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THE MAJOR CYANOGENIC GLYCOSIDE IN *THALICTRUM AQUILEGIFOLIUM*

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Key Word Index—*Thalictrum aquilegifolium*; Ranunculaceae; cyanogenic glucoside; triglochinin.

Abstract—The isolation and structure elucidation of the third and major cyanogenic glycoside of *T. aquilegifolium* as (II) is described.

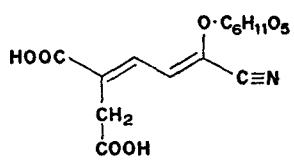
INTRODUCTION

IN A previous publication[1] we reported the presence of three cyanogenic glycosides in the leaves of *Thalictrum aquilegifolium* (Ranunculaceae), one major glycoside accounting for 90% of the cyanogenic content of the plant and two minor glycosides each accounting for 5% of the cyanogenic content. The structures of the two minor glycosides have been shown to be *p*-glucosyloxymandelonitrile and *p*-glucoxyloxymandelonitrile β-glucoside. The structure of the third, and major glycoside is now reported.

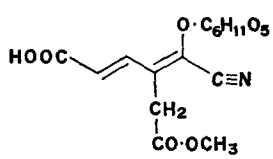
RESULTS

Structure Elucidation

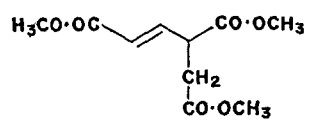
The major cyanogenic glycoside was isolated and purified from crude extracts of *T. aquilegifolium* by column chromatography and obtained as a pale yellow syrup which could not be crystallized. The UV spectrum showed one broad absorption peak at 275 nm and exhibited no bathochromic shift with alkali.



(I)



(II)



(III)

The IR spectrum of the glycoside in KBr exhibited major peaks at 2500–3600 cm^{-1} (OH stretching) 2220 cm^{-1} (C≡N stretching), 1700–1740 cm^{-1} (C=O stretching) and 1620–1640 cm^{-1}(C– C multiple bond stretching). The C≡N stretching peak is interesting since it is not only lower than in other cyanogenic glycosides (e.g. prunasin 2270 cm^{-1}) but is also relatively strong since it is usually quenched by the glycoside hydroxyl groups.[2] This indicates the presence of an α,β-unsaturated nitrile grouping in the molecule. The IR and UV

[1] D. SHARPLES and J. R. STOKER, *Phytochem.* **8**, 597 (1969).
[2] L. J. BELLAMY, *Infra-red Spectra of Complex Molecules*, p. 263, Methuen, London (1958).

spectra are in fact very similar to those exhibited by the cyanogenic glycoside triglochinin (I) contained in the flowers of *Triglochini maritinum*.[3] Thus triglochinin exhibits a broad peak in the UV spectrum at 275 nm and major peaks in the IR (as KBr disc) at 2500–3600 (s), 2200 (m), 1715 (s) and 1625 cm^{-1} (m).

From considerations of the UV, and IR spectra of the glycoside and the NMR spectrum of the glycoside acetate, structure (II) is proposed for the major glycoside of *T. aquilegifolium*. The NMR spectrum of the acetate showed two low field doublets centred at τ 2·97 and τ 3·58, each integrating for 1 proton, due to the vinylic protons H_B and H_A respectively in the aglycone, with coupling constant (J_{AB}) of 13 Hz typical of the system C=CH–CH=C. The methylene protons H_C coupling with the methyl ester protons gave rise to two closely spaced singlets integrating for 5 protons at τ 6·2 typical of the system $CH_2 \cdot CO \cdot OCH_3$. A broad signal at about τ 4·6 which disappeared on the addition of D_2O was assigned to the carboxylic acid proton. The remaining peaks on the spectrum were assigned to tetraacetyl glucose.[4] A broad doublet centred on τ 4·86 integrating for 4 protons was assigned to the hydrogens at C-1, 2, 3 and 4 of the glucose molecule, a broad doublet at τ 5·8 integrating for 2 protons to the methylene hydrogens at C-6 and an ill-defined multiplet at τ 6·5 integrating of 1 proton, to the hydrogen at C-5. The doublet at τ 7·9 integrating for 12 protons was assigned to the 4 acetoxyl groups.

The proposed structure was substantiated by the MS of the major glycoside acetate. In general, the mass peaks of glycoside acetates are not easily detectable since they immediately split to give an aglycone fragment and a tetraacetyl glucose fragment.[5] The MS of the major glycoside acetate showed a large number of peaks, the majority of which could be assigned to the fragmentation of tetraacetylglucose. However, a number of peaks remained which could only be attributed to the aglycone. A large peak at *m/e* 210 corresponding to the elimination of tetraacetylglucose and peaks at *m/e* 168 and *m/e* 151 corresponding to the loss of NCO (42 *m/e*) and $COOCH_3$ (59 *m/e*) from the aglycone.

Methylation of the glucoside acetate under conditions in which only the carboxylic acid would be methylated and re-examination of the MS shows the disappearance of the peak at *m/e* 210 and the appearance of a peak at *m/e* 224 (14 *m/e* CH_2).

Enzymic Hydrolysis

On enzymic hydrolysis, the major glycoside yields HCN, glucose (identified by co-chromatography and specific glucose assay[6]) and an ether extractable compound which on chromatography could be detected by spraying with alkaline $KMnO_4$ but gave no reaction with 2,4-dinitrophenylhydrazine. This tends to confirm the presence of an α,β-unsaturated nitrile grouping since this type of compound yields on hydrolysis a ketene which adds water to yield a carboxylic acid (cf. triglochinin).

Acid Hydrolysis

On acid hydrolysis the major glycoside yields the same hydrolysis products as enzymic hydrolysis. The aglycone isolated by ether extraction showed one sharp peak in the UV

[3] R. EYJOLFSSON, *Phytochem.* **9,** 854 (1970).
[4] G. H. N. TOWERS *et al.*, *Tetrahedron* **20,** 71 (1964).
[5] I. A. PEARL and S. F. DARLING, *Phytochem.* **7,** 831 (1968).
[6] J. D. FLEMING and H. F. PEGLER, *Chem. Analyst.* **88,** 967 (1963).

spectrum at 210 nm and peaks in the IR spectrum (KBr disc) at 2600–3400 cm^{-1} (OH stretching), 1720 cm^{-1} (C=O stretching), 1420 cm^{-1} (CH_2 stretching), indicating the presence of an aliphatic carboxylic acid. Methylation with CH_2N_2 resulted in a pale yellow oil which in the mass spectrum displayed a weak mass peak at *m/e* 230 due to the aglycone trimethyl ether (III).

As can be seen from structure (II), a number of isomers of the glycoside are possible. The stereochemistry of the isolated glycoside has not been finally determined although the large coupling constant (13 Hz) observed for the vinylic protons H_A and H_B would indicate that this bond at least is *trans*.

EXPERIMENTAL

Plant. material. Thalictrum aquilegifolium plants were grown from seed either in the greenhouse or outdoors.

Isolation of the major glycoside (II). Fresh leaves of *T. aquilegifolium* (200 g) were ground to a powder with liquid N_2. The powder was immediately transferred to boiling 80%, v/v EtOH (3 l.) for 15 min. The suspension was filtered and the filtrate evaporated to dryness below 40°. The residue was dissolved in 20%, v/v EtOH (200 ml), stored at 4° for 24 hr, centrifuged and the supernatant evaporated to dryness below 40°. The extract was dissolved in 50%, v/v EtOH and streaked out onto many sheets of Whatmann 3 MM paper and the chromatograms developed with *n*-BuOH–EtOH–H_2O (40:11:19). The major glycoside (R_f 0·06) was eluted with 50%, v/v EtOH and the eluate evaporated to dryness below 40°. The major glycoside was suspended in absolute MeOH, absorbed onto microcrystalline cellulose and dried *in vacuo* for 24 hr. The powder was then transferred to the top of a cellulose column (300 g) and eluted with EtOAc–HOAc–H_2O (8:1:1) using an LKB 'Radirac' fraction collector, taking 10 ml fractions. The fractions were monitored by TLC on silica gel in the same solvent and the spots visualized by spraying with 5%, v/v H_2SO_4 in EtOH and charring at 150°; in this solvent, the major glycoside has R_f 0·22. The relevant fractions were evaporated to dryness to obtain the major glycoside as a pale yellow syrup which could not be crystallized This syrup was concentrated repeatedly *in vacuo* with EtOH–benzene (1:1) to remove all traces of H_2O. This gave the glycoside as a pale yellow amorphous powder which delequesced extremely rapidly and was stored over P_2O_5. The homogeneity of the glycoside was verified by descending PC on Whatmann 1 paper; R_fs: *n*-BuOH–EtOH–H_2O (40:11:19) 0·06, *iso*-PrOH–H_2O (7:3) 0·72, *n*-BuOH–HOAc–H_2O (13:3:5) 0·18, 5% HOAc 0·80. IR spectrum (KBr disc), ν_{max}: 3500–2900 (s), 2220 (m), 1750 (s), 1650 (m), 1590 (m), 1230 (m), 1160 (m), 1030 (m), 800 (m), 720 (s), 680 (s) cm^{-1}. UV max 275 nm (H_2O).

Acetate. The major glycoside was acetylated with Ac_2O–pyridine.

NMR spectrum (*in* $CDCl_3$). τ 2·97, doublet, IH (H_B), 3·52, doublet, IH (HA), (J_{AB} 13 Hz), 4·6, IH, broad (COOH)—disappearing in D_2O, 6·2, two singlets, 5H ($CH_3O{\cdot}C{=}O{\cdot}CH_2$), 4·86, doublet, 4H (glucose $H_{1,2,3,4}$), 5·8, doublet, 2H (glucose H_6), 6·5, broad, IH, (glucose H_5), 7·9, doublet, 12H ($CH_3{\cdot}C{=}O{\cdot}O$). MS. *m/e* 210 (aglycone), 168 (aglycone–NCO), 151 (aglycone–$COOCH_3$). *m/e* 331, 271, 229, 169, 109 (tetra-acetyl glucose) on methylation, peak at *m/e* 210 disappears to be replaced by one at 224 (aglycone + CH_2).

Methylation. Methylations were carried out using an ethereal solution of CH_3N_2 at 0° for 1 hr.

Enzymic hydrolysis of the major glycoside. Small amounts of the major glycoside (1 mg) were hydrolysed with β-glucosidase (10 mg) at pH 5·5 for 2 hr in an enclosed system. The HCN liberated was removed by aeration with CO_2 free N_2 and trapped in 10 ml of 0·1 M NaOH. The aerated solution was extracted with ether and the ethereal extract examined by TLC. The aqueous solution remaining after ether extraction was shown to contain glucose by co-chromatography, and specific glucose assay.[6]

Acid hydrolysis of the major glycoside. The major glycoside (40 mg) was hydrolysed in distilled H_2O (10 ml), with 4 M HOAc (5 ml) under reflux for 1 hr. The solution was cooled, extracted with ether and the ethereal solution evaporated to dryness *in vacuo* to yield the aglycone (10 mg) as a pale brown solid. IR spectrum (KBr disc): ν_{max}: 3500–2400 (s), 1720 (m), 1410 (s), 1260 (s), 1050 (m), 800 (s) cm^{-1}. UV spectrum: sharp peak at 210 nm (H_2O). MS (methyl ester) weak signal *m/e* 230 (m^+).

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