Novel Molecular Conformation of (*R*,*S*)-Hesperetin in Anhydrous Crystal

Satoshi FUJII,* Yuriko YAMAGATA, Guang-Zhu JIN, and Ken-ichi TOMITA

Faculty of Pharmaceutical Sciences, Osaka University, 1–6 Yamadaoka, Suita, Osaka 565, Japan.
Received July 19, 1993; accepted December 26, 1993

Novel molecular conformation of racemic hesperetin, (±)-2,3-dihydro-5,7,-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one, was determined by X-ray analysis. A new form was crystallized from ethanol solution and its molecular conformation is quite different from that of monohydrate crystal (*Acta Crystallogr.*, C43,1946 (1987)). The aromatic ring part of benzopyrone and the phenyl ring form the twist orientation (dihedral angle of two rings, Φ is 53.1(3)°), in contrast to the parallel arrangement in the monohydrate form ($\Phi = 0.6°$). The pyrone ring forms a slightly flattened sofa conformation, where C(2) is displaced by 0.40(2) Å from the pyrone plane, in contrast to the large displacement in the monohydrate form (0.54 Å). There is a strong intramolecular hydrogen bond between keto O(4) atom and hydroxy H(O5)–O(5) group which forms a six-membered ring conjugated with benzopyrone rings. The degree of conjugation is also slightly different of the two forms and may relate to the difference of hydrogen bonding network and stacking mode of aromatic rings.

Keywords hesperetin; benzopyrone; sofa-conformation; X-ray analysis; conformational flexibility

The mixture of hesperidin and rutin is usually called vitamin P, and both are natural flavonoids widely distributed in plants. Hesperetin is a component of hesperidin and has a similar biological function of sweet taste.[1] To clarify the conformational properties and compare with the structure of (*R*,*S*)-naringenin, (±)-2,3-dihydro-5,7,-dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one,[2] we carried out an X-ray structure determination of racemic hesperetin. The crystal structure of racemic hesperetin monohydrate was reported.[3] We obtained a new crystal in an anhydrous form from a different solvent system. The new molecular conformation is quite different from the previous monohydrate form.

Experimental

Racemic hesperetin was purchased from Sigma Chemical Co., St. Louis, and was crystallized from ethanol solution. The crystal data are as follows: chemical formula $C_{16}H_{14}O_6$; $M_r = 302.29$; space group $P2_1/c$; $a = 12.464(2)$, $b = 16.226(3)$, $c = 7.102(1)$ Å, $\beta = 104.24(2)°$, $V = 1392.2$ Å^3; $Z = 4$; observed density $D_m = 1.440(5)$ Mgm^{-3} by flotation in benzene–chloroform–ethylene dibromide mixture; calculated density $D_x = 1.442$ Mgm^{-3}; $\mu(CuK_\alpha) = 0.89$ mm^{-1}. The intensity data were measured on a Rigaku automatic four-circle diffractometer (AFC-5R-300) as follows: ω–2θ scanning technique; $2\theta_{max} = 126°$; graphite- monochromatized CuK_α radiation at the 10 °C; crystal dimensions $0.3 \times 0.2 \times 0.15$ mm; intensity fluctuation (< 1.0%) monitored periodically by three reflections; total of 2037 reflections measured; 1285 unique reflections observed with $F_O > 6\sigma(F_O)$ ($-14 \le h \le 13$, $0 \le k \le 18$, $0 \le l \le 8$); Lorentz and polarization corrections, but no absorption correction; unit-cell dimensions by least-squares procedure based on 2θ values ($48° < 2\theta < 60°$) of 40 reflections. The structure was solved by direct methods SHELXS86[4] and was refined by full-matrix least squares, SHELX76[5] with anisotropic temperature factors for all non-hydrogen atoms. Hydrogen atoms except three atoms of methoxy group were found at the calculated position in a difference Fourier map. The final refinement by fixing H atoms at the calculated position with constant isotropic temperature factor reduced R to 0.077 ($R_\omega = 0.077$, $s = 1.829$) for 1285 observed reflections and 199 variables with the weighting scheme $\omega(F_O - F_C)^2$ where $\omega = 1.0$. The average and maximum of shift/deviation ratios are $(\Delta/\sigma)_{ave} = 0.10$, $(\Delta/\sigma)_{max} = 0.31$ (thermal parameter U_{33} of atom C(2)). The maximum and minimum peak heights in the final difference Fourier map are 0.40 and $-0.30\,e$Å^{-3}, respectively. All calculations were performed with program system UNICS[6] on the ACOS 930 computer of the Protein Engineering Research Center, Institute for Protein Research, Osaka University. Atomic scattering factors were taken from the International Tables for X-ray Crystallography.[7]

Results and Discussion

Final atomic parameters are given in Table I. An ORTEP drawing of the molecule with numbering is shown in Fig. 1.[8] Bond lengths, bond angles and selected torsion angles are listed with those of related compounds in Table II. The crystal packing is shown in Fig. 2. The thermal parameters for C(2) and C(7′) atoms are large. In the case of C(2), the large vibration along normal vector of the pyrone ring, which intersects *a*–*b* plane at almost 40°, shows a widely spread puckering amplitude of C(2) (Fig. 2). No close contact between the terminal methoxy group and the adjacent molecule was observed in this crystal (Fig. 2) and the large value of thermal parameters of C(7′) is also consistent with the freedom of this group.

The most significant difference between the anhydrous

TABLE I. Fractional Coordinates of Non-H Atoms and Equivalent Isotropic Temperature Factors with e.s.d.'s in Parentheses
$U_{eq} = (1/3)\Sigma_i\Sigma_j U_{ij} a_i^* a_j^* \boldsymbol{a}_i \boldsymbol{a}_j$

Atom	x	y	z	U_{eq} (Å^2)
O(1)	0.1673 (4)	0.1749 (3)	0.4461 (8)	0.052 (2)
C(2)	0.2325 (8)	0.1059 (6)	0.4616 (19)	0.107 (5)
C(3)	0.1781 (6)	0.0251 (5)	0.4512 (13)	0.049 (3)
C(4)	0.0644 (6)	0.0213 (5)	0.3112 (13)	0.044 (3)
O(4)	0.0196 (4)	−0.0464 (3)	0.2634 (9)	0.053 (2)
C(5)	−0.0963 (6)	0.1031 (5)	0.1274 (11)	0.041 (3)
O(5)	−0.1520 (4)	0.0334 (3)	0.0586 (9)	0.052 (2)
C(6)	−0.1463 (6)	0.1782 (5)	0.0759 (12)	0.045 (3)
C(7)	−0.0905 (6)	0.2498 (5)	0.1477 (13)	0.047 (3)
O(7)	−0.1435 (4)	0.3212 (3)	0.0956 (8)	0.054 (2)
C(8)	0.0148 (6)	0.2480 (5)	0.2702 (12)	0.039 (3)
C(9)	0.0659 (6)	0.1739 (5)	0.3196 (11)	0.040 (3)
C(10)	0.0135 (6)	0.0982 (4)	0.2489 (11)	0.039 (3)
C(1′)	0.3296 (7)	0.1156 (5)	0.6411 (16)	0.075 (4)
C(2′)	0.4262 (7)	0.0749 (6)	0.6404 (15)	0.068 (4)
C(3′)	0.5146 (6)	0.0799 (5)	0.8021 (13)	0.054 (3)
O(3′)	0.6100 (5)	0.0386 (4)	0.7946 (10)	0.082 (3)
C(4′)	0.5080 (6)	0.1244 (5)	0.9621 (12)	0.048 (3)
O(4′)	0.6030 (4)	0.1248 (4)	1.1067 (9)	0.062 (2)
C(5′)	0.4121 (7)	0.1666 (6)	0.9644 (14)	0.062 (4)
C(6′)	0.3224 (7)	0.1604 (6)	0.8022 (16)	0.072 (4)
C(7′)	0.6022 (8)	0.1684 (8)	1.2748 (15)	0.089 (5)

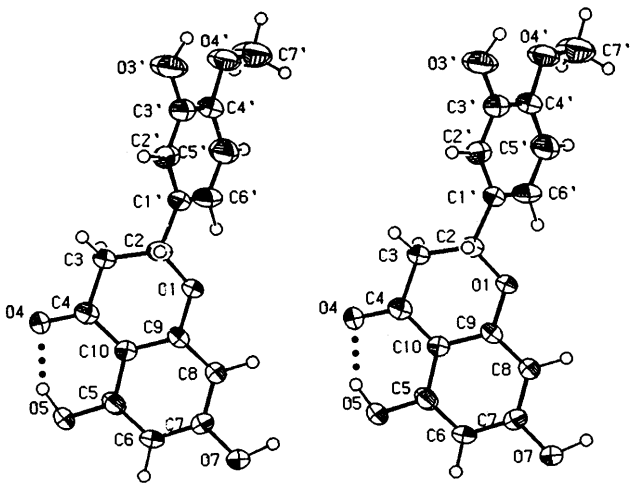

Fig. 1. ORTEP Drawing of Hesperetin with Atom Labelling
Dotted line is intramolecular hydrogen bond.

and monohydrate forms is the relative orientation between the aromatic ring part (ring A) of benzopyrone and phenyl ring (ring C). The two rings in the monohydrate form are nearly parallel to each other (dihedral angle between them, Φ is 0.6°), but in the anhydrous form the two rings have a twist orientation ($\Phi = 53.1(3)°$). In naringenin, a perpendicular arrangement is observed ($\Phi = 85.7°$). The perpendicular arrangement of rings A and C seems energetically more favorable than the parallel or the twist one owing to repulsive steric hindrance between O(1), C(3) and phenyl H(C2') or H(C6'). The intermediate orientation in the anhydrous form is stabilized by aromatic ring stacking among benzopyrone rings and among 4-methoxyphenyl rings as discussed later. There are several reports on the structure–activity relationships of flavone compounds as inhibitors of enzymes such as aldose reductase[9] and cyclic AMP phosphodiesterase.[10] They show that the relative position of the overall methoxy, oxy-substituted is important. As two rotamers were observed in hesperetin crystal, there exist multiple relative positions of O(3') to benzopyrone and a variety of hydrogen bond modes. These possibilities may indicate the functions of this compound and its interactions with many enzyme systems.[11]

The pyrone ring (ring B) has a sofa conformation. C(2) is displaced by 0.40(2) Å from the planar pyrone ring plane, where O(1), C(9), C(10), C(4), and C(3) are displaced by 0.001(8), 0.002(9), −0.007(10), 0.009(10) and −0.006(11) Å, respectively. Comparison of the torsion angles of the pyrone ring with the related compounds listed in Table II, indicates that the buckling amplitude of C(2) is smaller than naringenin (0.44 Å displacement) or monohydrated hesperetin (0.54 Å). This shows the considerable flexibility of the benzopyrone ring.

The keto O(4) and hydroxy O(5) form a strong intramolecular hydrogen bond (2.615(9) Å) as observed in narigenin and related compounds. As shown in Fig. 1, the favored hydrogen bonded six-membered ring is formed. The C(4)–O(4) bond of 1.241(10) Å is slightly longer than the normal carbonyl C–O bond of 1.23 Å, and the C(9)–C(10) bond of 1.423(11) Å is 0.03 Å longer than the

Table II. Bond Length (Å), Bond Angle (°) and Selected Torsion Angle (°) with e.s.d.'s in Parentheses

Bond length			
O(1)–C(2)	1.372 (14)	1.442[a]	
O(1)–C(9)	1.359 (10)	1.361	
C(2)–C(3)	1.470 (16)	1.481	
C(2)–C(1')	1.535 (17)	1.512	
C(3)–C(4)	1.519 (12)	1.504	
C(4)–O(4)	1.241 (10)	1.231	
C(4)–C(10)	1.420 (12)	1.430	
C(5)–O(5)	1.354 (10)	1.361	
C(5)–C(6)	1.377 (12)	1.373	
C(5)–C(10)	1.429 (11)	1.406	
C(6)–C(7)	1.385 (12)	1.410	
C(7)–O(7)	1.340 (11)	1.343	
C(7)–C(8)	1.385 (12)	1.384	
C(8)–C(9)	1.365 (11)	1.377	
C(9)–C(10)	1.423 (11)	1.406	
C(1')–C(2')	1.374 (16)	1.389	
C(1')–C(6')	1.377 (16)	1.381	
C(2')–C(3')	1.385 (14)	1.375	
C(3')–O(3')	1.377 (11)	1.382	
C(3')–C(4')	1.366 (13)	1.390	
C(4')–O(4')	1.363 (11)	1.352	
C(4')–C(5')	1.381 (13)	1.381	
O(4')–C(7')	1.390 (14)	1.437	
C(5')–C(6')	1.398 (15)	1.372	
Bond angle			
C(2)–O(1)–C(9)	118.7 (8)	115.4[a]	
O(1)–C(2)–C(3)	117.9 (10)	112.1	
O(1)–C(2)–C(1')	108.3 (10)	107.0	
C(3)–C(2)–C(1')	113.1 (10)	114.6	
C(2)–C(3)–C(4)	114.4 (8)	111.6	
C(3)–C(4)–O(4)	120.0 (7)	120.7	
C(3)–C(4)–C(10)	116.2 (7)	116.0	
O(4)–C(4)–C(10)	123.8 (8)	123.3	
O(5)–C(5)–C(6)	119.0 (7)	118.8	
O(5)–C(5)–C(10)	120.1 (7)	119.2	
C(6)–C(5)–C(10)	120.9 (7)	122.0	
C(5)–C(6)–C(7)	119.4 (8)	118.5	
C(6)–C(7)–O(7)	117.0 (8)	120.7	
C(6)–C(7)–C(8)	121.7 (8)	121.3	
O(7)–C(7)–C(8)	121.2 (8)	118.0	
C(7)–C(8)–C(9)	119.3 (8)	118.7	
O(1)–C(9)–C(8)	117.3 (7)	117.2	
O(1)–C(9)–C(10)	121.0 (7)	120.6	
C(8)–C(9)–C(10)	121.6 (7)	122.2	
C(4)–C(10)–C(5)	121.7 (7)	121.2	
C(4)–C(10)–C(9)	121.1 (7)	121.4	
C(5)–C(10)–C(9)	116.9 (7)	117.3	
C(2)–C(1')–C(2')	117.4 (10)	120.9	
C(2)–C(1')–C(6')	123.0 (11)	121.2	
C(2')–C(1')–C(6')	119.5 (11)	117.7	
C(1')–C(2')–C(3')	119.2 (10)	120.0	
C(2')–C(3')–O(3')	117.3 (8)	118.7	
C(2')–C(3')–C(4')	121.5 (9)	122.0	
O(3')–C(3')–C(4')	121.2 (8)	119.4	
C(3')–C(4')–O(4')	114.2 (8)	116.9	
C(3')–C(4')–C(5')	120.1 (8)	117.6	
O(4')–C(4')–C(5')	125.6 (8)	125.4	
C(4')–O'4')–C(7')	117.5 (8)	117.7	
C(4')–C(5')–C(6')	118.3 (9)	120.4	
C(1')–C(6')–C(5')	121.4 (10)	122.2	
Torsion angle			
C(9)–O(1)–C(2)–C(3)	−38.1 (13)	−52.6[a]	−49.8[b]
C(2)–O(1)–C(9)–C(10)	18.9 (12)	23.9	24.8
O(1)–C(2)–C(3)–C(4)	36.0 (14)	52.3	50.6
O(1)–C(2)–C(1')–C(2')	−152.8 (10)	−27.4	−120.5
C(3)–C(2)–C(1')–C(6')	−103.1 (13)	33.5	−62.5
C(2)–C(3)–C(4)–C(10)	−15.9 (12)	−25.4	−28.3
C(3)–C(4)–C(10)–C(9)	−1.8 (11)	−2.4	3.1
O(1)–C(9)–C(10)–C(4)	1.3 (12)	4.1	0.4

a) Hesperetin in monohydrate form[3]; The average e.s.d.'s for this crystal are 0.006 Å and 0.4°. *b*) Naringenin; The average e.s.d. for torsion angle is 0.3°.

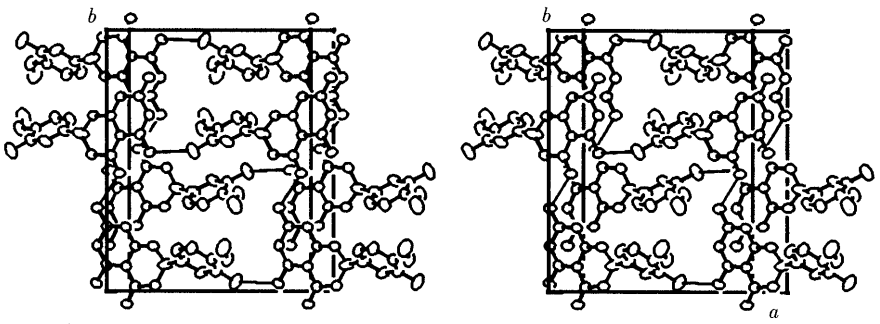

Fig. 2. Stereoscopic Drawing of the Molecular Packing Viewed along *c*-Axis
The lined hydrogen bonds are also indicated.

benzene C–C bond length. The tendency to lengthen in these bonds indicates that the hydrogen bonded ring is considerably conjugated with benzopyrone rings. This conjugate may flatten the sofa conformation noted above. In the case of the monohydrate form, the C(4)–O(4) bond of 1.231 Å and the C(9)–C(10) bond of 1.406 Å indicate that this conjugate is not effective in flattening the displacement of C(2). In the anhydrous form keto O(4) is involved in the intermolecular hydrogen bond network as O(4)···O(7) (2.691(8) Å), while in the monohydrate form, O(4) does not participate in intermolecular hydrogen bonding. The bond length O(1)–C(2) is significantly different between the two forms (1.372(14) Å in anhydrous form and 1.442(6) Å in monohydrate form). We do not yet know the reason for the short length in the anhydrous form. The sp^2 character at C(2) cannot be observed from bond angles around C(2).

Planar rings of benzopyrone face the *a*–*b* plane in crystalline lattice. Benzopyrone moieties are connected through *a* strong O(4)···O(7) hydrogen bond along the *b* direction and through π–π interactions of aromatic rings perpendicular to the *a*–*b* plane. Cyclic tetrametric formation by the additional O(3′)–O(4) hydrogen bond along the *a*-axis is a notable packing feature. The methoxyphenyl rings are also clustered and form weak π–π interaction with each other. This packing mode is quite different from that of the monohydrate form.

References and Notes

1) M. Yamato, K. Hashigaki, K. Mito, T. Koyama, *Chem. Pharm. Bull.*, **26**, 2321 (1978).
2) W. Shin, M. S. Lah, *Acta Crystallogr., Sect. C*, **C42**, 626 (1986).
3) W. Shin, S. Kim, K. S. Chun, *Acta Crystallogr., Sect. C*, **C43**, 1946 (1987).
4) Sheldrick, G. M. (1986), SHELXS86, Program for crystal structure solution, Univ. of Göttingen, Germany.
5) Sheldrick, G. M. (1976), SHELX76, Program for crystal structure determination, Univ. of Cambridge, England.
6) T. Ashida, N. Yasuoka (eds.), "The Universal Crystallographic Computing System: Osaka," Library of Programs, Computation Center, Osaka Univ., 1979.
7) J. A. Ibers, W. C. Hamilton (eds.) "International Tables for X-Ray Crystallography," Vol. IV, Kynoch Press, Birmingham, 1974, p. 72.
8) C. K. Johnson, ORTEP II, Report ORNL-5138, Oak Ridge National Laboratory, Tennessee, U.S.A., 1976.
9) S. D. Varma, J. H. Kinoshita, *Biochem. Pharmacol.*, **25**, 2505 (1976).
10) J. E. Ferrell Jr., P. D. G. Chang Sing, G. Loew, R. King, J. M. Mansour, T. E. Mansour, *Mol. Pharmacol.*, **16**, 556 (1979).
11) M. Rossi, L. F. Rickles, W. A. Halpin, *Bioorganic Chem.*, **14**, 55 (1986).