Plant Proanthocyanidins. Part II.[1] Proanthocyanidin-A2 and its Derivatives

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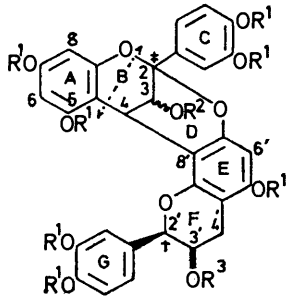
The 2,7"-epoxy-4,8"-biflavan structure (1) has been deduced for the natural proanthocyanidin-A2 on the basis of spectroscopic (^{13}C and ^{1}H n.m.r.) and chemical evidence. Spectroscopic data are also outlined which lead, in association with biogenetic arguments, to a proposal (1a) for the absolute stereochemistry of the natural product. Hydrogenolysis of proanthocyanidin-A2 leads to fission of the C–O and C–C inter-flavan bonds and formation of the phenols (20) and (22). The same technique also leads to rupture of the inter-flavan linkage of dimers of the B group and hence to a novel micro-procedure for their identification. The isolation and characterisation of three trimeric proanthocyanidins based on A2 is also discussed.

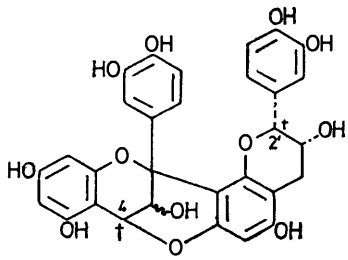
ALTHOUGH phenolic proanthocyanidins readily complex with and precipitate proteins [2,3] they nevertheless comprise one of the major groups of plant polyphenols which occur in the free state in the vegetative tissues of plants.[1,4] Their biosynthesis [5] is intimately connected with the metabolism of (+)-catechin (9) and (−)-epicatechin (8), and, as a preliminary to such studies and wider ones concerning their possible physiological function in plants, structural work has been undertaken on the two principal groups of naturally occurring oligomeric proanthocyanidins. That on the dimers and trimers of the B-group [6] has been reported,[1] and investigations on proanthocyanidin-A2 and its derivatives which are typical of the second class are discussed here.[7]

[1] Part I, R. S. Thompson, D. Jacques, E. Haslam, and R. J. N. Tanner, *J.C.S. Perkin I*, 1972, 1387.
[2] E. C. Bate-Smith, *Phytochemistry*, 1973, **12**, 907.
[3] E. Haslam, *Biochem. J.*, 1974, **139**, 285.
[4] E. Haslam, *J. Chem. Soc.* (*C*), 1969, 1824.
[5] D. Jacques and E. Haslam, *J.C.S. Chem. Comm.*, 1974, 231.
[6] K. Weinges, W. Kaltenhauser, H.-D. Marx, E. Nader, J. Perner, and D. Seiler, *Annalen*, 1968, **711**, 184.
[7] Preliminary report, D. Jacques, E. Haslam, G. R. Bedford, and D. Greatbanks, *J.C.S. Chem. Comm.*, 1973, 518.

Mayer and his collaborators [8] first isolated the crystalline proanthocyanidin-A2, $C_{30}H_{24}O_{12},2H_2O$, from the seed shells of horse chestnut (*Aesculus hippocastanum*). With concentrated hydrochloric acid both cyanidin (10) and (—)-epicatechin (8) were detected by paper chromatography as hydrolysis products. On the basis of this evidence and some spectroscopic data Mayer [8] postulated three structures including (1) and (7) for the natural



(1) R^{1-3} = H
(2) R^{1-3} = Ac
(3) R^1 = Me, R^2 = R^3 = H
(4) R^1 = R^3 = Me, R^2 = H
(5) R^1 = Me, R^2 = R^3 = Ac
(6) R^1 = R^3 = Me, R^2 = Ac



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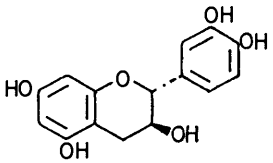

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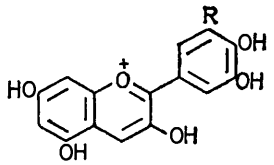


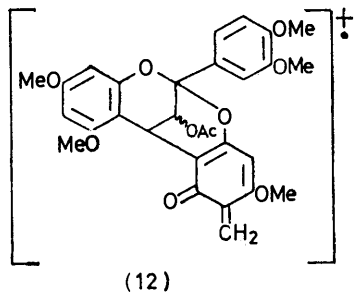
(10) R = H
(11) R = OH

product; of these structure (7) was favoured. Weinges [6] later isolated other proanthocyanidins of the A type as their acetate derivatives and concluded similarly that they were doubly linked structures of the type (1) or (7).

Proanthocyanidin-A2 was isolated from *Aesculus hippocastanum* where it comprises some 8—10% of the total phenolic extract. It formed a nona-acetate (2) and with diazomethane gave both a heptamethyl (3) and an octamethyl ether (4). These ethers themselves formed, respectively, a diacetate (5) and a monoacetate (6), and details of the ^{1}H n.m.r. spectra of these derivatives and of the parent phenol are collated in Table 1. This evidence and mass spectrometric analysis of the ether acetates (5) and (6) confirmed the previous structural proposals of Mayer [8] and Weinges [6] and limited the alternatives for A2 to (1) and (7). The mass spectral fragmentations of both (5) and (6) thus showed only one characteristic retro-Diels–Alder fission of the flavan ring [9] and in the case of the octamethyl ether monoacetate the two fragments resulting from this mode of fission were formulated as (12) and (13) in accord with earlier related work.[9] These observations therefore confirmed the assignment of a structure such as (4) to the octamethyl ether. The formation of this aliphatic ether by the action of diazomethane is therefore rather unusual. In contrast to the observations of Mayer,[8] the acid-catalysed degradation of proanthocyanidin-A2 gave (—)-epicatechin (8) and cyanidin (10) as only very minor products (*ca.* 15%). The major product (*ca.* 70%) was a pigment, discussed below, which had chromatographic characteristics very similar to those of delphinidin (11).[10]

A decision between the two structures (1) and (7) for proanthocyanidin-A2 was made by ^{13}C n.m.r. analysis, utilising the model compounds (8) and (14)—(16). Subsequent to the completion of this study a ^{13}C n.m.r. analysis of proanthocyanidin-A2 has been published.[11] To the extent to which comparison is possible the results of these two studies are in accord, though they differ in experimental detail.

The proposed structure (7) contains two carbon atoms (C-2′ and -4) § with very similar chemical environments to that of C-2 in (—)-epicatechin (8) (†), whereas the alternative structure (1) has only one such centre but in addition a carbon atom (‡) with the distinctive characteristics of an acetal centre. This analysis is based on the different ^{13}C n.m.r. chemical shifts associated with such nuclei. Spectra were obtained by using the pulse Fourier transform technique and assignments of the individual resonances were facilitated by broad band and off-resonance decoupling [12] and by the use of the limited



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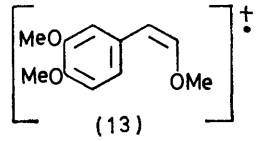


(13)

data available from the ^{13}C n.m.r. spectra of related compounds. Particular use was thus made of the known ^{13}C substituent effects on a phenyl ring [13,14] and on a cyclo-

§ In order to avoid using a multiplicity of primes, locants are not assigned to the phenyl rings; thus the locants of one chroman unit are unprimed and those of the other carry single primes.

[8] W. Mayer, L. Goll, E. V. Arndt, and A. Mannschreck, *Tetrahedron Letters*, 1966, 429.
[9] J. W. Clark-Lewis, *Austral. J. Chem.*, 1968, **21**, 3025.
[10] J. B. Harborne, 'Comparative Biochemistry of the Flavonoids,' Academic Press, London and New York, 1967, p. 7.
[11] G. Schilling, K. Weinges, O. Muller, and W. Mayer, *Annalen*, 1973, 673.
[12] E. Breitmaier, G. Jung, and W. Voelter, *Angew. Chem. Internat. Edn.*, 1971, **10**, 673.
[13] H. Spiesecke and W. G. Schneider, *J. Chem. Phys.*, 1961, **35**, 731.
[14] G. E. Mariel and R. V. James, *J. Amer. Chem. Soc.*, 1964, **86**, 3893.

TABLE 1

^{1}H N.m.r. chemical shifts (τ values) and coupling constants (Hz) for proanthocyanidin-A2 and its derivatives

' Upper ' flavan unit

Proton(s)	3	4	6	8	Ring c	OH (phenolic)	OMe	OAc
Proanthocyanidin-A2 [a]	6·0br (s)	5·56 (d)	4·0 (d)	3·90 (d)	2·8—3·2 (m)	0·6—1·6 (3H, m) 1·78 (1H, s)		
Nona-acetate [b]	4·80 (d)	5·40 (d)	3·20 (d)	3·50 (d)	2·4—2·85 (m)			7·74(3) 8·26(1) 8·50(1)
Heptamethyl ether [3]	5·75 (d)	5·05 (d)	3·93 (d)	3·68 (d)	2·6—3·1 (m)		6·08(1) 6·10(1) 6·26(1) 6·53(1)	
Heptamethyl ether diacetate [b]	4·50 (d)	5·04 (d)	3·94 (d)	3·70 (d)	2·7—3·2 (m)		6·09(1) 6·10(1) 6·29(1) 6·50(1)	8·26(1)
Octamethyl ether [b]	5·78 (d)	5·10 (d)	4·01 (d)	3·74 (d)	2·66—3·3 (m)		6·12(1) 6·14(1) 6·30(1) 6·62(1)	
c	5·24 (d)	4·52 (d)	3·75 (d)	3·32 (d)	2·2—3·05 (m)		6·20(1) 6·23(1) 6·42(1) 6·66(1)	
Octamethyl ether acetate [b]	4·52 (d)	5·10 (d)	4·03 (d)	3·74 (d)	2·7—3·27 (m)		6·12(2) 6·30(1) 6·61(1)	8·26(1)
Thioether (20) [d]	5·8—6·2 (m)	5·54 (d)	3·98 (d)	3·92 (d)	2·8—3·2 (m)	1·75—2·1 (m)		
Thioether (29) nona-acetate [b]	4·78br (s)	5·40 (d)	3·46 (d)	3·16 (d)	2·4—2·9 (m)			7·64(3) 8·24(1) 8·54(1)
	$J_{3,4}$ 3·5		$J_{6,8}$ 2·5					

'Lower' flavan unit

Proton(s)	2′	3′	4′	6′	Ring G	OH (phenolic)	OMe	OAc	Ph	S·CH$_2$
Proanthocyanidin-A2 [a]	5·06 (s)	5·75br (s)	7·18 (m)	3·87 (s)	2·8—3·2 (m)	0·6—0·13 (m)				
Nona-acetate [b]	4·78 (m)	4·78 (m)	7·15 (m)	3·48 (s)	2·4—2·85 (m)			7·74(3) 8·06(1)		
Heptamethyl ether [b]	4·97 (s)	5·53 (m)	7·05 ‡ (dd) 7·30 (dd)	3·78 (s)	2·62—3·11 (m)		6·10(1) 6·20(1) 6·26(1)			
Heptamethyl ether diacetate [b]	4·80 (s)	4·40 (m)	7·10 (m)	3·82 (s)	2·7—3·2 (m)		6·10(1) 6·22(1) 6·27(1)	8·10(1)		
Octamethyl ether [b]	4·76 † (d)	6·04 (m)	7·02 * (dd) 7·40 (dd)	3·84 (s)	2·7—3·3 (m)		6·14(1) 6·16(1) 6·30(1) 6·44(1)			
c	4·58 † (d)	5·85 (m)	7·0 (m)	3·53 (s)	2·2—3·05 (m)		6·20(1) 6·23(1) 6·32(1) 6·34(1)			
Octamethyl ether acetate [b]	4·76 † (d)	6·04 (m)	7·10 * (dd) 7·48 (dd)	3·83 (s)	2·7—3·3 (m)		6·12(1) 6·16(1) 6·27(1) 6·42(1)			
Thioether (29) [d]	4·52br (s)	5·89 (m)	5·90 (m)	3·80 (s)	2·8—3·2 (m)	1·75—2·1 (m)			2·5—2·8 (m)	5·8—6·2 (m)
Thioether (29) nona-acetate [b]	4·20br (s)	4·82br (s)	5·84 (s)	3·52 (s)	2·4—2·9 (m)			7·66(1) 8·02(1) 8·14(1) 8·70(1)	2·4—2·9 (m)	5·92, 6·14 (*J* 14)

* $J_{3',4'}$ 4·0 and 5·0, $J_{4'R,4'S}$ 16·0. † $J_{2',3'}$ 1·5. ‡ $J_{3',4'}$ 2·5 and 3·5, $J_{4'R,4'S}$ 16·5.

[a] In $(CD_3)_2SO$. [b] In $CDCl_3$. [c] In C_5D_5N. [d] In $(CD_3)_2CO$.

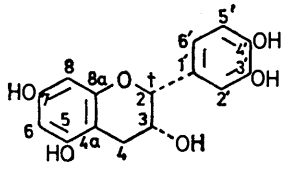
hexane ring [15-17] to confirm the assigned chemical shift values in the model compounds (8), (14), and (15) (Table 2). Assignments were made analogously of the twenty-four resonances (93·9—156·0 p.p.m. from Me_4Si) of the carbon atoms associated with the aromatic rings in procyanidin-B2 (16). The remaining six resonances were then assigned to the six carbon atoms in the two heterocyclic rings on the procyanidin (Table 2). Three of these were associated, by analogy with (—)-epicatechin, with the corresponding carbon atoms in the ' lower ' flavan unit in procyanidin-B2 (C-2', -3', and -4'; Table 2). Although the geometry of the heterocyclic ring of flavan-3-ols is significantly different from that of cyclohexane, broad corroboration of the assignment of the remaining three resonances (C-2, -3, and -4) was nevertheless gained by comparison with the known effects of substitution of an *o*-hydroxyphenyl group on a cyclohexane ring.[15] The shifts in the resonances of C-3 (4·2 p.p.m.) and C-4 (10·0 p.p.m.) in comparison with those in (—)-epicatechin (Table 2) thus accords with the predicted effects as suggested by Pehk and Lippmaa.[15]

TABLE 2

^{13}C N.m.r. chemical shifts of (—)-epicatechin and related proanthocyanidins (p.p.m. relative to Me_4Si)

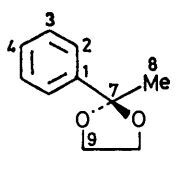
Compound	Carbon atoms 2	3	4	2'	3'	4'
(—)-Epicatechin (8)	78·1	65·1	25·1			
Procyanidin-B2 (16)	74·9	69·3	35·1	77·5	64·2	27·2
Proanthocyanidin-A2 (1a)	102·6	66·0	29·0	79·3	64·4	27·6

The ^{13}C n.m.r. spectrum of A2 showed signals due to twenty four carbon atoms (94·6—156·5 p.p.m. from Me_4Si), one resonance of the type previously associated with C-2 of (—)-epicatechin (8) (†, 79·3 p.p.m.) and four signals (66·0—27·6 p.p.m.) which have been assigned as shown (Table 2). One additional resonance (102·6 p.p.m. from Me_4Si) was, by comparison with the model acetals (14) and (15), assigned to a carbon atom with a very similar environment in A2 (1) (‡) and hence this observation leads unequivocally to an overall structure such as (1) for the natural product. The substituents at C-2 and -4 in (1) necessarily occupy quasi-axial positions on the heterocyclic ring and the change in chemical shift of the carbon atoms C-3 (3·3 p.p.m.) and C-4 (6·1 p.p.m.) in comparison with those in procyanidin-B2 may be rationalised satisfactorily (within the framework of assumptions outlined above) in terms of the effect of the substitution of an axial oxygen substituent at C-2.[17]



(8)

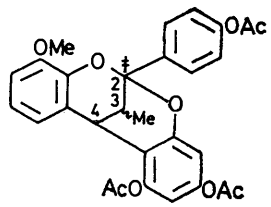
C-8	94·3	C-5'	114·8	C-4'	144·3
C-6	95·4	C-6'	118·0	C-7	155·7
C-4a	98·6	C-1'	130·6	C-5	156·2
C-2'	114·8	C-3'	144·3	C-8a	156·4



(15)

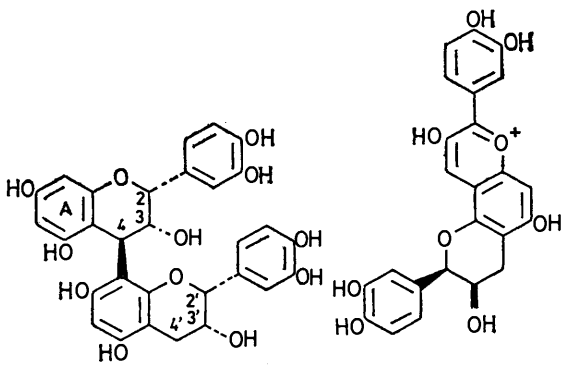
C-8	27·4	C-4	127·7		
C-9	64·2	C-3	127·9		
C-7	108·3	C-1	128·1		
C-2	125·1				

(p.p.m. from Me_4Si)



(14)

C-2	101·5	O-Me	56·0
C-3	34·2	C-Me	13·4
C-4	34·5	CO-Me	20·9



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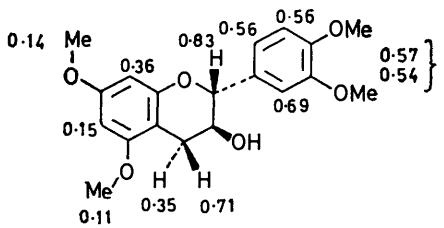

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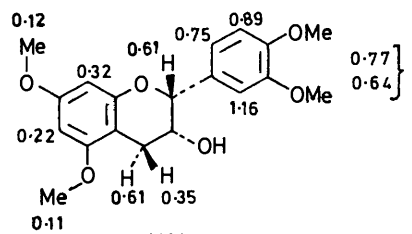


(18)

Lanthanide [Eu(fod)$_3$] induced shifts (p.p.m). of (+)-catechin tetramethyl ether (17) and (—)-epicatechin tetramethyl ether (18): shifts relative to H-3 shift (1·0 p.p.m.).

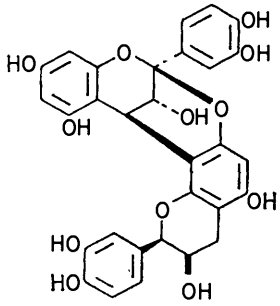
[15] T. P. Pehk and E. Lippmaa, *Org. Magnetic Resonance*, 1971, **3**, 679.

[16] D. K. Dalling and D. M. Grant, *J. Amer. Chem. Soc.*, 1967, **89**, 6612.

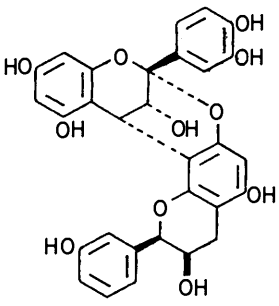
[17] J. D. Roberts, F. J. Weigert, J. I. Kroschwitz, and H. J. Reich, *J. Amer. Chem. Soc.*, 1970, **92**, 1338.

This evidence limits the possible structures for A2 to (1) and two alternatives in which the inter-flavan bonds are between (i) C-4 and -6′ and (ii) C-2 and one of the phenolic oxygen atoms at C-5′ and -7′ in the ' lower ' flavan unit. Molecular models and the ^{1}H n.m.r. data (Table 1), however, strongly support structure (1). In the spectra of the phenol, its nona-acetate, and both its hepta- and octa-methyl ethers distinctive signals arise due, respectively, to *one* phenolic hydroxy-group (τ 1·78), a phenolic acetate (τ 8·50), and a phenolic methyl ether (τ 6·53 and 6·62), all of which occur at unusually high field. Models show that these effects are most probably due to the shielding influence of the aromatic ring G on the oxygen substituent at C-5. In the rigid structure of the natural product (1) this substituent is held above and close to the aromatic ring G. This possibility is not present in either of the alternative formulations with a C-4 to -6′ inter-flavan link.

On biogenetic grounds it seems highly probable that the ' upper ' flavan unit of A2 is derived from a molecule with the (−)-epicatechin absolute stereochemistry (8). Thus only trace quantities of (+)-catechin (9) have been found in *Aesculus hippocastanum* fruit shells [1] whilst (−)-epicatechin (8) (*ca.* 25%), procyanidins-B2 (16) (*ca.* 8%) and -B5 (*ca.* 2%) [both dimers of (−)-epicatechin] are major constituents of the phenolic extract. With this biogenetic argument in mind the stereochemical configurations of proanthocyanidin-A2 are limited to (1a) and (1b). Earlier work [18] has shown that pyridine-induced ^{1}H n.m.r. shifts are valuable for establishing the



(1a)



(1b)

chemical and stereochemical position of protons neighbouring hydroxy-groups. In (+)-catechin tetramethyl ether (17) and (−)-epicatechin tetramethyl ether (18) the relative changes in n.m.r. chemical shift in [^{2}H]chloroform as compared with [$^2H_5$]pyridine for the two aromatic protons H-6 and -8 were observed as 1 : 1·26 and 1 : 1·66, respectively. With proanthocyanidin-A2 octamethyl ether (4) the change in chemical shift induced by pyridine for H-6 and -8 was found (Table 1) to be as 1 : 1·60, which mirrors closely that observed for (−)-epicatechin tetramethyl ether (18). This observation suggests therefore that the hydroxy-group at C-3 in (1) bears a similar stereochemical relationship to the aromatic ring A in (1) as does the aliphatic hydroxy-group in (−)-epicatechin (8). Hence (1a) represents the complete structure and stereochemistry of the natural product.

Attempts to elucidate details of the relative stereochemistry of the hydroxy-group at C-3 in proanthocyanidin-A2 by use of the lanthanide shift reagent Eu(fod)$_3$ were unsuccessful. The effects of increasing concentrations of Eu(fod)$_3$ on the spectra of the model compounds (17) and (18) were rationalised in terms of the presence of two principal sites for the association of the paramagnetic ion with these substrates—namely the hydroxy-group at C-3 and the *ortho*-dimethoxy-system in the pendant aromatic ring. Changes in chemical shift were related to that of the C-3 proton (relative shift 1·0). In agreement with the observations of Wright and Tang Wei [19] aromatic methyl ethers appear to bind strongly to Eu(fod)$_3$ only when there are at least two ether groups *ortho* to one another. The accidental magnetic equivalence [20] of the methylene protons at C-4 in (−)-epicatechin tetramethyl ether is removed in the presence of Eu(fod)$_3$ and on the basis of the changes in chemical shift of the two protons [3] it was possible to deduce $J_{3,4S}(syn) = 4{\cdot}0$ and $J_{3,4R}(anti) = 2{\cdot}0$ Hz. In the case of the two methyl ether derivatives of proanthocyanidin-A2 [(3) and (4)] a comparable analysis was not possible owing to the multiplicity of binding sites in these ethers.

Little success has attended efforts specifically to degrade proanthocyanidin-A2. The acid-catalysed decomposition gave as its major product (*ca.* 70%) a typical anthocyanidin pigment whose R_F values [10] were very similar to those of the natural floral pigment delphinidin (11) but which showed $\lambda_{max.}$ (1% v/v HCl–EtOH) 535 and 282 nm. (−)-Epicatechin (8) was detected transiently during the early stages of the reaction and small quantities (*ca.* 10%) of cyanidin (10) were also detectable amongst the hydrolysis products. The major pigment has been provisionally formulated as (19) and it is proposed that this is formed by the normal acid-catalysed fission of the bicyclo[3.3.1]nona-2,8-diene at the points shown (◄---►) in ring B of (1). The other product of this mode of breakdown is phloroglucinol and this was readily identified during the hydrolysis. Presumably splitting at these points takes precedence over the alternative mode across ring D [to yield cyanidin and (−)-epicatechin] owing to the preferred initial protonation at the more basic resorcinol type ring A as compared with the monophenolic ring E. The decomposition to give (19) occurred in the absence of oxygen in contrast to the breakdown of procyanidins of the B-class [1] and was not accompanied by the usual phlobaphen formation. Prothocyanidin-A2 was, surprisingly, resistant to degradation by toluene-α-thiol and acetic acid.[1]

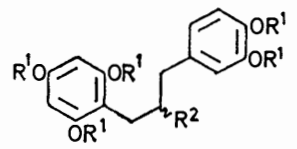
Catalytic hydrogenolysis of proanthocyanidin-A2 gave two major phenolic products, (20) and (22), which were identified by ^{1}H n.m.r. and by conversion into their

[18] T. N. Huckberry, *Ann. Reports N.M.R. Spectroscopy*, ed. E. F. Mooney, vol. 3, Academic Press, London and New York, 1970, p.19.

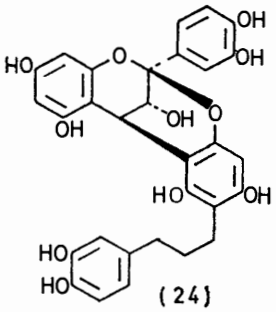
[19] G. E. Wright and T. Y. Tang Wei, *Tetrahedron*, 1973, **29**, 3775.

[20] D. T. Coxon, W. D. Ollis, and A. Holmes, *Tetrahedron Letters*, 1970, 5241.

phenolic methyl ethers, (21) and (23). These had been obtained previously by reduction of (−)-epicatechin tetramethyl ether (18) with sodium in ethanol [21] or



(20) $R^1 = R^2 = H$
(21) $R^1 = Me, R^2 = H$
(22) $R^1 = H, R^2 = OH$
(23) $R^1 = Me, R^2 = OH$

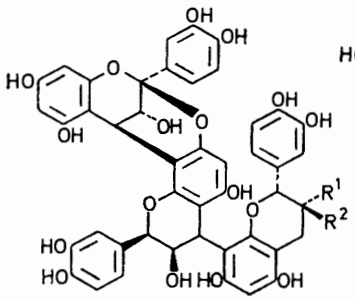


(24)

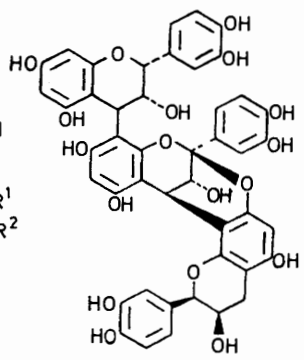
ethanol–liquid ammonia [22] and were key compounds in the earlier stereochemical study of the catechins. Both phenols were also obtained by catalytic hydrogenolysis of (−)-epicatechin (8) and the small and variable optical rotation of the alcohol (22) (from different experiments) suggests that partial racemisation occurs during the reduction. Hydrogenolysis of (+)-catechin also gave the diarylpropane (20) and the alcohol (22) which had a small but opposite optical rotation. These results contrast with those of Clark-Lewis and Ramsay [23] who hydrogenolysed the catechin ethers under pressure but only obtained the alcohol (23). Interruption of the hydrogenolysis of A2 showed the formation of a complex mixture of products from which one major product was isolated and tentatively identified of the basis of ^1H n.m.r. and mass spectroscopic analysis of the phenolic methyl ether as (24).

The formation of both (20) and (22) from A2 (1a) must involve the unusual hydrogenolysis of a benzylic carbon–phenyl linkage. The ease of this particular mode of cleavage in this class of compound has been further demonstrated by examinations of procyanidins of the B type. In all cases examined (B1, B2, B3, B4, and B8) the major final products were the phenols (20) and (22). However at an early stage of the hydrogenolysis (15—30 min) both ' halves ' of these dimers were readily detected by paper chromatography [*e.g.* (+)-catechin and (−)-epicatechin from B8 and B4] and this procedure therefore provides a simple and alternative method to the toluene-α-thiol degradation [1] for the chemical identification of procyanidin dimers of the B class.

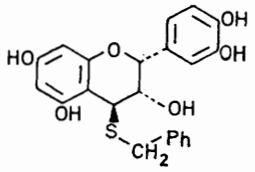
Three trimeric proanthocyanidins, derivatives of the dimer A2, have also been obtained from natural sources. The principal proanthocyanidin of avocado seed (*Persea gratissima*) was first obtained by Geissman and Dittmar [24] and on the basis of the R_F value of its peracetate Weinges [6,25] later proposed that it was identical with procyanidin-B4 [(+)-catechin-(−)-epicatechin]. The principal procyanidin constituents (D1 and D2) [1] of avocado seed were obtained as an inseparable mixture by chromatography on Sephadex LH-20. With diazomethane the procyanidin mixture gave an undecamethyl ether ($M^{+\cdot}$ 1018) which formed a triacetate ($M^{+\cdot}$ 1144). With acetic anhydride a tetradeca-acetate was produced. Acidic treatment of the procyanidins gave approximately equal amounts of (+)-catechin (9) and (−)-epicatechin (8) and cyanidin (10). Treatment with toluene-α-thiol–acetic acid in pentanol also gave (+)-catechin and (−)-epicatechin and the thioether (29), whose structure was deduced from ^1H n.m.r. analysis (Table 1), its conversion with Raney nickel into proanthocyanidin A2 (1a), and the formation of a nona-acetate and a heptamethyl ether. The ^1H n.m.r. spectrum of the nona-acetate allowed a complete analysis of the structure of the thioether and the stereochemistry at C-4′ in the molecule was formulated as in (29) on the basis of analogy with the (−)-epicatechin derivative (28) and its probable mode of formation.[1] With this evidence in mind the avocado procyanidin mixture has been formulated as a mixture of approximately equal amounts of the two trimeric structures (25) [A2-(−)-epicatechin] and (26) [A2-(+)-catechin].



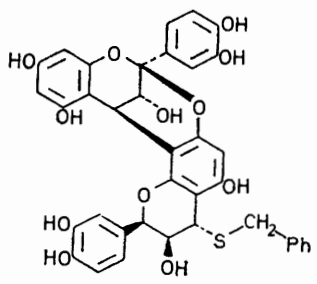
(25) $R^1 = OH, R^2 = H$
(26) $R^1 = H, R^2 = OH$



(27)



(28)



(29)

A mixture of trimeric proanthocyanidins (25) [A2-(−)-epicatechin] and (27) [(−)-epicatechin-A2] was similarly obtained from the seed shells of *Aesculus hippocastanum*. Hydrolysis with acid gave (−)-epicatechin (8), cyanidin (10), and the pigment (19) and degradation with toluene-α-thiol–acetic acid yielded the two thioethers (28) and (29), (−)-epicatechin (8), and A2 (1a). On the basis of this evidence and the formation of an undecamethyl ether

[21] K. Freudenberg and E. Cohn, *Ber.*, 1923, **56**, 2127.
[22] A. J. Birch, J. W. Clark-Lewis, and A. V. Robertson, *J. Chem. Soc.*, 1957, 3586.
[23] J. W. Clark-Lewis and G. C. Ramsay, *Austral. J. Chem.*, 1965, **18**, 389.
[24] T. A. Geissman and F. Dittmar, *Phytochemistry*, 1965, **4**, 267.
[25] K. Weinges, H. Gorissen, and R. Lontie, *Ann. Physiol. Veg.*, 1969, **11**, 67.

($M^{+\cdot}$ 1018), an undeca-*O*-methyl triacetate ($M^{+\cdot}$ 1144), and a tetradeca-acetate the proanthocyanidin mixture has been formulated as composed of (25) and (27) in approximately equal parts.

The presence of the structural unit of A2 (1a) in polyphenols of the proanthocyanidin class may be readily inferred from its characteristic o.r.d. and c.d. spectra. Both trimeric proanthocyanidin mixtures thus gave similar o.r.d. and c.d. data but no structural conclusions have yet been deduced from this information owing to the absence of appropriate models.

EXPERIMENTAL

Mass spectra were obtained with A.E.I. MS9 and MS12 instruments. O.r.d. and c.d. measurements were made with a Thorn-Bendix instrument. Paper chromatographic analysis (two-dimensional) was carried out at 20 ± 2° on Whatman No. 2 paper (27·5 cm^2) in the solvent systems (A) 6% acetic acid (v/v) and (B) butan-2-ol–acetic acid–water (14 : 1 : 5 v/v). Proanthocyanidins and other *o*-dihydroxyphenols were detected as described previously.[1] Anthocyanidins were determined by measurement of their u.v. absorption at 530—550 nm (ethanolic 1% HCl) and by chromatography on cellulose pre-coated plastic sheets in Forestal solvent.[10]

^{13}C *N.m.r. spectroscopy.*—^{13}C N.m.r. spectra were recorded at 25·1 MHz with a Varian HA 100D instrument, with Varian pulse box linked to a G.E.C. M1240 computer. The protons were decoupled at 100 MHz by using a Varian heteronuclear decoupler V 3512-1 at 100 MHz. The sample (200 mg) was dissolved in [$^2H_6$]dimethyl sulphoxide (1 ml) in an 8 mm tube. The reference for the homonuclear stabilisation channel was a capillary of ^{13}C-enriched methyl iodide. The spectra were referenced to Me_4Si by using the relationship $\delta(Me_4Si) = \delta(Me_2SO) + 39{\cdot}6$.

Phenols from Aesculus hippocastanum.—The crude extract of phenols (25 g) from the freshly picked fruit shells of *Aesculus hippocastanum* was obtained as described previously.[1] Chromatography in ethanol on Sephadex LH-20 gave products from the following fractions (15 ml):

Fractions 38—65. (−)-Epicatechin (6·1 g).

Fractions 70—85. Procyanidin-B2 (0·75 g) was obtained as a buff powder after freeze-drying from t-butyl alcohol–water; R_F(A) 0·58, R_F(B) 0·42; c.d. (*c* 0·70 in MeOH): $[\theta]_{323}$ +528, $[\theta]_{312}$ +1056, $[\theta]_{303}$ +1320, $[\theta]_{294}$ +3696, $[\theta]_{286}$ +3696, $[\theta]_{278}$0, $[\theta]_{270}$ −4488, $[\theta]_{263}$ −3960, $[\theta]_{256}$ −1056, $[\theta]_{250}$ +3700, $[\theta]_{244}$ +6864. Methylation with diazomethane in methanol and purification by t.l.c. on silica [chloroform–methanol, 3% v/v] followed by precipitation from acetone by methanol gave the octamethyl ether as a white amorphous powder (Found: C, 66·6; H, 5·7. Calc. for $C_{38}H_{42}O_{12}$: C, 66·1; H, 6·1%), c.d. (*c* 0·78 in ethanol): $[\theta]_{323}$ +2211, $[\theta]_{312}$ +3564, $[\theta]_{303}$ +3570, $[\theta]_{294}$ +3993, $[\theta]_{286}$ 6000, $[\theta]_{278}$ +2673, $[\theta]_{270}$ −1835, $[\theta]_{263}$ +891, $[\theta]_{256}$ +8038, $[\theta]_{250}$ +21,450, $[\theta]_{244}$ +36,300, $[\theta]_{238}$ +38,610, $[\theta]_{232}$ +19,140.

Acetylation with acetic anhydride–pyridine of procyanidin-B2 gave an amorphous deca-acetate which was purified by precipitation from ethanol–water (Found: C, 60·1; H, 4·8. Calc. for $C_{50}H_{46}O_{22}$: C, 59·9; H, 4·8), R_F 0·48 (silica; benzene–acetone, 8 : 2), $[\alpha]_{578}^{20}$ +40° (*c* 1·5 in Me_2CO), τ ($CDCl_3$) 2·6—3·4 (6 Ar-H,m), 3·34 (1H, s, H-6′), 3·76 and 4·0 (2H, q, *J* 2·0 Hz, H-6, -8), 4·40 (1H, s, H-2′), 4·78—4·90 (2H, m, H-3, -3′), 5·42 (1H, s, H-2), 5·53 (1H, d, *J* 1·5 Hz, H-4), 6·95—7·15 (2H, m, H-4′), and 7·66, 7·74, 7·83, 7·78, 8·02, and 8·10 (30H, OAc).

Fractions 85—160. Proanthocyanidin-A2 (1·78 g) was obtained as fine white needles, m.p. >300°, after trituration with water and crystallisation from acetone–water (Found: C, 58·6; H, 4·8. Calc. for $C_{30}H_{24}O_{12}$,$2H_2O$: C, 58·8; H, 4·6%), $[\alpha]_{578}^{20}$ +56·9° (*c* 0·62 in MeOH), R_F(A) 0·24, R_F(B) 0·51, c.d. (*c* 0·62 in MeOH): $[\theta]_{233}$ +65,030, $[\theta]_{238}$ +74,320, $[\theta]_{244}$ +40,920, $[\theta]_{250}$ +9290, $[\theta]_{256}$ −8360, $[\theta]_{263}$ −18,580, $[\theta]_{270}$ −37,110, $[\theta]_{278}$ −20,460, $[\theta]_{286}$ −4645, $[\theta]_{294}$ +919. The *nona-acetate* of A2, prepared in acetic anhydride–pyridine, crystallised from methanol as small prisms, m.p. 156—157° (Found: C, 60·5; H, 4·2. $C_{48}H_{42}O_{21}$ requires C, 60·4; H, 4·4%), $[\alpha]_{278}^{20}$ −88·6° (*c* 0·60 in MeOH); c.d. (*c* 1·8 in MeOH): $[\theta]_{303}$ −1584, $[\theta]_{294}$ −2640, $[\theta]_{286}$ −9504, $[\theta]_{278}$ −7392, $[\theta]_{270}$ +30,624, $[\theta]_{263}$ +25,512, $[\theta]_{256}$ +12,672, $[\theta]_{250}$ 0, $[\theta]_{244}$ −13,728. Methylation of proanthocyanidin-A2 (1·0 g) in methanol (20 ml) with diazomethane (excess in ethereal solution; 24 h) gave a product which was separated by t.l.c. (silica; methanol–chloroform, 2% v/v) to give the heptamethyl ether (R_F 0·45) and an octamethyl ether (R_F 0·55). The *heptamethyl ether* was obtained after precipitation from ethanol–water as an amorphous powder (0·65 g) (Found: C, 65·6; H, 5·9. $C_{37}H_{38}O_{12}$ requires C, 65·9; H, 5·6%), $M^{+\cdot}$ 674, $[\alpha]_{578}^{20}$ +39·6° (*c* 0·68 in Me_2CO), c.d. (*c* 0·60 in MeOH): $[\theta]_{303}$ +1120, $[\theta]_{294}$ +1320, $[\theta]_{286}$ +2640, $[\theta]_{278}$ −7128, $[\theta]_{270}$ −15,576, $[\theta]_{263}$ −13,365, $[\theta]_{256}$ −10,692, $[\theta]_{250}$ −26,730, $[\theta]_{244}$ −39,270. The *heptamethyl ether diacetate* was prepared with acetic anhydride–pyridine and crystallised from acetone–methanol as small prisms, m.p. 184—185° (Found: C, 64·6; H, 5·9. $C_{41}H_{42}O_{14}$ requires C, 64·9; H, 5·5%), $[\alpha]_{578}^{20}$ +21·6° (*c* 0·5 in MeOH), $M^{+\cdot}$ 758. The *octamethyl ether* was obtained after precipitation from ethanol–water as an amorphous powder (0·15 g) (Found: C, 66·0; H, 6·1. $C_{38}H_{40}O_{12}$ requires C, 66·3; H, 5·8%), $M^{+\cdot}$ 688, $[\alpha]_{578}^{20}$ +21·8° (*c* 0·7 in MeOH). The octamethyl ether acetate crystallised from methanol as minute prisms, m.p. 139—141° (Found: C, 66·0; H, 5·9. $C_{40}H_{42}O_{13}$ requires C, 65·8; H, 5·8%), $M^{+\cdot}$ 730.

Proanthocyanidin-A2 (0·05 g) was heated at 60° in ethanol (5 ml) containing concentrated hydrochloric acid (1%) and samples were taken after 15, 30, 60, 120, 240, and 480 min. Analysis after 240 min by paper chromatography showed cyanidin, (−)-epicatechin, and phloroglcuinol to be present. The major anthocyanidin pigment had R_F(A) 0·05, R_F(B) 0·29, R_F(Forestal) 0·43, and $\lambda_{max.}$ (EtOH–1% HCl) 535 nm.

Fractions 190—240. These gave, after rechromatography on Sephadex LH-20, a mixture of proanthocyanidins-D2 and -D3 (0·35 g), R_F(A) 0·32, R_F(B) 0·36, $[\alpha]_{578}^{20}$ +71·4° (*c* 1·05 in Me_2CO), τ[$(CD_3)_2CO$] 2·1br (11H, s), 3·0 (9H, m), 4·1 (4H, m), 4·7 (1H, s), 6·0 (9H, m), and 7·2 (2H, m). Treatment with diazomethane in methanol gave a mixture of undeca-*O*-methylproanthocyanidins-D2 and -D3 which was isolated by t.l.c. (silica; methanol–chloroform, 3% v/v; R_F 0·30), $M^{+\cdot}$ 1018 ($C_{56}H_{58}O_{18}$), $[\alpha]_{578}^{20}$ +44·6° (*c* 0·65 in Me_2CO), c.d. (*c* 1·04 in MeOH) $[\theta]_{250}$ +14,690, $[\theta]_{256}$ −15,660, $[\theta]_{263}$ −21,540, $[\theta]_{270}$ −27,450, $[\theta]_{278}$ −13,706, $[\theta]_{286}$ −100, τ ($CDCl_3$) 3·0 (9H, m), 3·9 (4H, m), 4·3 (1H, s), 5·6 (6H, m), 6·3 (36H, m), and 7·15 (2H, m). The mixture of tri-*O*-acetylundeca-*O*-methylproanthocyanidins-D2 and -D3 was prepared with acetic anhydride–pyridine and purified by t.l.c. (silica; methanol–chloroform, 3% v/v; R_F 0·53) as an amorphous powder, $M^{+\cdot}$ 1144 ($C_{62}H_{64}O_{21}$), $[\alpha]_{578}^{20}$ +39·7° (*c* 0·40 in Me_2CO), τ ($CDCl_3$) 3·0 (9H, m), 3·8 (4H, m), 4·4 (1H,

s), 5·3 (6H, m), 6·3 (33H, m), 7·10 (2H, m), and 8·2 (9H, m). The mixture of tetradeca-*O*-acetylpropanthocyanidins-D2 and -D3 was prepared with pyridine–acetic anhydride and purified by t.l.c. (R_F 0·68; silica gel; methanol–chloroform, 3% v/v), $[\alpha]_{578}^{20}$ +31·1° (*c* 0·96 in Me_2CO), c.d. (*c* 1·0 in MeOH): $[\theta]_{227}$ +145,200, $[\theta]_{238}$ +87,120, $[\theta]_{251}$ +14,520, $[\theta]_{263}$ +58,080, $[\theta]_{270}$ +75,500, $[\theta]_{278}$ +43,560, $[\theta]_{282}$ 0, $[\theta]_{286}$ −5810, $[\theta]_{290}$ 0.

Proanthocyanidins-D1 and -D2.—The proanthocyanidin mixture was obtained as an amorphous powder after chromatography of the phenolic extract of *Persea gratissima* on Sephadex LH-20 in ethanol and had R_F(A) 0·29, R_F(B) 0·35, $[\alpha]_{578}^{20}$ +87·3° (*c* 0·75 in Me_2CO), c.d. (*c* 1·23 in MeOH): $[\theta]_{250}$ +15,440, $[\theta]_{263}$ −26,280, $[\theta]_{267}$ −37,910, $[\theta]_{270}$ −36,500, $[\theta]_{278}$ −19,600, $[\theta]_{286}$ −11,230, $[\theta]_{294}$ 0, $[\theta]_{323}$ +2110, τ [$(CD_3)_2CO$] 2·0br (11H, s), 3·0 (9H, m), 4·0 (4H, m), 4·45—4·65 (1H, m), 6·0 (9H, m), and 7·25 (2H, m). The mixed undeca-*O*-methylproanthocyanidins-D1 and -D2 were obtained after treatment with diazomethane and t.l.c. (silica; methanol–chloroform, 3% v/v; R_F 0·52), $M^{+\cdot}$ 1018 ($C_{56}H_{58}O_{18}$), $[\alpha]_{578}^{20}$ +58·2° (*c* 0·6 in Me_2CO), c.d. (*c* 0·28 in MeOH): $[\theta]_{250}$ −25,950, $[\theta]_{256}$ −29,940, $[\theta]_{263}$ −33,930, $[\theta]_{270}$ −45,910, $[\theta]_{278}$ −33,930, $[\theta]_{286}$ −13,970, $[\theta]_{294}$ −2000, $[\theta]_{323}$ 0. The mixture of tri-*O*-acetylundeca-*O*-methylproanthocyanidins-D1 and -D2 was obtained after treatment with acetic anhydride–pyridine and purified by t.l.c. (silica; methanol–chloroform, 3% v/v; R_F 0·65), $M^{+\cdot}$ 1144 ($C_{62}H_{64}O_{21}$), $[\alpha]_{578}^{20}$ +47·9°. Treatment of the original proanthocyanidin mixture with acetic anhydride–pyridine and t.l.c. (silica; methanol–chloroform, 3% v/v; R_F 0·72) gave the mixed tetradeca-*O*-acetylproanthocyanidins-D1 and -D2 as an amorphous powder after precipitation from ethanol–water, $[\alpha]_{578}^{20}$ +37·8° (*c* 0·84 in Me_2CO), c.d. (*c* 1·8 in MeOH): $[\theta]_{246}$ +25,740, $[\theta]_{250}$ +19,130, $[\theta]_{256}$ +27,360, $[\theta]_{263}$ +53,100, $[\theta]_{270}$ +80,450, $[\theta]_{278}$ +53,100.

Degradation of Proanthocyanidins-D1—3.—(*a*) Proanthocyanidins-D1 and -D2 (*Persea gratissima*) (1·5 g) were dissolved in ethanol (10 ml) containing toluene-α-thiol (3·0 ml) and acetic acid (1 ml) and the solution was refluxed under nitrogen for 48 h. The solvents were removed and the residual oil applied to a Sephadex LH-20 column (35 × 3·5 cm) in chloroform–ethanol (4 : 1). Elution gave 10 ml fractions which were grouped as follows: fractions 101—160 gave a mixture of (+)-catechin and (−)-epicatechin (0·04 g), fractions 230—260 gave proanthocyanidin-A2 thiobenzyl ether (29), (0·150 g) from fractions 280—440 and proanthocyanidin-A2 (0·07 g) from fractions 641—735.

Proanthocyanidin-A2 thiobenzyl ether (29) obtained as described above was an amorphous powder, c.d. (*c* 0·72 in MeOH): $[\theta]_{263}$ −7760, $[\theta]_{267}$ −11,630, $[\theta]_{270}$ −9690, $[\theta]_{286}$ −7760, $[\theta]_{294}$ −1940, R_F (A) 0·20, R_F(B) 0·75. Treatment of the thioether (2 mg) in ethanol (5 ml) with Raney nickel (3 ml of ethanolic slurry) for 2 h gave proanthocyanidin-A2, which was detected by paper chromatography and identified by co-chromatography with an authentic sample.

The *heptamethyl ether* was prepared with diazomethane in methanol (2 ×) and purified by t.l.c. (silica; chloroform–methanol, 1% v/v; R_F 0·36) to give, after precipitation from ethanol with water, an amorphous powder (Found: C, 66·0; H, 5·1; S, 3·6. $C_{44}H_{44}O_{12}S$ requires C, 66·3; H, 5·5; S, 4·0%), $M^{+\cdot}$ 796, c.d. (*c* 0·8 in MeOH): $[\theta]_{247}$ −30,620, $[\theta]_{250}$ −40,820, $[\theta]_{256}$ −26,530, $[\theta]_{263}$ −22,450, $[\theta]_{270}$ −20,140, $[\theta]_{278}$ −20,410, $[\theta]_{286}$ −12,250, $[\theta]_{294}$ −6120.

Nona-O-acetylproanthocyanidin-A2 thiobenzyl ether was obtained as an amorphous powder after precipitation from ethanol–water (Found: C, 61·0; H, 4·8; S, 2·6. $C_{55}H_{48}O_{21}$ requires C, 61·4; H, 4·5; S, 3·0%), $(M^{+\cdot}-60)$ 1016, R_F (silica; methanol–chloroform, 1% v/v) 0·48, c.d. (*c* 1·58 in MeOH) $[\theta]_{247}$ −96,270, $[\theta]_{250}$ −46,130, $[\theta]_{254}$ 0, $[\theta]_{256}$ +8170, $[\theta]_{263}$ +49,030, $[\theta]_{267}$ +68,100, $[\theta]_{270}$ +65,380, $[\theta]_{278}$ +40,860, $[\theta]_{282}$ 0, $[\theta]_{286}$ −5450, $[\theta]_{294}$ −4090, $[\alpha]_{578}^{20}$ −54·8° (*c* 0·4 in Me_2CO).

Hydrogenolysis of (−)-Epicatechin.—(−)-Epicatechin (1·0 g) in ethanol (20 ml) was hydrogenated over palladium–charcoal (0·25 g; 10%) for 72 h. Paper chromatography showed that no starting material was present at this stage. After removal of the catalyst the product was chromatographed in ethanol on Sephadex LH 20 (35 × 2·5 cm) to give two phenols, Fractions 72—85 gave as a gum 1-(3,4-dihydroxyphenyl)-3-(2,4,6-trihydroxyphenyl)propan-2-ol (0·24 g), τ [$(CD_3)_2CO$] 1·5—2·5 (5H, m), 3·35—3·60 (3H, m), 4·12 (2H, s), 5·18 (OH, m), 6·0 (1H, m), and 7·02—7·60 (4H, m), R_F(A) 0·52, R_F(B) 0·62. Methylation (diazomethane) and purification by t.l.c. (silica; methanol–chloroform, 2% v/v) gave 1-(3,4-dimethoxyphenyl)-3-(2,4,6-trimethoxyphenyl)propan-2-ol, m.p. 87—88° (from methanol) (lit., 88—89°) (Found: C, 66·0; H, 7·5. Calc. for $C_{20}H_{26}O_6$: C, 66·3; H, 72%), $[\alpha]_{578}^{20}$ +0·5 to +1·2° (*c* 0·8 in $CHCl_3$), τ [$(CD_3)_2CO$] 3·25—3·36 (3H, m), 3·82 (2H, s), 6·10 (1H, m), 6·22—6·36 (15H, m), and 7·15—7·40 (4H, m).

Fractions 96—108 gave, as a gum, 1-(3,4-dihydroxyphenyl)-3-(2,4,6-trihydroxyphenyl)propane (0·12 g), τ [$(CD_3)_2CO$] 2·2—2·7 (50H, m), 3·35—3·65 (3H, m), 4·18 (2H, s), 7·41—7·68 (4H, m), and 8·25—8·40 (2H, m), R_F(A) 0·40, R_F(B) 0·71. Methylation (diazomethane) and purification by t.l.c. (silica; methanol–chloroform, 1% v/v) gave 1-(3,4-dimethoxyphenyl)-3-(2,4,6-trimethoxyphenyl)propane, m.p. 87—88° (from ethanol) (lit., 88—89°) (Found: C, 69·0; H, 7·6. Calc. for $C_{29}H_{26}O_5$: C, 69·4; H, 7·5%), τ [$(CD_3)_2CO$] 3·2—3·38 (3H, m), 3·83 (2H, s), 6·2—6·4 (15H, m), 7·36—7·58 (4H, m), and 8·20—8·40 (2H, m). Rechromatography of the intermediate fractions 86—95 gave further quantities of both phenols. Hydrogenation of (+)-catechin (1·0 g) gave similarly, after methylation, the diphenylpropane (0·16 g) and 1-(3,4-dimethoxyphenyl)-3-(2,4,6-trimethoxyphenyl)propan-2-ol (0·23 g), m.p. 86—87°, $[\alpha]_{578}^{20}$ −1·0 to −2·3° (*c* 0·6 in $CHCl_3$).

Hydrogenolysis of Proanthocyanidin-A2.—(*a*) Proanthocyanidin-A2 (1·0 g) was hydrogenated over palladium–charcoal (0·25 g; 10%) for 72 h in ethanol (25 ml). The product was worked up as above to give, after methylation, 1-(3,4-dimethoxyphenyl)-3-(2,4,6-trimethoxyphenyl)propan-2-ol (0·15 g), m.p. 87—88°, $[\alpha]_{578}^{20}$ +0·6° (*c* 0·8 in $CHCl_3$), and 1-(3,4-dimethoxyphenyl)-3-(2,4,6-trimethoxyphenyl)propane (0·6 g), m.p. 84—86°.

(*b*) Proanthocyanidin-A2 (1·0 g) was hydrogenated over palladium–charcoal (0·25 g; 10%) for 24 h in ethanol (25 ml). The product, after removal of the catalyst, was chromatographed on Sephadex LH-20 (35 × 3·5 cm) in ethanol. Fractions 37—42 gave 1-(3,4-dihydroxyphenyl)-3-(2,4,6-trihydroxyphenyl)propan-2-ol. Fractions 90—124 gave, after trituration with water, unchanged proanthocyanidin-A2 (0·32 g). Fractions 125—165 were combined and chromatographed on thin layers of cellulose in solvent system (B). From bands of R_F 0·25—0·40 was isolated, as a gum, a phenol, R_F(A) 0·35, R_F(B) 0·33, τ [$(CD_3)_2CO$] 1·88—2·58 (5H, m), 2·0—2·53 (6H, m), 2·0—2·53 (6H, m), 3·80 (1H, d, *J* 2 Hz), 3·96 (1H, s), 4·08 (1H, d, *J* 2 Hz), 4·20 (1H, d, *J* 2 Hz), 5·16 (2H, m), 5·44 (1H, s), 5·60 (1H, d, *J* 3·5 Hz), 5·90 (1H, d, *J* 3·5 Hz), 6·15 (1H, m), and 7·28 (2H, m). From

bands of R_F 0·60—0·75 was isolated, as a gum, a phenol, R_F(A) 0·30, R_F(B) 0·66, τ [$(CD_3)_2CO$] 1·2—2·6 (OH, m), 3·0—3·65 (8H, m), 3·94 (1H, d, J 2 Hz), 4·02 (1H, s), 4·20 (1H, d, J 2 Hz), 4·63 (10H, s), 5·02br (2H, s), 5·87 (1H, d, J 3·5 Hz), 7·48—7·74 (4H, m), and 8·25—8·49 (2H, m). Methylation (diazomethane–methanol) gave a product isolated by t.l.c. (methanol–chloroform, 2% v/v; R_F 0·60), *m/e* 674 ($M^{+\cdot}$), 151, and 509.

*Hydrogenolysis of Procyanidin-B*2.—Procyanidin-B2 (0·68 g) was hydrogenated over palladium–charcoal (0·25 g; 10%) for 48 h in ethanol (25 ml). The product, after removal of the catalyst, was worked up as described above to give, after methylation, 1-(3,4-dimethoxyphenyl)-3-(2,4,6-trimethoxyphenyl)propane (0·07 g), m.p. 84—85°, and 1-(3,4-dimethoxyphenyl)-3-(2,4,6-trimethoxyphenyl)propan-2-ol (0·10 g), m.p. 87—88°, $[\alpha]_{578}^{20}$ +0·8° (*c* 1·0 in $CHCl_3$). Interruption of the hydrogenation after 0·5 h and analysis by paper chromatography showed the presence of (−)-epicatechin.[1]

*Hydrogenolysis of Procyanidins-B*1 *and -B*4.—The procyanidin (0·07 g) was hydrogenated as above over palladium–charcoal (0·01 g) in ethanol (5 ml). Paper chromatography after 0·5 h showed the presence of both (+)-catechin and (−)-epicatechin.

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