The Crystal and Molecular Structures of Esculetin 6-Glucoside and 7-Glucoside

Katsuhiko UENO,* Masaru SHIRAKI, 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 158*
††*Chemical Laboratory, Meiji-gakuin University, Minato-ku, Tokyo 108*
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The structures of two dihydroxycoumarin glucosides, esculetin 6-glucoside (**E6G**) and escultin 7-glucoside (**E7G**) have been determined by the X-ray analysis. The crystal data are: **E6G**, $C_{15}H_{16}O_9 \cdot 1.5H_2O$, orthorhombic $P2_12_12_1$ with a=11.358(2), b=36.850(9), c=7.663(1) Å and Z=8: and **E7G**, $C_{15}H_{16}O_9 \cdot 2H_2O$, orthorhombic $P2_12_12_1$ with a=4.507(1), b=13.077(3), c=27.748(5) Å and Z=4. Both structures were solved by the direct method and refined by the least-squares to the final R values of 0.073 (2036 reflections) for **E6G** and 0.069 (1037 reflections) for **E7G**. The geometries of the glucosyl linkages in **E6G** and **E7G** are different: the torsion angles around the glucosidic C–O bond are −58.2° and 14.3° for the two crystallographically independent **E6G** molecules and 9.9° for **E7G**. The glucosidation at the 6-hydroxyl group causes little effect on the conjugation of the coumarin moiety, whereas the conjugation is greatly decreased in the glucosidation at 7-hydroxyl group. This fact suggests that **E6G** is energetically more stable than **E7G**. The enzymatic transglucosidation from 7- to 6-hydroxyl group may proceed utilizing such an energy difference.

In the course of investigation on the biosyntheses of hydroxylated coumarin glucosides, characteristic transglucosidases were isolated from *Daphne odora* and *Cichorium intybus*. In *C.intybus*, it is considered that 7-*O*-(β-D-glucosyloxy)-6-hydroxycoumarin (or esculetin 7-glucoside: **E7G**) is formed from *p*-glucosyloxycinnamic acid, and that it is changed into 6-*O*-(β-D-glucosyloxy)-7-hydroxycoumarin (or esculetin 6-glucoside: **E6G**) by the action of transglucosidase.[1] A similar reaction has been observed for 7,8-dihydroxycoumarin (or daphnetin: **D**), where glucose is transferred from 7- to 8-hydroxyl group.[2] The transglucosidation to 7-hydroxyl group from 6- or 8-hydroxyl group was undetectable in either case. Figure 1 shows the above reactions schematically. In order to elucidate the mechanism, the structures of esculetin(**E**),[3] **D**,[4] daphnetin 8-glucoside(**D8G**)[5] and daphnetin 7-glucoside(**D7G**)[6] have been determined. The coumarin moiety of **D8G** has approximately the same structure as those of **D** and **E**, whereas that of **D7G** is significantly different from that of **D8G**. The glucosidation at 7-hydroxyl group has an important role in the resonance system of the coumarin moiety. Moreover, the geometries of the glucosyl linkages of **D8G** and **D7G** are different. It has been proposed that such structural difference is a factor for the transglucosidation from **D7G** to **D8G**. Present paper reports the structures of **E6G** and **E7G** and discusses the mechanism of another transglucosidation from **E7G** to **E6G** on the basis of the two structures.

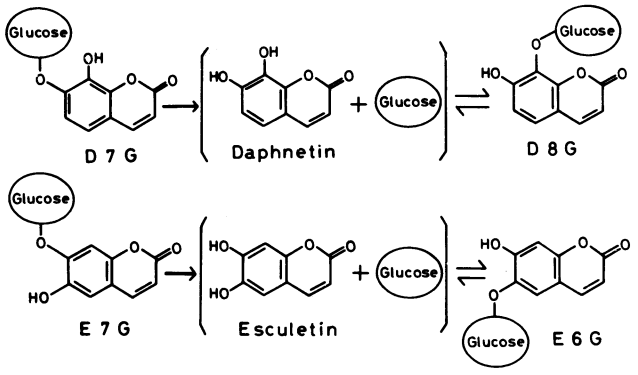


Fig. 1. Interconversions of dihydroxycoumarin 7-glucoside to 6- or 8-glucoside.

Experimental

Both crystals were prepared by slow evaporation from an aqueous ethanol solution. The lattice parameters and the intensity data up to 2θ=120° were obtained on a Nicolet P3/F four-circle diffractometer with graphite monochromated Cu $K\alpha$ radiation. An $\omega/2\theta$ scanning mode was applied with the scanning range and rate of 1.0° (ω) plus α_1—α_2 divergence and 1° (2θ)min^{-1}, respectively. Space groups were assigned from systematic absences. No correction was made for absorption. The densities were measured by flotation method in a mixture of dichloromethane and carbon tetrachloride. Crystal data of **E6G**; $C_{15}H_{16}O_9 \cdot 1.5H_2O$, F.W=367.3, orthorhombic $P2_12_12_1$, a=11.358(2), b=36.850(9), c=7.663(1) Å, U=3207.3 (11) Å^3, Z=8, needle, 0.08×0.04×0.18 mm^3 in size, D_x=1.524 g cm^{-3}, D_m=1.52 g cm^{-3}, μ(Cu $K\alpha$)=13.0 cm^{-1}, 2552 reflections measured of which 2036 had net intensities. Crystal data of **E7G**; $C_{15}H_{16}O_9 \cdot 2H_2O$, F.W=376.3, orthorhombic $P2_12_12_1$, a=4.507(1), b=13.077(3), c=27.748(5) Å, U=1635.4(6) Å^3, Z=4, thin plate, 0.08×0.04×0.02 mm^3 in size, D_x=1.528 g cm^{-3}, D_m=1.53 g cm^{-3}, μ(Cu $K\alpha$)=13.1 cm^{-1}, 1354 reflections measured of which 1037 had net intensities.

Structure Determination

Both structures were solved by the direct method[7] and the structural parameters were refined by the block-diagonal least-squares method. Anisotropic thermal parameters were applied for the non-hydrogen atoms. All the hydrogen atoms were located on difference electron density maps. The refinement including the

contribution of the hydrogen atoms with isotropic temperature factors converged to the R values of 0.073 and 0.069 for **E6G** and **E7G**, respectively. The weighting scheme used in the final stages were $w=((\sigma|F_o|)^2+(0.00012|F_o|)^2)^{-1}$ and $w=((\sigma|F_o|)^2+(0.00016|F_o|^2)^{-1}$ for **E6G** and **E7G**, respectively. Atomic scattering factors were taken from Ref. 8. The final atomic parameters are shown in Table 1.[9]

Results

The perspective drawings of the two crystallographically independent **E6G** molecules, **E6G(A)** and **E6G(B)**, and **E7G** with the numbering system of the atoms are shown in Fig. 2. Bond lengths and angles are listed in Table 2. The coumarin moieties are nearly planar.[9] The conformations of glucosyl linkages are different among the three molecules. In **E6G(B)** and **E7G**, the torsion angles around the glucosidic C–O bond, C(5)–C(6)–O(1′)–C(1′) for **E6G(B)** and C(8)–C(7)–O(1′)–C(1′) for **E7G**, are 14.3° and 9.9°, respectively, whereas the corresponding angle in **E6G(A)** is −58.2°. The former "in-plane" conformation appears to cause considerable intramolecular repulsion between the coumarin and glucose moieties, compared with the latter "out-of-plane" conforma-

Table 1. Positional and thermal parameters with standard deviations in parentheses

E6G

	x	y	z	B_{eq}/Å²
O(1)A	0.2430(5)	0.2613(2)	0.0654(9)	3.01
C(2)A	0.1834(9)	0.2297(2)	0.0264(14)	3.12
C(3)A	0.0584(9)	0.2293(3)	0.0687(14)	3.37
C(4)A	0.0052(8)	0.2574(3)	0.1456(15)	3.34
C(5)A	0.0205(8)	0.3207(2)	0.2673(15)	2.99
C(6)A	0.0866(8)	0.3508(2)	0.2938(13)	2.36
C(7)A	0.2050(8)	0.3516(2)	0.2480(13)	2.54
C)8)A	0.2585(8)	0.3211(2)	0.1710(13)	2.40
C(9)A	0.1874(8)	0.2911(2)	0.1424(13)	2.49
C(10)A	0.0696(8)	0.2899(2)	0.1889(13)	2.32
O(2)A	0.2396(6)	0.2058(2)	−0.0385(11)	4.27
O(1′)A	0.0404(6)	0.3815(2)	0.3792(9)	2.95
O(7)A	0.2660(5)	0.3829(2)	0.2794(10)	3.50
C(1′)A	−0.0587(7)	0.3971(2)	0.2926(12)	2.00
C(2′)A	−0.1254(8)	0.4180(2)	0.4305(13)	2.48
C(3′)A	−0.2292(7)	0.4376(2)	0.3430(11)	1.68
C(4′)A	−0.1824(7)	0.4609(2)	0.1941(13)	2.20
C(5′)A	−0.1090(8)	0.4378(2)	0.0683(13)	2.16
C(6′)A	−0.0539(9)	0.4588(2)	−0.0736(13)	2.77
O(2′)A	−0.1716(6)	0.3950(2)	0.5595(9)	3.95
O(3′)A	−0.2904(5)	0.4597(2)	0.4664(9)	2.85
O(4′)A	−0.2840(5)	0.4755(2)	0.1009(9)	2.65
O(5′)A	−0.0141(5)	0.4208(1)	0.1644(9)	2.36
O(6′)A	0.0013(6)	0.4346(2)	−0.1943(8)	3.27
O(1)B	0.0308(5)	0.2453(2)	0.6352(10)	3.12
C(2)B	0.0946(10)	0.2183(3)	0.5556(14)	3.66
C(3)B	0.2136(10)	0.2259(3)	0.5053(16)	3.89
C(4)B	0.2626(9)	0.2594(2)	0.5348(14)	2.84
C(5)B	0.2429(7)	0.3212(2)	0.6654(14)	2.26
C(6)B	0.1727(8)	0.3458(2)	0.7479(13)	2.33
C(7)B	0.0544(8)	0.3382(2)	0.7848(13)	2.64
C(8)B	0.0064(8)	0.3041(2)	0.7510(14)	2.58
C(9)B	0.0806(8)	0.2795(2)	0.6696(14)	2.52
C(10)B	0.1941(8)	0.2864(2)	0.6231(13)	2.52
O(2)B	0.0406(7)	0.1900(2)	0.5301(11)	4.83
O(1′)B	0.2058(5)	0.3798(2)	0.8065(9)	2.78
O(7)B	−0.0175(6)	0.3626(2)	0.8637(11)	4.25
C(1′)B	0.3129(8)	0.3946(2)	0.7459(14)	2.82
C(2′)B	0.3136(8)	0.4341(2)	0.8128(13)	2.53
C(3′)B	0.4321(8)	0.4521(2)	0.7839(13)	2.34
C(4′)B	0.5322(8)	0.4272(2)	0.8465(13)	2.24
C(5′)B	0.5175(8)	0.3899(2)	0.7630(12)	1.94
C(6′)B	0.6154(8)	0.3630(2)	0.8182(13)	2.50
O(2′)B	0.2227(6)	0.4555(2)	0.7335(10)	3.30
O(3′)B	0.4416(5)	0.4856(2)	0.8791(10)	3.26
O(4′)B	0.6442(5)	0.4418(1)	0.7987(9)	2.45
O(5′)B	0.4068(5)	0.3756(1)	0.8200(8)	2.08
O(6′)B	0.6285(6)	0.3621(2)	1.0006(10)	3.57
O(1)W	0.4955(7)	0.3747(2)	0.2853(10)	4.23
O(2)W	0.2282(6)	0.4585(2)	0.3752(11)	4.26
O(3)W	0.4502(7)	0.4673(2)	0.2487(11)	5.61

E7G

	x	y	z	B_{eq}/Å²
O(1)	0.6308(15)	0.1098(4)	0.3482(2)	3.57
C(2)	0.5803(21)	0.0217(7)	0.3209(3)	3.41
C(3)	0.3831(20)	0.0308(7)	0.2802(3)	3.83
C(4)	0.3393(20)	−0.0352(7)	0.2598(3)	3.32
C(5)	0.1584(20)	0.3024(7)	0.2885(3)	4.02
C(6)	0.2103(18)	0.3855(7)	0.3174(3)	2.70
C(7)	0.4106(19)	0.3749(5)	0.3556(3)	2.45
C(8)	0.5455(18)	0.2829(5)	0.3671(3)	2.58
C(9)	0.4870(16)	0.2011(5)	0.3367(3)	2.34
C(10)	0.3031(18)	0.2111(7)	0.2970(3)	3.23
O(2)	0.7150(15)	−0.0526(5)	0.3337(2)	4.74
O(6)	0.0790(15)	0.4759(5)	0.3073(2)	3.63
C(1′)	0.6616(17)	0.4726(7)	0.4160(3)	2.35
C(2′)	0.7473(17)	0.5864(5)	0.4215(3)	1.88
C(3′)	0.9345(18)	0.6056(5)	0.4658(3)	2.16
C(4′)	0.8036(17)	0.5541(6)	0.5096(3)	2.45
C(5′)	0.7494(18)	0.4413(6)	0.4988(3)	2.33
C(6′)	0.6015(20)	0.3835(6)	0.5402(3)	3.38
O(1′)	0.4454(14)	0.4654(4)	0.3806(2)	2.95
O(2′)	0.8838(13)	0.6233(4)	0.3796(2)	2.45
O(3′)	0.9561(13)	0.7134(4)	0.4729(2)	2.75
O(4′)	1.0524(13)	0.5638(4)	0.5478(2)	2.62
O(5′)	0.5472(13)	0.4331(4)	0.4593(2)	2.25
O(6′)	0.5567(14)	0.2777(4)	0.5293(2)	3.32
O(W1)	0.6246(16)	0.6556(5)	0.6108(2)	4.40
O(W2)	0.4833(17)	0.7322(5)	0.3268(3)	5.60

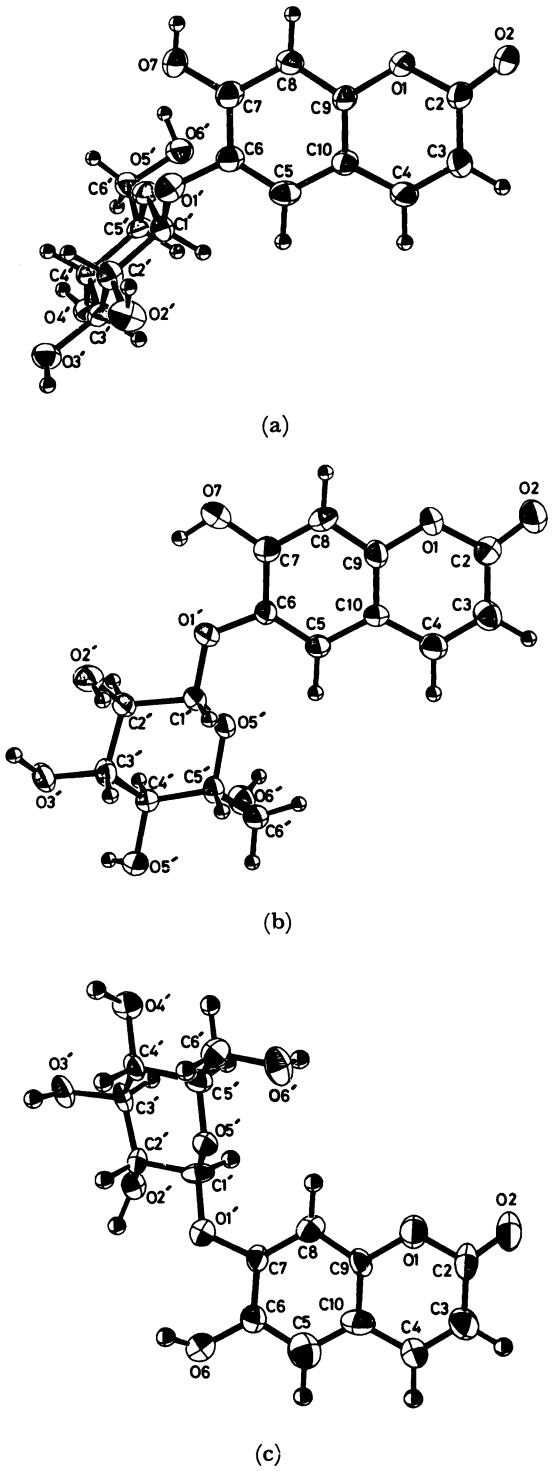


Fig. 2. Perspective drawing of the molecules with the numbering systems; (a): **E6G(A)**, (b): **E6G(B)**, (c): **E7G**.

tion. The angles of C(6)-O(1')-C(1') and C(5)-C(6)-O(1') in **E6G(B)** are 118.5(8)° and 126.2(8)°, which are significantly larger than those in **E6G(A)**, 113.7(7)° and 121.4(9)°, respectively. The corresponding angles in **E7G**, C(7)-O(1')-C(1') and C(8)-C(7)-O(1'), are 119.5(6)° and 125.4(7)°, respectively, and are similar to those of **E6G(B)**. They must be expanded to avoid the short contacts between the coumarin and glucose moieties.

In the benzene rings of **E6G(A)** and **E6G(B)**, the C(5)-C(6) bonds are significantly shorter than C(5)-C(10) and C(6)-C(7), and C(8)-C(9) is slightly shorter than C(7)-C(8). Such a quinonoid structure has been found in the structures of **D8G** and other 7-hydroxylated coumarins. In **E7G**, however, the six bonds in the benzene ring are approximately equivalent, as observed in **D7G**.

The glucose moieties in the three molecules are in C_1 chair conformations. The endo- and exocyclic torsion angles in the glucose portions are in good agreement with each other. The conformations around the C(5')-C(6') bond are trans-gauche in **E6G(A)** and **E7G** and gauche-gauche in **E6G(B)**. Each conformation corresponds to either of the two favored arrangement of the primary alcohol group in glucopyranoside.[10]

The bond lengths in the sequence of C(5')-O(5')-C(1')-O(1')-R have been discussed as an anomeric effect. Sundaralingam claimed that in α-pyranose C(5')-O(5')>O(5')-C(1')≃C(1')-O(1'), while in β-anomer C(5')-O(5')≃O(5')-C(1')>C(1')-O(1').[11] In **E6G** and **E7G**, however, C(5')-O(5') are longer by 0.019—0.041 Å than O(5')-C(1') and C(1')-O(1'). The same trend is observed in other aromatic β-glucosides so far determined such as in **D7G**, **D8G** and 1-naphthyl tetra-*O*-acetyl-β-D-glucoside,[12] and even in the α-anomer such as in *p*-nitrophenyl α-D-glucoside.[13] This indicates that in aromatic glucopyranosides, not only in α-anomer but also in β-anomer, C(5')-O(5') is longer than O(5')-C(1') and C(1')-O(1').

Figure 3 shows the crystal structure of **E6G** viewed along the c axis. The coumarin planes of **A** and **B** molecules are stacked alternately along the c axis. All the hydroxyl groups and water molecules make hydrogen bonds and form a three-dimensional network. The hydrogen bonding schemes around the glucose moieties are different between **A** and **B** as shown in Fig. 4. All the hydroxyl groups of **E6G(B)** participate in hydrogen bonds as a donor and an acceptor. In **E6G(A)**, on the other hand, O(2')H acts as a very weak donor and not an acceptor. The hydrogen bonding system of **E6G(B)** is apparently more favorable than that of **E6G(A)**.

The crystal structure of **E7G** viewed along the a axis is shown in Fig. 5. The molecules are connected by the hydrogen bonds to form sheets perpendicular to the c axis. The sheets are stacked along the c axis with the van der Waals contacts.

Discussion

The glucose moiety at the 7-position takes the "in-plane" conformation in **E7G** as in **D7G**. The torsion

Table 2. Bond lengths (l/Å) and angles (ϕ/°) with estimated standard deviations in parentheses

	E6G(A)	E6G(B)	E7G		E6G(A)	E6G(B)	E7G
O(1)-C(2)	1.379(13)	1.374(13)	1.395(11)	C(9)-C(10)	1.384(14)	1.361(14)	1.384(11)
O(1)-C(9)	1.396(12)	1.404(12)	1.393(10)	C(1')-C(2')	1.509(13)	1.543(15)	1.542(12)
C(2)-C(3)	1.455(15)	1.431(16)	1.441(13)	C(1')-O(1')	1.427(11)	1.410(13)	1.387(11)
C(2)-O(2)	1.196(14)	1.224(14)	1.198(11)	C(1')-O(5')	1.407(11)	1.396(12)	1.405(11)
C(3)-C(4)	1.337(16)	1.374(16)	1.320(13)	C(2')-C(3')	1.536(13)	1.513(14)	1.511(11)
C(4)-C(10)	1.442(15)	1.430(15)	1.464(13)	C(2')-O(2')	1.401(12)	1.435(12)	1.400(10)
C(5)-C(6)	1.353(15)	1.363(14)	1.369(13)	C(3')-C(4')	1.521(13)	1.536(14)	1.508(11)
C(5)-C(10)	1.399(15)	1.435(14)	1.379(13)	C(3')-O(3')	1.428(11)	1.438(12)	1.424(10)
C(6)-C(7)	1.389(14)	1.400(14)	1.398(13)	C(4')-C(5')	1.532(14)	1.525(13)	1.522(11)
C(6)-O(1')[a]	1.408(12)	1.384(12)	1.349(11)	C(4')-O(4')	1.458(12)	1.427(12)	1.422(10)
C(7)-C(8)	1.405(14)	1.391(14)	1.383(12)	C(5')-C(6')	1.532(14)	1.548(14)	1.527(12)
C(7)-O(7)[b]	1.366(13)	1.354(13)	1.379(11)	C(5')-O(5')	1.446(11)	1.430(11)	1.428(10)
C(8)-C(9)	1.384(14)	1.387(15)	1.386(11)	C(6')-O(6')	1.429(12)	1.405(12)	1.428(11)

a) C(6)-O(6) in **E7G**, b) C(7)-O(1') in **E7G**.

	E6G(A)	EG(B)	E7G		E6G(A)	EG(B)	E7G
C(2)-O(1)-C(9)	122.3(8)	121.3(8)	120.3(8)	C(5)-C(10)-C(9)	118.0(9)	118.3(9)	120.0(8)
O(1)-C(2)-C(3)	116.1(9)	118.4(10)	117.2(8)	C(6)-O(1')-C(1')[c]	113.7(7)	118.5(8)	119.5(6)
O(1)-C(2)-O(2)	116.8(10)	115.0(10)	115.1(8)	O(1')-C(1')-C(2')	106.0(7)	105.0(8)	108.1(7)
C(3)-C(2)-O(2)	127.1(10)	126.6(11)	127.6(8)	O(1')-C(1')-O(5')	106.8(7)	109.3(8)	108.9(6)
C(2)-C(3)-C(4)	121.9(10)	120.9(11)	122.2(8)	C(2')-C(1')-O(5')	110.7(7)	109.5(8)	111.1(7)
C(3)-C(4)-C(10)	121.1(10)	118.8(10)	121.3(8)	C(1')-C(2')-C(3')	108.6(8)	111.5(8)	112.4(6)
C(6)-C(5)-C(10)	120.4(10)	118.2(9)	120.3(9)	C(1')-C(2')-O(2')	111.9(8)	111.9(8)	111.0(6)
C(5)-C(6)-C(7)	121.0(10)	121.5(9)	118.4(8)	C(3')-C(2')-O(2')	107.9(8)	109.6(8)	111.9(6)
C(5)-C(6)-O(1')[a]	121.4(9)	126.4(9)	119.8(7)	C(2')-C(3')-C(4')	109.0(7)	110.6(8)	111.3(6)
C(7)-C(6)-O(1')[a]	117.4(9)	112.2(8)	121.8(7)	C(2')-C(3')-O(3')	110.7(7)	111.6(8)	108.3(6)
C(6)-C(7)-C(8)	120.5(9)	121.2(9)	122.9(7)	C(4')-C(3')-O(3')	110.1(7)	107.3(8)	110.9(6)
C(6)-C(7)-O(7)[b]	117.6(9)	122.3(9)	111.6(6)	C(3')-C(4')-C(5')	110.3(8)	108.9(8)	109.5(6)
C(8)-C(7)-O(7)[b]	121.9(9)	116.4(9)	125.4(7)	C(3')-C(4')-O(4')	107.4(7)	110.8(8)	107.6(6)
C(7)-C(8)-C(9)	116.9(9)	115.9(9)	116.4(7)	C(5')-C(4')-O(4')	109.1(8)	109.1(8)	109.9(6)
O(1)-C(9)-C(8)	115.5(9)	115.3(9)	115.5(6)	C(4')-C(5')-C(6')	113.7(8)	112.6(8)	113.5(7)
O(1)-C(9)-C(10)	121.3(9)	120.0(9)	122.7(7)	C(4')-C(5')-O(5')	109.0(8)	107.5(7)	109.1(6)
C(8)-C(9)-C(10)	123.2(9)	124.8(10)	121.7(7)	C(6')-C(5')-O(5')	106.6(8)	108.0(7)	105.2(6)
C(4)-C(10)-C(5)	124.7(10)	121.2(9)	123.5(7)	C(1')-O(5')-C(5')	110.8(7)	111.1(7)	113.2(7)
C(4)-C(10)-C(9)	117.2(9)	120.6(9)	116.0(8)	C(5')-C(6')-O(6')	109.6(8)	111.3(8)	112.3(7)

a) O(1') should be replaced by O(6) in **E7G**. b) O(7) should be replaced by O(1') in **E7G**. c) C(7)-O(1')-C(1') angle in **E7G**.

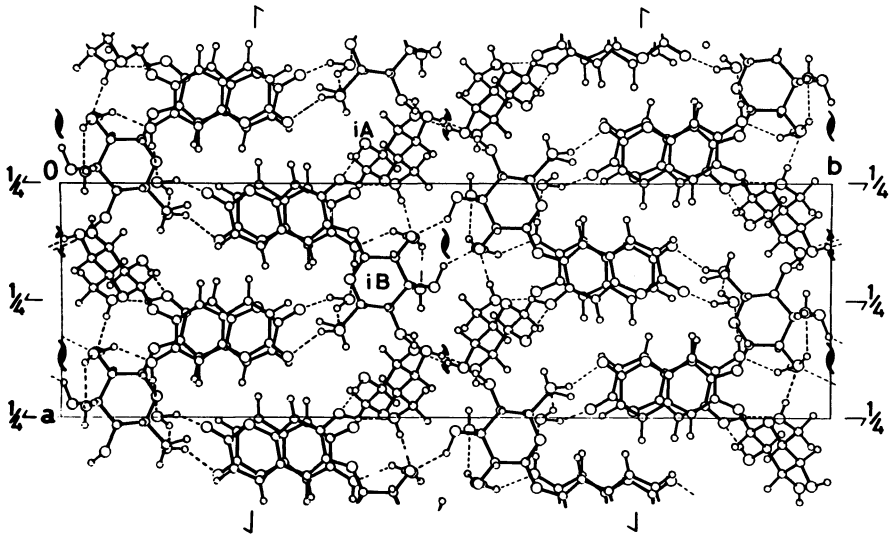


Fig. 3. The crystal structure of **E6G** viewed down the c axis. Hydrogen bonds are indicated by broken lines.

angles in the aromatic β-glucosides so far determined are summarized in Table 3. The glucose or alkyl moiety bonded to the hydroxyl group tend to take the "in-plane" conformation, in which π electrons of the oxygen atom of glucosyl linkage would take part in the resonance of the aromatic ring. Such an "in-plane" conformation would cause the intramolecular repulsion between the coumarin and glucose moieties as observed in the structures of **E7G** and **D7G**. The resonance energy brought about by the "in-plane" conformation may be compensated by the distorsion of the structure. **E6G** or **D8G**, however, is not required to take the "in-plane" conformation, since 6- and 8-hydroxyl group has little effect on the resonance of the coumarin moiety.

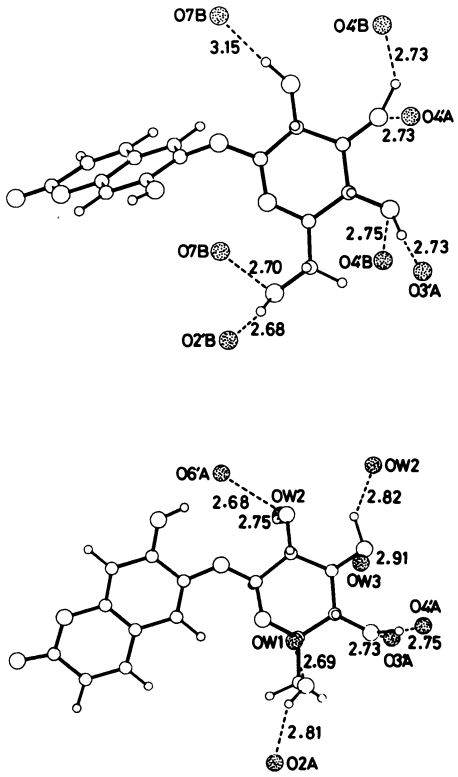


Fig. 4. Hydrogen bonding systems around the glucose moieties of **E6G**(upper; **A**, lower; **B** molecule).

Although **E6G(A)** has an "out-of-plane" conformation, **E6G(B)** is in an "in-plane" conformation. This may be explained by the difference of the hydrogen bond systems of the two molecules as shown in Fig. 4. The intramolecular repulsion in **E6G(B)** would be stabilized by the fairly strong intermolecular hydrogen bonds. In solution, therefore, **E6G** would have the structure close to that of **E6G(A)**.

The structures of the 7-hydroxylated coumarins are well explained by the resonance shown in Fig. 6. The benzene ring apparently takes a quinonoid structure in 7-hydroxycoumarin (umbelliferone: U),[16] 4-methylumlbelliferone,[17] **D**[4] and **E**.[3] Since **E6G** and **D8G** have essentially the same structure as those observed in the 7-hydroxylated coumarins, the glucosidation at the 8-hydroxyl group or at the 6-hydroxyl group causes little effect on the structure of the coumarin moiety. In **E7G** and **D7G**, however, the contributions of the limiting structure(II) is significantly smaller than those in **E6G** and **D8G**, respectively. In the structure of 7-ethoxycoumarin, recently determined, the contribution of (II) are apparently lowered compared with the structure of 7-hydroxycoumarin.[16] The glucosidation at the 7-hydroxyl group tends to decrease the cojugation effect. This seems to indicate that **E6G** and **D8G** are energetically more stable than **E7G** and **D7G**, respectively.

Although the structure of the active site of the enzyme is still unknown, the mechanism of the transglucosidation, as shown in Fig. 1, can be explained by the thermodynamic properties of the substrates and products. From the intermediate stationary state, both of forward and backward reactions could occur.

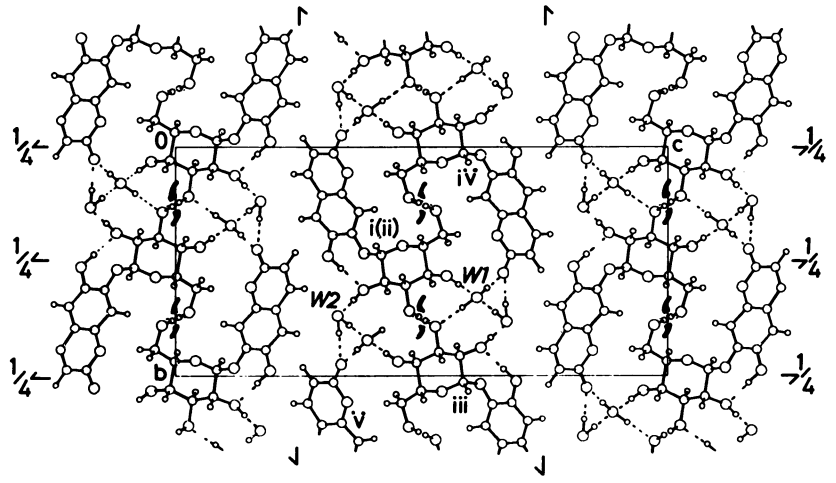
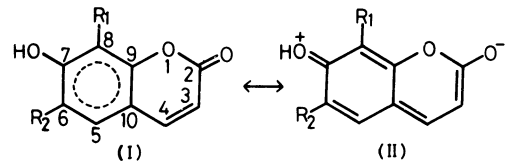


Fig. 5. The crystal structure of **E7G** viewed down the a axis. Hydrogen bonds are indicated by broken lines.



	E	D	U
R_1	H	OH	H
R_2	OH	H	H

Fig. 6. The conjugation system expected in 7-hydroxylated coumarins.

Table 3. Geometry of glycosyl linkages in aromatic glycosides

	ϕ/° a)	α/° b)	
E7G	9.9	119.6	This work
E6G(A)	−58.2	113.7	This work
E6G(B)	14.3	118.5	This work
D7G	13.8	118.5	6
D8G	−78.2	114.4	5
PNPNAG c)	16.4	120.0	14
PNPX d)	23.6	118.5	15
NTAG e)	11.2	119.7	12
U7E f)	2.6	117.7	16

a) Torsion angle around the bond between glycosidic O and aromatic C atoms (e.g., C(1′)-O(1′)-C(7)-C(8) in E7G). b) Valence angle of glycosidic oxygen atom. c) *p*-Nitrophenyl *β*-*N*-acetylglucosaminide. d) *p*-Nitrophenyl *β*-D-xylopyranoside. e) 1-Naphthyl tetraacetyl-*β*-D-glucoside. f) 7-Ethoxycoumarin.

However, the formation of **D8G** or **E6G** would be more favorable than that of **D7G** or **E7G**, since the former is energetically more stable than the latter. This should be a reason why glucose is transferred from 7-hydroxyl group to 8- or 6-hydroxyl group by the action of transglucosidase and the reverse is undetectable.

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