prep. PC was repeated. Triglochinin obtained in this way was chemically and radiochemically homogeneous as shown by PC and TLC in several solvent systems and was used for radioactivity determination and enzymatic degradation.

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