Structures of Dimeric, Trimeric, and Tetrameric Procyanidins from *Areca catechu* L.

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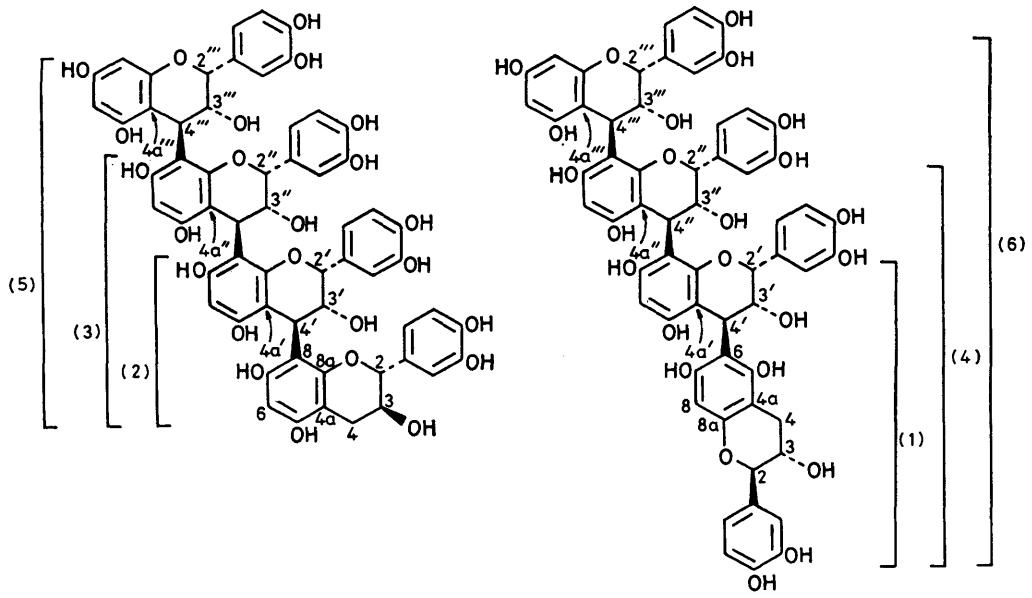
Summary Two procyanidin tetramers, two trimers, and a dimer which is a structural isomer of procyanidin B-1, along with (+)-catechin, (−)-epicatechin, and procyanidins A-1, B-1, and B-2, have been isolated pure from the seed of *Areca catechu* L., and their ^{1}H and ^{13}C n.m.r. spectral data, combined with degradative studies on their reactions with toluene-α-thiol, have established that they all, except for procyanidin B-2, have the C(4)-to-C(8) [or C(6)]-linked (−)-epicatechin stereochemistry [C(2), C(3): *cis*] in the upper units, and the (+)-catechin stereochemistry [C(2), C(3): *trans*] in the terminal (lower) units.

DURING the past ten years the chemistry of the proanthocyanidins, which are widely distributed in the plant kingdom, has attracted considerable interest from chemists and biologists; in particular, the chemistry of proanthocyanidin dimers has been almost completely established by the use of Sephadex LH-20 dextran gel for separation and purification, and of ^{1}H and ^{13}C n.m.r. spectroscopic techniques for their structural determination.[1–5] However, the structures of trimeric, tetrameric, and higher oligomeric procyanidins are not yet fully known.

Our interest in the chemistry of astringent tannins in the seeds of *Areca catechu* L. has resulted in the isolation and characterization of two procyanidin tetramers, two trimers, and a dimer which is presumably identical with procyanidin B-7 whose structure has been proposed by Haslam *et al.*[1] but not fully characterized, together with (+)-catechin, (−)-epicatechin, and procyanidins A-1,[6] B-1, and B-2.[1]



Structures of the procyanidins (**1**)—(**6**).

A procyanidin dimer (**1**), $[\alpha]_D$ + 129·4°, afforded approximately equal quantities of the 4-benzylthio-derivatives of (+)-catechin and (−)-epicatechin[1] on treatment with toluene-α-thiol in ethanolic acetic acid.[1] Although the ^{1}H n.m.r. spectrum of (**1**) is similar to that of procyanidin B-1 (**2**), the C(2)-H signal of the catechin moiety in the lower unit is observed at higher field (δ 4·52) compared with that of procyanidin B-1 (δ 4·76). Since the chemical shift of the C(2)-H signal of the lower unit in (**1**) is similar to that in (+)-catechin, suggesting the heterocyclic ring (c-ring) in the lower unit is magnetically less affected by the upper unit than that of 4′,8-linked procyanidin B-1, this enabled us to characterize the structure of (**1**) as the C(4′)-to-C(6)-linked (−)-epicatechin-(+)-catechin dimer. The stereochemistry at C(4′) in (**1**) was determined as shown from the chemical shift and coupling constant of C(4′)-H in the ^{1}H n.m.r. spectrum of (**1**), which are analogous to those in procyanidin B-1.

Procyanidin trimers [(**3**), $[\alpha]_D$ + 76·9° and (**4**), $[\alpha]_D$ + 130·0°], obtained homogeneously by repeated chromatography on Sephadex LH-20 and reversed-phase polystyrene gels, yielded the 4-benzylthio-derivatives of (+)-catechin and (−)-epicatechin on similar treatment as for (**1**). In the ^{1}H n.m.r. spectra of (**3**) and (**4**) a complete assignment of the aliphatic protons is possible, and the most notable point to distinguish between these two trimeric isomers is a difference of the C(2)-H chemical shifts in the terminal catechin units [that of (**3**) being at δ 4·91 and that of (**4**) at δ 4·58]. These observations are in close agreement with the relationship between procyanidin B-1 and its structural isomer (**1**), and on the basis of this evidence the two trimeric procyanidins are formulated as structures (**3**) and (**4**) respectively.

A tetrameric procyanidin (**5**), $[\alpha]_D$ + 98·6°, has an additional epicatechin unit compared with (**3**), as revealed by the ^{1}H n.m.r. spectrum which exhibits three C(2)-H broad singlet signals (δ 5·12, 5·20, and 5·31) for the epicatechin moieties. The appearance of a doublet signal (δ 4·97) at lower field, due to C(2)-H of the lower catechin unit, indicates that the interflavan linkage in the lower two units is of C(4′), C(8)-type, analogous to procyanidin B-1 and (**3**). Since rotational isomerism is not observed in the ^{1}H n.m.r. spectrum,[1] three epicatechin units are considered to be linearly linked in the upper part of the tetramer, and therefore the structure of the tetramer is represented as (**5**).

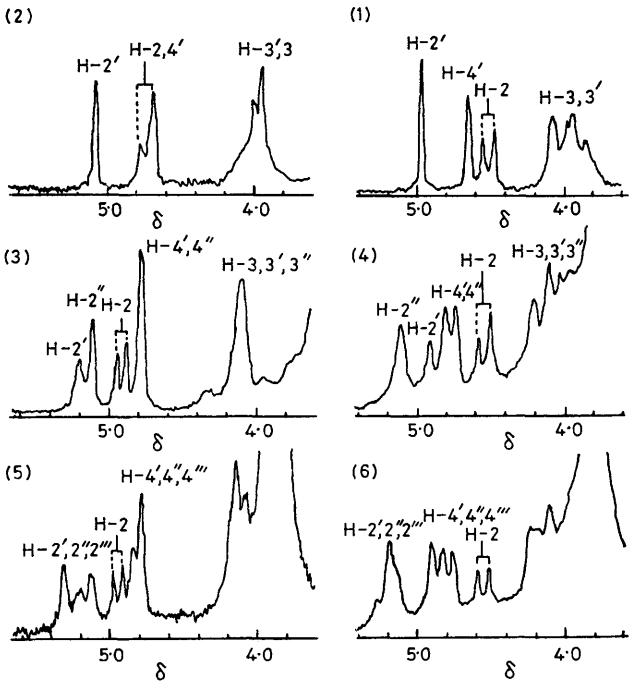


FIGURE. 100 MHz ^{1}H n.m.r. spectra [$(CD_3)_2CO$–D_2O] of procyanidins (**1**)—(**6**) isolated from *Areca catechu* L., with $SiMe_4$ as internal standard.

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

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‴)
(1)	82·2	68·0	[a]	76·7	71·8	37·1						
(2)	81·9	67·7	[a]	76·7	72·6	36·7						
(3)	81·7	67·8	[a]	76·7	71·5	36·7	76·7	72·8	36·7			
(4)	82·1	68·0	[a]	76·5	71·7	36·9	76·5	72·9	36·9			
(5)	81·3	67·4	[a]	76·5	71·4	36·8	76·5	71·8	36·8	76·5	72·8	36·8
(6)	82·1	68·0	[a]	76·5	71·0	37·1	76·5	72·2	37·1	76·5	72·7	37·1

	C(4a)	C(4a′)	C(4a″)	C(4a‴)
(1)	98·6	101·0		
(2)	100·7	100·7		
(3)	101·5	100·5	100·5	
(4)	99·4	101·5	101·2	
(5)	101·4	100·9	100·4	100·4
(6)	100·0	101·2	101·0	101·0

[a] Overlapped with solvent signal.

Another tetramer (**6**), $[\alpha]_D$ + 134·8°, likewise reveals in the ^1H n.m.r. spectrum three proton signals (δ 5·13, 5·18, and 5·28) attributable to the C(2)-H atoms of the epicatechin units, along with a C(2)-H doublet (δ 4·55) of the terminal (lower) catechin unit, and these chemical shifts may be correlated with those in (**1**) and (**4**). The occurrence of three epicatechin units and one catechin unit in the molecule of (**4**) is further confirmed by the ^{13}C n.m.r. spectrum (Table) which shows signals due to three epicatechin C(3)-atoms (δ 71·0, 72·2, and 72·7 p.p.m.) and one catechin C(3)-atom (δ 68·0 p.p.m.).[3] The appearance of resonances at δ 76·5 (three carbon atoms) and 82·1 p.p.m. ascribable to the C(2)-atoms of the epicatechin and catechin moieties, respectively, is also highly diagnostic.[3] From these spectral data the structure of the tetramer is formulated as (**6**).

It should be noted that from accumulated ^1H and ^{13}C n.m.r. spectral data the points of linkages in the terminal catechin units can be determined not only by the chemical shifts of C(2)-H in the catechin units, but also by the carbon signals due to the C(4a)-atoms of the catechin moieties, which lie within the ranges δ100·4—101·5 [C(4′), C(8)-linkages] and 98·6—100·0 p.p.m. [C(4′), C(6)-linkages].

(*Received*, 27*th April* 1981; *Com.* 503.)

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