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A CYANOGENIC GLUCOSIDE FROM *ILEX AQUIFOLIUM*

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Key Word Index—*Ilex aquifolium*; Aquifoliaceae; cyanogenic glucoside.

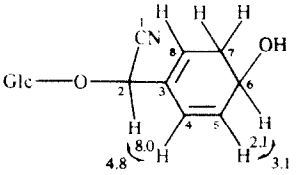
Abstract—A novel cyanogenic glucoside (2-β-D-glucopyranosyloxy-*p*-hydroxy-6,7-dihydromandelonitrile) has been isolated from the ethanolic extract of ripe fruits of *Ilex aquifolium*. Its structure has been established, primarily on the basis of IR, NMR and mass spectral data and its corresponding acetate.

INTRODUCTION

Well-known as a poisonous plant, *Ilex aquifolium* (Holly) is often found in parks and ornamental gardens [1]. Children are regularly poisoned following ingestion of the fruits [2, 3]. Although many compounds have been isolated, the toxin has not yet been identified [1]. The present investigation led to the isolation and identification of a novel cyanogenic glucoside (**1**), occurring in ripe fruits, leaves and stems.

RESULTS AND DISCUSSION

In general, the highest concentrations of cyanogenic glycosides in plants are found in leaves [4]. In this case leaves and stems showed lesser amounts, so **1** was isolated from ripe fruits. Separation of an EtOH extract by column chromatography on silica gel and purification by low pressure column chromatography on RP-18 yielded colourless crystals with a mp of 166–168° (uncorr.). The presence of glucose was established by enzymatic (β-glucosidase) and acidic hydrolysis and TLC. The FABMS spectrum shows a pseudomolecular ion peak at m/z 314 $[M+H]^+$ and after addition of LiJ at m/z 320 $[M+Li]^+$ indicating the M_r to be 313, corresponding to the molecular formula $C_{14}H_{19}NO_7$. **1** readily forms a penta-acetate showing a molecular ion peak in the mass spectrum at m/z 523. The UV spectrum showed λ_{max} (MeOH) at 259 nm. It did not shift on addition of alkali. First indications of the presence of a nitrile was given in the IR spectrum by the characteristic peak at 2212 cm^{-1}, which is in agreement with the signal at δ117.65 in the ^{13}C NMR spectrum [5, 6]. Other important absorptions are at 3200–3600 cm^{-1} (*s*, hydroxyl), 2900–3000 cm^{-1} (*m*, C-H stretching), 1630 cm^{-1} (*m*, conjug. olefins) and at 1010–1190 cm^{-1} (*s*, ether stretching). The ^1H NMR (DMSO) shows a AB-System of two olefinic protons at δ6.23 and 6.14 with a coupling constant of $J = 10$ Hz according to H-4 and H-5. The signal of H-5 is split slightly into a double doublet caused by coupling with the vicinal proton H-6 at δ4.2, $J = 2.3$ Hz (Dieder angle *ca* 90°). Irradiation of the broad doublet of H-6 simplified the double doublet of H-5 to a doublet, collapsed the doublet of the geminal hydroxy group at δ5.15 ($J = 6.6$ Hz) to a singlet and simplified the multiplets at δ2.26 and 1.65 according to the vicinal methylene group H-7a and H-7b ($J = 11.4$ Hz). D_2O exchange caused, a



1

NOEs in per cent.

simplification of the doublet H-6 to a broad singlet and led to the disappearance of the doublet at δ5.15 due to the hydroxy group. A broad doublet at δ4.73 (J = 10.2 Hz) was attributed to a further olefinic proton (H-8). Irradiation of this signal simplified the multiplets of the protons according to the vicinal methylene group due to H-7a and H-7b while irradiation of the multiplet at δ1.65 (H-7b) collapsed the doublet of H-8 to a singlet and simplified the multiplet of H-7a as well as the signal of H-6. The sharp singlet at δ5.6 has to be assigned to H-2. The signal of the anomeric proton was observed at 4.4. The coupling constant J = 7.14 Hz is indicative of β-linked glucosides [7]. The remaining signals are consonant with a glucoside 3.01–3.2 (3H, *m*, H-2′-H-5′), 3, 42 (1H, *dd*, J = 12.2; 5.6 Hz, H-6′a) and 3.69 (1H, *d*, J = 12.2 Hz, H-6′b). The assignment of the signals in the ^{1}H NMR as well as those of the ^{13}C NMR spectrum, listed in Table 1, additionally were based on ^{1}H–^{1}H and ^{1}H–^{13}C COSY experiments [8]. Furthermore the assignments of H-4 and H-5 were established on NOE-measurements. Effects could only be observed between H-2 and H-4 or H-5 and H-6, but not between H-2 and H-5 or H-4 and H-6. All data identify **1** as 2-β-D-glucopyranosyloxy-2-*p*-hydroxy-6,7-dihydromandelonitrile. Whether its trivial name should be 6,7-dihydrodhurrin or 6,7-dihydrotaxiphyllin [9] is not yet clear because the absolute configuration of C-2 has not yet been established.

Table 1. ^{13}C NMR chemical shifts of 2-β-D-glucopyranosyloxy-*p*-hydroxy-6,7-dihydromandelonitrile (**1**) in DMSO and for its corresponding acetate in $CDCl_3$

C	1	1-Acetate	Multiplicity
1	117.2	116.3	*s*
2	95.0	102.2	*d*
3	155.9	152.9	*s*
4	126.3	129.0	*d*
5	141.6	133.0	*d*
6	64.4	64.0	*d*
7	36.2	32.8	*t*
8	72.9	71.1	*d*
1′	99.5	99.3	*d*
2′	76.9*	73.5*	*d*
3′	77.0*	72.8*	*d*
4′	70.4	68.1	*d*
5′	76.9*	72.2*	*d*
6′	61.7	61.6	*t*
-OCOMe	—	20.5, 20.5	
		20.5, 20.7	
		20.9	*q*
-COMe	—	168.9, 169.3	
		170.1, 170.5	
		170.9	*s*

* These assignments are interchangeable.

EXPERIMENTAL

IR: KBr; 1H NMR: 300 MHz (DMSO) TMS as int. standard; ^{13}C NMR: 300 MHz (DMSO or $CHCl_3$); $[\alpha]_D^{20}$ **1** = −0.620 (MeOH; *c* 0.10).

Plant material. Ilex aquifolium L. was obtained from the Botanic Garden of Frankfurt/Main, collected in Dec. 1985.

Extraction and isolation of **1**. Dried (airing cupboard 40°) fruits (50 g) were powdered and extracted with MeOH (5 × 200 ml/5 min) at room temp. (Ultraturrax). After centrifuging and concng under vacuum, the residue (14.5 g) was chromatographed on silica gel (300 g, KG 60 (Merck) 70–230 mesh) with EtOAc–EtOH 70% (9:1) resulting in 4 fractions. The cyanogenic fraction III, eluted within 2800–5700 ml, was concd. under vacuum and purified by low pressure CC on LiChroprep RP-18 (2.5 × 31 cm) using EtOH 5%, 1 ml/min, UV detection at 254 nm (R_t of **1** 45 min).

Hydrolysis with β-glucosidase (Serva, Heidelberg) from almonds 24 hr at 37°, pH 6, acidic in 1 M HCl 20 min at 100°.

TLC systems. Silica gel–EtOAc–EtOH 70%, (4:1), detection (**1**) by UV 254 and Komarowsky reagent [10] (green-yellow spots); silica gel–EtOAc–EtOH 70%, 7:3, detection (glucose) Komarowsky reagent.

Acetylation of **1**. Treatment of **1** with Ac_2O–pyridine gave the pentaacetate crystallized from EtOH–H_2O as colourless crystals mp 175–177°, analysed for $C_{24}H_{29}NO_{12}$. IR ν_{max} cm^{-1} 2968, 2218, 1746, 1440, 1377, 1248 (*br*), 1158, 1068, 921, 846, 804. ^{1}H NMR 0.91 (1H, *q*, $J_{7a,7b}$ = 7.52 Hz, $J_{7\beta,8}$ = 4.7 Hz, H-7b), 1.32 (1H, *m*, $J_{7a,7b}$ = 7.52 Hz, H-7a), 1.99 (3H, *s*, Ac), 2.02 (3H, *s*, Ac), 2.04 (3H, *s*, Ac), 2.093 (3H, *s*, Ac), 2.095 (3H, *s*, Ac), 4.76 (1H, *t*, $J_{5,6}$ = 4.5 Hz, $J_{6,7}$ = 3.8, H-6), 5.35 (1H, *s*, H-2), 6, 18 (1H, *dd*, $J_{4,5}$ = 9.9 Hz, $J_{5,6}$ = 4.5 Hz, H-5), 6.35 (1H, *d*, $J_{4,5}$ = 4.5 Hz, H-4).

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