

Plant Proanthocyanidins. Part 6.^{1a} Chiroptical Studies. Part 95.^{1b} Circular Dichroism of Procyanidins

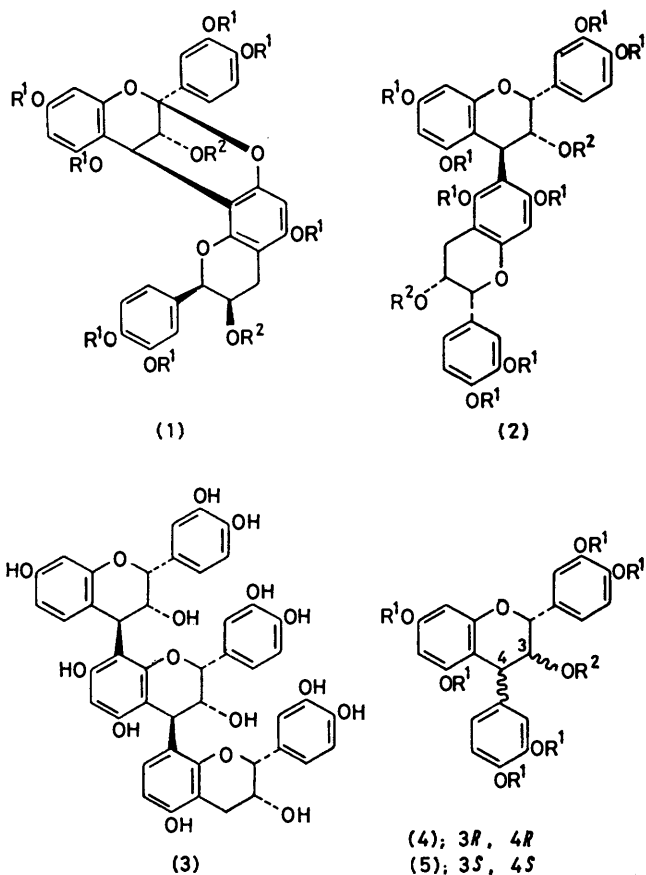
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Circular dichroism spectra of 26 procyanidins and their derivatives have been measured. All exhibit a strong positive or negative couplet at 200–220 nm and this has been correlated with the absolute stereochemistry at C-4 on the interflavan linkage.

THE histological reaction for vegetable tannins in plant tissues is most commonly due to the presence of proanthocyanidins,² and surveys show^{3,4} that of these the procyanidins are the most widely distributed, and are generally associated with plants which show a 'woody' habit of growth. Earlier work defined the structure, stereochemistry,⁵⁻⁹ and mode of biosynthesis¹⁰ of the naturally occurring procyanidins and the closely related proanthocyanidin A-2.^{11,12} During this work some o.r.d. and c.d. measurements were noted^{8,12} and the possibility of utilising these data to determine the stereochemistry of the interflavan linkage was alluded to. Recent publications^{13,14} prompt us to disclose our more detailed results on this subject.

Previous work demonstrated⁸ that because of hindered rotation about the interflavan bond each of the four principal dimeric procyanidins [B-1 to B-4; compounds (9), (6), (11), and (8), respectively; $R^1 = R^2 = H$] and their minor isomers [C-4 to C-6 interflavan bond, *e.g.* procyanidin B-5 (2; $R^1 = R^2 = H$)] has a preferred conformation. Significantly those derived from B-1 (9) and B-2 (6) bear a quasi-mirror image relationship to those from B-3 (11) and B-4 (8), and procyanidin polymers derived by addition of further flavan-3-ol units to B-1 or B-2 [(–)-epicatechin units, 4*R*-stereochemistry] or to B-3 or B-4 [(+)-catechin units, 4*S*-stereochemistry] adopt linear, helical conformations with, respectively, a left-handed and right-handed screw. The c.d. measurements on the procyanidins, their derivatives, and related model compounds⁹ are shown in the Table and they both support and confirm the results obtained earlier.⁸ The principal distinguishing feature of all the c.d. spectra is a very large positive or negative couplet at short wavelength (200–220 nm). Furthermore procyanidins and model compounds related to B-1 or B-2 with 4*R*-absolute stereochemistry all show a strong positive couplet whilst those related to procyanidins B-3 or B-4 (4*S*-absolute stereochemistry) exhibit a negative couplet. In addition the couplet increases in amplitude with an increased degree of polymerisation of the procyanidin [*cf.* Table, procyanidin B-2 (6; $R^1 = R^2 = H$), procyanidin B-5 (2; $R^1 = R^2 = H$), and procyanidin C-1 (3)]. The parent 'monomer' flavan-3-ols, (–)-epicatechin and (+)-catechin, both show negative couplets at short wavelengths although the $\Delta\epsilon$ values are very much smaller than those of their derivatives with C-4

aryl substituents. This supports the view that the strong positive and negative couplets observed in the c.d. spectra of the procyanidins originate from the interaction of the two aryl chromophores [rings A, A' for example in procyanidin B-2, (6)] and thus confirms the



conclusion⁹ derived earlier that the two groups of dimeric procyanidins (B-1, B-2 and B-3, B-4) and their derived higher oligomers have structures of a quasi-enantiomeric type.

Proanthocyanidin A-2 (1; $R^1 = R^2 = H$), which is closely related to (–)-epicatechin (7; $R^1 = R^2 = H$) and procyanidin B-2 (6; $R^1 = R^2 = H$),^{11,12} is held in a rigid bridged molecular conformation by the presence of both the C-4 to C-8 and C-2 to O-7 interflavan bonds. This fixed conformation is analogous to the preferred

Circular dichroism of procyanidins and related compounds

Formula		Solvent	$\Delta\epsilon$	λ/nm	$\Delta\epsilon$	λ/nm	$\Delta\epsilon$	λ/nm	$\Delta\epsilon$	λ/nm	$\Delta\epsilon$	λ/nm	$\Delta\epsilon$	λ/nm	Sign of short wavelength couplet	
(a) Compounds with 4 <i>R</i> -stereochemistry																
Proanthocyanidin A-2	(1; R ¹ = R ² = H)	M			-0.84	287		-5.05	271	+13.80s	237	+22.2	221	-38.0	205	+
		A										+22.3	222	-38.6	204	
Proanthocyanidin A-2 heptamethyl ether	(1; R ¹ = Me, R ² = H)	M						-4.46	270	+6.60s	232	+19.8	219	-18.2	206	+
		A										+43.9	220	-48.7	205	
Proanthocyanidin A-2 nona-acetate	(1; R ¹ = R ² = Ac)	M			-4.15	284		+9.50	269	-3.41	235	0.0	227	-52.0	210	+
		A										-1.9	225	-53.1	207	
(2 <i>R</i> ,3 <i>R</i> ,4 <i>R</i>)-4-(2,4,6-Trihydroxyphenyl)flavan-3,3',4',5,7-pentaol	(4; R ¹ = R ² = H)	M	+0.34	317	+0.85	285				+10.58	235	+22.0	213	-15.7	203	+
(2 <i>R</i> ,3 <i>R</i> ,4 <i>R</i>)-4-(2,4,6-Trihydroxyphenyl)flavan-3,3',4',5,7-pentaol heptamethyl ether	(4; R ¹ = Me, R ² = H)	M			+0.44	280				+4.14	245	+20.8	211	-14.1	199	+
		A										+8.8	212	-8.8	203	+
(2 <i>R</i> ,3 <i>R</i> ,4 <i>R</i>)-4-(2,4,6-Trihydroxyphenyl)flavan-3,3',4',5,7-pentaol octa-acetate	(4; R ¹ = R ² = Ac)	M			+2.14	278		+1.14s	268	+16.70	240	+26.7	212	-15.0	202	+
		A										+51.2	200	-25.6	189	
Procyanidin B-1 deca-acetate	(9; R ¹ = R ² = Ac)	M			+1.31	276		+1.27	267	+10.95s	236	+67.0	200	0.0!	199	+
		A										+60.0	208	-15.0!	188	
Procyanidin B-2	(6; R ¹ = R ² = H)	M	+0.25	306	-0.43	288		-1.13	275	+9.25s	235	+23.9	215	-32.4	205	+
		A										+24.2	213	-18.8	204	
Procyanidin B-2 octamethyl ether	(6; R ¹ = Me, R ² = H)	M			+0.13	286		-1.46	273	+13.37	240	+13.0	215!		202	+
		A										+34.2	212	-10.3	202	
Procyanidin B-2 deca-acetate	(6; R ¹ = R ² = Ac)	M			-0.51	282		-0.55	272	+2.75s	235	+14.2	210	+4.7!	201	+
		A										+46.8	204	-39.0	189	
Procyanidin B-5	(2; R ¹ = R ² = H)	M	+0.80	307	+1.20	290		-0.95	275	+23.50	237	+23.0	215	-22.0	203	+
		A										+23.8	214	-23.2	198	
Procyanidin C-1	(3)	M	+0.94	302	+0.47	284		-1.25	276	+16.40	230	+34.5	215	-43.9	204	+
		A										+49.1	215	-43.7	203	
(b) Compounds with 4 <i>S</i> -stereochemistry																
(2 <i>R</i> ,3 <i>S</i> ,4 <i>S</i>)-4-(2,4,6-Trihydroxyphenyl)flavan-3,3',4',5,7-pentaol	(5; R ¹ = R ² = H)	M			-0.22	286		+0.72	274	-7.70s	233	-44.3	209	+19.5!	199	-
		A										-56.3	206	+25.6	195	
(2 <i>R</i> ,3 <i>S</i> ,4 <i>S</i>)-4-(2,4,6-Trihydroxyphenyl)flavan-3,3',4',5,7-pentaol heptamethyl ether	(5; R ¹ = Me, R ² = H)	M			-0.97	282		+0.81	269	-25.40	240	-72.1	208	+35.5!	195	-
		A										-67.7	208	+35.5!	195	
(2 <i>R</i> ,3 <i>S</i> ,4 <i>S</i>)-4-(2,4,6-Trihydroxyphenyl)flavan-3,3',4',5,7-pentaol octa-acetate	(5; R ¹ = R ² = Ac)	M			-4.21	277		-3.11s	270	-7.66s	230	-40.7!	201		186	-
		A										-57.5	199	+34.8!	186	
Procyanidin B-3	(11; R ¹ = R ² = H)	M			-1.37	235		+0.43	270	-13.83s	235	-49.2	211	+36.6!	196	-
		A										-61.3	212	+41.0	196	
Procyanidin B-3 octamethyl ether	(11; R ¹ = Me, R ² = H)	M			-1.96	284		+0.36	270	-34.49	233	-65.3	210	+67.4!	198	-
		A										-81.9	210	+70.2	198	
Procyanidin B-3 acetate	(11; R ¹ = R ² = Ac)	M			-5.78	276		-5.20s	266	-8.92	232	-67.2	206		188	-
		A										-60.9	205	+38.0	188	
Procyanidin B-4	(8; R ¹ = R ² = H)	M	+0.24	309	-0.64	286		-0.78	267	-12.20s	231	-39.1	212	+26.8	199	-
		A										-50.1	212	+27.3	196	
Procyanidin B-4 acetate	(8; R ¹ = R ² = Ac)	M			-7.82	278		-6.81s	271	-8.20	234	-94.0	206	0.0!	195	-
		A										-81.8	204	+38.0	189	
(c) (-)-Epicatechin and its derivatives																
(-)-Epicatechin	(7; R ¹ = R ² = H)	M	+0.81	308	-0.71	281		-0.65	275	+1.57	239	-13.8	206	+8.9	197	-
		A										-28.9	204	+26.2	195	
(-)-Epicatechin tetramethyl ether	(7; R ¹ = Me, R ² = H)	M			-0.80	277		-0.71s	274	-4.68	230	-20.1	206	+13.6	198	-
		A										-15.8	206	+14.5	196	
(-)-Epicatechin penta-acetate	(7; R ¹ = R ² = Ac)	M			-2.15	275		-2.15	270	+3.71	299	-7.5	204	0.0!	196	-
		A										-4.8	204	+3.7!	194	
(d) (+)-Catechin and its derivatives																
(+)-Catechin	(10; R ¹ = R ² = H)	M			-0.87	282		-0.52s	275	-2.23	227	-4.7	205	+21.5!	197	-
		A										-8.3	205	+29.5	194	
(+)-Catechin tetramethyl ether	(10; R ¹ = Me, R ² = H)	M			-0.96	279		-0.43s	272	-2.48	234	-7.4	206	+14.2	196	-
		A										-8.2	206	+20.7	196	
(+)-Catechin penta-acetate	(10; R ¹ = R ² = Ac)	M			-1.76	277		-1.21s	270	+3.05	230	-5.0	214	+9.4	202	-
		A										-2.9	213	+9.5!	201	

M = Methanol; A = acetonitrile. All $\Delta\epsilon$ values are for c.d. maxima, except those indicated by s (=shoulder) or ! (= $\Delta\epsilon$ at lowest wavelength measured).

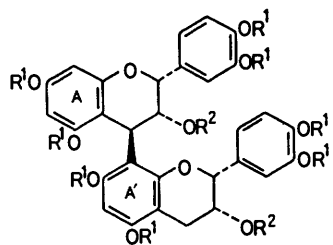
conformations adopted by procyanidins B-1 and B-2 and this relationship is strongly supported by the c.d. spectra of proanthocyanidin A-2 and its derivatives which all exhibit a strong positive couplet at short wavelengths. These results are therefore broadly similar to those obtained by Roux and his collaborators^{13,14} with proanthocyanidins related to the (2*R*,3*S*)-3',4',7-trihydroxyflavan-3-ol system, although in this work as in our earlier measurements⁸ only one half of the characteristic short wavelength doublet described above was observed. The c.d. method as outlined and with appropriate standards therefore clearly permits the determination of the absolute configuration at C-4 on the interflavan linkage of unknown proanthocyanidins.

In addition to the short wavelength couplet, the c.d. spectra also show bands at longer wavelengths corresponding to the ¹L_b and ¹L_a transitions of the aromatic chromophores. The results for (2*R*,3*R*)-(-)-epicatechin (7) and (2*R*,3*S*)-(+)-catechin (10) may be compared with those published by Korver and Wilkins¹⁵ in 1971; the signs of the c.d. bands are in agreement but the present

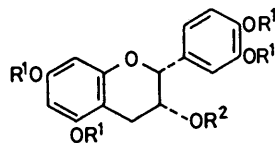
$\Delta\epsilon$ values are significantly greater. In the earlier work Korver observed that the ¹L_b bands for these two compounds were split into two distinct maxima at low temperatures. He suggested that these two separate ¹L_b bands were due to the two aromatic chromophores and that the band at slightly longer wavelength (*ca.* 283 nm) should be attributed to the more rigid fused aryl ring and the band at slightly shorter wavelength (*ca.* 272 nm) to the C-2 aryl substituent. In our present work, two bands have been observed at room temperature for epicatechin and catechin and for most of the procyanidins and related compounds; the second ¹L_b band usually appears as a shoulder. Six typical compounds were selected for c.d. study at temperatures from 0 to -140 °C but no significant variation was observed in the spectra.

EXPERIMENTAL

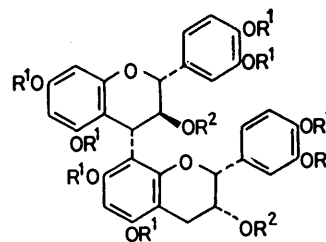
Procyanidins and proanthocyanidin A-2 and their derivatives were obtained as described earlier.^{8,9,12} C.d. measurements were recorded on a Cary-61 instrument using



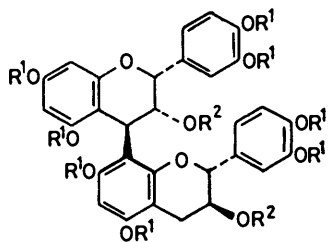
(6) B-2



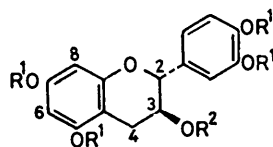
(7) epicatechin



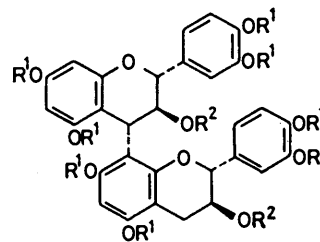
(8) B-4



(9) B-1



(10) catechin



(11) B-3

Procyanidins

methanol or acetonitrile solutions with a concentration of procyanidin < 0.5 mg per ml and in cells of path length 5.0, 2.0, 1.0, or 0.5 mm.

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