The Crystal and Molecular Structure of Daphnin Dihydrate: 7-(β-D-Glucopyranosyloxy)-8-hydroxycoumarin Dihydrate

Katsuhiko Ueno,* Mitsuhiko Sato,† and Norio Saito††

Research Institute for Polymers and Textiles, Yatabe-Higashi 1-1-4, Tsukuba, Ibaraki 305

†*Department of Biology, Faculty of Science, Tokyo Metropolitan University, Setagaya-ku, Tokyo 154*

††*Chemical Laboratory, Meiji-gakuin University, Minato-ku, Tokyo 108*

(Received March 13, 1982)

The title compound, $C_{15}H_{16}O_9 \cdot 2H_2O$, crystallizes in orthorhombic space group $P2_12_12_1$ with $a=7.082(2)$, $b=34.789(5)$, $c=6.867(2)$ Å, and $Z=4$. The structure was solved by direct method and refined by least-squares to a final R of 0.038 based on 1841 observed reflections measured on a four-circle diffractometer. The coumarin ring is approximately planar and the torsion angle around the glucosidic C–O bond is 13.5°; this is much smaller than the corresponding value in 8-glucosyloxy-7-hydroxycoumarin, and causes considerable intramolecular repulsion around the glucosidic linkage. The valence angle of the oxygen atom linking the coumarin and glucose moieties is quite large, 118.5°. The glucopyranose portion is in a C_1 chair conformation. Glucosidation at the 7-hydroxyl group brings about considerable decrease of the resonance system of the 7-hydroxylated coumarin moiety. Such an effect seems to play an important role in the transglucosidation of dihydroxycoumarin glucosides.

In the course of investigation on the biosyntheses of hydroxylated coumarins and their glucosides, some characteristic transglucosidases were isolated from *Daphne odora* and *Cicholium intybus*.[1] In *D. odora*, it is considered that 7-(β-D-glucosyloxy)-8-hydroxycoumarin (or daphnetin 7-glucoside: **D7G**) is formed from *p*-glucosyloxycinnamic acid and that this is changed into 8-(β-D-glucosyloxy)-7-hydroxycoumarin (or daphnetin 8-glucoside: **D8G**) by the action of transglucosidase. This reaction proceeds *via* two steps: *i.e.*, hydrolysis of **D7G** to 7,8-dihydroxycoumarin (or daphnetin: **D**) and glucose, and glucosidation of **D**, as shown in Fig. 1.[2] The back reaction, the formation of **D7G** from **D8G**, could not be detected. In order to elucidate the structural significance of these substrates for the interconversion reactions, the crystal structures of **D**[3] and **D8G**[4] have been determined. In **D8G** the glucosidation at 8-hydroxyl group of **D** caused little effect on the coumarin structure and the glucosyl moiety takes a conformation so as to minimize the intramolecular repulsion. This paper deals with the crystal structure of **D7G** to determine the effect of glucosidation at 7-hydroxyl group of **D**.

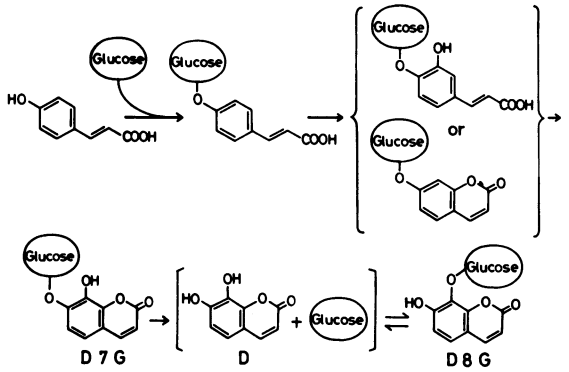


Fig. 1. Biosynthesis of **D7G** and conversion into **D8G**.

Experimental

Single crystals were prepared by slow evapolation from aqueous ethanol solution. They are colorless prisms elongated along the c axis. The space group was determined to be $P2_12_12_1$ from photographs. The density was measured by flotation method in a mixture of carbon tetrachloride and hexane. The lattice constants and intensity data were obtained on a Rigaku four-circle diffractometer with graphite monochromated Cu $K\alpha$ radiation. Of 1940 independent reflections measured within $2\theta<153°$, 1842 had intensities greater than 3σ ($|F_o|$) and these were used for the structure analysis. No correction was made for absorption. The crystallographic data are: $C_{15}H_{16}O_9 \cdot 2H_2O$, F.W.=376.3. Orthorhombic $P2_12_12_1$, $a=7.082(2)$, $b=34.789(5)$, $c=6.867(2)$ Å, $Z=4$, $D_x=1.48$ g cm^{-3}, $D_m=1.48$ g cm^{-3}, μ(Cu $K\alpha$)=12.7 cm^{-1}.

Structure Determination

The structure was solved by the direct method[5] and the structural parameters were refined by block-diagonal least-squares method. All the hydrogen atoms were located on a difference electron density map and their positional and isotropic thermal parameters were included in the refinement. In the later stage of refinement the 012 reflection seemed to be extinct and was given zero weight. The weighting scheme for the other reflections was $w=(10.0/|F_o|)$ if $|F_o|\geq 10.0$, $w=1.0$ if $10.0\geq|F_o|>3.0$, and $w=(|F_o|/3.0)$ if $|F_o|<3.0$. The final R value was 0.038 for 1841 observed reflections. Atomic scattering factors were taken from Ref. 6. The final atomic parameters are shown in Table 1 with equivalent isotropic temperature factors.[7]

Results

Bond Lengths and Angles. The perspective drawing of the molecule with the numbering system is shown in Fig. 2. The daphnetin moiety is nearly planar.[7] The bond lengths and angles are given in Fig. 3. The bond length of carbonyl C=O, 1.226 Å, is larger than those values in coumarin (1.203 Å),[8] gnidicoumarin (1.204 Å),[9] amyrolin (1.210 Å),[10] and xanthotoxin (1.198 Å),[11] and this is close to those of 7-hydroxylated coumarins such as **D** (1.219 Å), **D8G** (1.222 Å), and esculetin (1.227 Å).[12] In the benzene ring, the C(5)–C(6) and C(8)–C(9) bonds are somewhat shorter than the other four C–C bonds. In the 7-hydroxylated

TABLE. 1. FINAL POSITIONAL AND THERMAL PARAMETERS WITH STANDARD DEVIATIONS IN PARENTHESES

Atom	x	y	z	B_{eq}/Å^2
O(1)	0.9588(3)	0.46956(4)	0.6373(3)	2.36
C(2)	0.9211(4)	0.43149(6)	0.6367(3)	2.50
C(3)	0.7293(5)	0.41895(6)	0.6555(4)	2.90
C(4)	0.5871(4)	0.44451(7)	0.6610(4)	2.58
C(5)	0.4862(4)	0.51350(7)	0.6526(4)	2.41
C(6)	0.5355(3)	0.55198(6)	0.6457(4)	2.26
C(7)	0.7259(3)	0.56240(6)	0.6422(4)	1.86
C(8)	0.8685(4)	0.53467(6)	0.6406(4)	1.96
C(9)	0.8148(3)	0.49651(6)	0.6439(4)	1.94
C(10)	0.6258(4)	0.48515(6)	0.6512(4)	2.06
O(2)	1.0578(3)	0.41018(5)	0.6181(3)	3.46
O(8)	1.0547(2)	0.54355(5)	0.6322(4)	3.06
C(1′)	0.6645(3)	0.62976(6)	0.6751(4)	1.94
C(2′)	0.7813(4)	0.66600(6)	0.7045(4)	2.08
C(3′)	0.6495(4)	0.70052(6)	0.7261(4)	1.94
C(4′)	0.5086(4)	0.70284(6)	0.5606(4)	2.07
C(5′)	0.4087(4)	0.66413(6)	0.5406(4)	2.10
C(6′)	0.2741(4)	0.66150(7)	0.3703(5)	2.95
O(1′)	0.7913(2)	0.59970(4)	0.6341(3)	2.11
O(2′)	0.8932(3)	0.66158(5)	0.8749(3)	2.99
O(3′)	0.7648(3)	0.73416(4)	0.7336(3)	2.49
O(4′)	0.3737(3)	0.73202(5)	0.6095(3)	2.94
O(5′)	0.5474(3)	0.63445(4)	0.5103(3)	2.09
O(6′)	0.3679(3)	0.66581(5)	0.1877(3)	3.21
O(W1)	1.1749(3)	0.71713(5)	0.9644(3)	3.02
O(W2)	1.1732(3)	0.60535(5)	0.8236(3)	3.20

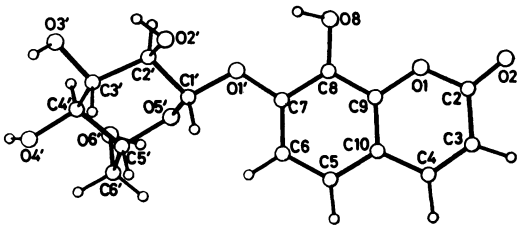


Fig. 2. Perspective drawing of the molecule with the numbering system used.

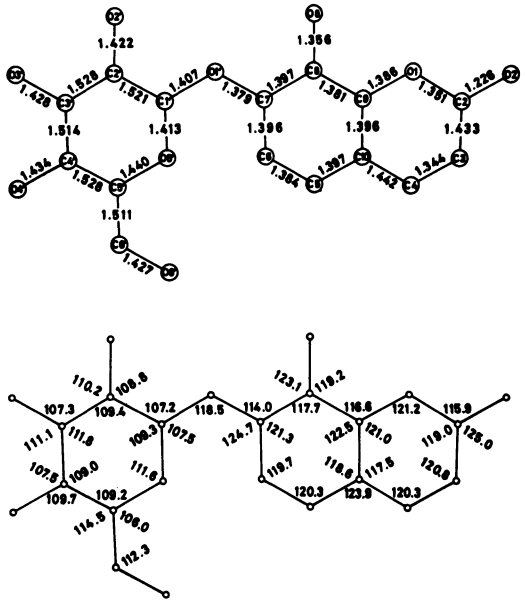


Fig. 3. Bond lengths (l/Å) and angles (ϕ/°). The e.s.d.s are 0.003—0.004 Å for lengths and 0.2—0.3° for angles.

coumarins so far determined by X-rays, the differences in length between C(5)–C(6) and C(6)–C(7) are significantly large. They are 0.024, 0.036, 0.039, and 0.032 Å for **D**, **D8G**, esculetin, and 4-methylumbelliferone,[13] respectively. However, the difference in this molecule, 0.012 Å, is rather small.

The glucopyranose ring is in the usual C_1 chair conformation; the endo- and exocyclic torsion angles are similar to those of **D8G** (Table 2). The anomeric effect in the bond lengths of the sequence C(5′)–O(5′)–C(1′)–O(1′) is unusual: C(5′)–O(5′)>O(5′)–C(1′)≃C(1′)–O(1′), which is similar to the case in **D8G**, whereas in usual β-glucosides C(5′)–O(5′)≃O(5′)–C(1′)>C(1′)–O(1′).[14]

Conformation of the Glucosyl Linkage. The anomeric C(1′)–O(1′) bond is twisted only by 13.8° against the daphnetin plane with the valence angle at O(1′) of 118.5°. The corresponding values in **D8G** are 101.8° and 114.4°, respectively, as shown in Fig. 4. While the glucosyl moiety in **D8G** takes the conformation so as to minimize intramolecular repulsion, the small torsion angle of glucosidic bond in **D7G** results in unusually short intramolecular distances between C(1′)···C(6) (2.863 Å) and C(1′)···H(C6) (2.59 Å). A great difference between the bond angles of C(6)–C(7)–O(1′) and C(8)–C(7)–O(1′), 10.7°, also indicates steric hindrance, while the corresponding values in **D** and **D8G** are only 6.0° and 6.3°, respectively. The angle at the glucosyl oxygen in **D7G** is definitely larger than that in **D8G** and is approximately the same as the value of the sp^2 angle. The geometries of the glycosyl linkage in the aromatic β-glycopyranosides so far determined are listed in Table 3. All of them, except for **D8G**, are similar to the

TABLE 2. ENDO- AND EXOCYCLIC TORSION ANGLES. ONLY FOR EXOCYCLIC ANGLES IS THE ENTIRE SEQUENCE OF ATOMS GIVEN

Endocyclic	ϕ/°		Exocyclic	ϕ/°	
	D7G	**D8G**		**D7G**	**D8G**
C(1′)C(2′)	57.3(2)	54.3(2)	O(1′)C(1′)C(2′)O(2′)	−66.1(2)	−69.3(2)
C(2′)C(3′)	−52.2(2)	−48.2(2)	O(2′)C(2′)C(3′)O(3′)	66.1(2)	69.4(2)
C(3′)C(4′)	52.1(2)	50.0(2)	O(3′)C(3′)C(4′)O(4′)	−69.2(2)	−69.7(2)
C(4′)C(5′)	−57.0(2)	−58.0(2)	O(4′)C(4′)C(5′)C(6′)	66.9(2)	62.5(3)
C(5′)O(5′)	65.6(2)	66.0(2)	C(4′)C(5′)C(6′)O(6′)	62.6(3)	69.4(3)
O(5′)C(1′)	−65.6(2)	−63.9(2)	O(5′)C(5′)C(6′)O(6′)	57.8(3)	−49.9(3)

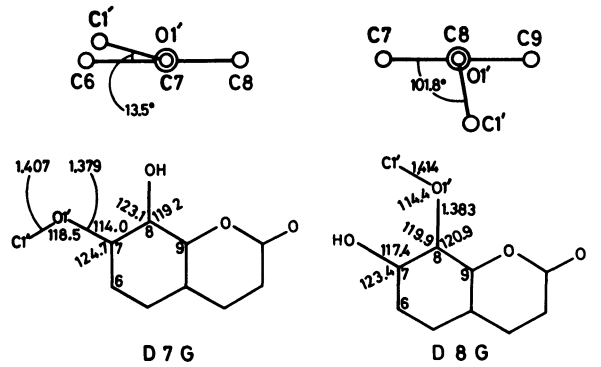


Fig. 4. Comparison of the glucosyl linkage in **D7G** and **D8G**.

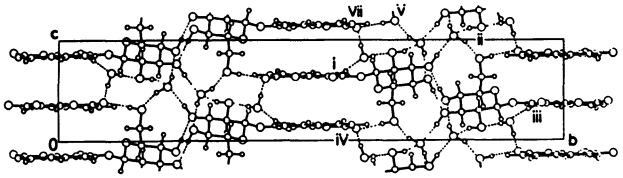


Fig. 5. Crystal structure viewed down the a axis. Hydrogen bonds are indicated by broken lines.

Table 3. Geometry of glycosidic linkage in aromatic β-glycopyranoside

	θ/° a)	φ/°b)	δ/°c)	Ref.
D7G	13.8	118.5	10.7	This work
D8G	78.2	114.4	6.0	4
p-Nitrophenyl β-*N*-acetylglucosaminide	16.0	120.0	9.7	17
p-Nitrophenyl β-D-xylopyranoside	23.6	118.5	9.3	18
p-Br-2-naphtyl β-D-glucoside	0.7	117.5	15.5	19
D	—	—	6.3	3

a) Torsion angle of glycosidic bond and aromatic plane. b) Valence angle at the bridge oxygen atom. c) Difference of the valence angles between each side of the glycosidic bond connecting to aromatic ring (between C(8)–C(7)–O(1′) and C(6)–C (7)–O(1′) in **D7G** and corresponding values in the others).

Table 4. Hydrogen bond distances and angles

Donor	Acceptor	Distance *l*/Å		Angle φ/°
(D)	(A)	D···A	H···A	D–H–A
O(8)H	O(W2)^{i}	2.656(3)	1.71(4)	157(4)
O(2′)H	O(W1)^{i}	2.845(3)	2.04(4)	146(4)
O(3′)H	O(W1)^{ii}	2.752(3)	1.79(4)	172(4)
O(4′)H	O(3′)^{iii}	2.744(3)	1.83(4)	179(4)
O(6′)H	O(2)^{iv}	2.738(3)	1.96(4)	154(4)
O(W1)Ha	O(6′)^{v}	2.721(3)	1.80(4)	160(4)
O(W1)Hb	O(4′)^{vi}	2.862(3)	2.04(4)	168(4)
O(W2)Ha	O(2)^{vii}	2.830(3)	1.94(4)	156(4)
O(W2)Hb	O(2′)^{i}	2.808(3)	2.08(4)	164(4)

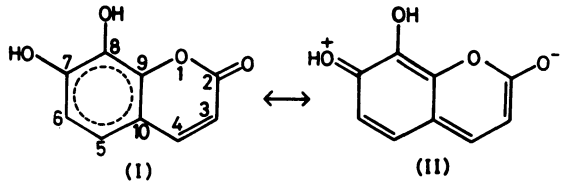
Symmetry codes: (i) x, y, z; (ii) $-1/2+x, 3/2-y, 2-z$; (iii) $-1/2+x, 3/2-y, 1-z$; (iv) $3/2-x, 1-y, -1/2+z$; (v) $1+x, y, 1+z$; (vi) $1+x, y, z$; (vii) $5/2-x, 1-y, 1/2+z$.

structure in **D7G**. It is thus plausible that the glycosyl bond of aromatic β-glycopyranoside usually takes a conformation with small torsion angle of θ. π Electrons of the oxygen atom of glycosyl linkage may take part in the resonance of the aromatic ring and stabilize the molecular structure in spite of the large intramolecular repulsion; this will be explained in the discussion.

Crystal Structure. Figure 5 shows the crystal structure viewed along the a axis. The coumarin moieties are stacked along the c axis and a three-dimensional network of hydrogen bonding connects the molecules. The hydrogen bonds O(3′)–H···O(W1)–H···O(4′) form infinite arrays along the c axis and O(4′)–H···O(3′) connects the glucose moieties related by 2_1 axis along the a axis. The remaining six hydrogen bonds link the three molecules at i, v, and vii. The hydrogen bond parameters are listed in Table 4.

Discussion

It has been found that the structure of **D** can be well explained by the following resonance:



The contribution from the limiting structure(II) is also apparent in the related structures: esculetin, which has a hydroxyl group at the 6 position instead of 8, and 4-methylumbelliferone, which contains a methyl group bonded to C(4) instead of the 8-hydroxyl group. Such a conjugation effect has been postulated to account for the characteristic nature of the spectral[15] and chemical[16] properties in 7-hydroxylated coumarins.

The structure of **D8G** reveals that the glucosidation at 8-hydroxyl group of **D** causes little effect on the resonance of daphnetin moiety. The C(5)–C(6) bond is significantly shorter than C(6)–C(7) and the C(2)–O(2) bond is longer than the corresponding one of coumarin.

In the present **D7G** molecule, however, the contribution from the structure(II) is expected to be less as a result of the glucosidation at the 7-hydroxyl group. The difference between C(5)–C(6) and C(6)–C(7) is significantly smaller than that in **D8G**. Although a slight contribution from the structure(II) is observed for **D7G**, most of the resonance energy may be compensated by the intramolecular repulsion between the coumarin ring and the glucose moiety. These results clearly indicate that **D8G** is more stable energetically than **D7G**.

Action of transglucosidase for dihydroxycoumarin glucosides has already been shown in Fig. 1. Although the structure of the active site of the enzyme is still unknown, the structures of substrate and product can explain the reaction mechanism. At the intermediate stationary state of the enzymatic reaction, both **D7G** and **D8G** may be formed from **D** and glucose; however the formation of **D8G** is considered to be far more favorable in terms of the stability of the molecule, so

that glucose is transferred from **D7G** to **D8G**.

The authors are grateful to Professor Yoshio Sasada and Dr. Yuji Ohashi of Tokyo Institute of Technology for their valuable discussions and encouragement.

References

1) M. Sato and M. Hasegawa, *Phytochemistry*, **10**, 2367 (1971); **11**, 3149 (1972).
2) M. Sato and M. Hasegawa, *Phytochemistry*, **11**, 657 (1972).
3) K. Ueno and N. Saito, *Acta Crystallogr., Sect. B*, **32**, 946 (1976).
4) K. Ueno, N. Saito, and M. Sato, *Bull. Chem. Soc. Jpn.*, **51**, 3170 (1978).
5) J. Karle and H. Hauptman, *Acta Crystallogr.*, **9**, 635 (1956); J. Karle and I. L. Karle, *ibid.*, **21**, 849 (1966); G. Germaine and M. M. Woolfson, *ibid., Sect. B*, **24**, 91 (1968).
6) "International Tables for X-Ray Crystallography," Kynoch Press, Birmingham (1974), Vol. IV, pp. 72—73.
7) Lists of structure factors, anisotropic thermal parameters, atomic parameters for hydrogen atoms and the equation of least-squares plane have been deposited as Document No. 8319 at the Office of the Chemical Society of Japan.
8) E. Gavuzzo, F. Mazza, and E. Giglio, *Acta Crystallogr., Sect. B*, **30**, 1351 (1974).
9) M. S. Shen and R. F. Bryan, *Acta Crystallogr., Sect. B*, **31**, 2907 (1975).
10) K. Kato, *Acta Crystallogr., Sect. B*, **26**, 2022 (1970).
11) N. R. Stemple and W. H. Watson, *Acta Crystallogr., Sect. B*, **28**, 2485 (1972).
12) K. Ueno and N. Saito, *Acta Crystallogr., Sect. B*, **33**, 283 (1977).
13) S. Shimizu, S. Kashino, and M. Haisa, *Acta Crystallogr., Sect. B*, **31**, 1287 (1975).
14) M. Sundaralingam, *Biopolymers*, **6**, 189 (1968); S. Arnott and W. E. Scott, *J. Chem. Soc., Perkin Trans. 2*, **1972**, 324.
15) W. E. Sherman and E. Robins, *Anal. Chem.*, **40**, 803 (1968); G. Y. Yakatan, R. J. Juneau, and S. G. Schulman, *ibid.*, **44**, 1044 (1972); G. S. Beddard, S. Carlin, and R. S. Davidson, *J. Chem. Soc., Perkin Trans. 2*, **1977**, 262.
16) F. M. Dean, "Naturally Occurring Oxygen Ring Compounds," Butterworths, London (1973), Chap. 6, pp. 177—180.
17) L. Brehm and J. Moult, *Proc. R. Soc. London, Ser. B*, **188**, 425 (1975).
18) K. Harata, *Acta Crystallogr., Sect. B*, **32**, 1932 (1975).
19) K. Harata, private communication.