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# STRUCTURE OF A NEW CYANOGLUCOSIDE FROM ILEX WARBURGII LOESN.

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A cyanoglucoside (1) has been isolated from the fruits of Ilex warburgii Loesn., and its structure has been elucidated by chemical and spectral means as (Z)-6α-(β-D-glucosyloxy)-4α,5α-dihydroxy-2-cyclohexene-$\Delta^{1,\alpha}$-acetonitrile. A related compound, Menisdaurin (3)[1] was also isolated.

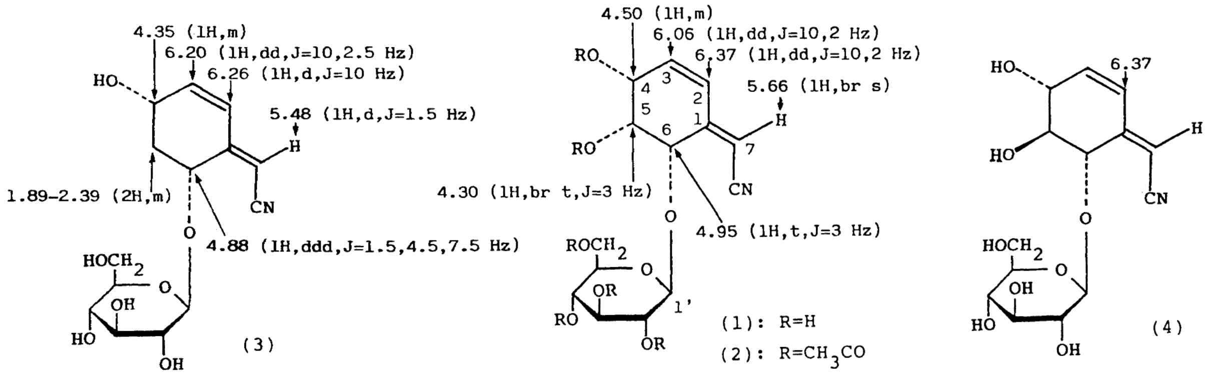
In the continuation of our chemical studies on endemic plants of the Ryukyus, We investigated the constituents of Ilex warburgii Loesn.. This plant bears the conspicuously red fruits which, as we noticed, are rarely eaten by birds. This observation prompted us to investigate the constituents of the fruits, and a new cyanoglucoside (1) and a related compound, Menisdaurin (3) were isolated.

The fruits collected in Iriomote island were crushed with dioxane in a blender. After removal of the solid residue by filtration, the filtrate was concentrated under reduced pressure. The residue was partitioned between chloroform and water. The aqueous portion was separated by reversed phase chromatography to give compounds (1) and (3). Recrystallization from aqueous methanol afforded a pure sample of (1): $C_{14}H_{19}NO_8$; mp 221-223 °C; $[\alpha]_D^{25}$ -247° (c 0.611, $CH_3OH$); MS, m/e 330 ($M^+$+1), 329, 311, and 178.

A characteristic, sharp band at 2220 $cm^{-1}$ in the IR spectrum and a signal at δ117.1 in the $^{13}C$-NMR spectrum showed the presence of an α,β-unsaturated nitrile. Some other $^{13}C$-NMR signals (δ61.3, 68.3, 69.1, 70.2, 73.0, 75.8, 76.4, 76.9, and 100.6) and the IR absorption bands at 3600-3100 and 1100-980 $cm^{-1}$ indicated a glycoside. Acid hydrolysis of (1) with HCl gave D-glucose which was confirmed by paper chromatography. (1) was hydrolyzed with β-glucosidase (from almonds), and then the mixture was concentrated under reduced pressure. Acetylation ($Ac_2O$/pyridine) of the oily residue gave D-glucose pentaacetate and the triacetate of the aglycon (5): MS, m/e 293 ($M^+$), 233, 191, and 149; $UV_{max}$ (EtOH) 255 nm; IR (neat) 2950, 2220, 1760, 1390, and 1220 $cm^{-1}$; NMR ($CDCl_3$) δ2.03 (3H, s), 2.05 (3H, s), 2.25 (3H, s), 5.3 (1H, m), 5.4-6.0 (4H, m), and 6.30 (1H, br d, J=10 Hz). Acetylation of (1) ($Ac_2O$/pyridine) yielded a hexaacetate (2): MS, m/e 581 ($M^+$), 521 ($M^+$-AcOH), and 132; $UV_{max}$ (EtOH) 253 nm; IR (neat) 2950, 2210, 1750, and 1040 $cm^{-1}$; NMR ($CDCl_3$) δ2.00-2.10 (18H, 6xAcO-), 4.10 (1H, dd, J=13, 4 Hz), 4.33 (1H, dd, J=13, 3 Hz), 4.8 (2H, m), 5.2 (3H, m), 5.37 (1H, br s), 5.63 (1H, br t, J=3.5 Hz), 5.99 (1H, dd, J=10.5, 3.5 Hz), and 6.34 (1H, br d, J=10.5 Hz). Therefore, the aglycon moiety has two hydroxyl groups. Signals at δ96.3, 126.2, 138.1, and 154.9 in the $^{13}C$-NMR spectrum and at δ5.66 (1H, br s), 6.06 (1H, dd, J=10, 2 Hz), and 6.37 (1H, dd, J=10, 2 Hz) in the $^1H$-NMR spectrum of (1) suggested the presence of four $sp_2$-carbons, of which two carbons constituted a di-substituted cis double bond. Considering the UV spectrum (257 nm) of (1) and the above results, the aglycon moiety proved to be a monocyclic structure containing $\alpha,\beta,\gamma,\delta$-unsaturated nitrile and two hydroxyl groups.

The structure of the cyanoglucoside (1) was determined by detailed analysis of the $^1$H-NMR spectra of (1) and (2) with aid of decoupling procedures. The chemical shifts and coupling constants of each proton in the aglycon moiety of (1) are shown in the figure, indicating that the sugar moiety is attached to C6, because the chemical shift of C6-H in (1) is virtually unchanged by acetylation of (1) [$\delta$ 4.95 in (1) and $\delta$4.8 in (2)] . The configuration of the cyanomethylene moiety was established by the following reasons: 1. About 7 % NOE was observed between C2-H and C7-H in (2), 2. One allylic proton, C6-H showed a resonance at lower field than the other allylic proton, C4-H, owing to the diamagnetic anisotropy of the nitrile group, 3. If the configuration were E, C2-H in (1) would exhibit a resonance at even lower field than C2-H in (3) or (4).[2] Considering coupling constants ($J_{3,4}$=2 Hz, $J_{4,5}$=3 Hz and $J_{5,6}$= 3 Hz) in (1), it could be presumed that C4-H, C5-H, and C6-H are cis to each other. This was confirmed by the following chemical degradation. After ozonolysis of (5) followed by the reduction with $NaBH_4$, the mixture was concentrated under reduced pressure. The oily residue was successively treated with $NaIO_4$, $NaBH_4$, and $Ac_2O$/pyridine to give ribitol pentaacetate. Consequently, the structure of the cyanoglucoside was determined as shown in (1).

Though the stereoisomer of (1), Lithospermoside (4)[4] has been isolated as the constituent of Lithospermum officinale Linn., only a few such compounds have been reported so far.[1), 4), 5)]



(3)

(1): R=H

(2): R=$CH_3CO$

(4)

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## References

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