Structure of the Dimeric Proanthocyanidin-A2 and its Derivatives

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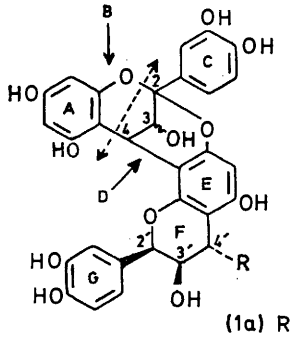
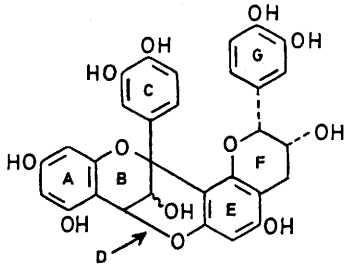
Summary The determination of the structure of the proanthocyanidin A2 and three derivatives is described.

MAYER, GOLL, VON ARNDT, and MANNSCHREK[1] first isolated a novel proanthocyanidin from the shells of unripe horse chestnuts (*Aesculus hippocastanum*) and Weinges and his associates[2] later isolated the same compound from other fruit, including the mountain cranberry (*Vaccinium vitis-idaea*). The substance was named procyanidin A2 but neither group of workers was able to distinguish[1–3] between the alternative doubly bonded dimeric structures (**1a** and **2**).



(1a) R = H
(1b) R = S·CH_2·Ph



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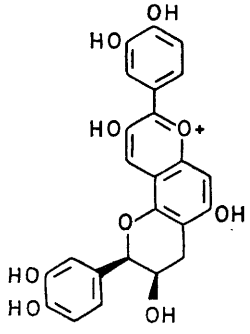

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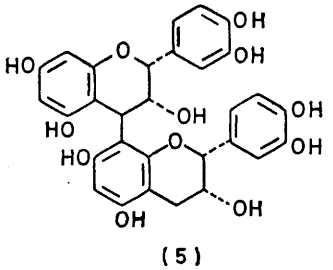

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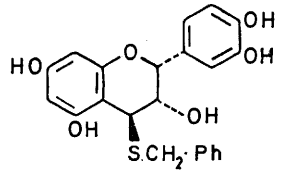
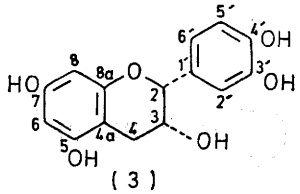


(7)

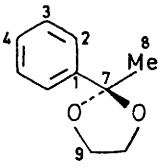
The crystalline proanthocyanidin ($C_{30}H_{24}O_{12}$, $[\alpha]_{578}$ + 56·9°, m.p. > 280°) was obtained from horse chestnuts and mountain cranberry by chromatography on Sephadex LH-20.[4] The ^{1}H n.m.r. spectrum of the proanthocyanidin [$(CD_3)_2SO$] combined with spectroscopic and analytical data on the nona-acetate (m.p. 158°, $[\alpha]_{578}$ — 88·6°) and diacetyl heptamethyl ether (m.p. 186°, $[\alpha]_{578}$ + 26·5°, M^+ 758) confirmed the previous structural proposals[1–3] and established[4] that the 'lower half' of the dimer had the (—)-epicatechin (**3**) stereochemistry at C-2 and C-3.



(3)

C-8	94·3	C-5′	114·8	C-4′	144·3
C-6	95·4	C-6′	118·0	C-7	155·7
C-4a	98·6	C-1′	130·6	C-5	156·2
C-2′	114·8	C-3′	144·3	C-8a	156·4

(p.p.m. from Me_4Si)



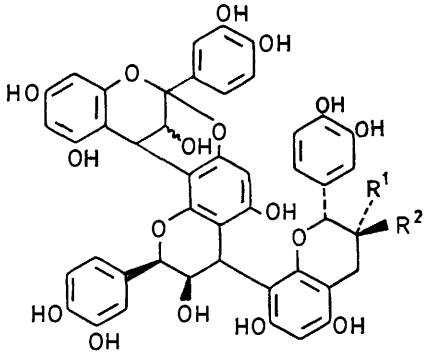
(4)

C-8	27·4	C-2	125·1
C-9	64·2	C-4	127·7
C-7	108·3	C-3	127·9
		C-1	128·1

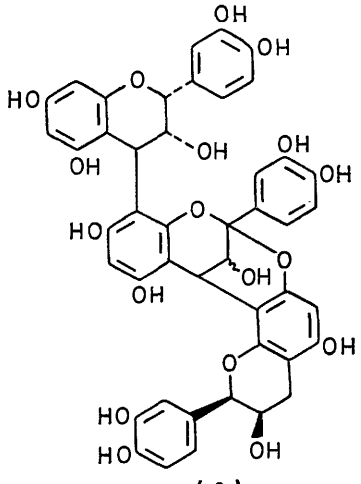
(p.p.m. from Me_4Si)

The application of ^{13}C n.m.r. spectroscopy has permitted a choice to be made between the two alternative structures (**1a** and **2**) for the proanthocyanidin A2. The ^{13}C n.m.r. spectra of (—)-epicatechin (**3**), procyanidin B2 (**5**), proanthocyanidin A2, and the model compound (**4**) were obtained in [$(CD_3)_2SO$] and the assignments for (—)-epicatechin (**3**) and compound (**4**), shown in p.p.m. relative to Me_4Si, were based on values calculated from related compounds[5] and proton decoupling experiments. The ^{13}C n.m.r. spectrum of the procyanidin B2 (**5**), obtained at 70°, is conveniently discussed in terms of three broad regions and individual assignment of the signals was made on the basis of the analysis of the (—)-epicatechin spectrum. In the region 156·0—114·3 p.p.m. eighteen signals due to the twelve carbon atoms associated with the catechol nuclei (**1a** or **2**, rings C and G) plus the six aromatic carbon atoms attached to oxygen in the phloroglucinol nuclei (**1a** and **2**, rings A and E) were observed. The six signals due to the residual carbon atoms in the phloroglucinol rings were readily assigned to the second region of the spectrum,

106·6—93·9 p.p.m. The remaining six resonances (77·5—27·2 p.p.m.) were designated, by comparison with the (−)-epicatechin spectrum and on the basis of the known effects of phenyl and hydroxy-substitution on a cyclohexane ring,[6] to the six sp^3 hybridised carbon atoms in the two heterocyclic rings of the natural product (Table). The spectrum of the proanthocyanidin A2 showed several similarities to that of the procyanidin B2 but the major difference between the spectra of the two compounds lay in the two regions 79·3—27·6 p.p.m. and 105·5—94·6 p.p.m. which contained for proanthocyanidin A2, resonances due to five and seven carbon atoms respectively. The assignment of the five resonances (79·3—27·6 p.p.m.) was made by reference to the spectra of (−)-epicatechin (**3**) and the procyanidin B2 (**5**) and is shown in the Table. The additional resonance (105·6 or 102·6 p.p.m.) in the remaining region (105·5—94·6 p.p.m.) was attributed, by reference to the model compound (**4**), to the acetal type carbon atom C-2 in structure (**1a**) and leads to the confirmation of this structure for the natural product. As with the procyanidins of the B group[2,4] it has not been possible to distinguish between the structure shown (**1a**) and the two alternative 4,6′ linked structures which are possible within the context of the present results.



(8a) R^1 = OH, R^2 = H
(8b) R^1 = H, R^2 = OH



(9)

The doubly linked dimeric proanthocyanidin structure (**1a**) is rigid and does not possess the possibilities for conformational isomerism associated with the procyanidins of the B group.[2,4] It is not decomposed by toluene-α-thiol–acetic acid[4] and the reaction to give cyanidin and (−)-epicatechin (**3**), which occurs in acid media in the absence of oxygen, is only a minor pathway of degradation. The major product of this reaction is an anthocyanidin pigment [λ_{max}. 535 nm, R_F (Forestal) 0·43; R_F (butan-2-ol–acetic acid–water) 0·25] which has been assigned the structure (**6**) and which is presumably formed by alternative cleavage (←----→) of the bicyclic ring system B and D in structure (**1a**).

Three trimeric proanthocyanidins, derivatives of the dimer A2, have also been obtained from natural sources. The major proanthocyanidin from avocado seed (*Persea gratissima*) was first isolated by Geissman and Dittmar[7] and Weinges[2,8] later proposed that it was identical with procyanidin B-4 [(+)-catechin-(−)-epicatechin].[2] The major procyanidin constituents (D_1 and D_2)[4] of avocado seed were obtained as an inseparable mixture, $[\alpha]_{578}$ + 87·3° by chromatography on Sephadex LH-20. Acid treatment of the procyanidin gave cyanidin and approximately equal quantities of (+)-catechin and (−)-epicatechin. With diazomethane the procyanidin mixture gave an undecamethyl ether {$C_{45}H_{25}O_7(OMe)_{11}$, $[\alpha]_{578}$ + 58·2°, M^+ 1018} which formed a triacetate {$C_{45}H_{22}O_4(OMe)_{11}(OAc)_3$, $[\alpha]_{578}$ + 47·9°, M^+ 1144}, and with acetic anhydride a tetradecaacetate ($[\alpha]_{578}$ + 37·8°) was produced. Treatment of the procyanidin with toluene-α-thiol–acetic acid[4] gave (+)-catechin and (−)-epicatechin in equal quantities and the thioether (**1b**, $C_{37}H_{30}O_{12}S$) whose structure was deduced from ^{1}H n.m.r. analysis (hexadeuterioacetone), its conversion with Raney nickel into the proanthocyanidin A2 (**1a**), and the formation of a heptamethyl ether with diazomethane [$C_{37}H_{23}O_5S(OMe)_7$, M^+ 796] and a nona-acetate {$C_{37}H_{21}O_3S$ $(OAc)_9$, $[\alpha]_{578}$ − 58·4°, $(M - 60)^+$ 1016}. In the ^{1}H n.m.r. spectrum of the nona-acetate a complete analysis of the aliphatic protons was possible and this led to the structure (**1b**) for the thioether. The stereochemistry at C-4 in the thioether was formulated on the basis of the analogy with the (−)-epicatechin derivative (**7**) and its probable mode of formation.[4,9] With this evidence and the knowledge of previous degradative work[4] the avocado procyanidins have been formulated as a mixture of equal proportions of the two trimeric species [(**8a**), A2-(−)-epicatechin] and [(**8b**), A2-(+)-catechin].

TABLE

^{13}C *N.m.r. spectra of* (−)-*epicatechin and related proanthocyanidins: chemical shifts in p.p.m. relative to* Me_4Si

Compound	Carbon atoms 2	3	4	2′	3′	4′
(−)-Epicatechin (**3**)	78·1	65·1	25·1	—	—	—
Procyandin B2 (**5**)	74·9	69·3	35·1	77·5	64·2	27·2
Proanthocyanidin A2 (**1a**)	105·5 or 102·6	66·0	29·0	79·3	64·4	27·6

A similar mixture of trimeric proanthocyanidins, $[\alpha]_{578}$ + 71·4°, was obtained from the shells of horse chestnut (*Aesculus hippocastanum*).[4] Hydrolysis with acid gave (−)-epicatechin and both cyanidin and the pigment (**6**), previously derived from the proanthocyanidin A2 (**1a**).

The proanthocyanidin likewise gave an undecamethyl ether {$C_{45}H_{25}O_7$[(OMe)$_{11}$, $[\alpha]_{578}$ + 44·6°, M^+ 1018], which formed a triacetate [$C_{45}H_{27}O_4$(OMe)$_{11}$[(OAc)$_3$, $[\alpha]_{578}$ + 39·7°, M^+ 1144} and a tetradeca-acetate {$C_{45}H_{22}O_4$·(OAc)$_{14}$, $[\alpha]_{578}$ + 31·3°}. Degradation with toluene-α-thiol–acetic acid gave the thioethers (**1b** and **7**), (−)-epicatechin and the proanthocyanidin A2 (**1a**) and on the basis of these observations and analogies with previous work[4] the proanthocyanidin has been formulated as composed of approximately equal parts of the trimeric phenols [(**8a**), A2-(−)-epicatechin] and [(**9**), (−)-epicatechin-A2]. As with the procyanidins of the B group[2,4] it has not yet been possible to distinguish between the structures (**8a**, **8b**, and **9**) and the alternative structures in which the interflavan linkages are of the 4,6′-type.

The evidence which is now available[2,4,8,10] regarding the procyanidins and the isolation of several derivatives of the proanthocyanidin A2 suggests a novel biogenetic hypothesis which links their formation to that of the pigment cyanidin. This theory will be elaborated in more detail in the full paper.

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