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Studies on the Constituents of Medicinal Plants. XX.[1] The Constituent of the Vines of *Menispermum dauricum* DC.

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Menisdaurin, a new nitrile glucoside was isolated from the vines of *Menispermum dauricum* DC (Menispermaceae). The structure I, (*Z*)-6(*S*)-(β-D-glucopyranosyloxy)-4(*R*)-hydroxy-2-cyclohexene-$\Delta^{1,\alpha}$-acetonitrile has tentatively proposed for menisdaurin and the structure III, (*Z*)-4(*R*)-6(*S*)-dihydroxy-2-cyclohexene-$\Delta^{1,\alpha}$-acetic acid γ-lactone for menisdaurilide, the hydrolysis product of menisdaurin, on the basis of the chemical and spectral evidences.

Keywords——*Menispermum dauricum* DC; Menispermaceae; menisdaurin; menisdaurilide; ^{1}H- and ^{13}C-NMR; ORD; CD

There have been some reports[3] regarding the alkaloidal components of *Menispermum dauricum* DC (Menispermaceae). This paper deals with the isolation and the structural elucidation of a new nitrile glucoside, named menisdaurin. The methanolic extract of the vines afforded menisdaurin $C_{14}H_{19}NO_7$ (I), colorless plates of mp 175—176°. $[\alpha]^{15°}=-185.4°$ (c=1.00, methanol). Menisdaurin (1) formed a penta-acetate $C_{24}H_{29}NO_{12}$ (II), colorless needles of mp 178—179°. Menisdaurin (I) bears a D-glucose unit which was removed hydrolytically by incubation with β-glucosidase (emulsin). By the hydrolysis with 20% sulfuric acid, I afforded menisdaurilide $C_8H_8O_3$ (III), colorless needles of mp 113° together with D-glucose. Attempt to isolate the aglycone from the hydrolysates failed and the genuine aglycone was not isolated. Since there are four free hydroxyl groups on the glucose moiety, the other one hydroxyl group must, therefore, be borne on the aglycone moiety. The infrared (IR) spectrum of I shows a sharp and strong band at 2220 (C≡N) and bands at 1625, 1620 (cm^{-1}) (unsaturation), which are to be expected for a conjugated nitrile group.[4] These data and ultraviolet (UV) absorption maximum at 260.9 nm (log ε 5.54) suggest the presence of αβγδ-unsaturated nitrile group[5] in the molecule of menisdaurin (I). These functionalities taken with the molecular formula indicate that I is a monocyclic nitrile glucoside. The ^{1}H-nuclear magnetic resonance (NMR) spectra of I, II and III (Table I and II) furnish sufficient information to establish the structures of I and III and also to establish the relative configuration of the substituents and the position of attachment of the glucose moiety, as shown in Chart 1.

Menisdaurilide (III) shows the UV absorption maximum at 256.5 nm (log ε 4.24) (αβγδ-unsaturated γ-lactone)[5] and the IR bands at 3400 (OH), 1715, 1635, 1580 cm^{-1} (αβγδ-unsaturated γ-lactone) and the NMR signals (δ-value) due to three olefinic protons at 5.86 (d, J=1.5 Hz, Cα-H), 6.42 (d, J=10 Hz, C_2-H) and 6.63 (dd, J=10 and 2 Hz, C_3-H), which could respectively be assigned to α, γ, δ protons of the αβγδ-unsaturated γ-lactone. Menisdaurilide

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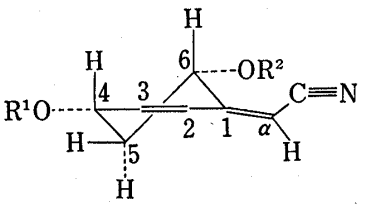
Table I. The ^{1}H-NMR Data (δ value, J in Hz, 100 MHz),

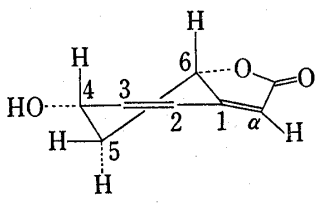
	Menisdaurin (I) (CD_3OD)	Menisdaurin penta-acetate (II) ($CDCl_3$)	Menisdaurilide (III) ($CDCl_3$)
C_α-H	5.48 (d), $J_{\alpha,6}=1.5$	5.35 (d), $J_{\alpha,6}=1$	5.86 (d), $J_{\alpha,6}=1.5$
C_2-H	6.27 (d), $J_{2,3}=10$	6.36 (d), $J_{2,3}=10$	6.42 (d), $J_{2,3}=10$
C_3-H	6.20 (dd), $J_{2,3}=10$, $J_{3,4}=2.5$	6.17 (dd), $J_{2,3}=10$, $J_{3,4}=4$	6.63 (dd), $J_{2,3}=10$, $J_{3,4}=2$
C_4-H	4.35 (m), $W_{h/2}=12.5$	5.33 (m)	4.68 (ddd), $J_{3,4}=2$, $J_{4,5\alpha}=11$, $J_{4,5\beta}=5$
C_5-$H_{ax(\alpha)}$	1.87—2.39 (m) (2H)	2.17 (m) (2H)	1.69 (ddd), $J_{4,5\alpha}=11$, $J_{5\alpha,5\beta}=11$, $J_{5\alpha,6}=13.5$
C_5-$H_{eq(\beta)}$			2.96 (ddd), $J_{4,5\beta}=5$, $J_{5\alpha,5\beta}=11$, $J_{5\beta,6}=5$
C_6-H	4.91 (ddd), $J_{\alpha,6}=1.5$, $J_{5\alpha,6}=8$, $J_{5\beta,6}=4$	4.77 (m)	4.94 (ddd), $J_{\alpha,6}=1.5$, $J_{5\alpha,6}=13.5$, $J_{5\beta,6}=5$
		2.11 (6H, s), 2.05 (3H, s), 2.03 (3H, s), 1.99 (3H, s): five acetyl groups.	2.94 (s, C_4-OH, exchangeable with D_2O)
$C_{1'}$-H	4.54 (d), $J_{1',2'}=7$	4.84 (d), $J_{1',2'}=7$	
$C_{2'}C_{3'}C_{4'}$-H	3.31 (m) (4H)	4.93—5.19 (m) (3H)	
$C_{5'}$-H		3.71—3.87 (m)	
$C_{6'}$-H	3.68 (dd), $J_{5',6'H}=4$, $J_{6'H,6'H'}=11.5$	4.13 (dd), $J_{5',6'H}=4$, $J_{6'H',6'H'}=12.5$	
$C_{6'}$-H′	3.88 (dd), $J_{5',6'H'}=1.5$, $J_{6'H,6'H'}=11.5$	4.30 (dd), $J_{5',6'H'}=2.5$ $J_{6'H,6'H'}=12.5$	

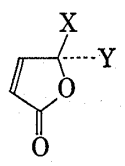
Abbreviation: s, singlet; d, doublet; m, multiplet.

Table II. ^{1}H-NMR Decoupling Data of Menisdaurilide (III) (J in Hz)

Irradiated at	Change of multiplicity		
C_α-H	C_6-H (ddd)	Becomes	dd, $J_{5\alpha,6}=13.5$, $J_{5\beta,6}=5$
C_3-H	C_4-H (ddd)	Becomes	dd, $J_{4,5\alpha}=11$, $J_{4,5\beta}=5$
C_4-H	C_3-H (dd)	Becomes	d, $J_{2,3}=10$
	C_5-H_β (ddd)	Becomes	dd, $J_{5\alpha,5\beta}=11$, $J_{5\beta,6}=5$
C_5-H_β	C_4-H (ddd)	Becomes	dd, $J_{3,4}=2$, $J_{4,5\alpha}=11$
	C_6-H (ddd)	Becomes	dd, $J_{5\alpha,6}=13.5$, $J_{\alpha,6}=1.5$
C_6-H	C_α-H (d-like)	Becomes	s
	C_5-H_α (ddd)	Becomes	dd, $J_{4,5\alpha}=11$, $J_{5\alpha,5\beta}=11$
	C_5-H_β (ddd)	Becomes	dd, $J_{4,5\beta}=5$, $J_{5\alpha,5\beta}=11$
C_3-H and C_5-H_α	C_4-H (ddd)	Becomes	d, $J_{4,5\beta}=5$
	C_5-H_β (ddd)	Becomes	dd, $J_{4,5\beta}=5$, $J_{5\beta,6}=5$
	C_6-H (ddd)	Becomes	dd, $J_{5\beta,6}=5$, $J_{\alpha,6}=1.5$
C_3-H and C_5-H_β	C_4-H (ddd)	Becomes	d, $J_{4,5\alpha}=11$
	C_6-H (ddd)	Becomes	dd, $J_{5\alpha,6}=13.5$, $J_{\alpha,6}=1.5$
C_5-H_α and C_5-H_β	C_4-H (ddd)	Becomes	d, $J_{3,4}=2$
	C_6-H (ddd)	Becomes	d, $J_{\alpha,6}=1.5$
C_4-H and C_6-H	C_5-H_β (ddd)	Becomes	d, $J_{5\alpha,5\beta}=11$







I: R^1=H, R^2=β-D-glucopyranosyl III
II: R^1=Ac, R^2=tetra-acetyl β-D-glucopyranosyl

Chart 1

(III) shows furthermore the NMR signals of the methine protons at 4.68 (ddd, J=11, 5, 2 Hz, $C_4H_{quasi-ax}$), 4.94 (ddd, J=1.5, 13.5, 5 Hz, $C_6H_{quasi-ax}$) and of the methylene protons at 1.69 (ddd, J=11, 11, 13.5 Hz, C_5H_{ax}) and at 2.96 (ddd, J=5, 11, 5 Hz, C_5H_{eq}). Each coupling constant (J) was established by the spin-spin decoupling technique as shown in Table II. Beecham[6] has proposed the rules relating the sign of n–π^* circular dichroism (CD) curve to the C=C–C=O chirality in endo-α, β-unsaturated lactones. These rules, however, are ambiguous when applied to butanolides. Besides the n–π^* Cotton effect, α,β-unsaturated γ-lactones usually show a Cotton effect associated with π-π^* transition. Uchida and Kuriyama[7] have recently reported that the chirality at the γ-carbon atom of the butenolide ring is the sign-determing factor for the π–π^* CD, that is, if the γ-carbon is asymmetrically substituted (X≠Y), the sign of the π–π^* Cotton effect is negative when X>Y in polarizability, and positive when X<Y, as shown in Chart 1. They have also reported that this new empirical rule can applied to all compounds with the exception of the compounds containing an allylic oxygen substituent. The optical rotatory dispersion (ORD) curve of III exhibits a positive n–π^* Cotton effects,[8] and its n–π^* CD and π–π^* CD curves exhibit a positive and a negative effect, respectively. On the basis of these data and on the assumption that the extended allylic C_4-OH group of III affects the sign of π–π^* CD as in the case of linderenolide[7] with an allylic oxygen substituent ,we tentatively formulate the structure of menisdaurilide as (*Z*)-4(*R*)-6-(*S*)-dihydroxy-2-cyclohexene-$\Delta^{1,\alpha}$-acetic acid γ-lactone (III). At present, however, the effect of the C_4-OH group on the CD curve is uncertain, and further work is needed to confirm the absolute stereochemistry of this new unsaturated lactone, menisdaurilide.

Menisdaurin (I) shows the NMR signals due to three olefinic protons at 5.48, 6.20 and 6.27. The 1.5 Hz doublet occurring at 5.48 assignable to the C_α-H is considered to be coupled with the C_6-H at 4.91 through the exocylic double bond system and the C_6-H shows a doublet doublet doublet signal with J of 8 Hz ($J_{C_6H_{quasi-ax}-C_5H_{ax}}$) and with J of 4 Hz ($J_{C_6H_{quasi-ax}-C_5H_{eq}}$) and with J of 1.5 Hz ($J_{C_6H_{quasi-ax}-C_\alpha H}$), indicating that the C_6-H is quasi-axial. The signal of I at 6.27 assignable to the C_2-H is coupled with the C_3-H to form a doublet with J of 10 Hz and the signal at 6.20 assignable to the C_3-H is coupled with the C_2-H and the C_4-H to form doublet doublet with J of 10 Hz ($J_{C_3H-C_2H}$) and with J of 2.5 Hz ($J_{C_3H-C_4H_{quasi-ax}}$). The signal at

Table III. The ^{13}C-NMR Data (ppm from TMS, 25.15 MHz)

Carbon	I (in D_2O, Dioxane)	III (in $CDCl_3$)
C_α	96.9(d)	144.1(d)
C_1	156.4(s)	163.4(s)
C_2	127.6(d)	111.1(d)
C_3	139.3(d)	119.6(d)
C_4	64.5(d)	66.6(d)
C_5	35.2(t)	39.8(t)
C_6	73.8(d)	78.3(d)
C≡N or C=O	118.7(s)	173.8(s)
$C_{1'}$	101.2(d)	
$C_{2'}$	73.1(d)	
$C_{3'}$	76.9(d)	
$C_{4'}$	70.7(d)	
$C_{5'}$	76.9(d)	
$C_{6'}$	61.9(t)	

The letter in parentheses designates the multiplicity of signal with off-resonance decoupling. Abbreviation: s, singlet; d, doublet; t, triplet

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4.35 (1H, m) assignable to the C_4-H is coupled with the C_5-methylene protons and the C_3-H to form a multiplet with the line width at half height ($W_{h/2}$) of 12.5 Hz, indicating that the C_4-H is quasi-axial. The other signals of I and III could be assigned to their appropriate protons as shown in Table I. In the glucose moiety of menisdaurin (I), a value of 7 Hz (diaxial) for $J_{C_1'H\text{-}C_2'H}$ corroborates the enzymatic result in assigning a β-linkage at the anomeric center, whose proton resonates at 4.54. Penta-acetate (II) shows the NMR signals of two methine protons at 5.33 and 4.77. The large acetylation shift in the resonance of the C_4-H (5.33—4.35=0.98 ppm) indicates that the hydroxyl group at the C_4-carbon is unsubstituted in the menisdaurin itself. In the process of the conversion of I to III, the β-D-glucose linkage was hydrolysed leaving a hydroxyl group and the nitrile group was converted to a carboxyl group to form the lactone, namely menisdaurilide (III) as the ultimate product of the reaction, indicating that menisdaurin could be elucidated to be (*Z*)-6(*S*)-(β-D-glucopyranosyloxy)-4(*R*)-hydroxy-2-cyclohexene-$\Delta^{1,\alpha}$-acetonitrile (I). The ^{13}C-NMR of I and III could be interpreted as shown in Table III.

The cyanogenic glycosides,[9] glucosidic derivatives of α-hydroxy-nitriles are widely distributed in the plant kingdom, but only three compounds of this nitrile glycoside type, simmondsin,[10] griffonin[5] and lithospermoside[11] have recently been reported.

Experimental

Melting point was taken in a Kofler-type hot plate and uncorrected. The IR spectra were taken in KBr pellet with JASCO IR-G spectrometer, the NMR spectra with JNM-PS-100 high resolution instrument at 100 (^{1}H) and 25.15 (^{13}C) MHz with $(CH_3)_4Si$ as internal reference, the MS spectra with JMS-OlSG mass spectrometer, the UV spectra with Hitachi 323 spectrometer, the ORD and CD curves with JASCO J-20 automatic recording spectropolarimeter and the optical rotation at 589 nm with JASCO automatic polarimeter DIP-SL. The thin-layer chromatogram (TLC) was obtained on a glass plate coated with Kieselgel-GF_{254} (Stahl). Column chromatography was carried out using silica gel (Merck, Kieselgel 60).

Isolation of I——Dried, cut vines (19 kg) were extracted with hot methanol (36 l) and the extract was concentrated *in vacuo* to yield resinous material, which was adsorbed on celite (5 kg) and the celite was extracted with *n*-hexane, benzene, ethyl acetate and methanol, successively. The methanol soluble fraction was, after being concentrated, adsorbed on silica gel (1 kg) and was chromatographed on silica gel (1.6 kg) with $CHCl_3$–MeOH (4: 1). The fraction of *Rf* 0.37 (TLC, $CHCl_3$–MeOH=4: 1) afforded colorless plates (I) of mp 175—176° from $CHCl_3$–MeOH mixture. Yield: 3.08 g. MS (*m/e*, relative intensity): 313 (M^+), 295 M^+-H_2O, 1), 206 (2), 133 (aglycone–H_2O, 100), 106 (133-HCN, 16), 78 (16), 77 (11), 44 (21) and as fragment ions from glucose moiety, 162 (10), 145 (11), 127 (8), 116 (16), 104 (7), 96 (6). *Anal.* Calcd. for $C_{14}H_{19}NO_7$: C, 53.67; H, 6.11; N, 4.47. Found: C, 53.40; H, 6.08; N, 4.48.

Acetylation of I——A solution of I (100 mg) in a mixture of pyridine (1.5 ml) and acetic anhydride (2 ml) was kept at room temperature overnight and the solution was poured into ice-water to afford colorless powder, which was crystallised from dil. MeOH to afford colorless needles of penta-acetate (II) of mp 178—179°. Yield: 50 mg. $[\alpha]^{25°}=-160.2°$ (c=1.00, MeOH). *Rf*: 0.50 (TLC, $CHCl_3$–CH_3COCH_3=6: 1). UV λ_{max}^{EtOH}: 255.5 nm (log ε 4.34). IR ν_{max}^{KBr} (cm^{-1}): 2200 (C≡N), 1750 (acetate), 1630, 1600 (C=C). MS: 523 (M^+) and as fragment ions from tetra-acetyl glucose moiety, 331 (20), 229 (5), 211 (3), 169 (63), 109 (30). *Anal.* Calcd. for $C_{24}H_{29}NO_{12}$: C, 55.06; H, 5.58; N, 2.68. Found: C, 54.52; H, 5.58; N, 2.96.

Acid Hydrolysis of I——Menisdaurin (I) (200 mg) in 20% H_2SO_4 (3 ml) was heated at 100° for 3 hr and the mixture was extracted with ethyl acetate. The extract was concentrated and was column chromatographed on silica gel with $CHCl_3$–CH_3COCH_3 (6: 1). The fraction of *Rf* 0.32 (TLC, $CHCl_3$–CH_3COCH_3=6: 1) afforded colorless needles (III) of mp 113° from $CHCl_3$–C_6H_6 mixture. Yield: 15 mg. $[\alpha]^{23°}=-31.4°$ (c=1.00, MeOH). ORD (c=0.001, MeOH, 25°): $[\phi]_{403}=0$, $[\phi]_{280}^{p}=5776$, $[\phi]_{271}=0$, $[\phi]_{249}^{t}=-20976$, $[\phi]_{218}=-3040$. CD (c=0.001, MeOH, 25°): $[\theta]_{302}=304$, $[\theta]_{267}^{p}=25536$, $[\theta]_{247}=0$, $[\theta]_{227}^{t}=-18544$, $[\theta]_{215}=-11248$. MS: 152 (M^+, 16), 134 (M^+-H_2O, 5), 124 (M^+-CO, 3), 123 (15), 110 (M^+-CH_2CO, 25), 106 (134-CO, 11), 95 (123-CO, 39), 77 (33), 67 (95-CO, 46). *Anal.* Calcd. for $C_8H_8O_3$: C, 63.15; H, 5.30. Found: C, 63.41; H, 5.33. The aqueous layer of the reaction mixture, after being treated with ion-exchange resine Amberlite IR-SB

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was concentrated to afford a syrup, which showed a spot of *Rf* 0.17 (glucose) on the paper chromatogram (*n*-butanol–acetic acid–H_2O=4: 1: 5) and afforded D-glucose phenylosazone of mp 206° (mixed fusion).

Enzymatic Hydrolysis of I——To a solution of I (200 mg) in a small amount of methanol were added acetate buffer (pH 4.8, 200 ml) and emulsion (330 mg, sigma). The mixture was incubated for 4 days under stirring at 30° and then the mixture was extracted with ethyl acetate. The aqueous layer showed a spot of D-glucose at *Rf* 0.15 on a paper chromatogram (*n*-butanol–acetic acid–H_2O=4: 1: 5). From the ethyl acetate solution, the genuine aglycone was not isolated.

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