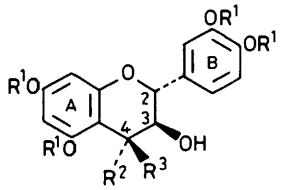
The Molecular and Crystal Structure of (+)-2,3-*trans*-3,4-*trans*-Leucocyanidin [(2*R*,3*S*,4*R*)-(+)-3,3',4,4',5,7-Hexahydroxyflavan] Dihydrate, and Comparison of its Heterocyclic Ring Conformation in Solution and the Solid State

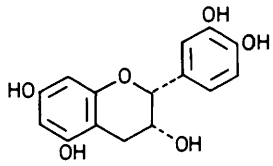
Lawrence J. Porter,† Rosalind Y. Wong, and Bock G. Chan
U.S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center, 800 Buchanan Street, Berkeley, California 94710

The crystal structure of (2*R*,3*S*,4*R*)-(+)-3,3',4,4',5,7-hexahydroxyflavan (**1**) dihydrate has been determined; the crystals are orthorhombic, space group $P2_12_12_1$, and have unit cell dimensions, a = 4.862(1), b = 15.796(4), c = 19.362(5) Å, U = 1 487.0(5) Å^3, Z = 4. The structure was solved by direct methods, refinements being to R = 0.031 for 1 247 independent reflections. The crystals are bound by two infinite networks of hydrogen bonds centred on the solvated water molecules. The conformation of the heterocyclic ring in the solid state is intermediate between a half-chair and a C(3)-sofa, whereas that in solution (acetone or water) is considered to be a C(2)-sofa.

Leucocyanidins (diastereoisomers of 3,3',4,4',5,7-hexahydroxyflavan) are compounds of central importance to the chemistry and biochemistry of procyanidins, the most widely distributed class of condensed tannin. Until the recent synthesis[1] of (2*R*,3*S*,4*R*)-3,3',4,4',5,7-hexahydroxyflavan (**1**) from (2*R*,3*R*)-taxifolin (**2**) there was some doubt as to their existence in the phenolic form because of the extreme acid-lability of the benzylic 4-hydroxy group.[2,3] The synthesis of (**1**) and its



(**1**),(**2**),(**3**),(**5**),(**6**),(**7**)



(**4**)

(**1**) $R^1 = R^3 = H$; $R^2 = OH$
(**2**) $R^1 = H$;R_2, $R_3 = O$
(**3**) $R^1 = R^2 = H$; $R^3 = OH$
(**4**) Epicatechin
(**5**) $R^1 = R^2 = R^3 = H$; Catechin
(**6**) R^1 = Me; R^2 = OH; $R^3 = H$
(**7**) R^1 = Me; R^2 = 2,4,6-$(MeO)_3C_6H_2$; $R^7 = H$

isolation as an acetone solvate showed that the usual methods of work-up used in proanthocyanidin chemistry lead to polymerization, but careful exclusion of traces of acid enabled its isolation.[1] More recently (2*R*,3*S*,4*S*)-3,3',4,4',5,7-hexahydroxyflavan (**3**) has been shown to be the major enzymic reduction product from (2*R*,3*R*)-taxifolin (**2**) in Douglas fir cell suspension cultures.[4] It still remains for a leucocyanidin to be isolated from intact plant tissue.

Problems associated with conformational analysis of the pyran ring of flavanoids has remained an enduring problem ever since the absolute stereochemistries of catechin and epicatechin were first established. The introduction of ^{1}H n.m.r. enabled Clark-Lewis and co-workers[5] to argue for ring conformations at least approximating a half-chair, with the B-ring in an equatorial position, for a series of flavans with phenolic groups protected by methylation and with various heterocyclic ring substituents. Since that time numerous ^{1}H n.m.r. studies have generally borne out these findings. More recently, three *X*-ray crystallographic structures, of epicatechin (**4**),[6] and the 8-bromo-3',4',5,7-tetramethyl ether derivatives of catechin (**5**)[7] and epicatechin[8] have been published which generally support the n.m.r. conclusions.

Of more interest, however, is the conformation of the heterocyclic ring of flavanoids substituted at C(4), since such knowledge is essential to an understanding of the preferred conformation of polyflavanoid chains and also the relative reactivity of substituents at C(4).

In the course of a re-investigation of the chemistry of the *trans*,*trans*-isomer (**1**) of leucocyanidin, we found that this compound could be readily crystallized from water. We therefore determined the crystal and molecular structure of compound (**1**), and compared the preferred conformation of the heterocyclic ring in solution and the solid-state.

Experimental

Compound (**1**) was prepared by either one of the following methods. (i) Isolation as an acetone solvate as described previously.[1] This preparation when suspended in water and warmed to *ca.* 80 °C on a steam-bath initially dissolved and then precipitated a yellow impurity. This was filtered off from the solution which then rapidly deposited colourless elongated *prisms* on cooling. (ii) (2*R*,3*R*)-Taxifolin (**2**) (1 g) was reduced with $NaBH_4$ (0.5 g) in absolute ethanol (200 ml) as described previously.[1] On completion of the reaction the mixture was poured into 0.2M-acetate buffer (pH 4.5; 500 ml). The pH was adjusted to 4 with 1M-HOAc and the solution stirred with pre-washed (methanol) XAD-2 resin (100 ml). The ethanol was removed on a rotary evaporator (*ca.* 40 °C) and the resin filtered off and washed with distilled water (500 ml). The resin was sucked dry and washed with absolute ethanol (3 × 40 ml). The initial solution was retreated with the same resin, which was

† *Permanent address:* Chemistry Division, D.S.I.R., Petone, New Zealand.

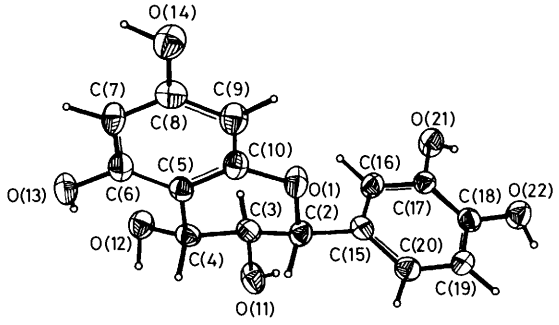


Figure 1. Perspective drawing of (2*R*,3*R*,4*S*)-3,3′,4,4′,5,7-hexahydroxyflavan (**1**) with the atom numbering used in the crystallographic analysis. Thermal ellipsoids are at the 50% probability level and the H-atoms with 0.1 Å radii.

then re-washed with water and re-eluted with ethanol. The combined ethanol eluates were evaporated, without drying, on a rotary evaporator at 40 °C and crystalline leucocyanidin separated from the residual water when the flask contents reached a low volume. This material was collected and recrystallized from water to yield pure, single crystals, m.p. (sealed tube), started to discolour at 125 °C and decomposed to a maroon solid by 170 °C, $\lambda_{max.}$(MeOH) 280 nm (ε 3 860), $[\alpha]_D^{25}$ +20.6° [*c* 0.24 in MeOH–H_2O (1:1, v/v)], (lit.[1] value for acetone solvate, +15.5°); c.d. [*c* 0.013 in MeOH–H_2O (1:1, v/v) λ nm, ($\Delta\varepsilon_{max.}$)] 288 (−0.17), 272 (−0.58), 233 (−6.65), and 216 (−6.95).

Crystal Data.—$C_{15}H_{14}O_7 \cdot 2H_2O$, M = 342.3, orthorhombic, space group $P2_12_12_1$, a = 4.862(1), b = 15.796(4), c = 19.362(5) Å, U = 1 487.0(5) Å^3. Z = 4, $D_{calc.}$ = 1.53 g cm^{-3}, μ = 10.52 cm^{-1}, F(000) = 720. Intensity data were measured[9] on a Nicolet R3 diffractometer* with Ni-filtered monochromated Cu-K_α radiation (λ = 1.5418 Å) by a θ—2θ scan technique with variable scan speed at room temperature. A total of 1 247 independent reflections (3° ≤ 2θ ≤ 114°) were measured, of which 1 193 ($|F_o| \geq 2\sigma|F_o|$) were significant.

Corrections were not made for absorption and extinction. The crystal structure was solved using the direct methods program SHELXTL.[10] Full-matrix least-squares refinement of atomic positional and anisotropic thermal parameters for non-H atoms, and positional and isotropic temperature factors for H atoms, gave a final R = 0.031 and R_w = 0.036. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$, where $w = [\sigma^2(F_o) + 0.001|F_o|^2]^{-1}$. A final difference Fourier map showed no residuals greater than 0.14 e Å^{-3}. The absolute configuration was determined by least-squares refinement of the parameters of both enantiomers, giving a ratio of the two final R_w values of 1.294. According to Hamilton's statistical test,[11] the enantiomer with the lower R_w value has a probability of being correct to a significance level better than 5%.

Full observed and calculated structure factors are available on request from the editorial office and the isotropic thermal parameters are available as a Supplementary Publication [SUP No. 56233 (2 pp.)].†

* Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval of the product to the exclusion of others which may also be suitable.

† For details of the Supplementary publications scheme, see Instructions for Authors (1985), *J. Chem. Soc., Perkin Trans. 1*, 1985, Issue 1.

Table 1. Atom co-ordinates (× 10^4) and temperature factors (A × 10^3)

Atom	*x*	*y*	*z*
O(1)	5 081(4)	2 235(1)	2 868(1)
C(2)	7 397(6)	2 814(2)	2 911(1)
C(3)	7 376(6)	3 434(2)	2 312(1)
C(4)	7 536(6)	2 953(2)	1 637(1)
C(5)	5 430(6)	2 261(2)	1 614(1)
C(6)	4 468(6)	1 894(2)	1 000(1)
C(7)	2 516(7)	1 258(2)	992(1)
C(8)	1 549(6)	941(2)	1 612(1)
C(9)	2 444(6)	1 269(2)	2 232(1)
C(10)	4 347(6)	1 924(1)	2 223(1)
O(11)	9 719(5)	3 969(1)	2 339(1)
O(12)	7 149(4)	3 517(1)	1 060(1)
O(13)	5 448(5)	2 153(1)	370(1)
O(14)	−342(5)	300(1)	1 647(1)
C(15)	7 235(6)	3 212(1)	3 617(1)
C(16)	5 243(6)	3 828(2)	3 754(1)
C(17)	5 081(5)	4 187(2)	4 409(1)
C(18)	6 898(6)	3 940(2)	4 921(1)
C(19)	8 807(6)	3 323(2)	4 789(1)
C(20)	9 024(6)	2 963(2)	4 132(1)
O(21)	3 092(5)	4 787(1)	4 521(1)
O(22)	6 613(4)	4 329(1)	5 555(1)
Ow(1)	1 271(6)	4 254(2)	6 297(1)
Ow(2)	12 107(5)	4 091(2)	590(1)
H(2)	9 061(62)	2 468(15)	2 796(12)
H(3)	5 650(64)	3 696(17)	2 211(13)
H(4)	9 272(63)	2 714(16)	1 568(11)
H(7)	2 010(62)	1 019(16)	545(13)
H(9)	1 630(58)	1 072(16)	2 654(13)
H(11)	9 481(56)	4 310(14)	2 608(11)
H(12)	9 030(80)	3 866(20)	1 000(16)
H(13)	6 176(71)	2 531(17)	418(14)
H(14)	−1 023(78)	194(19)	1 233(16)
H(16)	3 907(56)	4 029(15)	3 386(11)
H(19)	9 989(64)	3 158(16)	5 152(13)
H(20)	10 369(61)	2 555(15)	3 999(11)
H(21)	3 347(76)	5 000(20)	4 832(15)
H(22)	8 026(83)	4 229(21)	5 736(15)
HOw(1a)	1 076(83)	3 815(23)	6 654(16)
HOw(1b)	3 237(111)	4 202(28)	6 067(23)
HOw(2a)	11 998(75)	4 378(19)	967(17)
HOw(2b)	13 936(187)	3 854(58)	475(36)

N.m.r. Measurements.—^{1}H and ^{13}C N.m.r. measurements were performed at 200 and 50.3 MHz respectively on a Nicolet NT 200 spectrometer in either D_2O or [^{2}H]$_6$-acetone.

Results and Discussion

Figure 1 depicts the absolute configuration and molecular conformation of compound (**1**) in the solid state. The final atomic co-ordinates are listed in Table 1. The observed bond lengths and angles (Table 2) for compound (**1**) are all consistent with those predicted from standard values. The ring plane of the B-ring is orientated almost orthogonally to the chroman ring [torsion angle O(1)–C(2)–C(15)–C(16) = −74.3], *i.e.* the B-ring is in an eclipsed conformation with respect to the C(2)–H(2) bond. The other three flavan crystallographic structures which have been reported,[6,7,12] with an equatorially disposed B-ring, also have the C(2)–aryl group in a similar conformation.

Table 3 gives the torsion angles defining heterocyclic ring, and Figure 2 illustrates the possible heterocyclic ring conformations.

Absolute Configuration and Chiroptical Properties.—The absolute configuration of compound (**1**), confirmed by *X*-ray crystallography, is (2*R*,3*S*,4*R*)-3,3′,4,4′,5,7-hexahydroxyflavan.

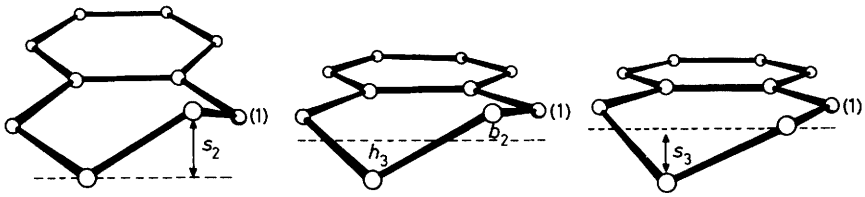


Figure 2. The thermodynamically favoured heterocyclic ring conformations of a flavanoid unit. The hatched line indicates the projection of the A-ring plane.

Table 2.

Bond lengths (Å)			
O(1)–C(2)	1.452(4)	O(1)–C(10)	1.389(4)
C(2)–C(3)	1.518(5)	C(2)–C(15)	1.507(4)
C(3)–C(4)	1.514(4)	C(3)–O(11)	1.419(4)
C(4)–C(5)	1.499(5)	C(4)–O(12)	1.441(4)
C(5)–C(6)	1.402(4)	C(5)–C(10)	1.396(4)
C(6)–C(7)	1.381(5)	C(6)–O(13)	1.373(4)
C(7)–C(8)	1.383(4)	C(8)–C(9)	1.379(4)
C(8)–O(14)	1.369(4)	C(9)–C(10)	1.389(5)
C(15)–C(16)	1.398(5)	C(15)–C(20)	1.381(5)
C(16)–C(17)	1.390(4)	C(17)–C(18)	1.385(5)
C(17)–O(21)	1.371(4)	C(18)–C(19)	1.370(5)
C(18)–O(22)	1.380(4)	C(19)–C(20)	1.397(4)
Bond angles (°)			
C(2)–O(1)–C(10)	118.2(2)	O(1)–C(2)–C(3)	111.0(2)
O(1)–C(2)–C(15)	105.9(2)	C(3)–C(2)–C(15)	115.0(2)
C(2)–C(3)–C(4)	109.6(2)	C(2)–C(3)–O(11)	110.5(2)
C(4)–C(3)–O(11)	106.8(2)	C(3)–C(4)–C(5)	110.9(2)
C(3)–C(4)–O(12)	110.6(2)	C(5)–C(4)–O(12)	109.8(2)
C(4)–C(5)–C(6)	123.7(2)	C(4)–C(5)–C(10)	120.7(2)
C(6)–C(5)–C(10)	115.6(3)	C(5)–C(6)–C(7)	122.7(3)
C(5)–C(6)–O(13)	121.0(3)	C(7)–C(6)–O(13)	116.3(2)
C(6)–C(7)–C(8)	119.1(3)	C(7)–C(8)–C(9)	120.9(3)
C(7)–C(8)–O(14)	122.6(3)	C(9)–C(8)–O(14)	116.5(2)
C(8)–C(9)–C(10)	118.6(3)	O(1)–C(10)–C(5)	121.9(3)
O(1)–C(10)–C(9)	115.0(2)	C(5)–C(10)–C(9)	123.1(2)
C(2)–C(15)–C(16)	120.0(3)	C(2)–C(15)–C(20)	120.2(3)
C(16)–C(15)–C(20)	119.8(2)	C(15)–C(16)–C(17)	119.8(3)
C(16)–C(17)–C(18)	120.1(3)	C(16)–C(17)–O(21)	117.8(3)
C(18)–C(17)–O(21)	122.1(2)	C(17)–C(18)–C(19)	119.9(3)
C(17)–C(18)–O(22)	116.6(3)	C(19)–C(18)–O(22)	123.5(3)
C(18)–C(19)–C(20)	120.7(3)	C(15)–C(20)–C(19)	119.6(3)

Table 3. Torsion angles measured for (2*R*,3*S*,4*R*)-3,3′,4,4′,5,7-hexahydroxyflavan (**1**) compared with angles calculated for equivalent conformations of cyclohexane.

Torsion angle	Compd. (**1**)	Half-chair*	C(2)-Sofa*	C(3)-Sofa*
O(1)–C(2)–C(3)–C(4)	−59.7	−61(+1)	−56(−4)	−56(−4)
C(2)–C(3)–C(4)–C(5)	+50.2	+44(+6)	+28(+22)	+53(−3)
C(3)–C(4)–C(5)–C(10)	−23.0	−15(−8)	0(−23)	−27(+4)
C(4)–C(5)–C(10)–O(1)	+2.6	0(+3)	0(+3)	0(+3)
C(5)–C(10)–O(1)–C(2)	−11.5	−15(+3)	−27(+13)	0(−12)
C(10)–O(1)–C(2)–C(3)	+40.1	+44(−4)	+53(−13)	+28(+12)

* Numbers in parentheses are the deviation of the torsion angles of compound (**1**) from those theoretically predicted, rounded to the nearest degree.

The structure is therefore the same as that expected on steric grounds[1] from sodium borohydride reduction of optically pure (2*R*,3*R*)-taxifolin (**2**). The absolute configuration is the same as catechin (**5**) at C(2) and C(3), as is the phenolic hydroxylation pattern.

As expected, the molar extinction coefficient for the diol (**1**), $\lambda_{max.}$ 280 nm (ε 3 860 in methanol) is close to that for catechin at the same wavelength in the same solvent (ε = 3 740). Additionally the c.d. spectrum for compound (**1**) is very similar to the Δε values observed for catechin in methanol,[13] although the amplitude of the band near 230 nm is much greater for the diol. Such a close correlation of c.d. spectra of the two compounds is expected as the direction of the bands are thought to be largely controlled by the absolute configuration at C(2)[14] and hence the conformation of the B-ring chromophore.

Hydrogen Bonding.—*X*-Ray analysis indicated that compound (**1**) crystallizes from aqueous solution as the dihydrate. The pair of solvated water molecules enhances the formation of a more complete system of intermolecular hydrogen bonds in the crystal structure. The flavanoid molecules related by the centre of symmetry are linked together by a network of hydrogen bonds between the hydroxy groups and the two solvated water molecules. The first water molecule, Ow(1), forms an approximately trigonal arrangement of hydrogen bonds about itself; one of its hydrogen atoms is shared with the heterocyclic ring ether oxygen O(1), while the second hydrogen atom is shared almost equally by two different O(22) atoms of the adjacent flavanoids molecules, forming a slightly asymmetric bifurcated[15] hydrogen bond. The second water molecule, Ow(2), assumes the usual distorted tetrahedral water co-ordination pattern; one of its hydrogen atoms is shared with O(13), and the other hydrogen atom forms almost equal bifurcated[15] hydrogen bonds to two O(12) atoms of two neighbouring flavanoid molecules. Ow(2) Forms the fourth hydrogen bond by sharing the hydrogen atom from O(21) of an adjacent molecule. In addition to these intermolecular hydrogen bonds, there are two others in the crystal structure: O(11)–O(14) and O(14)–O(21). The intermolecular contacts less than 3.0 Å between two heavy atoms are given in Table 4, together with their symmetry transformations.

Heterocyclic Ring Conformation.—The only attempt so far to correlate the solid-state and solution conformation of the heterocyclic ring of flavans, and to carry out a detailed conformational analysis, was the study of Sliwa and co-workers of *cis*- and *trans*-diphenylflavan-3-ol.[12] In this study very bulky groups were introduced at C(3) with the intention of forcing the B-ring to adopt an axial orientation, which was indeed observed.[12] In contrast, it is generally considered that naturally occurring flavan-3-ols, and their derivatives, adopt dihydropyran ring conformations with the C(2)–aryl substituent equatorial.[8]

As pointed out by Sliwa and co-workers[12] cyclohexene presents an appropriate model for possible conformations of a dihydropyran ring, and the terminology utilized by Bucourt[16] (Figure 2) may therefore be used to describe these conformational states. As Bucourt[16] pointed out the various conformations of six membered rings may most easily be described by consideration of the sign and magnitude of the torsion angles.

Table 4. Intermolecular heavy atom close contact distances (≤3.0 Å). E.s.d's = 0.005 Å

Atoms *	Distance (Å)
Ow(1)–O(22)[a]	2.969
Ow(1)–O(22)[b]	2.684
Ow(1)–O(1)[c]	2.913
Ow(2)–O(12)[a]	2.732
Ow(2)–O(12)[d]	2.768
Ow(2)–O(13)[e]	2.822
Ow(2)–O(21)[f]	2.726
O(21)–O(14)[g]	2.750
O(14)–O(11)[h]	2.893

* Superscript refers to the symmetry transformations, as follows: [a] x,y,z. [b] $-1+x,y,z$. [c] $-\frac{1}{2}+x, \frac{1}{2}-y, 1-z$. [d] $1+x,y,z$. [e] $\frac{1}{2}+x, \frac{1}{2}-y, \bar{z}$. [f] $\frac{3}{2}-x, 1-y, -\frac{1}{2}+z$. [g] $\bar{x}, \frac{1}{2}+y, \frac{1}{2}-z$. [h] $1-x, -\frac{1}{2}+y, \frac{1}{2}-z$.

Although half-chair conformations are energetically the most favoured for both cyclohexane and cyclohexene, the presence of two sp^2–sp^3 bonds in the latter means that flexible (boat-twist) forms are not significant for cyclohexene and the energy of the sofa form is considerably lowered and is expected to constitute 20% of the conformational equilibrium populations.[16]

As pointed out by Sliwa and co-workers,[12] arguments cannot be transferred directly from cyclohexene to the dihydropyran ring of flavans, because of the introduction of oxygen. As C–O bonds are shorter than C–C bonds, the ring is no longer symmetrical. An additional source of distortion is the fact that the C(2)–O(1)–C(10) bond is nearer to the angle of an sp^2 than sp^3 carbon centre (see Table 2). As further pointed out by Sliwa and co-workers[12] it can be predicted that this asymmetry will result in the following relationship for the out-of-A-ring-plane distances for C(2) and C(3), where h_2,h_3 and s_2,s_3 are the out-

$$|h_2| < |h_3| < |s_3|$$
$$|h_2| < |s_2| < |s_3|$$

of-plane distances for the half-chair and sofa conformations respectively (see Figure 2).

These predictions are fully supported by three of the flavan structures so far solved by crystallography. Firstly, epicatechin (**5**) exists in the solid-state with an almost perfect half-chair conformation, the torsion angles deviating by a maximum of 3° from those of the corresponding conformation of cyclohexene.[6] The observed out-of-plane distances are $h_2 = +26.3$ pm and $h_3 = -49.5$ pm, thus h_3 is almost double h_2. Secondly, both 8-bromoepicatechin 3′,4′,5,7-tetramethyl ether[8] and *cis*-diphenylflavan-3-ol[12] have conformations very close to a C(3)-sofa conformation and, once again, in either case, the torsion angles differ by a maximum of ±3° from those predicted from cyclohexene. The observed values for s_3 are −70 and −67 pm out-of-plane respectively, much larger values than h_3 observed for the half-chair epicatechin conformation.[6]

The observed torsion angles for the solid-state dihydropyran ring conformation of the diol (**1**) are compared with those predicted for cyclohexene for a half-chair, and C(2)- and C(3)-sofa conformation in Table 3. As may be seen there are quite significant deviations of the observed torsion angles of the diol from all three cyclohexene conformations. The largest deviations are from a C(2)-sofa, and it may be concluded that the actual conformation is in fact described as intermediate between a half-chair and C(3)-sofa conformation as two torsion angles are consistent with either conformation, two with a half-chair and two with a C(3)-sofa.

The out-of-plane distances in the solid-state conformation were $h_2 = +17.6$ and $h_3 = -51.9$ pm, so that C(2) is considerably less above the A-ring plane than observed for epicatechin, but C(3) a similar distance below, so that the ring is somewhat flattened at C(2). Further evidence for strain due to bond twisting may be obtained from projected bond angles about the C(2)–C(3) bond. Here considerable deviations from tetrahedral symmetry are evident. The bond projections for H(2)–C(2)–O(1) = 111.7° and H(3)–C(3)–O(11) = 136.5° (calculated from the sum of torsion angles about this bond) are extreme, and may be compared with the theoretical values of 120°. In contrast, similar projections calculated from the crystallographic data for epicatechin[6] along this bond differ by no more than ±1° from the theoretical value.

The torsion angles defining the relationship of the heterocyclic ring protons are: H(2)–C(2)–C(3)–H(3) = +158.0° and H(3)–C(3)–C(4)–H(4) = +168.8°. If leucocyanidin (**1**) possesses the same conformation in solution, these values imply that the value of $J_{2,3} < J_{3,4}$, or at least their values should be similar. The observed values of the coupling constants in [$^2H_6$]acetone are 9.9 and 7.9 Hz,[1] and in D_2O are 9.5 and 7.5 Hz.[4] We had previously assigned the larger coupling constant to $J_{2,3}$ on the assumption that the diol (**1**) possessed a half-chair conformation.[1] Since the crystallographic and ^1H n.m.r. experiments are in apparent contradiction of one another, it became necessary to assign unequivocally the proton-proton coupling constants.

This was done in two ways. In the 50.3 MHz ^{13}C n.m.r. spectrum of (**1**) in [$^2H_6$]acetone the C(2), C(3), and C(4) chemical shifts are at δ 81.7, 73.9, and 72.6 p.p.m. In the ^1H n.m.r. in the same solvent, the 9.9 Hz coupling is associated with a chemical shift at δ 4.62 whereas 7.9 Hz coupling is associated with a signal at δ 5.00.[1] Selective proton irradiation at δ 4.62 decoupled the ^{13}C n.m.r. signal at δ 81.7, whereas a similar irradiation experiment at δ 5.00 decoupled the δ 72.6 signal. Therefore, these results are consistent with the earlier assignment. Further, temperature dependent ^1H n.m.r. studies of compound (**1**) (see later), resulted in O–H exchange being slowed sufficiently to observe H–C–O–H couplings. As may be seen from a consideration of the structure of (**1**) two of the heterocyclic ring carbons are bonded to oxygen possessing exchangeable protons, C(3) and C(4), whereas C(2) is not. As expected the ^1H n.m.r. signal for H(2), δ 4.62, remains a simple doublet, whereas the signals for H(3) and H(4) at δ 3.86 and 5.00 form multiplets.

The magnitude of vicinal proton-proton coupling constants remains one of the standard and generally most useful methods of assigning the conformation of organic compounds. The major problem in applying coupling constants results to a conformational analysis of the heterocyclic ring of compound (**1**) is that the orientation of electronegative substituents, in this case O(1) and the hydroxy groups attached to C(3) and C(4), may have a considerable effect on the magnitude of J, generally diminishing its value.[17]

Recent workers have attempted to overcome this problem by developing empirical parameters which modify the Karplus equation in such a way as to predict accurately the magnitude of these effects on J, using data based on a large number of experiments.[18] This approach has already been used, with some success to deduce the heterocyclic ring conformation of 2,3-*cis*-flavanoid units in procyanidins.[19]

However, when a similar approach was applied to calculating the diaxial proton-proton coupling constants to 2,3-*trans* flavan-3-ols, it was evident that the empirical equations[18] overestimated the effect of electronegative substituents on J. For example, the maximum value of $J_{2,3}$ [*i.e.* setting H(2)–C(2)–C(3)–H(3) at 180°] calculated from compound (**1**) was 9.0 Hz, whereas the observed value is 9.9 Hz in [$^2H_6$]acetone,[1] and 10.2 Hz for the 3′,4′,5,7-tetramethyl ether derivative (**6**) of this

Table 5. Predicted and observed values for torsion angles across the C(2)–C(3) and C(3)–C(4) bonds for the diol (**1**)

	H(2)–C(2)–C(3)–H(3)		O(1)–C(2)–C(3)–C(4)		H(3)–C(3)–C(4)–H(4)		C(2)–C(3)–C(4)–C(4)	
Conformation	Pred.	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.	Obs.
C(2)-Sofa	−176		−56		+148		+28	
Half-chair	+179	+177	−61	−62	+164	+163	+44	+47
C(3)-Sofa	−176	−179	−56	−54	+173	+174	+53	+54

compound.[1] These latter values correlate well with ^{1}H n.m.r. results for compounds with similarly substituted pyran rings—for instance the diaxial coupling constants between proton on the secondary alcohol moieties of hexose sugars in D_2O solution are in the range 9.5—10.0 Hz.[20] It is therefore reasonable to assume that the torsion angle is approximately 180° in the conformation adopted by the heterocyclic ring of compound (**1**) in solution.

As pointed out earlier, available crystallographic studies include examples of flavanoids possessing near-perfect half-chair[6] and C(3)-sofa[8,12] conformations. Additionally, two other structures have been solved in which the compounds have ring conformations which approach a C(2)-sofa in the solid state: 8-bromo-catechin tetramethyl ether[7] and catechin-(4α ⟶ 2)-phloroglucinol heptamethyl ether [(**7**) Wong and Porter, unpublished results]. These studies show that the pyranose ring of flavanoids is capable of some flexibility, at least in the range C(2)-sofa ⇌ half-chair ⇌ C(3)-sofa (see Figure 2), and therefore the diol (**1**) *may* adopt significantly different ring conformations in the solid or solution states.

It may be predicted from the conformation of cyclohexene,[16] equivalent to those in Figure 2, that the torsion angle O(1)–C(2)–C(3)–C(4) deviates only ±1—4° from 60° in these three conformations (Table 3), and consequently the torsion angle H(2)–C(2)–C(3)–H(3) will remain essentially invariant in compound (**1**), close to 180°. Therefore, the magnitude of $J_{2,3}$ cannot be used as a conformational index, but will remain essentially invariant in each of the three conformations.

In contrast, it may be predicted that the torsion angle H(3)–C(3)–C(4)–H(4) in compound (**1**) will vary with conformation, and therefore $J_{3,4}$ may, potentially, be used as a conformational probe.

These predications may be tested by direct comparison with crystallographically determined data. Table 5 compares the predicted and observed ring and diaxial substituent torsion angles across the C(2)–C(3) and C(3)–C(4) bonds. It may be seen that agreement is very close between the experimental and theoretical values predicted from cyclohexene. Therefore, cyclohexene may be used as a model to accurately predict torsion angles in the heterocyclic ring of flavans.

From Table 5 the predicted H(3)–C(3)–C(4)–H(4) torsion angle for a C(2)-sofa is +148°, considerably lower than those for a half-chair (+164°) and C(3)-sofa (+173°). The observed value for $J_{3,4}$ for compound (**1**) is 7.9 Hz. Construction of a simple Karplus curve,[17] setting $J_{\text{diaxial}} \cos^2 180$ at 10.2 Hz, the maximum diaxial coupling constant observed for similar flavan-3,4-diols,[1,3,5] shows that a value of the torsion angle consistent with the observed value of $J_{3,4}$ is 151°, very close to the predicted value for a C(2)-sofa. In contrast, coupling constants obtained from the same Karplus plot for a half-chair and C(3)-sofa are 9.4 and 10.0 Hz respectively.

Although the above model cannot be considered an accurate quantitative model[17] for the relationship between $J_{3,4}$ and the H(3)–C(3)–C(4)–H(4) torsion angle, the data are sufficient to indicate that a C(2)-sofa is the conformation heavily favoured by the ^{1}H n.m.r. results. Evidence that this is a reasonable model for the conformation of (**1**) in solution is the fact that catechin-(4α ⟶ 2)-phloroglucinol heptamethyl ether (**7**) has a heterocyclic ring conformation closely approaching a C(2)-sofa in the solid state and also displays very similar proton-proton coupling constants to (**1**) in [$^2H_6$]acetone solution: $J_{2,3}$ 9.9 Hz and $J_{3,4}$ 8.1 Hz (Wong and Porter, unpublished results).

The ^{1}H n.m.r. spectrum of the diol (**1**) in [$^2H_6$]acetone was remeasured at +50, −50, and −90 °C. The chemical shifts, coupling constants, and signal linewidths were found to be essentially invariant with temperature, which implied that conformations other than the C(2)-sofa are not energetically significant over this temperature range.

The above results imply that compound (**1**) adopts different heterocyclic ring conformations in the solution and solid states. In solution the conformation is apparently locked in a near C(2)-sofa conformation, whereas in the solid state the conformation is a distorted half-chair, presumably induced by strong packing forces[21] in the crystal lattice. This is, therefore, a further example of conformational changes being induced by the different effects of lattice forces and solvation on a molecule. Several examples of this phenomenon have recently been demonstrated.[22–24]

Conclusion.—The current study shows that at least one isomer of leucocyanidin may exist in a highly crystalline form. This isomer, compound (**1**), is highly stable in the crystalline state and has been stored with no particular precaution against light, moisture or oxygen, for several months with no sign of red or brown pigmentation, which is generally so characteristic of polyhydroxyflavan-3-ol compounds. In contrast, the acetone solvate of this compound becomes red or red-brown after only a few days under similar conditions.

The study also shows that the preferred conformation of the heterocyclic ring of the diol (**1**) or its 3′,4′,5,7-tetramethyl ether derivative (**6**) in solution is a C(2)-sofa.

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