The Crystal and Molecular Structure of Daphnetin 8-β-D-Glucopyranoside Dihydrate Isolated from *Daphne odora*

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The crystal structure of daphnetin 8-β-D-glucopyranoside dihydrate has been determined by the X-ray method. The crystal is monoclinic, space group $P2_1$, with the lattice parameters $a=7.773(1)$, $b=22.478(5)$, $c=4.715(1)$ Å, $\beta=99.77(2)°$, and $Z=2$. The structure was determined by the direct method and refined by least-squares to a final R of 0.041 for 2690 observed reflexions. The daphnetin moiety is approximately planar, and little effect from glucosylation is observed in the bond lengths and angles, compared with daphnetin itself. The glucopyranoside is in C_1 chair conformation, with the ring torsion angles ranging from 48 to 66°. The anomeric C–O bond is twisted by 78.2° against the coumarin plane, which is the largest value in aromatic glycopyranosides so far reported and which is concomitant with a small valence angle, 114.4°, at the oxygen atom linking the coumarin and the glucose group.

During the course of studies on the biosyntheses of naturally occuring dihydroxycoumarins, Sato and Hasegawa found new enzymes from *Daphne odora* and *Cichorium intybus*. These enzymes show high substrate specificities for the hydrolysis and transglucosylation of dihydroxycoumarins and their glucosides.[1-4] As a part of investigations on the reaction mechanism of these enzymes, the structures of daphnetin (or 7,8-dihydroxycoumarin)[5] and esculetin(or 6,7-dihydroxycoumarin) have already been reported and some effects of the tautomerism were suggested in the molecular structures of 7-hydroxylated coumarins.[6] The present paper deals with the conformation of glucosylated coumarin and the effects of the glucosylation on the structure of the coumarin molecule.

Experimental

The crystals were obtained as transparent prisms by slow evaporation from an aqueous ethanol solution. The lattice constants and intensity data were obtained on a Rigaku four-circle diffractometer with graphite monochromated Mo $K\alpha$ radiation. A crystal $0.3\times0.2\times0.3$ mm in size was used. The scan mode of $2\theta/\omega$ was applied, with the scanning rate and range in ω of 2° min^{-1} and $(1.0+0.5\tan\theta)°$, respectively. Stationary background counts were made for 10 s before and after each scan. Of 3017 independent reflexions measured within $2\theta\leq65°$, 2690 had intensities greater than $3\sigma(|F_o|)$ and were used for the structure determination. No correction was made for absorption. The crystallographic data of daphnetin 8-β-D-glucopyranoside $2H_2O$ are: $C_{15}H_{16}O_9\cdot2H_2O$, $F.W.=376.3$. Monoclinic, $P2_1$, $a=7.773(1)$, $b=22.478(5)$, $c=4.715(1)$ Å, $\beta=99.77(2)°$. $Z=2$, $D_x=1.53$, $D_m=1.54$ g cm^{-3} (by flotation in a mixture of hexane and carbon tetrachloride). μ(Mo $K\alpha$) = 1.60 cm^{-1}. Systematic absence, $0k0$ with k odd.

Structure Determination

The structure was solved by the multi-solution method using 237 reflexions with $|E|\geq1.50$.[7,8] A phase set with the lowest R-value[9] gave an E-map which revealed the coumarin moiety. All the other non-hydrogen atoms emerged in successive Fourier maps. The structural parameters were refined by the block-diagonal least-squares method. The hydrogen atoms were located on a D-map at the stage of $R=0.09$ and their positional and isotropic thermal parameters were included in the refinement. The weighting scheme of $w=(20.0/|F_o|)$ if $|F_o|\geq20.0$, $w=1.0$ if $20.0>|F_o|\geq10.0$, and $w=(|F_o|/10.0)$ if $|F_o|<10.0$ was applied. The final R was 0.041 for 2690 observed reflexions. The atomic scattering factors were taken from International Tables for X-Ray Crystallography.[10] A list of observed and calculated structure factors is given in Table 1.[11] The final atomic parameters are shown in Table 2.

Results and Discussion

Molecular Geometry. The perspective drawing of the molecule with the numbering system is shown in Fig. 1. The bond lengths and angles are given in Fig. 2. The molecule is bent down at the glucosidic oxygen with a dihedral angle between the coumarin plane and the mean plane of the glucose ring of 116°. The daphnetin moiety is nearly planar. The equation of the best plane for the daphnetin moiety is given by

$$-0.3451X - 0.5255Y - 0.7777Z + 8.3748 = 0.0,$$

where X, Y, Z refer to the crystallographic axes a, b, c* in Å units. The C(3), O(2), and O(1′) atoms show somewhat large deviations from this plane (−0.046, 0.045, and −0.038 Å, respectively). Compared with daphnetin,[5] the corresponding endocyclic bond lengths and angles deviate by no more than 0.01 Å and 0.7°, respectively, except for the angles around C(7) and, C(8); the C(6)–C(7)–C(8) angle is smaller by 2.0° the C(7)–C(8)–C(9) is larger by 1.5°, and the C(7)–C(8)–O(1′) is markedly larger by 3.4° than in daphnetin. The C(7)–O(7) bond is significantly shorter than those in other 7-hydroxylated coumarins, such as daphnetin, esculetin,[6] and 4-methylumbelliferone[12] (1.366, 1.358, and 1.393 Å, respectively). The C(8)–O(1′) bond is close to the bond length of the 8-methoxy group in xanthotoxin(1.375 Å).[13]

The glucopyranose moiety is in C_1 chair conformation and C–C bond lengths have similar values to those in other pyranoses observed in the X-ray studies.[14] Some relevant torsion angles are listed in

TABLE 2. FINAL ATOMIC PARAMETERS ($\times 10^4$) WITH THEIR STANDARD DEVIATIONS
The anisotropic thermal factors have the form $\exp(-\beta_{11}h^2-\beta_{22}k^2-\beta_{33}l^2-\beta_{12}hk-\beta_{13}hl-\beta_{23}kl)$.

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
O(1)	7574(2)	4208.3(7)	4094(4)	105(3)	10.2(3)	359(8)	3(2)	136(8)	10(3)
C(2)	8007(3)	4633.9(11)	2248(6)	133(4)	11.2(5)	354(12)	−15(2)	120(12)	2(4)
C(3)	6847(4)	5128.5(12)	1576(6)	166(5)	11.4(5)	437(15)	−6(3)	137(14)	40(4)
C(4)	5340(4)	5161.7(11)	2620(6)	153(5)	10.4(4)	373(13)	15(2)	60(13)	25(4)
C(5)	3342(3)	4706.3(11)	5675(6)	104(4)	10.5(4)	374(12)	19(2)	39(11)	−1(4)
C(6)	3016(3)	4263.6(12)	7507(6)	92(4)	13.6(5)	368(12)	10(2)	103(11)	−14(4)
C(7)	4216(3)	3798.7(10)	8239(5)	93(3)	10.8(4)	256(10)	0(2)	46(9)	−11(4)
C(8)	5735(3)	3785.4(10)	7027(5)	84(3)	8.6(4)	244(10)	3(2)	40(9)	−9(3)
C(9)	6046(3)	4240.8(10)	5206(5)	90(3)	9.3(4)	264(10)	0(2)	77(9)	−12(3)
C(10)	4878(3)	4709.3(10)	4507(5)	122(4)	9.0(4)	295(11)	9(2)	62(11)	−7(4)
O(2)	9369(2)	4544.2(9)	1346(3)	140(3)	15.2(4)	529(11)	−7(2)	265(10)	24(4)
O(7)	3986(2)	3358.7(8)	10063(4)	118(3)	15.0(4)	380(9)	9(2)	187(8)	38(3)
O(8)	6956(2)	3340.5(7)	7795(3)	97(3)	8.9(3)	267(7)	10(1)	31(7)	−10(3)
C(1′)	6948(3)	2890.6(10)	5697(5)	86(3)	8.8(4)	257(10)	1(2)	73(9)	1(3)
C(2′)	8446(3)	2478.4(10)	6858(5)	72(3)	10.2(4)	257(10)	3(2)	74(9)	10(3)
C(3′)	8403(3)	1909.3(10)	5086(5)	88(3)	9.0(4)	308(11)	8(2)	128(10)	6(3)
C(4′)	6589(3)	1635.9(10)	4591(5)	92(4)	8.8(4)	368(12)	1(2)	89(10)	−4(4)
C(5′)	5224(3)	2106.6(10)	3459(5)	90(4)	10.2(4)	319(11)	2(2)	82(10)	−14(4)
C(6′)	3361(3)	1882.9(12)	3132(6)	85(4)	16.2(6)	462(14)	−9(2)	50(12)	−40(5)
O(2′)	10096(2)	2766.2(7)	6861(4)	82(3)	13.6(3)	380(9)	−12(2)	88(7)	−16(3)
O(3′)	9586(2)	1483.2(8)	6537(4)	88(3)	12.2(3)	560(11)	20(2)	138(9)	38(3)
O(4′)	6420(3)	1173.1(9)	2518(5)	132(3)	14.8(4)	731(14)	8(2)	91(11)	−107(4)
O(5′)	5343(2)	2585.1(7)	5492(4)	77(2)	10.3(3)	341(8)	1(2)	77(7)	−21(3)
O(6′)	2875(3)	1778.6(11)	5449(5)	115(4)	27.0(6)	633(13)	−23(2)	202(11)	−10(5)
O(W1)	856(2)	3394.7(8)	11974(4)	121(3)	14.9(4)	372(9)	3(2)	159(8)	−14(3)
O(W2)	9260(4)	637.2(11)	10761(6)	353(7)	20.9(6)	853(17)	50(3)	764(18)	35(5)

	x	y	z	B(Å^2)		x	y	z	B(Å^2)
H(C3)	0.716(4)	0.542(1)	0.010(7)	2.4(7)	H(C6′a)	0.243(4)	0.216(1)	0.212(6)	1.3(6)
H(C4)	0.459(4)	0.545(2)	0.200(7)	2.6(8)	H(C6′b)	0.322(4)	0.149(1)	0.202(7)	2.6(7)
H(C5)	0.258(4)	0.502(1)	0.500(7)	2.7(8)	H(O2′)	1.026(5)	0.297(2)	0.850(7)	3.6(8)
H(C6)	0.197(4)	0.424(2)	0.819(7)	2.3(7)	H(O3′)	1.056(4)	0.156(2)	0.624(7)	2.8(8)
H(O7)	0.312(4)	0.338(2)	1.079(7)	2.7(7)	H(O4′)	0.734(4)	0.105(1)	0.235(7)	2.1(7)
H(C1′)	0.705(4)	0.305(1)	0.388(6)	0.9(6)	H(O6′)	0.298(5)	0.215(2)	0.696(8)	5.4(10)
H(C2′)	0.835(3)	0.237(1)	0.886(6)	0.7(6)	H(W1a)	0.049(4)	0.320(2)	1.319(7)	2.8(8)
H(C3′)	0.876(4)	0.199(1)	0.333(6)	1.5(6)	H(W1b)	0.040(4)	0.373(2)	1.169(7)	2.9(8)
H(C4′)	0.635(4)	0.152(1)	0.647(6)	1.4(6)	H(W2a)	0.956(6)	0.035(2)	0.968(10)	5.7(11)
H(C5′)	0.542(4)	0.225(1)	0.150(6)	1.2(6)	H(W2b)	0.954(5)	0.089(2)	0.955(8)	4.4(9)

Table 3. The endocyclic torsion angles vary from 48 to 66°. The pyranose ring has an approximate mirror symmetry normal to the mean plane of the ring and passing through the ring O and C(3′) atoms.[15] The maximum deviation from this pseudo mirror palne in torsion angle is 3.7°(between C(1′)–C(2′) and C(4′)–C(5′).) The conformation of the primary alcohol group is in a gauche-gauche orientation. although slightly distorted from the ideal 60° staggered form.

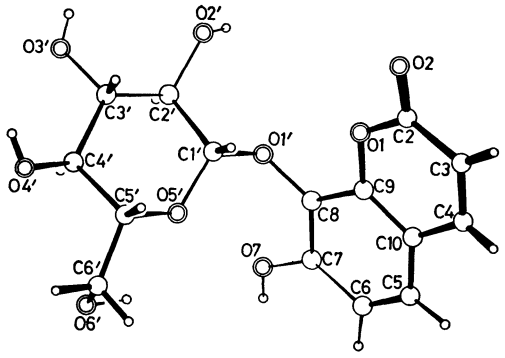


Fig. 1. Perspective drawing of the molecule with numbering system used.

Conformation of Glucosidic Bond. The bond lengths and angles in the sequence C(5′)–O(5′)–C(1′)–O(1′)–R have been discussed as an anomeric effect; it is expected that in the β-pyranose C(5′)–O(5′)≃O(5′)–C(1′)>C(1′)–O(1′), while in the α-anomer C(5′)–O(5′)>O(5′)–C(1′)≃C(1′)–O(1′).[14,16,17] In this crystal, however, C(5′)–O(5′) is significantly longer than O(5′)–C(1′) and C(1′)–O(1′). A similar geometry has been observed in some other aromatic β-pyranosides, such as *p*-nitrophenyl β-D-*N*-acetylglucosaminide[18] and 6-bromo-2-naphtyl β-D-glucopyranoside.[19] The relevant

valence angles are consistent with those in the β-anomer. Table 4 shows the geometry of the glycosidic bond lengths and angles in some pyranosides.

The valence angle of the oxygen atom linking the coumarin and glucose group is markedly smaller than those in the other aromatic glycopyranosides and is rather close to those in the methyl glucosides(Table 4).

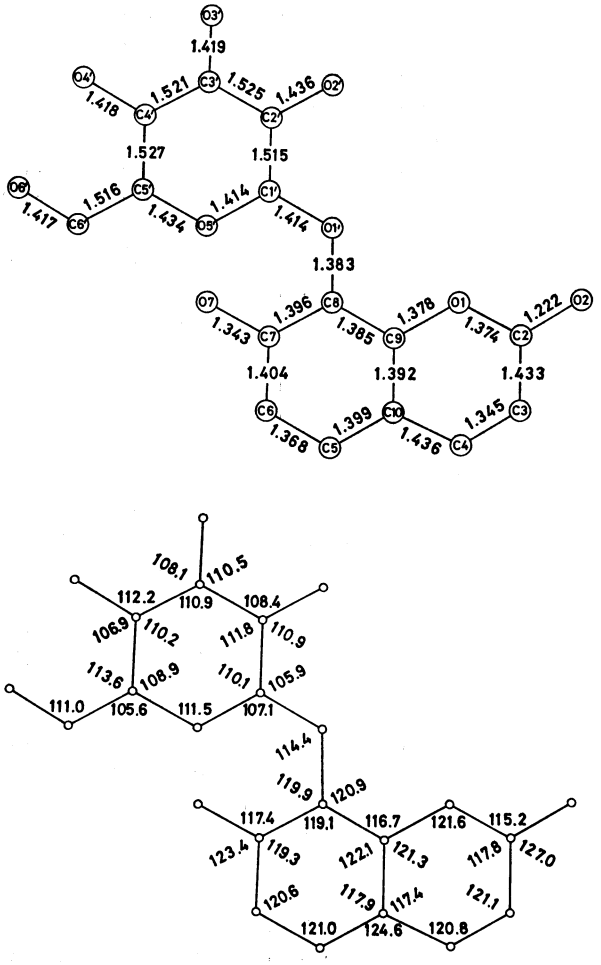
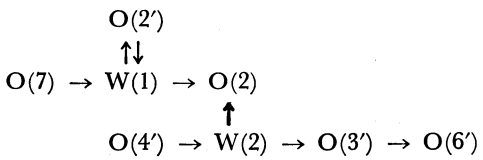


Fig. 2. Bond lengths (Å) and angles (°). The estimated standard deviations are 0.003—0.004 (Å) for lengths and 0.2—0.3 (°) for angles.

The O(1')–C(1') bond is twisted by 78.2° against the coumarin plane, which is the largest value in aromatic glycosides (ϕ in Table 4), probably owing to the repulsion between the O(7) and O(5') atoms. The orientation of the O(1')–C(8) bond is gauche and trans to the C(1')–O(5') and C(1')–C(2') bonds (−66.6 and 174.0°, respectively).

Crystal Structure. The crystal structure viewed down the c axis is shown in Fig. 3. The hydrogen bond lengths and angles are listed in Table 5. The O···O distances range from 2.716 to 2.918 Å. When a water molecule acts as a donor the lengths tends to be slightly larger than in other cases. This tendency is generally observed in the hydrogen bonds of amino acids and sugars.[24] The scheme of the three-dimensional hydrogen bond network is shown below:



where → indicates the donor direction. The water molecule W(1) is concerned with four hydrogen bonds in a tetrahedral arrangement, whereas W(2) is concerned with three hydrogen bonds in a pyramid. The difference in the hydrogen bond modes may reflect the discrepancy between the thermal parameters of these water molecules. The hydroxyl group O(6')H does not act as a hydrogen bond donor, although some weak interaction to intramolecular O(5')

Table 3. Endo- and exocyclic torsion angles
Only for exocyclic angles is the whole sequence of atoms given.

Endocyclic		Exocyclic	
C(1')C(2')	54.3°	O(8)C(1')C(2')O(2')	−69.3°
C(2')C(3')	−48.2	O(2')C(2')C(3')O(3')	69.4
C(3')C(4')	50.0	O(3')C(3')C(4')O(4')	−69.7
C(4')C(5')	−58.0	O(4')C(4')C(5')C(6')	62.5
C(5')O(5')	66.0	C(4')C(5')C(6')O(6')	69.4
O(5')C(1')	−63.9	O(5')C(5')C(6')O(6')	−49.9

Table 4. Geometry of anomeric C-O-C-O-R systems in some aromatic glycosides
Bond lengths and angles are denoted as $C(5')\overset{a}{-}O(5')\underset{\alpha}{\overset{b}{-}}C(1')\underset{\beta}{\overset{c}{-}}O(1')\underset{\gamma}{\overset{d}{-}}R$.

	a(Å)	b(Å)	c(Å)	d(Å)	α(°)	β(°)	γ(°)	θ(°)[a]	ϕ(°)[b]	Ref.
Daphnetin 8-β-glucoside	1.434	1.414	1.414	1.383	111.5	107.1	114.4	−66.6	78.2	this work
p-Nitrophenyl β-*N*-acetylglucosaminide	1.442	1.409	1.399	1.377	112.8	107.7	120.0	−80.9	16.4	18
p-Nitrophnyl β-xyloside	1.427	1.415	1.393	1.379	110.0	109.4	118.5	−75.1	23.6	19
6-Br-2-naphtyl β-glucoside	1.441	1.392	1.416	1.376	111.7	108.0	117.5	−78.3	0.7	20
Methyl β-glucoside	1.440	1.433	1.380	1.430	111.5	108.1	113.1	−73.2		21
Methyl α-glucoside	1.439	1.415	1.401	1.422	113.5	113.0	113.9	63.0		15
Novobiocin[c]	1.474	1.458	1.410	1.394	118.7	111.4	121.2	30	35	22
Phenyl α-maltoside	1.447 1.446	1.421 1.397	1.408 1.427	1.384 1.390	114.3 115.4	113.0 112.5	117.8 119.3	94 77	42 10	23

a) Torsion angle of O(5')-C(1')-O(1')-R. b) Rotation angle around O(1')-R. c) A derivative of coumarin 7-α-L-lyxoside.

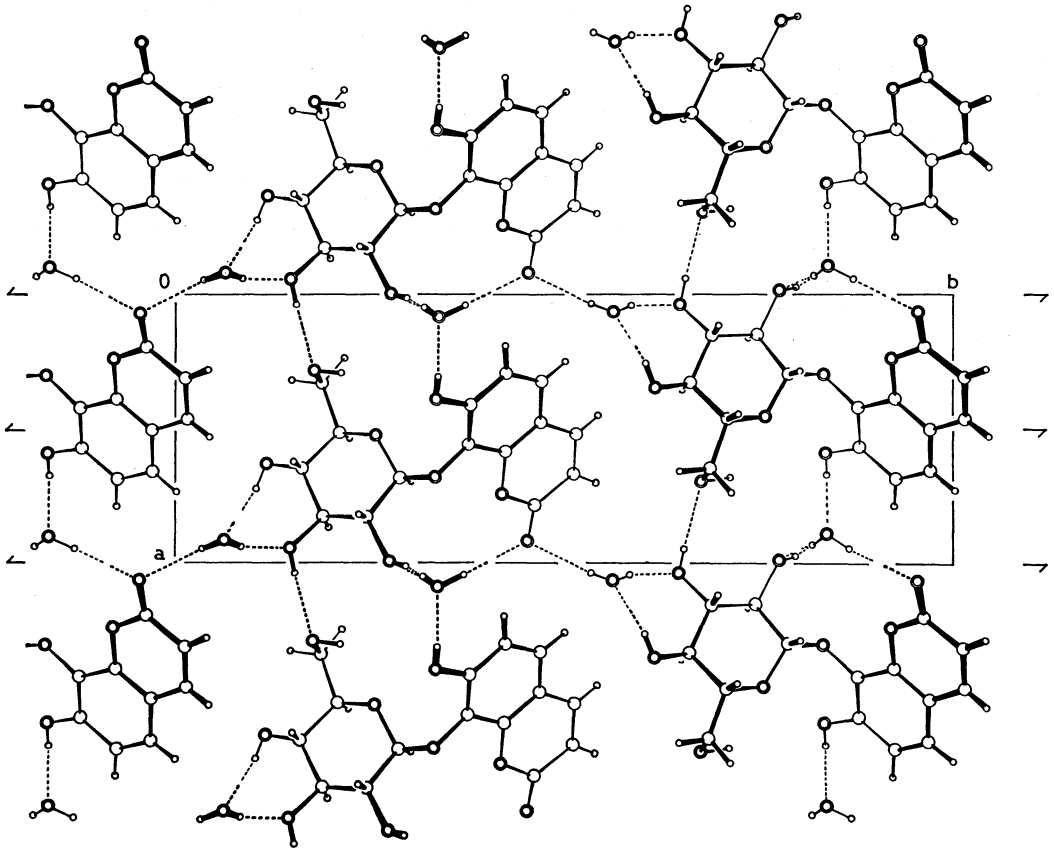


Fig. 3. The crystal structure viewed down the c axis. Hydrogen bonds are indicated by broken lines.

Table 5. Hydrogen bond distances (Å) and angles (°)

Donor atom (D)	(H)	Acceptor atom (A)		Distantances D···A	H···A	Angle D-H···A
O(7)	H(O7)	O(W1)	i	2.736(3)	1.94(4)	172(4)
O(2′)	H(O2′)	O(W1)	ii	2.770(3)	1.89(5)	174(3)
O(3′)	H(O3′)	O(6′)	ii	2.716(3)	1.91(4)	175(4)
O(4′)	H(O4′)	O(W2)	iii	2.764(4)	2.00(4)	162(4)
O(W1)	H(W1a)	O(2′)	iv	2.849(3)	2.05(4)	166(4)
O(W1)	H(W1b)	O(2)	v	2.826(3)	1.99(4)	175(4)
O(W2)	H(W2a)	O(2)	vi	2.918(4)	2.08(5)	157(4)
O(W2)	H(W2b)	O(3′)	i	2.797(4)	1.96(4)	166(4)

Symmetry code:
i) x, y, z, ii) $1+x, y, z$, iii) $x, y, -1+z$,
iv) $-1+x, y, z$, v) $-1+x, y, 1+z$, vi) $2-x, -1/2+y, 1-z$.

can be expected (distances of O(5′)–O(6′) and O(5′)–H(O6′) are 2.664 and 2.28 Å, respectively).

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