Synthesis of Condensed Tannins. Part 9.† The Condensation Sequence of Leucocyanidin with (+)-Catechin and with the Resultant Procyanidins

Jan. A. Delcour
Laboratorium voor Toegepaste Organische Scheikunde, Katholieke Universiteit Leuven, B-3030 Heverlee, Belgium
Daneel Ferreira and David G. Roux *
Department of Chemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300 South Africa

Molar equivalents of synthetic (2*R*,3*S*,4*R* or *S*)-leucocyanidin and (+)-catechin condense with exceptional rapidity at pH 5 under ambient conditions to give the all-*trans*-[4,8]- and [4,6]-bi-[(+)-catechins] (procyanidins B_3, B_6) the all-*trans*-[4,8:4,8]- and [4,8:4,6]-tri-[(+)-catechins] (procyanidin C_2, and novel isomer), and the presumed all-*trans*-[4,8]-linked tetraflavanoid analogue in the proportions 10 : 1 : 12 : 1 : 3. The facility and sequence of these condensations correlate with the observed absence of leucocyanidins (flavan-3,3',4,4',5,7-hexaols) and also the dominance of related condensed tannins in plant extracts.

Complex natural procyanidins have received considerable attention over the past decade through the application of analytical and degradative studies initiated by Haslam and his co-workers [1-3] as a sequel to the pioneering isolation and synthetic work by Weignes *et al.*[4,5] However, synthesis of the free phenolic forms of procyanidin dimers was hitherto based exclusively on 4-benzylthioflavan-3-ols, generated by thiolysis of complex natural procyanidins, as synthons for coupling with (+)-catechins and (−)-epicatechins.[2] This indirect method is symptomatic of the absence of natural leucocyanidins (flavan-3,4-diol analogues) from procyanidin tannin mixtures, a phenomenon which contributed partly to Haslam's postulate of protonated flav-3-en-3-ols as electrophilic intermediates in condensed tannin biosynthesis.[3] Although this concept was derived from ^3H and ^{13}C feeding experiments, his conclusion overlooked the predictably high reactivity of leucocyanidins (*cf.* Creasy and Swain [6]) on the grounds of effective delocalization over the phloroglucinol-type A-rings of the charge of the 4-carbenium ion generated under suitable conditions from these putative flavan-3,4-diol tannin precursors.

Our present work provides the first evidence of the remarkable ease of condensation which results in procyanidins in support of the flavan-3,4-diol postulate, demonstrates that in the presence of excess of the initial nucleophilic substrate [(+)-catechin] simultaneous condensations to higher and predominantly 'linear' [4,8]-linked oligomers occur under mild conditions, and also indicates that [4,6]-linkages are formed, albeit in minor proportion; this is a prognosis for their limited presence in natural procyanidins.

Thus, reduction of (2*R*,3*R*)-dihydroquercetin [(+)-taxifolin] with sodium borohydride in ethanol under nitrogen in the presence of a molar equivalent of (+)-catechin, and adjustment of the solution to pH 5 after the addition of an equal volume of water, results in the complete consumption of the unstable (2*R*,3*S*,4*R* or *S*)-3,3',4,4',5,7-hexahydroxyflavan. Low quantities of (+)-taxifolin (2.6%) and a substantial amount of (+)-catechin (42.5%) survive the reaction, while two biflavanoids, two triflavanoids, and a higher oligomer, presumably a tetraflavanoid, are also formed. The products were separated in the free phenolic form on Sephadex LH-20 [1] into the three oligomeric categories, the two biflavanoids being resolved individually under these conditions.

The products of condensation were successively methylated and acetylated in order to permit their final purification or separation as methyl ethers or as methyl ether acetates, to permit their unambiguous differentiation by ^1H n.m.r. spectroscopy at 80 MHz based on the excellent distribution of chemical shifts in the 'fingerprint' heterocyclic and aromatic regions (*cf.* Figure 1, and references 7—9), and in particular to allow definition of the points of bonding to (+)-catechin from chemical-shift data.[10] Choice of the methyl ether acetate derivatives is furthermore based on the convenient recognition of the degree of condensation in oligomers as judged by the number of sharply defined 3-acetoxy proton resonances, and on their temperature-dependent sharpening as an index for meeting the minimum-energy requirements for 'fast' rotation about the interflavanoid bonds.

Such detailed analysis of ^1H n.m.r. spectra recorded at high temperatures has proved to be essential for placing structural and stereochemical allocations of higher oligomers beyond doubt,[8-12] while providing reliable criteria as to the purity of tannin derivatives. ^{13}C N.m.r. spectroscopy is less adequate in this respect, owing to duplication and broadening of some of the resonances [13] of spectra recorded of necessity under ambient conditions, when considering the exacting combination of high temperatures and long accumulation times required.

Starting with the tetramethyl ether acetate (3) of (+)-catechin as the reference compound, a notable feature of the spectrum of the heterocyclic protons is the line broadening of 4-H_{ax} (δ 2.63,‡ dd, $J_{3,4}$ 6.75, $J_{4ax,4eq}$ 17.0 Hz) compared with its 4-equatorial counterpart (δ 2.91, dd, $J_{3,4}$ 5.5, $J_{4ax,4eq}$ 17.0 Hz) (*cf.* Figure 2). Decoupling of 4-H_{ax} leads to sharpening of 2-H_{ax} only, indicating coupling through space, since assumption of a half-chair conformation permits 1,3-*cis*-diaxial interaction rather than an exception to the W-rule.[14] The chemical-shift difference between 2-H and 3-H (Δδ 0.36) is similarly significant (*cf.* Table 1), and the characteristic chemical shift of the 3-acetoxy methyl function (δ 1.94) is reflected in all the higher oligomers formed during condensation, as is the above line broadening.

One of the two main products of direct condensation of (+)-leucocyanidin (1) with (+)-catechin (2) under conditions of low acidity (pH 5) is the all-*trans*-[4,8]-bi-[(+)-catechin] (4) (17.5% yield). Comparison of the ^1H n.m.r. spectrum of the octamethyl ether diacetate shows the introduction of an

† Part 8 is reference 12.

‡ Chemical shifts were measured for $CDCl_3$ solution at 100 °C (under pressure in firmly stoppered tubes).

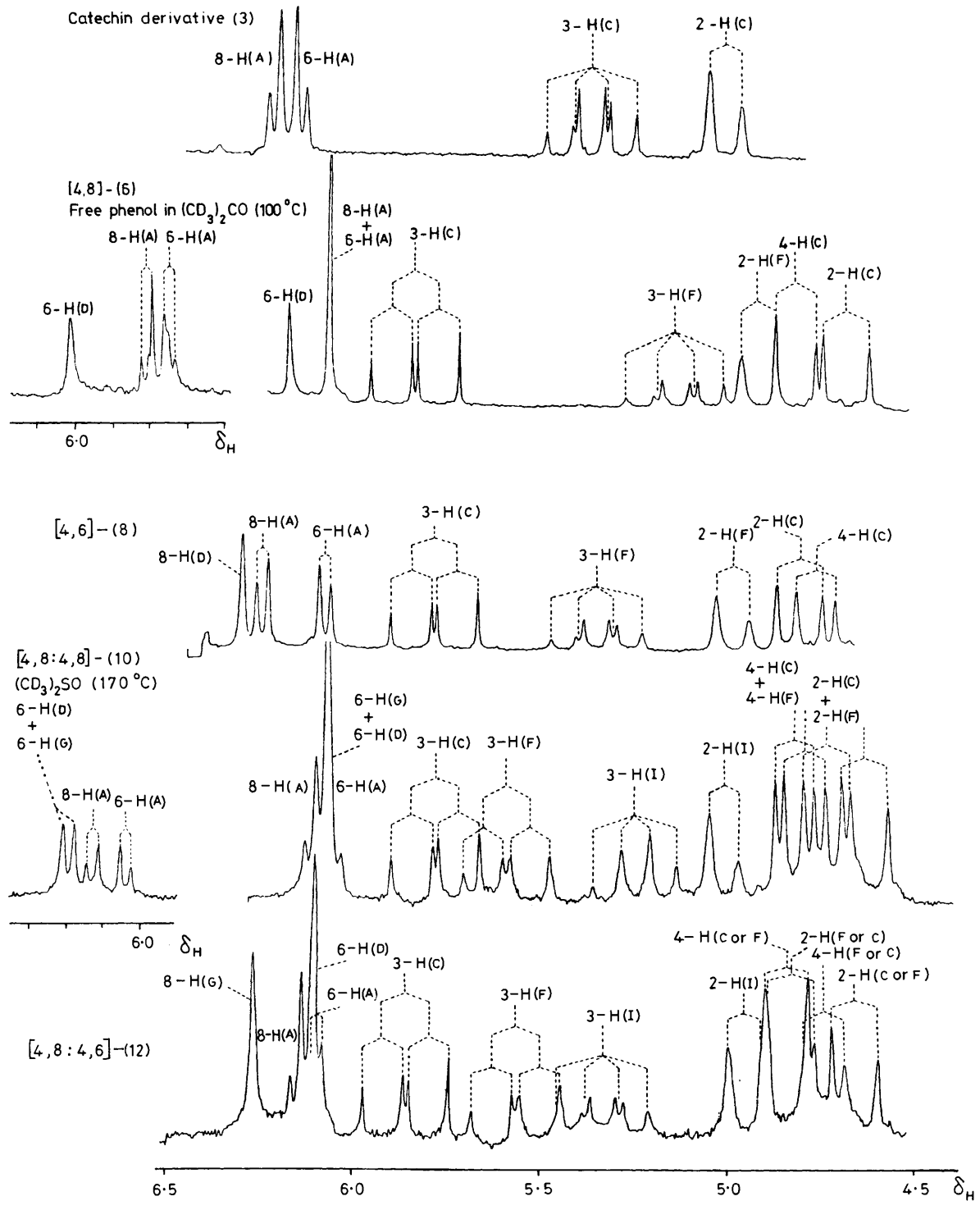


Figure 1. 80-MHz ^1H N.m.r. spectra in $CDCl_3$ (100 °C) of the high-field aromatic and the heterocyclic regions of the methyl ether acetate derivatives of (+)-catechin compound (3) and of the [4,8]- (6), [4,6]- (8), [4,8:4,8]- (10), and [4,8:4,6]-procyanidin (12) derivatives

AMX spin system superimposed upon the characteristic ABXY system of the catechin moiety (*cf.* Figure 1), with coupling constants ($J_{2,3}$ 10.0 and $J_{3,4}$ 8.75 Hz) indicative of a 2,3-*trans*-3,4-*trans* orientation of the 'upper' unit. C.d. (intense low-wavelength negative Cotton effects [15]) confirmed the 4*S* configuration (C-ring) at the point of bonding. The chemical shift of 6-H(D), δ 6.16, is in line with parameters previously established,[10] and defines the 8-substitution on the phloroglucinol unit of (+)-catechin. The overlap of signals due to 6-H(A) and 8-H(A) in $CDCl_3$ is resolved into the expected aromatic AB system (*J ca.* 2.5 Hz) in the spectrum of the unsubstituted biflavanoid in $(CD_3)_2CO$ at 100 °C (*cf.* Figure 1). The high-field aromatic resonances are accordingly much simplified compared with those of profisetinidin and prorobinetinidin analogues,[8] and were readily analysed at 80 MHz. The second acetyl group introduced [δ 1.61, 3-$COCH_3$-

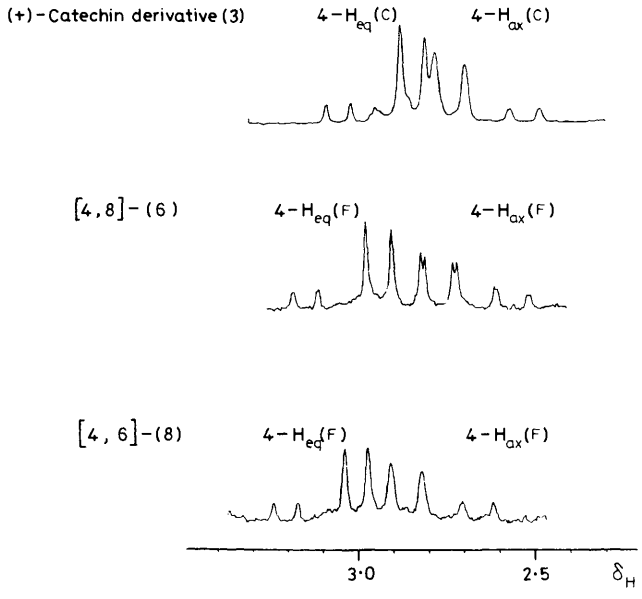


Figure 2. 80-MHz ^{1}H N.m.r. spectra in $CDCl_3$ (100 °C) of the heterocyclic methylene regions of the methyl ether acetate derivative of (+)-catechin [compound (3)] and of the [4,8]- (6) and [4,6]-procyanidin (8) methyl ether acetates

(C)] is shielded relative to that present on the (+)-catechin moiety [δ 1.88, 3-$COCH_3$(F)], probably due to its sustained proximity to the D and E aromatic rings during rotation about the interflavanoid bond at 100 °C. Line broadening of the 4-H_{ax}(F) signal ($J_{3,4ax}$ 7.1, $J_{4eq,4ax}$ 16.25 Hz) relative to that of 4-H_{eq}(F) in the biflavanoid derivative is accentuated with evidence of secondary coupling (J 0.9 Hz), compared with that of the flavan-3-ol cited above (*cf.* Figure 2), and spin decoupling again relates it to long-range 1,3-*cis*-diaxial effects.

During methylation with diazomethane a low proportion of the 3-OH(C) function is substituted as indicated (i) by the introduction of a high-field methoxy resonance (δ 2.83), (ii) by the upfield position (δ 3.91) of the doublet of doublets attributed to 3-H(C), and (iii) by the similarity of the chemical shifts of 3-H(F) (δ 5.19) and 3-OAc(F) (δ 1.89) relative to those (δ 5.11 and 1.88, respectively) of the diacetate. This 3-*O*-methyl derivative of the all-*trans*-[4,8]-procyanidin (4) is represented by structure (5); the free phenol (4) corresponds to the natural procyanidin B_3.[1]

The isomeric biflavanoid all-*trans*-[4,6]-bi-[(+)-catechin] (7), formed at about one-tenth of the concentration of its [4,8]-counterpart (4), was also characterized as its octamethyl ether diacetate (8) as follows. The upfield position of 8-H(D) (δ 6.28) defines [10] the point of bonding; the significance of the enhanced chemical-shift difference between 2-H(F) and 3-H(F) (Δδ 0.36) relative to that of the [4,8]-isomer (Δδ 0.19) (*cf.* Table 1) furnishes a parameter for determining the sequence of linkages in the 'trimeric' [4,8:4,6]-procyanidin derivative (12) (see below), and similarly the somewhat less shielded (δ 1.67) 3-OAc(C) resonance relative to that of the corresponding function (δ 1.61) of the [4,8]-isomer is of note. For the remainder the coupling constants reaffirm the all-*trans* relative configurations of constituent units, while c.d. supports the stereochemistry at the point of juncture of the interflavanoid bond (*cf.* Figure 3) as before.

With the detailed analyses of biflavanoids as a basis, those of the analogous triflavanoid pair fall into line. These procyanidins are represented by the predominant [4,8:4,8]-all-*trans*-tri-(+)-catechin (9) and its [4,8:4,6]-isomer (11), which are generated in the ratio 12 : 1 and at a level approximating to

Table 1. Chemical-shift differences between 2-H and 3-H of 'lower' terminal (+)-catechin units in oligomeric procyanidin methyl ether acetates

Procyanidin derivative		3-*O*-Substituents	Δδ $CDCl_3$	Δδ $(CD_3)_2SO$
Unsubstituted (+)-catechin	(3)	Ac	0.36	
Biflavanoids				
[4,8]	(5)	Me and Ac	0.23	
	(6)	Ac_2	0.19	
[4,6]	(8)	Ac_2	0.36	0.19 [a]
Triflavanoids				
[4,8:4,8]	(10)	Ac_3	0.23	0.16 [a]
[4,8:4,6]	(12)	Ac_3	0.33	
Tetraflavanoid				
[4,8:4,8:4,8]	(13)	Ac_4	0.21	

[a] The relative shifts are obviously solvent-dependent.

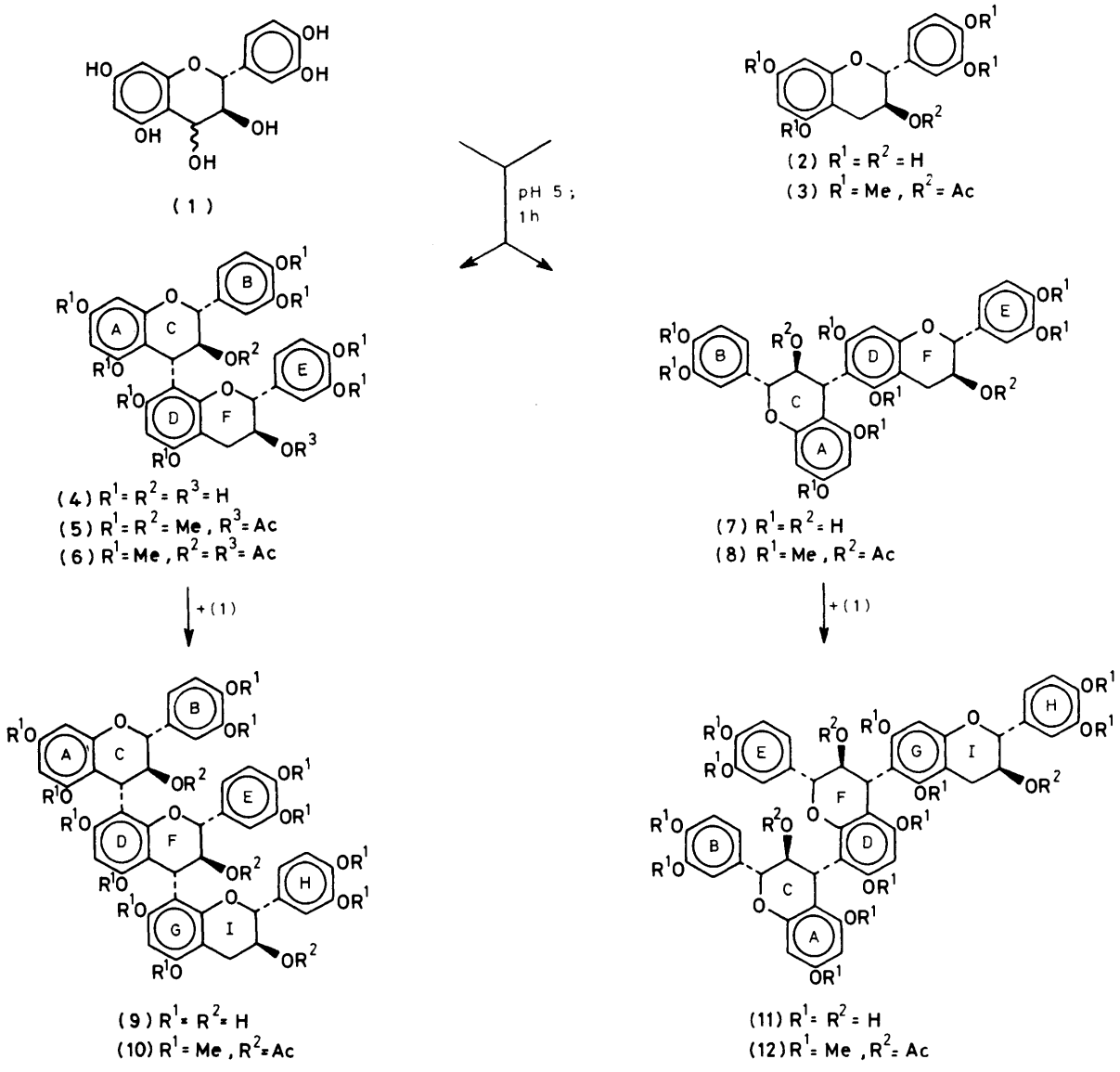
that of the biflavanoid pair. The following data support the former structure: analysis of the ^{1}H n.m.r. spectrum of the dodecamethyl ether triacetate (10) reveals *meta*-coupled doublets (A-ring) (δ 6.11 and 6.03, J 2.5 Hz) in addition to two overlapping singlets [δ 6.06, 6-H(D) and 6-H(G); peaks resolved in $(CD_3)_2SO$ at 170 °C] in the high-field aromatic region which denote [10] successive [4,8]-coupling of both 'upper' units. Another significant feature which correlates with that of the all-*trans*-[4,8]-biflavanoid derivative (6) is the reduced chemical-shift difference (Δδ 0.23) between 2-H(I) and 3-H(I) of the 'terminal' (+)-catechin moiety (*cf.* Table 1). Coupling constants of the heterocyclic systems of the 'upper' units [(C-ring) $J_{2,3}$ 10.0, $J_{3,4}$ 8.2 Hz; (F-ring) $J_{2,3}$ 10.0, $J_{3,4}$ 9.0 Hz] and c.d. (Figure 3) are in line with the proposed stereochemical assignments.

The minor triflavanoid procyanidin derivative (12) is differentiated from the aforementioned ones by the deshielding [δ 6.23, 8-H(G)] of one of two high-field aromatic proton singlets relative to the second [δ 6.07, 6-H(D)], signifying interflavanoid links to the 6- and 8-position, respectively,[10] of different flavanoid units, and also by the enhanced chemical-shift difference (Δδ 0.33) between 2-H(I) and 3-H(I) of the lower 'terminal' (+)-catechin unit as compared with that for the [4,6]-biflavanoid derivative (8) (*cf.* Table 1). The latter permits assignment of the [4,8:4,6] 'linear' sequence in interflavanoid links. Coupling constants again correlate with all-*trans* relative configuration.

Although the ^{1}H n.m.r. spectrum of the tetraflavanoid is poorly defined at 100 °C in $CDCl_3$, three aromatic singlets to high-field, δ 6.05, 5.97, and 5.89 (3 × 6-H), are discernible in addition to a *meta*-coupled AB system [δ 6.09 and 6.03, 8-H(A) and 6-H(A)], and also doublets (J *ca.* 10.0 Hz) in the high-field heterocyclic region (δ 4.72, 4.66, and 4.58), and three singlets [1.94, 1.81, and 1.62 (× 2)] representing four 3-OAc functions. The degree of condensation is supported by the ratios of the integral of the CH_2-group associated with the 'terminal' (+)-catechin unit to those of other (*e.g.* methoxy, heterocyclic, and aromatic) grouped-proton resonances. The product of highest mass may accordingly [10] be tentatively defined as an all-*trans*-[4,8:4,8:4,8] 'tetrameric' procyanidin.

The above series of condensations at ambient temperatures, and at a pH (5.0) representative of most natural tannin extracts, are significant in that the electrophilic flavan-3,4-diol condenses with a suitable nucleophilic flavan-3-ol, and then preferentially * in a secondary sequence with a succession of

* The sequence of secondary coupling operates almost as effectively in a 5 : 1 excess of the nucleophile [(+)-catechin].



Scheme.

oligomeric products (*cf.* Scheme), culminating in its quantitative removal from the solution in less than an hour. This implies that the leucocyanidin must be subject to a similar transient existence in nature, unlike its stable resorcinol- (leucofisetinidin, leucorobinetinidin) or pyrogallol-type (teracacidin, melacacidin) analogues, hence the inability by Haslam and his co-workers [3] to effect its isolation from the vegetative tissues of plants.

Both the primary and secondary condensations are highly regioselective, dominant 8-substitution of the nucleophilic substrates being attributed to lower steric hindrance encountered by the bulky electrophile with its attendant hydration sphere, rather than to very marked differences in nucleophilicity between C-8 and C-6 (*cf.* reference 16). However, evidence of significant 6-substitution of (+)-catechin correlates with previous evidence of [4,6]-links in natural procyanidins,[3,17] but the absence of commonly occurring 'angular' [4,6:4,8]-procyanidins in which (+)-catechin serves as the common nucleophile (*cf.* leucofisetinidins,[8] and leucorobinetinidins [11]) may be ascribed to a preference for 8-substitution due to a greater degree of steric approach control exercised by the additional presence of the 5-OH group close to the point of future linkage (C-4) on the parent electrophile.

The stereospecificity of the condensations at pH 5 in which the 'upper' units in all resultant procyanidins exhibit 2,3-*trans*-3,4-*trans* stereochemistry (Scheme) is unique when compared with the stereoselectivity encountered in the analogous formation of profisetinidins and prorobinetinidins under more acidic (pH 1) conditions. The difference may be rational-

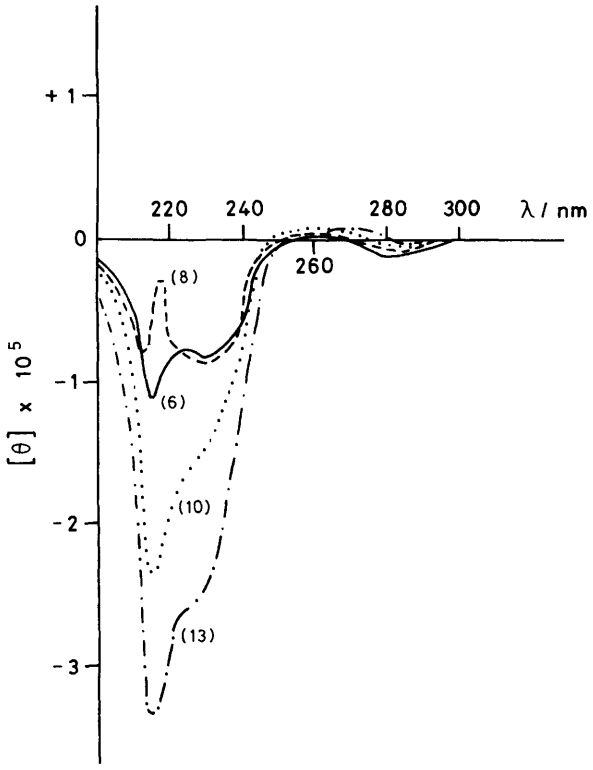


Figure 3. C.d. spectra of the procyanidin methyl ether acetates [4,8]-(6), [4,6]-(8), [4,8:4,8]-(10) and [4,8:4,8:4,8]-(13) in methanol

ized on the basis of an S_N1 mechanism for the latter, compared with predominantly an S_N2 mechanism for the former (as illustrated) under conditions much closer to neutrality, on the premise that $NaBH_4$ reduction of (+)-dihydroquercetin forms the thermodynamically more stable 2,3-*trans*-3,4-*cis*-flavan-3,3′,4,4′,5,7-hexaol (*cf.* reference 18).

The calculated percentages of leucocyanidin consumed in the production of various oligomers (Table 2) and also the high level of recovery of (+)-catechin indicate that, under conditions approximating to a 1 : 1 molar ratio of (+)-leucocyanidin to (+)-catechin, the resultant biflavanoids are stronger nucleophiles than the parent (+)-catechin. This is in line with our previous observation in connection with profisetinidins, which was tentatively ascribed to hyperconjugative effects.[12]

Condensations similar to the above were initially attempted by Geissman and Yoshimura [19] using a synthetic methyl ether of leucocyanidin, while Eastmond [20] identified procyanidin B_3 (4) in beer through chromatographic comparison with a product obtained by treating reduced dihydroquercetin * with (+)-catechin. Our present work represents a direct synthesis of procyanidins by a procedure modified from that of Eastmond. The ^1H n.m.r. data now available (Figure 1) should prove helpful in unambiguous structural determination of natural procyanidins.

Experimental

^1H N.m.r. spectra were recorded on a Bruker WP-80 FT spectrometer in $CDCl_3$ and $(CD_3)_2SO$ solutions with Me_4Si as

* Enzymic reduction of (+)-dihydroquercetin to the flavan-3,4-diol analogue was recently accomplished (H. A. Stafford and H. H. Lester, *Plant Physiol.*, 1982, **70**, 695).

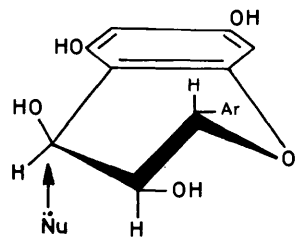


Table 2. Percentage of leucocyanidin consumed in the production of various procyanidin oligomers at pH 5

Oligomer	% Leucocyanidin used 1 : 1 [a,b]	1 : 5 [a,c]
[4,8]-Biflavanoid	17.5	52
[4,6]-Biflavanoid	5.5	8.5
[4,8:4,8]-Triflavanoid	30.5	29.5
[4,8:4,6]-Triflavanoid	7.7	*d*
[4,8:4,8:4,8]-Tetraflavanoid	28	*d*

[a] Molar ratio of (+)-dihydroquercetin to (+)-catechin. [b] 2.5% of (+)-Dihydroquercetin and 42.5% (+)-catechin recovered. [c] 1% of (+)-Dihydroquercetin recovered. [d] Isolation not attempted.

internal standard. N.m.r. tubes were firmly stoppered to avoid loss owing to pressure at temperatures above the boiling point of $CDCl_3$. Mass spectra were obtained with a Varian CH-5 instrument and c.d. data in methanol on a JASCO J-20 spectropolarimeter. T.l.c. was performed on precoated Merck t.l.c. plastic sheets (silica gel 60 PF_{254}, 0.25 mm) in ethyl acetate–formic acid–water (90 : 5 : 5 v/v) unless stated otherwise. The plates were sprayed with H_2SO_4–HCHO (40 : 1) after development. Paper chromatography was performed by upward migration at 20 °C on Whatman No. 1 paper (25 × 25 cm) using solvent A [butanol–acetic acid–water (4 : 1 : 5 v/v), upper phase] and solvent B (2% aqueous acetic acid). The polyphenols were detected by spraying air-dried paper with a freshly prepared aqueous solution of a mixture of $FeCl_3$ (0.2%) and $K_3Fe(CN)_6$ (0.2%). The papers were washed with 0.5M HCl to reveal blue spots on a white background. Preparative plates (p.l.c.) (20 × 20 cm; Kieselgel PF_{254}, 1.0 mm) were air-dried and used without prior activation. Methylations were performed with an excess of diazomethane in methanol–diethyl ether during 48 h at −15 °C, while acetylations were in acetic anhydride–pyridine at room temperature. Evaporations were done under reduced pressure at 50 °C in a rotary evaporator.

(+)-*Leucocyanidin*–(+)-*Catechin Condensation* (1 : 5 *Molar Ratio*)

(+)-Dihydroquercetin (2 g) and (+)-catechin (10 g) were dissolved in ethanol (200 ml) and treated with a solution of sodium borohydride (1.6 g) in ethanol (50 ml) added dropwise under nitrogen during 30 min. Water (250 ml) was then added and the pH adjusted to 5.0 with acetic acid solution (0.15M). After 1 h at room temperature (*ca.* 20 °C) under nitrogen, the solution was further diluted with water to a final volume of 800 ml. The mixture was extracted with ethyl acetate (1 × 500 ml and 5 × 250 ml). After evaporation of the solvent the product was separated on three Sephadex LH-20 columns (60 × 2.5 cm) in ethanol using 4 g of phenolics per separation. Fractions (12 ml each) were collected. Fractions 41—72 contained unchanged (+)-catechin (2) [R_F on silica 0.8; R_F (A) 0.69; R_F (B) 0.40], recovery 8.1 g. The

columns were subsequently eluted with methanol (5 l per column), and the solids (3.7 g) recovered were reseparated as above; 21-ml fractions were collected.

Fractions 27—40 contained (+)-catechin (50 mg); fractions 41—48 (+)-dihydroquercetin (20 mg) (R_F on t.l.c. silica gel 0.88); fractions 50—100 (2.01 g) [R_F (silica) 0.62, (A) 0.36, (B) 0.44]; fractions 101—114 (101 mg) [R_F (silica) 0.62 and 0.74 components]; fractions 117—160 contained a higher proportion of the R_F 0.74 component (286 mg) [R_F (A) 0.53, (B) 0.38]. The column was finally eluted with methanol (5 l) and gave a fraction (880 mg) [R_F (silica) 0.53, (A) 0.23, (B) 0.36].

[4,8]-*all*-trans-*Bi*-[(+)-*catechin*] (4) (*Procyanidin* B_3).—The R_F 0.62 fraction was methylated and resolved by p.l.c. [benzene–acetone (7 : 3 v/v)]. Acetylation of the octamethyl ether, R_F 0.37 (398 mg), followed by p.l.c. [benzene–acetone (9 : 1 v/v)] afforded the *octamethyl ether diacetate* (6), R_F 0.23, as a solid (251 mg) (Found: C, 65.1; H, 6.1. $C_{42}H_{46}O_{14}$ requires C, 65.1; H, 6.0%); δ (80 MHz; $CDCl_3$; 100 °C) 7.00—6.56 [m, 6 × ArH(B- and E-ring)], 6.16 [s, 6-H(D)], 6.05 [s, 8-H(A) + 6-H(A)], 5.84 [dd, Σ*J* 18.8 Hz, 3-H(C)], 5.11 [m, Σ*J* 20.5 Hz, 3-H(F)], 4.92 [d, $J_{2,3}$ 7.25 Hz, 2-H(F)], 4.80 [d, $J_{3,4}$ 8.75 Hz, 4-H(C)], 4.66 [d, $J_{2,3}$ 10.0 Hz, 2-H(C)], 3 85, 3.84, 3.81, 3.80, 3.79 3.78, 3.71, and 3.44 (8 × s, 8 × OMe), 3.03 [dd, *J* 16.25 and 5.75 Hz, 4-H_{eq}(F)], 2.66 [dd, *J* 16.25, 7.1, and 0.9 Hz, 4-H_{ax}(F)], 1.88 [s, 3-$COCH_3$(F)], and 1.61 [s, 3-$COCH_3$(C)]; c.d. spectrum (see Figure 3).

Methylation of the R_F 0.62 fraction also yielded a nonamethyl ether, R_F 0.48 (102 mg), which afforded its *monoacetate* (5), R_F 0.63, after purification by p.l.c. (× 3) with benzene–acetone (85 : 15 v/v) as a solid (30 mg) (Found: C, 65.8; H, 6.1. $C_{41}H_{46}O_{13}$ requires C, 65.9; H, 6.2%); δ (80 MHz; $CDCl_3$; 100 °C) 7.09—6.63 [m, 6 × ArH(B and E)], 6.19 [s, 6-H(D)], 6.04 [s, 8-H(A) + 6-H(A)], 5.19 [m, Σ*J* 19.4 Hz, 3-H(F)], 4.89 [d, $J_{2,3}$ 6.8 Hz, 2-H(F)], 4.73 [d, $J_{3,4}$ 8.0 Hz, 4-H(C)], 4.50 [d, $J_{2,3}$ 9.6 Hz, 2-H(C)], 3.91 [dd, 3-H(C)], 3.87, 3.85, 3.83, 3.82, 3.80, 3.75, 3.69, and 3.47 (8 × s, 8 × OMe), 3.02 [dd, *J* 16.2 and 5.6 Hz, 4-H_{eq}(F)], 2.66 [dd, *J* 16.2 and 6.8 Hz, 4-H_{ax}(F)], 2.83 [s, 3-OMe(C)], and 1.89 [s, 3-$COCH_3$(F)]; *m/z* 746 (M^+).

[4,6]-*all*-trans-*Bi*-[(+)-*catechin*] (7) (*Procyanidin* B_6).—The R_F 0.74 fraction was methylated and resolved by p.l.c. [benzene–acetone (3 : 1 v/v)]. Acetylation of the methyl ether, R_F 0.29 (92 mg), followed by p.l.c. [benzene–acetone (9 : 1 v/v)] afforded the *octamethyl ether diacetate* (8), R_F 0.34, as a solid (65 mg) (Found: C, 64.9; H, 6.0. $C_{42}H_{46}O_{14}$ requires C, 65.1; H, 6.0%); δ (80 MHz; $CDCl_3$; 100 °C) 6.72—7.16 [m, 6 × ArH(B and E)], 6.29 [s, 8-H(D)], 6.23 [d, *J* 2.2 Hz, 8-H(A)], 6.06 [d, *J* 2.2 Hz, 6-H(A)], 5.77 [dd, Σ*J* 18.6 Hz, 3-H(C)], 5.33 [m, Σ*J* 19.5 Hz, 3-H(F)], 4.97 [d, $J_{2,3}$ 7.0 Hz, 2-H(F)], 4.80 [d, $J_{2,3}$ 9.9 Hz, 2-H(C)], 4.73 [d, $J_{3,4}$ 8.7 Hz, 4-H(C)], 3.89, 3.87 (× 2), 3.84, 3.77, 3.65 (br), 3.55 (br), and 3.42 (8 × s, 8 × OMe), 3.09 [dd, *J* 16.1 and 5.25 Hz, 4-H_{eq}-(F)], 2.75 [dd, *J* 16.1 and 7.0 Hz, 4-H_{ax}(F)], 1.91 [s, 3-$COCH_3$-(F)], and 1.67 [s, 3-$COCH_3$(C)]; δ [80 MHz; $(CD_3)_2SO$; 170 °C] 6.31 [s, 6-H(D)], 6.20 [d, *J* 2.5 Hz, 8-H(A)], and 6.13 [d, *J* 2.5 Hz, 6-H(A)]; c.d. spectrum (see Figure 3).

Significant ions in the mass fragmentation spectra of the octamethyl ether diacetates (6) and (8) of procyanidins B_3 and B_6 and their respective relative abundances are *m/z* 774 (M^+, 1.1, 1.4%), 728 (14.0, 14.5), 714 (100, 100), 683 (4.6, 12.4), 654 (40, 8.4), 639 (11.4, 2.3), 623 (18.9, 4.5), 503 (35.7, 5.6), 492 (21, —), 477 (17.0, —), 461 (16.6, 6.6), 343 (28, 4.5), 328 (11.0, —), 327 (23, 24), 315 (10.6, 2.7), 300 (10.8, 6.9), 299 (48, 23), 246 (19.3, 3.9), 191 (13.3, 5.7), 181 (10.6, 5.8), 180 (39, 34), 179 (19.8, 8.1), 178 (10.9, 3.7), 167 (16.5, 14.8), 165 (25, 15), 152 (9.9, 8.0), and 151 (87, 72).

[4,8:4,8]-*all*-trans-*Tri*-[(+)-*catechin*] (9) (*Procyanidin* C_2).—The R_F 0.53 fraction was methylated and resolved by p.l.c. [benzene–acetone (3 : 1 v/v)]. Acetylation of the dodecamethyl ether, R_F 0.15 (114 mg), followed by p.l.c. [benzene–acetone (× 2) (4 : 1 v/v)] yielded the *dodecamethyl ether triacetate* (10) as a solid, R_F 0.59 (82 mg) (Found: C, 65.1; H, 6.0. $C_{63}H_{68}O_{21}$ requires C, 65.2; H, 5.9%); δ (80 MHz; $CDCl_3$; 100 °C) 7.09—6.56 [m, 9 × ArH(B, E, and H)], 6.11 [d, *J ca.* 2.5 Hz, 8-H(A)], 6.06 [s, 6-H(D) + 6-H(G)], 6.03 [d, *J ca.* 2 Hz, 6-H(A)], 5.77 [dd, Σ*J* 19.0 Hz, 3-H(C)], 5.58 [dd, Σ*J* 18.2 Hz, 3-H(F)], 5.23 [q, Σ*J* 15.75 Hz, 3-H(I)], 5.00 [br d, $J_{2,3}$ 6.25 Hz, 2-H(I)], 4.83 [d, $J_{3,4}$ 8.2 Hz, 4-H(F)], 4.80 [d, $J_{3,4}$ 9.0 Hz 4-H(C)]. 4.73 [d, $J_{2,3}$ 10.0 Hz, 2-H(F)], 4.64 [d, $J_{2,3}$ 10.0 Hz, 2-H(C)], 3.86, 3.84, 3.82 (× 4), 3.77, 3.74, 3.73, 3.52, 3.47, and 3.34 (12 × s, 12 × OMe), 2.95 [dd, *J ca.* 5.5 Hz, 4-H_{eq}(F)], 2.70 [dd, *J ca.* 6.3 Hz, 4-H_{ax}(F)], 1.94 [d, 3-$COCH_3$(I)], 1.63 [s, 3-$COCH_3$(D or C)], and 1.61 [s, 3-$COCH_3$-(C or D)]; δ [80 MHz; $(CD_3)_2SO$; 170 °C) 6.19 [s, 6-H(D or G)], 6.16 [s, 6-H(G or D)], 6.09 [d, *J* 2.1 Hz, 8-H(A)], and 6.02 [d, *J* 2.1 Hz, 6-H(A)]; *m/z* 1 100 (M^+ − 60, 10.7%), 1 069 (2.0), 1 040 (M^+ − 120, 11.1), 1 009 (9.8), 980 (M^+ − 180, 5.9), 949 (6.5), 327 (9.0), and 151 (100); c.d. spectrum (see Figure 3).

(+)-*Leucocyanidin*-(+)-*Catechin Condensation* (1 : 1 *Molar Ratio*)

(+)-Dihydroquercetin (1 g) and (+)-catechin (1 g) were dissolved in ethanol (40 ml). A solution of sodium borohydride (800 mg) in ethanol (40 ml) was added dropwise during 30 min under nitrogen. Water (200 ml) was added to the mixture and the pH adjusted to 5.0 with aqueous acetic acid (0.15M). After 1 h at room temperature (*ca.* 20 °C) under nitrogen, the solution was extracted with ethyl acetate (6 × 160 ml). Evaporation of the solvent gave a product which was separated on a Sephadex LH-20 column (60 × 2.5 cm) with ethanol. Fractions (10 ml) were collected. Fractions 35—52 contained (+)-catechin (425 mg), fractions 60—70 (+)-dihydroquercetin (26 mg), fractions 92—150 (350 mg) the procyanidin B_3, while fractions 160—190 yielded the procyanidin B_6 (38 mg). Fractions 191—245 contained both procyanidins C_2 and B_6 (400 mg). The column was then eluted with methanol (5 l) to yield the methanol fraction (1 090 mg).

A portion (400 mg) of the methanol fraction was methylated and the mixture resolved by p.l.c. [benzene–acetone (3 : 1 v/v) (× 2)] to give two fractions at R_F 0.14 and 0.25. The R_F 0.25 methyl ether fraction (80 mg) was acetylated and purified by p.l.c. [benzene–acetone (4 : 1 v/v)]; the resultant R_F 0.43 fraction (32 mg) consisted of the dodecamethyl ether triacetate (10) of procyanidin C_2. The R_F 0.14 methyl ether fraction (77 mg) was acetylated and resolved by p.l.c. [benzene–acetone (4 : 1 v/v)] to give two fractions at R_F 0.29 and 0.43.

[4,8:4,6]-*all*-trans-*Tri*-[(+)-*catechin*] (11).—The aforementioned R_F 0.43 fraction gave the *dodecamethyl ether triacetate* (12) as a solid (6 mg) (Found: C, 65.0; H, 6.0. $C_{63}H_{68}O_{21}$ requires C, 65.2; H 5.9%); δ (80 MHz; $CDCl_3$; 100 °C) 6.23 [s, 8-H(G)], 6.13 [d, *J* 2.5 Hz, 8-H(A)], 6.07 [s, 6-H(D)], 6.05 [d, *J* 2.0 Hz, 6-H(A)], 5.81 [dd, Σ*J* 19.0 Hz, 3-H(C)], 5.52 [dd, Σ*J* 19.0 Hz, 3-H(F)], 5.28 [m, Σ*J* 19.8 Hz, 3-H(I)], 4.95 [d, $J_{2,3}$ 7.3 Hz, 2-H(I)], 4.83 [d, $J_{3,4}$ 8.6 Hz, 4-H(C)], 4.81 [d, $J_{2,3}$ 10.0 Hz, 2-H(F)], 4.73 [br d, $J_{3,4}$ 9.0 Hz, 4-H(F)], 4.64 [d, $J_{2,3}$ 10.0 Hz, 2-H(C)], 3.85, 3.84 (× 2), 3.81 (× 3), 3.75, 3.72, 3.56, 3.48 (br), 3.47, and 3.34 (12 × s, 12 × OMe), 3.05 [dd, $J_{3,4}$ 5.5 Hz, 4-H_{eq}(I)], 2.70 [dd, $J_{3,4}$ 7.5 Hz, 4-H_{ax}(I)], 1.91

[s, 3-$COCH_3$(I)], 1.66 [s, 3-$COCH_3$(F or C)], and 1.64 [s, 3-$COCH_3$(C or F). The compound decomposed at 170 °C in $(CD_3)_2SO$.

[4,8:4,8:4,8]-*all*-trans-*Tetra*-[(+)-*catechin*].—The R_F 0.29 fraction gave the hexadecamethyl ether tetra-acetate (13) (tentative identification) as a solid (17 mg), δ (80 MHz; $CDCl_3$; 100 °C) 6.09 [d, 8-H(A)], 6.03 [d, 6-H(A)], 6.05, 5.97, and 5.89 [3 × s, 3 × 6-H(D, G, and J)], 4.72, 4.66, and 4.58 (3 × d, *J* 10.0 Hz, 3 × 2-H), 3.00 and 2.69 [dd, 2 × 4-H(L)], 1.94 [s, 3-$COCH_3$(L)], and 1.81 and 1.63 (× 2) [3 × 3-$COCH_3$-(C, F and I)]. The compound was unstable at 170 °C in $(CD_3)_2SO$.

Acknowledgements

Helpful discussions by Professor Dr. P. Dondeyne, Laboratorium voor Toegepaste Organische Scheikunde, University of Louvain and Dr. H. Kolodziej, the allocation of an overseas travel and subsistence grant to one of us (J. A. D.) by the Nationaal Fonds voor Wetenschappelijk Onderzoek and Vlaamse Leergangen, and financial support by the South African Council of Scientific and Industrial Research, Pretoria, and the Sentrale Navorsingsfonds of the University of the Orange Free State are acknowledged. Mass spectra were recorded by Dr. J. M. Steyn, Department of Pharmacology, University of the Orange Free State, and by Dr. A. Tuinman, National Chemical Research Laboratory, C.S.I.R., Pretoria.

References

1 R. S. Thompson, D. Jacques, E. Haslam, and R. J. N. Tanner, *J. Chem. Soc., Perkin Trans. 1*, 1972, 1387.
2 A. C. Fletcher, L. J. Porter, E. Haslam, and R. K. Gupta, *J. Chem. Soc., Perkin Trans. 1*, 1977, 1628.
3 D. Jacques, C. T. Opie, L. J. Porter, and E. Haslam, *J. Chem. Soc., Perkin Trans. 1*, 1977, 1637.
4 K. Weinges, W. Kaltenhauser, H.-D. Marx, E. Nader, J. Perner, and D. Seiler, *Liebigs Ann. Chem.*, 1968, **711**, 184; K. Weinges, K. Goritz, and F. Nader, *ibid.*, 1968, **715**, 164.
5 K. Weinges, J. Perner, and H.-D. Marx, *Chem. Ber.*, 1970, **103**, 2344.
6 L. L. Creasy and T. Swain, *Nature (London)*, 1965, **208**, 151.
7 J. J. Botha, D. Ferreira, and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1235.
8 J. J. Botha, P. M. Viviers, D. A. Young, I. C. du Preez, D. Ferreira, D. G. Roux, and W. E. Hull, *J. Chem. Soc., Perkin Trans. 1*, 1982, 527.
9 P. M. Viviers, D. A. Young, J. J. Botha, D. Ferreira, D. G. Roux, and W. E. Hull, *J. Chem. Soc., Perkin Trans. 1*, 1982, 535.
10 H. K. L. Hundt and D. G. Roux, *J. Chem. Soc., Chem. Commun.*, 1978, 696; *J. Chem. Soc., Perkin Trans. 1*, 1981, 1227.
11 P. M. Viviers, J. J. Botha, D. Ferreira, D. G. Roux, and H. M. Saayman, *J. Chem. Soc., Perkin Trans. 1*, 1983, 17.
12 J. A. Steenkamp, D. Ferreira, D. G. Roux, and W. E. Hull, *J. Chem. Soc., Perkin Trans. 1*, 1983, 23.
13 L. J. Porter, R. H. Newman, L. Y. Foo, H. Wong, and R. W. Hemingway, *J. Chem. Soc., Perkin Trans. 1*, 1982, 1217; E. V. Brandt, personal communication.
14 L. M. Jackman and S. Sternhill, 'Applications of Nuclear Magnetic Resonance in Organic Chemistry,' Pergamon, Oxford, 1969, p. 334.
15 J. J. Botha, D. Ferreira, and D. G. Roux, *J. Chem. Soc., Chem. Commun.*, 1978, 698; J. H. van der Westhuizen, D. Ferreira, and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1220.
16 H. Gheysens, 'Conformatiebepaling, Adsorptiegedrag en Zuurgekataliseerde Polymerisaties van Monomere Catechinen: Een Semi-Quantummechanische Studie,' Thesis for the Degree in Engineering, Catholic University of Louvain, 1982.
17 R. W. Hemingway, L. Y. Foo, and L. J. Porter, *J. Chem. Soc., Chem. Commun.*, 1981, 320; *J. Chem. Soc., Perkin Trans. 1*, 1982, 1209.
18 H. G. Krishnamurty, T. R. Seshadri, and D. G. Roux, *Tetrahedron Lett.*, 1965, 3689.
19 T. A. Geissman and N. N. Yoshimura, *Tetrahedron Lett.*, 1966, 2669.
20 R. Eastmond, *J. Inst. Brewing, London*, 1974, **88**, 188.

Received 29*th November* 1982; *Paper* 2/1992