Tannins and Related Compounds. Part 13.[1] Isolation and Structures of Trimeric, Tetrameric, and Pentameric Proanthicyanidins from Cinnamon

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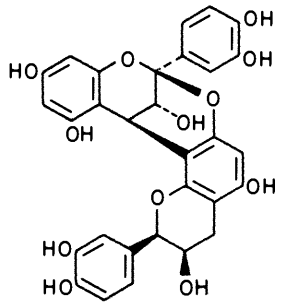
A proanthocyanidin trimer, two tetramers, and a pentamer have been isolated in their free phenolic forms from cinnamon, the bark of *Cinnamomum zeylanicum* (Lauraceae). Partial acid-catalysed degradation of these tannins with phenylmethanethiol, in conjunction with ^{1}H- and ^{13}C-n.m.r. analysis, has unequivocally established their structures as epicatechin-(4β→8, 2β→7)epicatechin-(4α→8)-epicatechin (5); epicatechin-(4β→8)epicatechin-(4β→8, 2β→7)-epicatechin-(4α→8)-epicatechin (6); epicatechin-(4β→6)-epicatechin-(4β→8, 2β→7)-epicatechin-(4α→8)-epicatechin (7); and epicatechin-(4β→8)-epicatechin-(4β→8)epicatechin-(4β→8, 2β→7)-epicatechin-(4α→8)-epicatechin (8).

Proanthocyanidins, one of the major classes of phenolic secondary metabolites, consist mainly of chains of 3′,4′,5,7-tetrahydroxy- and/or 3′,4′,5′,5,7-pentahydroxyflavan-3-ol units linked through carbon–carbon linkages at C(4)–C(8) or C(4)–C(6).[2-5] Much of the chemistry of the lower-molecular-weight proanthocyanidins, especially that of the singly linked proanthocyanidin dimers (B-type) and trimers, has now been elucidated.[6-8] The structures of polymeric proanthocyanidins consisting of linear chains of flavan-3-ol units have been studied on the basis of ^{13}C n.m.r. spectroscopy and analysis of the degradation products obtained from the cleavage of polymers by phenylmethanethiol or by phloroglucinol in acid medium,[4,9-11] although these studies have been made using polymer preparations probably still containing oligomers such as tetramers, pentamers, and hexamers. Until now there has been no report on the isolation of pure tetrameric, pentameric, and higher oligomeric proanthocyanidins in their free phenolic forms except for that of two structurally related tetramers from the seeds of *Areca catechu*.[2]

Proanthocyanidin A-2, which possesses a doubly bonded dimeric structure, was first isolated by Mayer and his collaborators from the seed of *Aesculus hippocastanum*.[12] The structure (1) was later deduced by Haslam and his co-workers on the basis of spectroscopic (^{13}C- and ^{1}H-n.m.r.) and chemical evidence [13] and, more recently, the structure has been unequivocally established by *X*-ray crystallography.[14] Trimeric proanthocyanidins containing this A-2 moiety in the molecule were obtained as an inseparable mixture by chromatography on Sephadex LH-20 from the seeds of *Persea gratissima* and *Aesculus hippocastanum*.[13] The assignments of the structures (2), (3), and (4) for these trimers were based mainly on ^{1}H n.m.r. analysis and on degradative studies including thiolysis, but evidence for the position of the linkage between the A-2 part and the remaining flavan-3-ol unit has not been described.

We had previously isolated the homologous series of singly linked procyanidin dimers, trimers, and tetramers by a combination of Sephadex LH-20 and high-porosity-polystyrene gel chromatography.[2] Application of similar separation techniques to proanthocyanidins from cinnamon (the bark of *Cinnamomum zeylanicum*) has now resulted in the isolation of trimeric, tetrameric, and pentameric proanthocyanidins, whose isolation and structures are fully discussed here.



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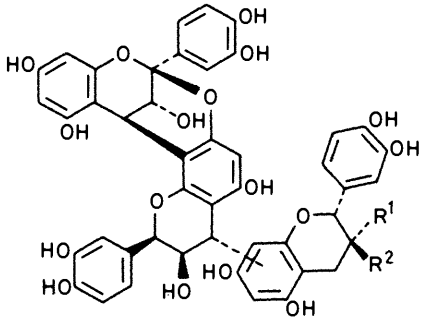
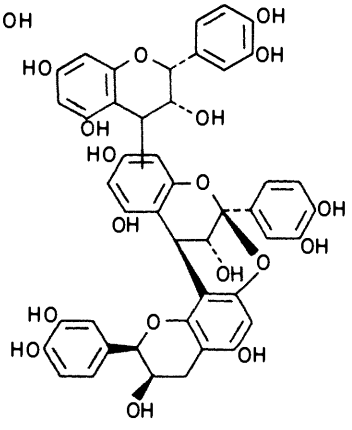


(2) R^1 = OH, R^2 = H
(3) R^1 = H, R^2 = OH



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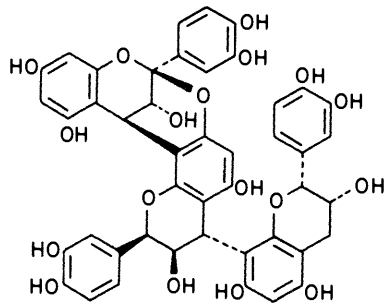
Results and Discussion

The aqueous acetone extract of commercial cinnamon contained, in addition to a large quantity of essential oil, proanthocyanidins whose t.l.c. pattern, showing that the major



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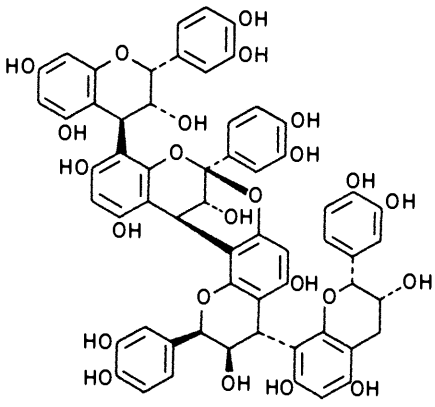

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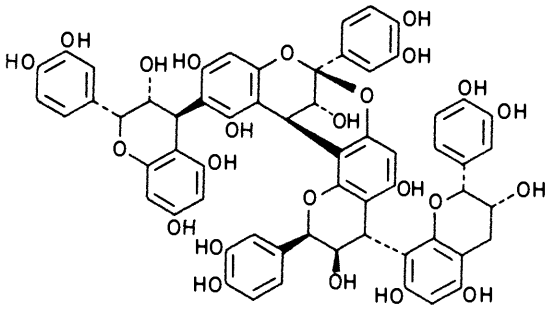

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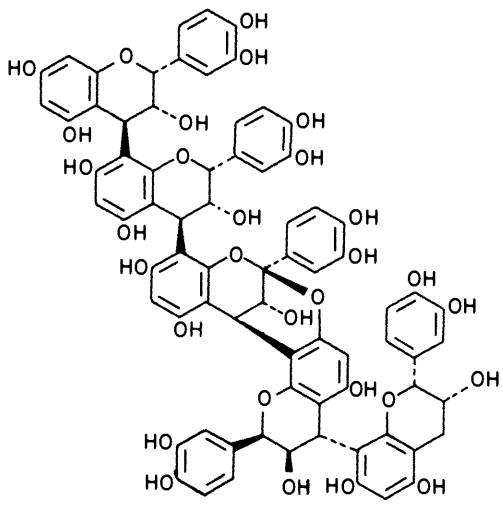

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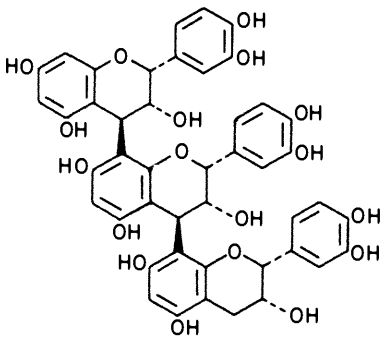

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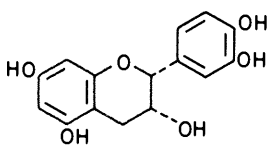

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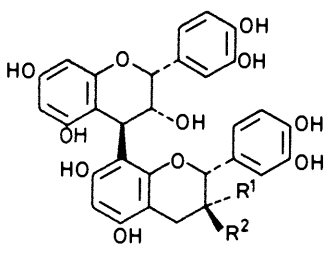
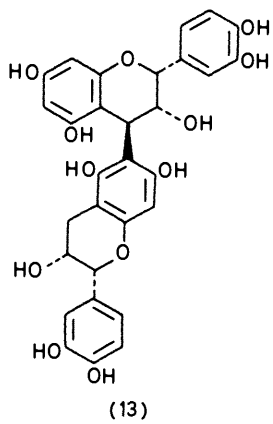


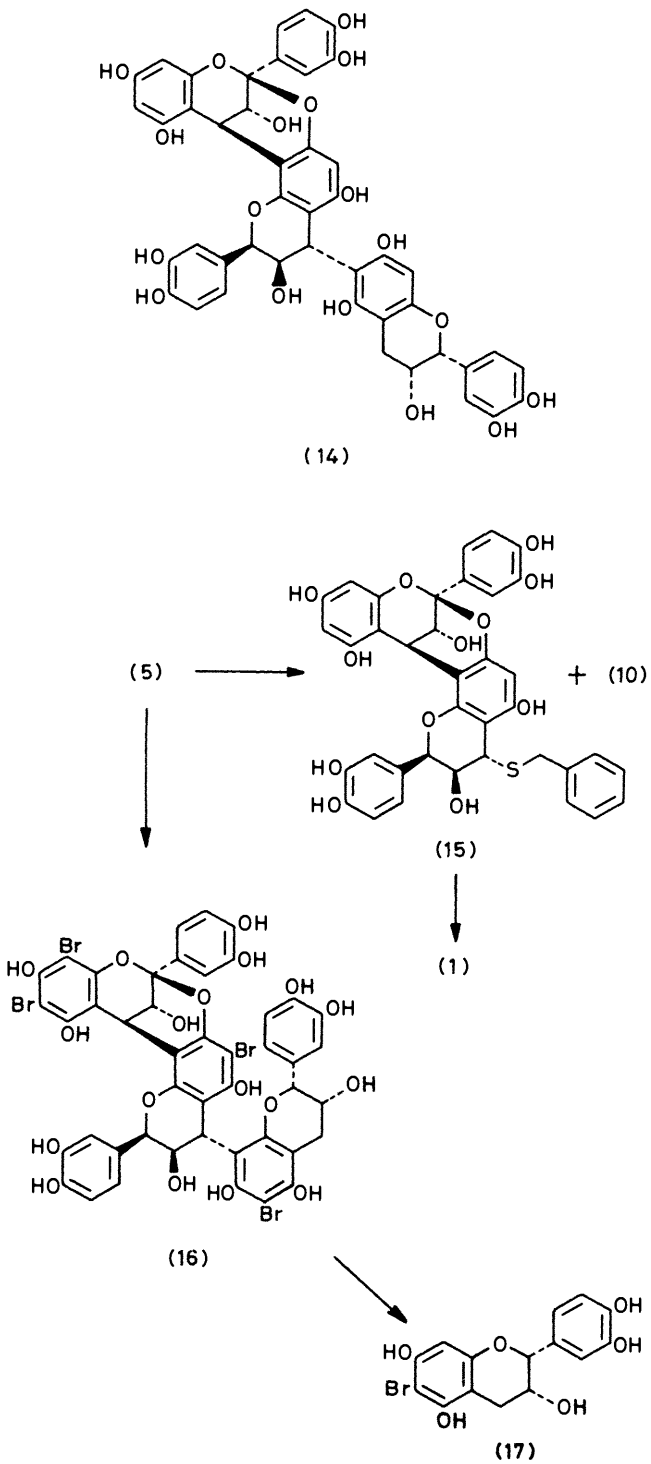
(11) R^1 = H, R^2 = OH
(12) R^1 = OH, R^2 = H



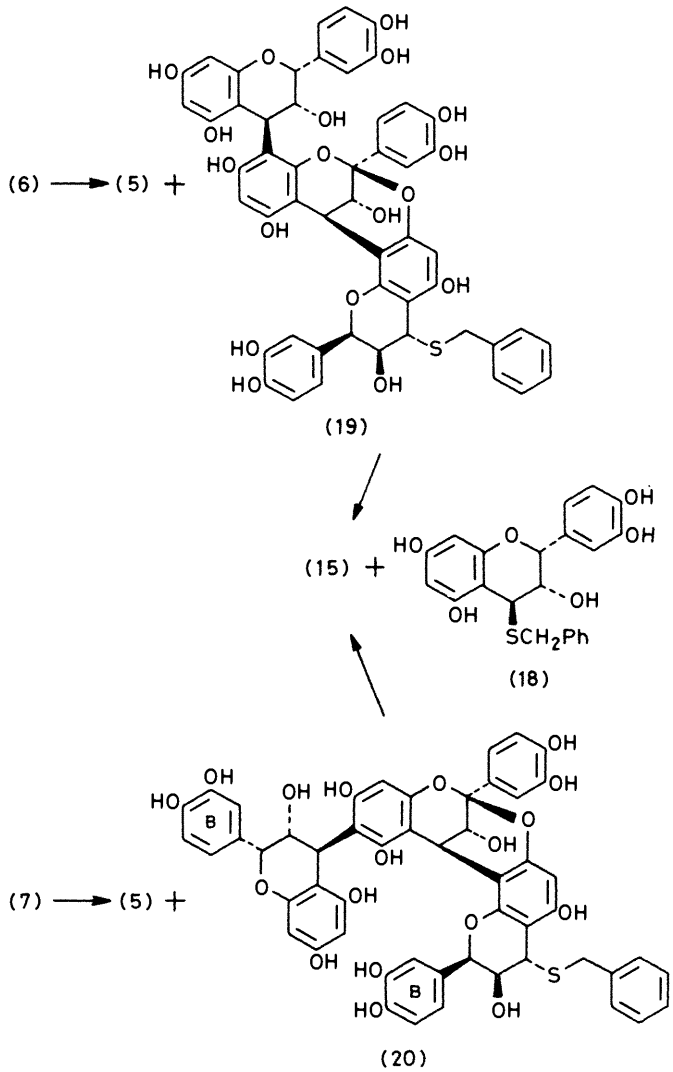
(13)

components were trimers and tetramers, was quite unlike those found in commonly distributed (singly linked) procyanidins. The procedure for the subsequent separation of proanthocyanidins from the extract was thus modified to include partition between n-butanol and water in place of ethyl acetate extraction. Chromatography of the n-butanol extract on Sephadex LH-20 afforded trimeric, tetrameric, and pentameric proanthocyanidin fractions. Each fraction was subjected to chromatography on high-porosity-polystyrene gel (Diaion HP-20 AG), to yield the trimer (5), the tetramers (6) and (7), and the pentamer (8), together with the known trimeric procyanidin C-1 (9).[6,15] (−)-Epicatechin (10) and the dimeric procyanidins B-1 (11), B-2 (12), B-5 (13), and A-2 (1) were also isolated in very small quantities from the fraction eluted earlier. Considering the low contents of the monomer (0.001%) and dimers (*ca.* 0.005% in total) and also of the higher oligomers (*e.g.* pentamer; 0.003%), the relatively high accumulation of the oligomers (5) and (6) in this plant (*ca.* 0.1 and 0.04%, respectively) was rather unusual.

The triflavanoid constitution of compound (5) was confirmed by field-desorption mass spectrometry of its methyl ether (M^+ at m/z 1 018). Acid-catalysed degradation of the trimer (5) with phenylmethanethiol [6] afforded (−)-epicatechin (10) and 4′-benzylthioproanthocyanidin A-2 (15) (Scheme 1). The structure of compound (15) was confirmed by ^{1}H n.m.r. analysis and its conversion into proanthocyanidin A-2 (1)



Scheme 1.



Scheme 2.

with Raney-nickel. From these results the alternative structures (5) and (14) were considered for this trimer.

To clarify the point of the interflavanoid linkage, bromination of the trimer, followed by acid-catalysed degradation, in which bromine may serve as the marker for determining the point of the linkage, was attempted. Application [16,17] of the already established methods for bromination of catechin and its derivatives to this trimer showed that, as expected, the introduction of the bromine atom took place exclusively at the A-rings of the flavan skeletons although the yield of the desired product (16) was less than 10%. With acetonitrile as the solvent instead of ethanol, dioxane, or pyridine, it was found that bromination (with pyridinium hydrobromide perbromide) was complete after 30 min at 0 °C and that the bromo derivative (16) could be prepared in 95% yield. The assignment of structure (16) was based on the fact that the mass spectrum of the undecamethyl ether of compound (16) showed the molecular ion peak at *m/z* 1 330, accompanied by $(M + 2)^+$, $(M + 4)^+$, $(M + 6)^+$, and $(M + 8)^+$ ions characteristic of the tetrabromo compound. Treatment of compound (16) with 0.2M hydrochloric acid [6] yielded, together with deeply coloured anthocyanidins, 6-bromo-(−)-epicatechin (17), the structure of which was confirmed by comparison of its physical and spectral data with those of a sample prepared from (−)-epicatechin. Attempts to cleave the interflavanoid linkage in compound (16) in the presence of phenylmethanethiol were unsuccessful because debromination easily occurred during the reaction, resulting in the formation of (−)-epicatechin as the only isolable product. In the case of the methyl ether of compound (16) it was also possible, under more drastic conditions (0.4M hydrochloric acid, 6 h reflux), to cleave the interflavanoid linkage, giving 6-bromo-3′,4′,5,7-tetra-*O*-methyl-(−)-epicatechin. The relative configuration of

Table. ^{13}C-N.m.r. chemical shifts of proanthocyanidins [p.p.m.; Me_4Si standard; $(CD_3)_2CO$–D_2O]

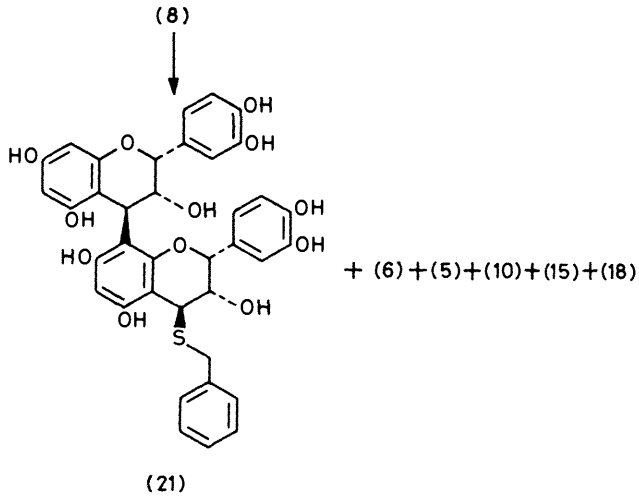
Ring	C			C′			C″			C‴			C⁗		
Compound	C(2)	C(3)	C(4)	C(2)	C(3)	C(4)	C(2)	C(3)	C(4)	C(2)	C(3)	C(4)	C(2)	C(3)	C(4
Proanthocyanidin A-2 (1)	103.6	67.1	29.9	81.1	65.7	28.5									
4′-Benzylthioproanthocyanidin A-2 (15)	103.7	67.3	28.5	77.2	70.3	44.3									
Trimer (5)	104.5	66.5	30.0	78.2	71.6	38.0	79.8	66.5	29.2						
Tetrabromo derivative (16)	106.4	66.3	30.1	79.0	71.3	39.9	80.1	66.3	29.5						
Tetramer (6)	75.8	70.7	37.1	104.3	66.5	29.4	77.9	71.3	38.0	79.4	66.5	28.1			
Tetramer (7)	77.2	72.5	38.0	104.8	66.8	29.4	79.2	72.5	38.0	79.9	66.8	28.6			
Pentamer (8)	76.0	70.9	37.4	77.1	71.7	37.4	104.3	66.5	29.4	78.2	71.7	38.3	79.6	66.5	28.7

the interflavanoid linkage between the A-2 and epicatechin units was determined on the basis of the ^{13}C n.m.r. chemical shifts of the C′-ring carbons,[18] thus establishing unequivocally the structure of this trimer as (5).

The tetrameric proanthocyanidins (6) and (7) were shown to possess the same constitution by field-desorption mass spectrometry of their methyl ethers (M^+ at m/z 1 362) and by complete acid-catalysed degradation with phenylmethanethiol which gave the same products, namely, 4-benzylthio-(−)-epicatechin (18),[6] 4′-benzylthioproanthocyanidin A-2 (15),[13,14] and (−)-epicatechin (10). Partial degradation of the tetramers afforded, in addition to the above products, a proanthocyanidin trimer identical with (5) and trimeric proanthocyanidin benzyl sulphides [(19) from (6) and (20) from (7), Scheme 2].

The structures of compounds (19) and (20) were determined as follows: since the trimeric proanthocyanidin (5) was formed upon partial degradation of both tetramers, each component unit in compounds (19) and (20) should be connected in the same sequence of (−)-epicatechin–(A-2)–SCH_2Ph, indicating that the only structural difference between these products is the point of the interflavanoid linkage. The ^{1}H n.m.r. spectrum of trimer (19) showed two broad singlets (δ 5.27 and 5.11) due to the flavan C(2) protons, whose chemical shifts were closely analogous to those of procyanidin C-1(9),[15] a C(4)–C(8) linearly linked trimer consisting of (−)-epicatechin units. On the other hand, the spectrum of trimer (20), taken at room temperature, was complicated by conformational isomerism. However, the C(2) proton signals (δ 5.66 and 4.88) arising from the major conformer were clearly distinguishable from other signals. The unusual downfield and rather upfield shifts of the respective C(2) proton may be interpreted in terms of the through-space interaction of the upper and terminal epicatechin units. Examination of a Dreiding model revealed that in the case of the C(4)–C(6)-linked isomer (20), the B- and B″-rings were close together and this may be responsible for the steric interference to free rotation. In addition, the abnormalities of the C(2) proton shifts in compound (20) were reasonably explained on the basis of a close approach of each ring to these protons. From these observations the points of the upper interflavanoid linkage in the trimers (19) and (20) were concluded to be C(4)–C(8) and C(4)–C(6), respectively, thus permitting the assignments of the structures (6) and (7) for these tetramers.

The proanthocyanidin (8) gave, on methylation, a nonadecamethyl derivative whose field-desorption mass spectrum (M^+ at m/z 1 706) confirmed its pentaflavanoid constitution. In the ^{13}C n.m.r. spectrum of the pentamer (8), four well-defined C(2) signals appeared in the aliphatic region (Table), in addition to five C(3) signals, suggesting the occurrence of a proanthocyanidin A-2 moiety in the molecule. Partial degradation with acid in the presence of phenylmethanethiol gave, after repeated chromatography, the proanthocyanidin



(21)

tetramer (6), the trimer (5), and the dimeric proanthocyanidin benzyl sulphides (21) and (15), together with (−)-epicatechin (10) and 4-benzylthio-(−)-epicatechin (18). Among these degradation products the formation of the tetramer (6) and the benzyl-sulphide (21) from the pentamer was of particular significance since the structure of this pentamer could thus be limited to (8).

The occurrence of minor pentameric proanthocyanidins and higher oligomeric proanthocyanidins in this plant material was detected by t.l.c., but these could not be isolated owing to their low contents and complexities.

It should be noted that among several plant materials of the genus *Cinnamomum* examined in our laboratory, only the bark of *C. zeylanicum* contained, as major phenolic metabolites, a series of proanthocyanidins with the doubly linked (A-type) unit, while in other materials (the barks of *C. burmanni* and *C. cassia*, and the root bark of *C. camphora*) the major penolic metabolites consisted of linearly linked proanthocyanidins.

Experimental *

Optical rotations were measured with a JASCO DIP-4 digital polarimeter (cell length: 0.5 dm). Field-desorption mass spectra were recorded on a JEOL DX-300 spectrometer, and ^{1}H- and ^{13}C-n.m.r. spectra on JEOL PS-100 and JEOL FX-100 spectrometers, respectively, with $SiMe_4$ as internal standard. Column chromatography was carried out using

* Throughout this section specific atoms are shown in the form C-*n*, *n*-H, *etc.*

Sephadex LH-20 (25—100 μ; Pharmacia Fine Chemicals), Diaion HP-20AG (75—150 μ; Mitsubishi Chemical Industries Ltd.) and Kieselgel 60 (70—230 mesh; Merck). T.l.c. was performed on precoated Kieselgel 60 F_{254} plates (0.20 mm; Merck) in the solvent systems (A) benzene–ethyl formate–formic acid (2 : 7 : 1) and (B) chloroform–ethyl formate–formic acid (1 : 7 : 1), and spots were detected by their blue fluorescence under u.v. light, and by a spray of iron(III) chloride or *p*-anisaldehyde–sulphuric acid reagent. H.p.l.c. was conducted on a Toyo Soda apparatus equipped with an SP 8700 solvent delivery system and a UV-8 model II spectrometer using an LS 410 column (5μ; 300 mm × 4 mm) [solvent : acetonitrile–water (1 : 4) or (3 : 7)].

Isolation of Proanthocyanidins.—Finely powdered commercial cinnamon (18.9 kg) was extracted at room temperature with acetone–water (7 : 3 v/v), and the extract, on concentration, yielded red-brown precipitates negative to the iron(III) chloride reagent. The filtrate was washed with diethyl ether and extracted with n-butyl alcohol to give a solid (960 g). The solid was treated with water, and insoluble materials were removed by filtration. The filtrate, after concentration, was applied to a column (11 cm i.d. × 54 cm) of Sephadex LH-20, pre-swollen in water, and the column was eluted with increasing amounts of methanol in water (1 : 0 ⟶ 1 : 9) to yield fractions I (0.82 g), II (2.5 g), III (95 g), and IV (83 g). Fraction I, after crystallisation from water, afforded (−)-epicatechin (10) as prisms (0.2 g). Chromatography of fraction II on a column (2 cm i.d. × 18 cm) of Diaion HP-20 AG eluted with water–methanol (7 : 3) gave procyanidins B-1 (11) (0.04 g) and B-2 (12) (0.8 g). Fraction III was further divided into two fractions (IIIa and IIIb) by chromatography over Sephadex LH-20 (6.5 cm i.d. × 40 cm) with ethanol and a mixture of methanol–water (1 : 1 as eluants. Procyanidins B-5 (13) (0.06 g) and A-2 (1) (0.05 g) were obtained from fraction IIIa by Sephadex LH-20 chromatography (2 cm i.d. × 18 cm) eluted with water–methanol (1 : 1), and procyanidin C-1 (9) (2.5 g) and the trimeric proanthocyanidin (5) (18.1 g) from fraction IIIb by Diaion HP-20 AG chromatography (8 cm i.d. × 36 cm) with water–methanol (7 : 3). Fraction IV, containing tetrameric and pentameric proanthocyanidins, was subjected to Sephadex LH-20 chromatography (4.5 cm i.d. × 35 cm) with ethanol as eluant to give the chromatographically pure pentamer (8) and a mixture of tetramers. Subsequent chromatography of the tetrameric proanthocyanidin fraction over Diaion HP-20 AG (4.5 cm i.d. × 40 cm) with water–methanol (7 : 3) yielded the tetramers (6) (6.6 g) and (7) (1.3 g).

The *trimeric proanthocyanidin* (5), obtained as an off-white amorphous powder, had $[\alpha]_D^{25}$ +70.3° (*c* 1.1 in acetone) (Found: C, 60.0; H, 4.5. $C_{45}H_{36}O_{18}\cdot 2H_2O$ requires C, 60.0 H, 4.5%); δ_H [$(CD_3)_2SO$; 150 °C] 2.83 (2 H, m, C″-ring 4-H_2), 3.76 (1 H, br s, C″-ring 3-H), 3.98 (1 H, s, C′-ring 3-H), 4.04 (1 H, d, *J* 4 Hz, C-ring 3-H), 4.24 (1 H, d, *J* 4 Hz, C-ring 4-H), 4.54–4.83 (2 H, m, C″-ring 2-H and C′-ring 4-H), 5.38 (1 H, s, C′-ring 2-H), 5.76—6.04 (4 H, m, A,A′,A″-ring 6-H and A-ring 8-H), and 6.40—7.20 (9 H, m, B, B′, and B″-ring H's).

A mixture of the trimer (5) (200 mg), anhydrous potassium carbonate (1.0 g), and dimethyl sulphate (0.5 ml) in dry acetone (8 ml) was stirred and refluxed for 2.5 h. After removal of inorganic salts, the filtrate was concentrated under reduced pressure to afford a syrup which was chromatographed over silica gel with benzene–acetone (17 : 3) as eluant to give the *undecamethyl ether* (120 mg) as a white amorphous powder, $[\alpha]_D^{22}$ +43.2° (*c* 1.0 in $CHCl_3$ (Found: C, 65.9; H, 5.8. $C_{56}H_{58}O_{18}$ requires C, 66.0; H, 5.7%); *m/z* 1 018 (M^+); δ_H ($CDCl_3$) 2.7—3.0 (2 H, m, C″-ring 4-H_2), 3.03 (3 H, s, A-ring 6-OCH_3), 3.30 (3 H, s, B′-ring 3-OCH_3), 3.7—3.9 (27 H, 9 × OCH_3), 5.92 (1 H, s, A′-ring 6-H), 5.94 and 6.18 (each 1 H, d, *J* 2 Hz, together A-ring 6- and 8-H), 6.24 (1 H, s, A″-ring 6-H), and 6.7—7.3 (9 H, m, B, B′, and B″-ring H's).

The trimer (5) (1.0 g) was treated with phenylmethanethiol (4 ml) and acetic acid (2 ml) in ethanol (20 ml) under reflux for 4 h. Removal of the solvents under reduced pressure left an oily residue which was chromatographed on a column of Sephadex LH-20. Elution with acetone furnished (−)-epicatechin (10) (85 mg), and 4′-benzylthioproanthocyanidin A-2 (15) (510 mg) as an off-white amorphous powder, $[\alpha]_D^{22}$ +59.5° (*c* 1.1 in acetone); δ_H (CD_3COCD_3) 4.04 (2 H, s, CH_2S), 4.12 (2 H, s, C′-ring 3- and 4-H), 4.14 (1 H, d, *J* 4 Hz, C-ring 3-H), 4.35 (1 H, d, *J* 4 Hz, C-ring 4-H), 5.32 (1 H, s, C′-ring 2-H), 5.96 (1 H, d, *J* 2 Hz, A-ring 6-H), 6.04 (1 H, d, *J* 2 Hz, A-ring 8-H), 6.16 (1 H, s, A′-ring 6-H), and 6.8—7.5 (11 H, ArH). Treatment of the thioether (15) (40 mg) in ethanol (5 ml) with Raney-nickel (W-4) at 60 °C for 1 h, followed by chromatography over Sephadex LH-20 with water–methanol (1 : 4) as eluant, afforded proanthocyanidin A-2 (1) (1 mg) which was identified by co-chromatography and ^1H n.m.r. comparison with a sample isolated from natural sources.

Bromination of the Trimer (5).—To an ice-cooled solution of the trimer (5) (150 mg) in acetonitrile (20 ml) was added dropwise a solution of pyridinium hydrobromide perbromide (200 mg) in acetonitrile (20 ml). The reaction mixture was kept at 0 °C for 30 min, and was then directly chromatographed over Sephadex LH-20, pre-swollen in water. Elution with the same solvent afforded the excess of reagent, and the adsorbed proanthocyanidin bromide was eluted with methanol. Purification by a column of Diaion HP-20 AG with water–methanol (1 : 1) as eluant gave the *tetrabromo derivative* (16) (195 mg) as an off-white amorphous powder, $[\alpha]_D^{23}$ +53.2° (*c* 1.1 in acetone) (Found: C, 44.4; H, 2.9. $C_{45}H_{32}Br_4O_{18}\cdot 2H_2O$ requires C, 44.5; H, 3.0%); δ_H (CD_3COCD_3) 2.68—3.08 (2 H, m, C″-ring 4-H_2), 5.75 (1 H, s, C′-ring 2-H), 6.6—7.5 (9 H, m, B, B′, and B″-ring H's). Methylation of the bromo compound (16) (150 mg) with ethereal diazomethane in ethanol and separation of the product by silica-gel chromatography with benzene–acetone (17 : 3) as eluant gave the *undecamethyl ether* (100 mg) as a white amorphous powder, $[\alpha]_D^{23}$ +29.5° (*c* 1.0 in $CHCl_3$) (Found: C, 50.3; H, 4.1. $C_{56}H_{54}Br_4O_{18}$ requires C, 50.4; H, 4.1%); *m/z* 1 330 (M^+), 1 332 ($M+2$)$^+$, 1 334 ($M+4$)$^+$, 1 336 ($M+6$)$^+$, and 1 338 ($M+8$)$^+$; δ_H ($CDCl_3$) 2.98—3.16 (2 H, m, C″-ring 4-H_2), 3.4—4.1 (33 H, 11 × OCH_3), 4.31 (1 H, d, *J* 4 Hz, C-ring 4-H), 4.44 (1 H, s, C′-ring 4-H), 4.71 (1 H, s, C″-ring 2-H), 5.71 (1 H, s, C′-ring 2-H), and 6.47—7.60 (9 H, m, B, B′, and B″-ring H's).

Treatment of the bromo derivative (16) (150 mg) with 0.2M-hydrochloric acid in ethanol (10 ml) under reflux for 3 h and separation of the product on a Sephadex LH-20 column with ethanol afforded needles (3.5 mg) which were shown to be identical with 6-bromo-(−)-epicatechin (17) prepared by bromination of (−)-epicatechin (see below).

Treatment of the undecamethyl ether of the bromo derivative (100 mg) with 0.4M-hydrochloric acid in ethanol (10 ml) under reflux for 6 h, followed by chromatography on a silica-gel column using benzene–acetone (9 : 1) as eluant, yielded a product (3.0 mg), identical with 6-bromo-3′,4′,5,7-tetra-*O*-methyl-(−)-epicatechin (see below) in its m.p., $[\alpha]_D$, and ^1H n.m.r. properties.

Bromination of (−)-Epicatechin.—(−)-Epicatechin (10) (500 mg) in acetonitrile (30 ml) was brominated with pyridinium hydrobromide perbromide (300 mg) in the same way as described above, and the reaction mixture was applied to a Diaion HP-20 AG column. Elution with water–methanol (3 : 2) afforded 8-*bromo*-(−)-*epicatechin* as needles (from

water) (82 mg), m.p. 205—208 °C; $[\alpha]_D^{20}$ −39.9° (*c* 0.9 in acetone) (Found: C, 48.7; H, 3.6. $C_{15}H_{13}BrO_6$ requires C, 48.8; H, 3.6%); δ_H (CD_3COCD_3) 2.7—3.1 (2 H, m, 4-H_2), 4.32 (1 H, m, 3-H), 5.01 (1 H, s, 2-H), 6.23 (1 H, s, 6-H), and 6.8—7.0 (3 H, m, B-ring H's); δ_C (CD_3COCD_3) 29.9 (C-4), 66.3 (C-3), 79.1 (C-2), 89.3 (C-8), 96.5 (C-6), 101.3 (C-4a), 114.9 and 115.5 (C-2′, 5′), 118.9 (C-6′), 131.5 (C-1′), 145.1 and 145.3 (C-3′, 4′), 152.9 (C-8a), and 153.7 and 156.3 p.p.m. (C-6, 8). Comparison of the 6-H shift with that of 8-bromo-(+)-catechin [17] established the structure. The *tetramethyl ether*, prepared with diazomethane, crystallised from water as needles, m.p. 163—165 °C, $[\alpha]_D^{21}$ −18.5° [*c* 0.9 in $CHCl_3$–MeOH (1 : 1)] (Found: C, 53.7; H, 5.1. $C_{19}H_{21}BrO_6$ requires C, 53.7; H, 5.0%); δ_H [$CDCl_3$–CD_3OD (1 : 1)] 2.8—3.0 (2 H, m, 4-H_2), 3.85, 3.86, and 3.89 (each 3 H, s, OCH_3), 4.20—4.46 (1 H, m, 3-H), 5.05 (1 H, s, 2-H), 6.28 (1 H, s, 6-H), and 6.9—7.2 (3 H, m, B-ring H's). The *penta-acetate* was prepared in pyridine–acetic anhydride and was purified on a silica-gel column with benzene–acetone (19 : 1) as eluant; it crystallised from ethanol as needles, m.p. 150—151 °C, $[\alpha]_D^{21}$ −30.3° (*c* 0.9 in $CHCl_3$ (Found: C, 51.7; H, 4.0. $C_{25}H_{23}BrO_{11}$ requires C, 51.8; H, 4.0%); δ_H ($CDCl_3$) 1.91 (3 H, s, 3-$COCH_3$), 2.30, 2.35, and 2.36 (each 3 H, s, OCH_3), 2.75—3.20 (2 H, m, 4-H_2), 5.19 (1 H, s, 2-H), 5.47 (1 H, m, 3-H), 6.65 (1 H, s, 6-H), and 7.2—7.4 (3 H, m, B-ring H's).

Further elution with water–methanol (7 : 3) yielded 6-*bromo*-(−)-*epicatechin* (17) as needles (from water) (82 mg), m.p. 215—217 °C; $[\alpha]_D^{20}$ −32.5° (*c* 1.1 in acetone) (Found: C, 48.9; H, 3.5. $C_{15}H_{13}BrO_6$ requires C, 48.8; H, 3.6%); δ_H (CD_3COCD_3) 2.7—3.1 (2 H, m, 4-H_2), 4.24 (1 H, m, 3-H), 4.90 (1 H, s, 2-H), 6.18 (1 H, s, 8-H, and 6.80—7.04 (3 H, m, B-ring H's); δ_C (CD_3COCD_3) 29.9 (C-4), 66.4 (C-3), 79.9 (C-2), 89.5 (C-6), 96.5 (C-8), 101.4 (C-4a), 115.0 and 115.4 (C-2′, 5′), 119.0 (C-6′), 131.6 (C-1′), 145.3 and 145.4(C-3′,4′), 153.6 (C-8a), and 153.8 and 156.4 p.p.m. (C-6, 8). The chemical shift of 6-H was in close agreement with that of 6-bromo-(+)-catechin.[17] The *tetramethyl ether*, produced by diazomethane methylation, was obtained as needles, m.p. 152 °C; $[\alpha]_D^{21}$ −19.2° (*c* 0.9 in acetone) (Found: C, 53.6; H, 5.1. $C_{19}H_{21}BrO_6$ requires C, 53.7; H, 5.0%); δ_H ($CDCl_3$) 2.8—3.2 (2 H, m, 4-H_2), 3.84, 3.88, and 3.91 (each 3 H, s, OCH_3), 4.12—4.40 (1 H, m, 3-H), 4.97 (1 H, s, 2-H), 6.43 (1 H, s, 8-H), and 6.9—7.1 (3 H, m, B-ring H's). The *penta-acetate* was prepared in pyridine–acetic anhydride and was purified by silica-gel chromatography with benzene–acetone (19 : 1) as eluant; it crystallised from ethanol as needles, m.p. 148 °C; $[\alpha]_D^{21}$ −25.2° (*c* 0.9 in $CHCl_3$) (Found: C, 51.8; H, 4.0. $C_{25}H_{23}BrO_{11}$ requires C, 51.8; H, 4.0%); δ_H ($CDCl_3$) 1.90 (3 H, s, 3-$COCH_3$), 2.30 and 2.37 (together 12 H, 4 × $COCH_3$), 2.75—3.20 (2 H, m, 4-H_2), 5.10 (1 H, s, 2-H), 5.38 (1 H, m, 3-H), 6.76 (1 H, s, 8-H), and 7.13—7.40 (3 H, m, B-ring H's).

Elution with the same solvent gave 6,8-*dibromo*-(−)-*epicathechin* as needles (from water), m.p. 205 °C; $[\alpha]_D^{20}$ −25.6° (*c* 1.1 in acetone) (Found: C, 40.3; H, 2.9. $C_{15}H_{12}Br_2O_6$ requires C, 40.0; H, 2.7%); δ_H (CD_3COCD_3) 2.8—3.2 (2 H, m, 4-H_2), 4.32 (1 H, m, 3-H), 5.04 (1 H, s, 2-H), and 6.76—7.12 (3 H, m, B-ring H's); δ_C (CD_3COCD_3) 29.8 (C-4), 65.9 (C-3), 79.9 (C-2), 90.8 and 91.7 (C-6, 8), 102.6 (C-4a), 114.8 and 115.6 (C-2′, 5′), 118.9 (C-6′), 131.0 (C-1′), 145.1 and 145.3 (C-3′, 4′), and 150.1, 151.9, and 152.6 p.p.m. (C-6, 8, 8a).

The *tetrameric proanthocyanidin* (6), obtained as a pale brown amorphous powder, had $[\alpha]_D^{22}$ +51.1° (*c* 1.0 in acetone) (Found: C, 59.5; H, 4.4. $C_{60}H_{48}O_{24}\cdot 3H_2O$ requires C, 59.7; H, 4.5%); δ_H (CD_3COCD_3) 2.60—3.00 (2 H, m, c‴-ring 4-H_2), and 5.80 (1 H, br s, c″-ring 2-H). Methylation of the tetramer (6) (300 mg) in the same way as described for (5) furnished the *pentadecamethyl ether* (150 mg) as a white amorphous powder, $[\alpha]_D$ +41.2° (*c* 1.0 in $CHCl_3$) (Found: C, 66.1; H, 5.7. $C_{75}H_{78}O_{24}$ requires C, 66.1; H, 5.8%); *m/z* 1 362 (M^+). Bromination of the tetramer (6) (102 mg) as before yielded the 6,6′,6″,6‴,8-*pentabromo derivative* (96 mg) as a pale brown amorphous powder, $[\alpha]_D$ +30.9° (*c* 1.0 in acetone) (Found: C, 44.8; H, 3.0. $C_{60}H_{43}Br_5O_{24}\cdot 3H_2O$ requires C, 45.0; H, 3.1%). No signal due to a flavan A-ring proton was observed in the ^1H n.m.r. spectrum.

The tetramer (6) (1.0 g) was heated for 2 h under reflux in ethanol (20 ml) containing phenylmethanethiol (3 ml) and acetic acid (2 ml). The reaction mixture, after concentration under reduced pressure, was subjected to Sephadex LH-20 chromatography. Elution with water–methanol (3 : 2) gave (−)-epicatechin (10) (35 mg), 4-benzylthio-(−)-epicatechin (18) (80 mg), and 4′-benzylthioproanthocyanidin A-2 (15) (135 mg). Further elution with the same solvent yielded a proanthocyanidin (450 mg) shown to be identical with the trimer (5) by $[\alpha]_D$ and ^1H n.m.r. comparison, and the *trimeric proanthocyanidin benzyl sulphide* (19) (30 mg) as a pale brown amorphous powder, $[\alpha]_D^{22}$ +32.4° (*c* 1.1 in acetone) (Found: C, 58.7; H, 4.7. $C_{52}H_{42}O_{18}S\cdot 4H_2O$ requires C, 59.0; H, 4.8%); δ_H (CD_3COCD_3) 3.25 (1 H, d, *J* 4 Hz, c′-ring 3-H), 4.40 (1 H, d, *J* 4 Hz, c′-ring 4-H), 4.76 (1 H, s, c-ring 4-H), 5.11 (1 H, s, c-ring 2-H), and 5.27 (1 H, s, c″-ring 2-H).

The *tetrameric proanthocyanidin* (7), obtained as a pale brown amorphous powder, had $[\alpha]_D$ +93.4° (*c* 1.2 in acetone) (Found: C, 59.7; H, 4.6. $C_{60}H_{48}O_{24}\cdot 3H_2O$ requires C, 59.7; H, 4.5%). The ^1H n.m.r. spectrum was duplicated owing to the occurrence of rotamers. Methylation of the tetramer (7) (300 mg) as before gave the *pentadecamethyl ether* as a white amorphous powder (150 mg), $[\alpha]_D$ +61.2° (*c* 0.9 in $CHCl_3$) (Found: C, 66.0; H, 5.7. $C_{75}H_{78}O_{24}$ requires C, 66.1; H, 5.8%); *m/z* 1 362 (M^+). Bromination of the tetramer (7) (105 mg) yielded the 6,6″,6‴,8,8′-*pentabromo derivative* (118 mg) as a pale brown amorphous powder, $[\alpha]_D^{21}$ +51.3° (*c* 1.1 in acetone) (Found: C, 44.9; H, 3.1. $C_{60}H_{43}Br_5O_{24}\cdot 3H_2O$ requires C, 45.0; H, 3.1%). The ^1H n.m.r. spectrum showed no signal arising from A-ring protons.

Treatment of the tetramer (7) (500 mg) with phenylmethanethiol (3 ml) and acetic acid (2 ml) in ethanol (10 ml), followed by separation on a Sephadex LH-20 column with water–methanol (1 : 4) as eluant, gave (−)-epicatechin (10) (5 mg), 4-benzylthio-(−)-epicatechin (18) (5 mg), 4′-benzylthioproanthocyanidin A-2 (15) (33 mg), the proanthocyanidin trimer (5) (120 mg), and the *trimeric proanthocyanidin benzyl sulphide* (20) (16 mg). Compound (20) had $[\alpha]_D^{22}$ +52.5° (*c* 1.0 in acetone) (Found: C, 60.2; H, 4.5. $C_{52}H_{42}O_{18}S\cdot 3H_2O$ requires C, 60.0; H, 4.7%); δ_H (CD_3COCD_3) 3.95 (2 H, s, CH_2S), 4.59 (1 H, s, c-ring 4-H), and 4.88, 5.28, 5.39, and 5.66 (2 H, in total, each s, together c, c″-ring 2-H).

The *pentameric proanthocyanidin* (8), obtained as a pale brown amorphous powder, had $[\alpha]_D^{25}$ +70.8° (*c* 1.1 in acetone) (Found: C, 60.2; H, 4.4. $C_{75}H_{60}O_{30}\cdot 3H_2O$ requires C, 60.2; H, 4.5%). Methylation of the pentamer (8) (200 mg) in the same way as for (5) gave the *nonadecamethyl ether* as a white amorphous powder (50 mg), $[\alpha]_D$ +42.3° (*c* 1.1 in $CHCl_3$) (Found: C, 65.4; H, 5.9. $C_{94}H_{98}O_{30}\cdot H_2O$ requires C, 65.4; H, 5.9%); *m/z* 1706 (M^+).

The pentamer (8) (300 mg) was heated for 1.5 h under reflux in ethanol (10 ml) containing acetic acid (2 ml) and phenylmethanethiol (4 ml). The reaction products were separated by repeated chromatography over Sephadex LH-20 with ethanol and a mixture of water–methanol as eluants to give (−)-epicatechin (10) (13 mg), 4-benzylthio-(−)-epicatechin (18) (8 mg), 4′-benzylthioproanthocyanidin A-2 (15) (32 mg), 4′-benzylthioprocyanidin B-2 (21) (5 mg), the trimer (5) (25 mg), and the tetramer (6) (153 mg).

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