

Electrophilic Aromatic Substitution of Catechins: Bromination and Benzylation

By Gerald W. McGraw and Richard W. Hemingway, USDA—Forest Service, Southern Forest Experiment Station, 2500 Shreveport Highway, Pineville, Louisiana 71360

Relative yields of C-6, C-8, and C-6 and C-8 substituted catechins obtained from the reaction of (+)-catechin or 3',4',5,7-tetra-*O*-methyl-(+)-catechin with pyridinium hydrobromide-perbromide, bromine, *p*-hydroxybenzyl alcohol, or *o*-hydroxybenzyl alcohol showed differing selectivities depending upon the electrophile used. Ratios of C-6 to C-8 substituted products were 1:1.2 for the reaction of *p*-hydroxybenzyl alcohol with (+)-catechin, 1:2–3 for reaction of either bromine or *o*-hydroxybenzyl alcohol with (+)-catechin, and essentially exclusive substitution at the C-8 position in the reaction of tetra-*O*-methyl-(+)-catechin with bromine. Comparatively high proportions of 6-substituted and 6- and 8-disubstituted products were obtained in the reaction of (+)-catechin with one equivalent of either *p*- or *o*-hydroxybenzyl alcohol.

SEVERAL important reactions of polymeric procyanidins (condensed tannins) involve the electrophilic aromatic substitution of catechin derivatives. In the biosynthesis of these polymers it is thought that flavanyl carbocations are produced by the stereospecific protonation of a flav-3-en-3-ol,^{1,2} and that consistent attack of the flavanyl carbocations at the C-8 position of flavan-3-ols and oligomeric procyanidins leads to linear polymers.²⁻⁴ If, however, substitution also occurred at the C-6 position, branched polymers would result.

One of the most promising approaches to the use of the condensed tannins as wood adhesives involves the use of polymethylolphenols (low molecular-weight phenol-formaldehyde resols) as cross-linking agents.^{5,6} This also involves the attack of hydroxybenzyl carbocations on catechin derivatives. Therefore, an understanding of the factors governing the reactivity of the nucleophilic centres of catechins is important for the study of the biosynthesis and structure of polymeric procyanidins as well as for the development of uses for these abundant natural products.

Estimates of the relative reactivity of the nucleophilic centres of (+)-catechin have been based on studies of the substitution of 3',4',5,7-tetra-*O*-methyl-(+)-catechin when brominated with pyridinium hydrobromide-perbromide. Roux *et al.*^{7,8} reported that bromination initially occurred exclusively at the C-8 position and that reaction at C-6 did not occur until all C-8 positions had been substituted. The B-ring was unreactive and an excess of reagent was required to obtain even small amounts of 6,6'-8-tribromo-3',4',5,7-tetra-*O*-methylcatechin. To expand our knowledge of these reactions we have examined the reaction of (+)-catechin and 3',4',5,7-tetra-*O*-methyl-(+)-catechin with *o*-hydroxybenzyl alcohol, *p*-hydroxybenzyl alcohol, and bromination reagents.

Replication of Roux's work^{7,8} on the bromination of 3',4',5,7-tetra-*O*-methyl-(+)-catechin with pyridinium hydrobromide-perbromide (1 or 2 mol equiv.) gave the reported results. In contrast, a mixture of products was obtained when (+)-catechin was treated with either pyridinium hydrobromide-perbromide (1 mol equiv.) or bromine (1 mol equiv.) in aqueous ethanol. After either

methylation or acetylation the 3',4',5,7-tetra-*O*-methyl or 3,3',4',5,7-penta-*O*-acetyl derivatives of 6-bromocatechin, 8-bromocatechin, and 6,8-dibromocatechin were obtained from the reaction products (Table). The

Summary of A-ring and benzyl CH₂ ¹H n.m.r. data
[τ(CDCl₃)]

	A-ring		Benzyl CH ₂
	6-H	8-H	
Acetates			
(+)-Catechin	3.41 (d)	3.33 (d)	
6-bromo-		3.34	
8-bromo-	3.39		
6-(<i>p</i> -acetoxybenzyl)-		3.34	6.26
8-(<i>p</i> -acetoxybenzyl)-	3.39		6.13
6,8-di-(<i>p</i> -acetoxybenzyl)-			6.13, 6.23
8-bromo-6-(<i>p</i> -acetoxybenzyl)-			6.23
6-(<i>o</i> -acetoxybenzyl)-		3.32	6.35
8-(<i>o</i> -acetoxybenzyl)-	3.38		6.19
6,8-bis(<i>o</i> -acetoxybenzyl)-			6.26, 6.37
Methyl ethers			
(+)-Catechin	3.91 (d)	3.87 (d)	
6-bromo-		3.64	
8-bromo-	3.80		
6-(<i>p</i> -methoxybenzyl)-		3.64	
8-(<i>p</i> -methoxybenzyl)-	3.80		
6-(<i>o</i> -methoxybenzyl)-		3.62	
8-(<i>o</i> -methoxybenzyl)-	3.85		

relative yields of products obtained from the reaction of (+)-catechin with either pyridinium hydrobromide-perbromide or bromine were similar. Although 8-bromocatechin (22 mol %) was obtained as the major product, substantial amounts of 6-bromocatechin (12 mol %) and 6,8-dibromocatechin (15 mol %) were also obtained and 51% of the catechin was recovered unchanged.

A mixture of products was obtained when 3',4',5,7-tetra-*O*-methylcatechin was refluxed with *p*-hydroxybenzyl alcohol (1.1 mol equiv.). Numerous attempts to separate the C-6 and C-8 monosubstituted products failed. However, the ratio of these isomers could be determined because of the differences in the ¹H n.m.r. chemical shifts of the catechin A-ring protons as described by Hundt and Roux.⁹ This indicated that the C-6 and C-8 substituted products were formed in the ratio 1:2 (Table).

A mixture of products was also obtained when (+)-catechin was refluxed with *p*-hydroxybenzyl alcohol (1 mol equiv.) for 7.5 h in dioxan-water. The acetate derivatives of the C-6 and C-8 substituted products were separated by preparative t.l.c. Assignments of the position of substitution for the two isomers were made by comparison of the ¹H n.m.r. shifts of the A-ring and benzyl-CH₂ protons with those of acetates of known 6-bromocatechin, 8-bromocatechin, 6-(*o*-acetoxybenzyl)-catechin, 8-(*o*-acetoxybenzyl)catechin (see below), and 8-bromo-6-(*p*-acetoxybenzyl)catechin which was synthesized from known 8-bromocatechin penta-acetate (Table).

To estimate the relative yields of the three products the acetate derivatives were separated (t.l.c.) into two fractions, one containing the two monosubstituted products and the second containing the disubstituted product. Integration of the two distinctly separated benzyl-CH₂ signals indicated that the C-6 and C-8 substituted derivatives were produced in the ratio 1 : 1. The yield of the disubstituted derivative was shown gravimetrically to be 17% of the total of unchanged catechin, mono- and disubstituted products, or 74% of the C-8 substituted derivative. The products of the reaction of *p*-hydroxybenzyl alcohol with catechin were also separated, by reverse phase h.p.l.c., and the yield of the phenols determined by measurement of the relative peak area (u.v. detector 280 nm), as well as gravimetrically by preparative h.p.l.c. These results also showed that the C-6 and C-8 substituted catechin derivatives were produced in the ratio 1 : 1.2.

When (+)-catechin was refluxed with *o*-hydroxybenzyl alcohol (1 mol equiv.) under the same conditions it was possible to isolate three major products as the free phenols both by chromatography on a silica-gel column and by reverse-phase h.p.l.c. This allowed the isolation of sufficient amounts of the phenols for both their methylated and acetylated derivatives to be prepared. The position of substitution of the two monosubstituted products was assigned by comparison of the ¹H n.m.r. shifts for the A-ring protons of the methylated derivatives with the data of Hundt and Roux.⁹ Acetylation of the same compounds permitted the assignment of the A-ring and benzyl-CH₂ protons for the two isomers (Table).

Determination of the relative yields of the C-6 and C-8 substituted isomers was made by integration of the two benzyl-CH₂ signals in the mixture of monosubstituted products isolated (preparative t.l.c.) from the acetate derivatives. This indicated that they were formed in the ratio 1 : 2. Gravimetric analysis of the ratio of the mono- and di-substituted products indicated that the 6,8-di-(*o*-hydroxybenzyl)catechin was produced in a yield of 67% of the 8-(*o*-hydroxybenzyl)catechin or 18% of the total of unchanged catechin and mono- and di-substituted products. Preparative reverse-phase h.p.l.c. indicated that the C-6 and C-8 substituted isomers were formed in the ratio 1 : 2.6–3.

The variations in relative yields of C-6 and C-8 substituted products obtained when either tetra-*O*-methyl-

catechin or (+)-catechin is treated with bromine, *p*-hydroxybenzyl alcohol, or *o*-hydroxybenzyl alcohol, together with recent work by Roux's group^{10,11} indicate that the varying degrees of regioselectivity exhibited in these reactions are undoubtedly determined by steric hindrance. In reactions of (+)-catechin with *p*-hydroxybenzyl alcohol substitution at the C-6 position is similar to that at C-8, but in reactions with a more bulky electrophile, such as *o*-hydroxybenzyl alcohol or bromine, substitution at the C-8 position is favoured with C-6 and C-8 substituted isomers being produced in the ratio *ca.* 1 : 2–3 in either case. Methylation of the C-5 and C-7 hydroxy-groups makes the A-ring inaccessible, particularly to the C-6 nucleophilic centre, as evidenced by the relative yields of the C-6 and C-8 substituted *p*-hydroxybenzyl- (1 : 2) and bromo- (exclusively C-8) derivatives.

The results are significant in an interpretation of the biosynthesis and structure of polymeric procyanidins. The ratio of 6-(*o*-hydroxybenzyl)catechin to 8-(*o*-hydroxybenzyl)catechin formed in the above reactions was much higher than the 1 : 9 ratio of C(4)–C(6) and C(4)–C(8) linked procyanidins reported by Fletcher *et al.*³ In studies of the dimeric procyanidins in loblolly pine phloem and those formed by the biogenetically-patterned synthesis by reaction of (+)-catechin with loblolly pine tannin we have found the procyanidins B-1 [(–)-epicatechin C4 → C8 (+)-catechin] and B-7 [(–)-epicatechin C4 → C6 (+)-catechin] in ratios of *ca.* 2.4 : 1.¹² Under conditions of biogenetically-patterned synthesis interflavanoid bonds are continually being formed, cleaved, and reformed so that the relative proportions of C(4)–C(6) and C(4)–C(8) linked procyanidins are dependent upon the relative rates of formation and cleavage of the two isomers. The C(4)–C(6) linked procyanidins are more resistant to acid-catalyzed cleavage than the C(4)–C(8) linked pairs.¹³ In addition, one would expect the proportion of unsubstituted C-6 positions to increase with increases in molecular weight. For these reasons it seems unlikely that condensed tannins would be linear polymers linked exclusively by C(4)–C(8) bonds. Indeed, recent work has shown that both C(4)–C(6) and C(4)–C(8) linked dimeric fragments are obtained by partial acid-catalyzed cleavage of polymeric procyanidins.^{12,14}

In early attempts to formulate wood adhesives from pine bark condensed tannins, through the use of polymethylphenols as cross-linking agents, we experienced difficulties with slow cure rates. When (+)-catechin was refluxed with *o*-hydroxybenzyl alcohol (1.1 mol equiv.) in dioxan-water (30 : 70) at 10% solids, and an initial pH of 7.0, t.l.c. showed small amounts of 6-(*o*-hydroxybenzyl)catechin and 8-(*o*-hydroxybenzyl)catechin after 1 h of heating. Small amounts of 6,8-di-(*o*-hydroxybenzyl)catechin appeared after 3 h of heating. After 7 h the intensities of 8-(*o*-hydroxybenzyl)catechin and 6,8-di-(*o*-hydroxybenzyl)catechin were approximately equal while 6-(*o*-hydroxybenzyl)catechin was approximately one-half as intense as the

8-(*o*-hydroxybenzyl)catechin. Large amounts of unchanged (+)-catechin and *o*-hydroxybenzyl alcohol remained after 7 h at reflux. If the initial pH was increased to 9.5 or 10.0 only small amounts of 8-(*o*-hydroxybenzyl)catechin were noted and neither 6-(*o*-hydroxybenzyl)catechin nor 6,8-di-(*o*-hydroxybenzyl)-catechin were detected after 7 h of reflux. A number of unidentified compounds appeared at the R_F region of 6-(*o*-hydroxybenzyl)catechin but large amounts of (+)-catechin and *o*-hydroxybenzyl alcohol were unchanged.

These results show that considerable energy is required to achieve a high degree of condensation of (+)-catechin with either *p*- or *o*-hydroxybenzyl alcohol when the pH is near neutrality. Substitution at the C-6 position was more favourable in reactions with *p*-hydroxybenzyl alcohol but the overall condensation rates were not substantially different from those in the reaction with *o*-hydroxybenzyl alcohol. Since the condensation rate is inherent in the stability of the hydroxybenzyl carbocation the pH conditions of these reactions should be held as low as practicable, within the constraints of tannin solubility and stability, to increase the condensation rates of these resins.

EXPERIMENTAL

The phenols (+)-catechin, *p*-hydroxybenzyl alcohol, and *o*-hydroxybenzyl alcohol were used as purchased (Fluka). Tetra-*O*-methyl-(+)-catechin was prepared from (+)-catechin by methylation with diazomethane, isolated by preparative t.l.c., and checked for purity by ^1H n.m.r. and mass spectroscopy and optical rotation measurements. Pyridinium hydrobromide-perbromide was prepared according to Fieser.¹⁵ Compounds were separated by preparative t.l.c. (silica gel, Baker 7GF, 0.75 mm), developed with (A) chloroform-methanol-acetic acid-water (85 : 15 : 10 : 3), or (B) benzene-acetone (9 : 1), or (C) benzene-acetone (8 : 2). Developed t.l.c. plates were viewed under u.v. light, sprayed with sulphuric acid-formalin (40%)–water (2 : 1 : 1), and heated for 5–10 min at 105 °C. Silica gel columns were 2 × 45 cm and were eluted with 15% methanol in chloroform or benzene-acetone (8 : 2). ^1H N.m.r. spectra were recorded in CDCl_3 containing 1% Me_4Si on a 60 MHz Hitachi-Perkin Elmer R24B spectrometer. Reverse-phase h.p.l.c. was performed on a Waters Associates liquid chromatograph equipped with a Microsorb RP-18 column (46 mm × 25 cm) using methanol-water (25 : 75, 30 : 70, and 50 : 50) flowing at 1.5 ml/min. Optical rotations were measured with a JASCO DIP-181 polarimeter. Mass spectra were provided by the Mass Spectroscopy Laboratory, Florida State University, Tallahassee. M.p.s were determined using a polarizing microscope with a hot stage and were uncorrected.

Bromination of Tetra-*O*-methyl-(+)-catechin.—Tetra-*O*-methyl-(+)-catechin was stirred with pyridinium hydrobromide-perbromide (1 or 2 mol equiv.) in dichloromethane (30 ml) at room temperature for 15 min. The reaction mixture was then washed with water (4 × 50 ml), dried (Na_2SO_4), and the solvent removed under reduced pressure.

8-Bromo-3',4',5,7-tetra-*O*-methyl-(+)-catechin.—Tetra-*O*-methyl-(+)-catechin (0.50 g, 1.45 mmol) was treated with pyridinium hydrobromide-perbromide (0.46 g, 1.45 mmol) and the solution was extracted into dichloromethane. One

product was shown by t.l.c. at R_F 0.41 [solvent system (C)]. The material was recrystallized from ethanol to give needles, m.p. 173–174 °C (lit.,⁸ 172–174 °C) (Found: C, 54.0; H, 5.1. Calc. for $\text{C}_{19}\text{H}_{21}\text{BrO}_6$: C, 53.6; H, 4.9%; $[\alpha]_{578} -98.4^\circ$ (c 1.74 in CHCl_3); m/e (175 °C) 424.426 (M^+ , $M^+ + 2$, 26%), 245, 247 (100), 180 (74), 165 (37), 152 (20), and 151 (43); τ (CDCl_3) 2.9–3.1 (3 H, m, B-ring), 3.80 (1 H, s, A-ring), 5.03–5.15 (1 H, d, 2-H), 5.80–6.00 (1 H, m, 3-H), 6.14–6.18 (12 H, 2 × s, ArOMe), and 7.05–7.30 (2 H, m, 4-H). Substitution at C-8 is clearly shown by the shift of the A-ring proton to τ 3.80.⁹

6,8-Dibromo-3',4',5,7-tetra-*O*-methyl-(+)-catechin.

Tetra-*O*-methyl-(+)-catechin (0.42 g, 1.2 mmol) was treated with pyridinium hydrobromide-perbromide (0.776 g, 2.4 mmol) and the product extracted into dichloromethane. T.l.c. showed mainly one product at R_F 0.49 [solvent system (C)]. It was purified by column chromatography on a silica column by elution with benzene-acetone (8 : 2). The product crystallized from methanol-water as needles, m.p. 186–188 °C (lit.,¹¹ 184–187 °C) (Found: C, 45.3; H, 4.0. Calc. for $\text{C}_{19}\text{H}_{20}\text{Br}_2\text{O}_6$: C, 45.2; H, 4.0%; $[\alpha]_{578} -74.5^\circ$ (c 2.04 in CHCl_3); m/e (175 °C) 502, 504, 506 (M^+ , $M^+ + 2$, $M^+ + 4$, 12%, 25%, 12%), 323, 325, 327 (8, 15, 8), 180 (100), 165 (42), and 151 (95); τ (CDCl_3) 3.0–3.2 (3 H, m, B-ring, no A-ring proton), 5.15–5.30 (1 H, m, 2-H), 5.8–6.05 (1 H, m, 3-H), and 6.15, 6.20 (12 H, 2 × s, ArOMe). The C-4 and hydroxy-protons were too broad to integrate accurately.

Bromination of (+)-Catechin.—Catechin hydrate (4.0 g, 11 mmol) was dissolved in dioxan (30 ml) and to this was added pyridinium hydrobromide-perbromide (3.53 g, 11 mmol). The reaction mixture was stirred under argon at room temperature for 10 min, frozen, and dried *in vacuo*. The product was dissolved in water and extracted with ethyl acetate (2 × 75 ml). The ethyl acetate extract was reduced in volume under reduced pressure, and subjected to preparative t.l.c. [solvent system (A), developed twice].

6,8-Dibromo-3',4',5,7-penta-*O*-acetyl-(+)-catechin.

Preparative t.l.c. yielded a compound of R_F 0.55 that was collected, dried overnight *in vacuo* and a portion acetylated (acetic anhydride-pyridine). The acetylated material was purified by preparative t.l.c. [solvent system (C), R_F 0.68] and crystallized from acetone, m.p. 173–175 °C (Found: C, 46.0; H, 3.5. $\text{C}_{25}\text{H}_{22}\text{Br}_2\text{O}_{11}$ requires C, 45.6; H, 3.3%), $[\alpha]_{578} -7.7^\circ$ (c 2.03 in CHCl_3); m/e (175 °C) 656, 658, 660 (M^+ , $M^+ + 2$, $M^+ + 4$, 3%, 6%, 3%), 428, 430, 432 (32, 61, 32), 295, 297, 299 (6, 13, 6), 194 (55), 152 (100), and 123 (97); τ (CDCl_3) 2.77–2.82 (3 H, m, B-ring, no A-ring proton), 4.61–4.76 (2 H, m, 2-H and 3-H), 7.10–7.27 (2 H, m, 4-H), 7.55, 7.64, 7.70 (12 H, 3 × s, ArOAc), and 7.92 (3 H, s, aliphatic OAc). Methylation of the free phenol with diazomethane gave a product with m.p., t.l.c., R_F values, and ^1H n.m.r. data consistent with those reported above for 6,8-dibromo-3',4',5,7-tetra-*O*-methyl-(+)-catechin.

6-Bromo-3',4',5,7-tetra-*O*-methyl-(+)-catechin.—A second major product with R_F 0.46 was isolated from the bromination reaction, dried overnight *in vacuo*, and a portion methylated with diazomethane. The methylated product was purified by t.l.c. [solvent system (C), R_F 0.48] and crystallized from ethanol to give needles, m.p. 127–128 °C (lit.,¹¹ 130–132 °C) (Found: C, 53.3; H, 5.0. Calc. for $\text{C}_{19}\text{H}_{21}\text{BrO}_6$: C, 53.6; H, 4.9%; $[\alpha]_{578} -11.9^\circ$ (c 1.72 in CHCl_3); m/e (175 °C) 424, 426 (M^+ , $M^+ + 2$, 26%), 245, 247 (57), 180 (95), 165 (55), 152 (100), and 151 (88); τ (CDCl_3) 3.0–3.35 (3 H, m, B-ring), 3.64 (1 H, s, 8-H), 5.15–5.95 (2 H, m, 2-H and 3-H), and 6.05–6.40 (12 H, 2 × s, ArO-

Me); 4-H and OH were too broad to integrate accurately. The shift of the A-ring proton to τ 3.64 is consistent for 8-H of 6-bromo-3',4',5,7-tetra-*O*-methyl-(+)-catechin.⁹

6-Bromo-3,3',4',5,7-penta-*O*-acetyl-(+)-catechin.—The phenol with R_F 0.46 was also acetylated and purified by preparative t.l.c. [solvent system (C), R_F 0.67]. It was isolated as an oil and crystallized with time, m.p. 149—151 °C (Found: C, 51.6; H, 3.9. $C_{25}H_{23}BrO_{11}$ requires C, 51.8; H, 4.0%); $[\alpha]_{578}^{20} + 42.2^\circ$ (c 1.48 in $CHCl_3$); m/e (175 °C) 578, 580 (M^+ , $M^+ + 2$, 9%), 475, 477 (77), 434, 436 (94), 392, 394 (96), 350, 352 (72), 217, 219 (19), 152 (100), and 123 (89); τ ($CDCl_3$) 2.7—2.9 (3 H, m, B-ring), 3.34 (1 H, s, 8-H), 4.8—5.1 (2 H, m, 2-H and 3-H), 7.22—7.35 (2 H, m, 4-H), 7.77—7.82 (12 H, 2 \times s, ArOAc), and 8.1 (3 H, s, aliphatic OAc).

8-Bromo-3,3',4',5,7-penta-*O*-acetyl-(+)-catechin.—The third major product from the bromination reaction occurred as a band at R_F 0.35. It was methylated with diazomethane and identified as the 8-bromo-derivative by comparison of its R_F values and 1H n.m.r. spectrum with those reported above. The 8-bromocatechin was also acetylated and purified by preparative t.l.c. [solvent system (C), R_F 0.63]. Crystallization from ethanol gave needles, m.p. 123—125 °C (Found: C, 52.0; H, 4.1. $C_{25}H_{23}BrO_{11}$ requires C, 51.8; H, 4.0%); $[\alpha]_{578}^{20} - 28.8^\circ$ (c 0.93 in $CHCl_3$); m/e (175 °C) 578, 580, (M^+ , $M^+ + 2$, 3%), 475, 477 (63), 434, 436 (65), 392, 394 (63), 350, 352 (50), 217, 219 (25), 152 (100), and 123 (80); τ ($CDCl_3$) 2.70—2.84 (3 H, m, B-ring), 3.39 (1 H, s, A-ring), 4.61—4.75 (2 H, m, 2-H and 3-H), 7.10—7.25 (2 H, m, 4-H), 7.67—7.77 (12 H, 2 \times s, ArOAc), and 8.00 (3 H, s, aliphatic OAc).

Reaction of (+)-Catechin with Bromine.—Bromination was also affected by dissolving (+)-catechin hydrate (5.0 g) in ethanol (80 ml) and dropping a solution of bromine (3.0 g) in ethanol-water (2 : 1; 75 ml) into it over a period of 25 min. The reaction mixture was stirred at room temperature for an additional 30 min after which the ethanol was removed under reduced pressure. The aqueous mixture was diluted with water, extracted with ethyl acetate, and the extracts were combined, dried, concentrated at 40 °C under reduced pressure; the residue was then subjected to preparative t.l.c. [solvent system (A)] to yield products identical with those reported above. This bromination method (1 mol equiv. of bromine) was used to determine the yield data. Visual comparison of t.l.c. plates from the two bromination methods indicated very similar product ratios.

Reaction of Tetra-*O*-methyl-(+)-catechin with *p*-Hydroxybenzyl Alcohol.—Tetra-*O*-methylcatechin (195 mg, 0.56 mmol), and *p*-hydroxybenzyl alcohol (76 mg, 0.62 mmol) were refluxed for 3.5 h in *t*-butyl alcohol. The reaction mixture was freeze-dried and then separated on a silica column by elution with benzene-acetone (8 : 2). Elution of the monosubstituted product was monitored by t.l.c. [solvent system (C), R_F 0.22]. Methylation with diazomethane gave 100 mg of the expected monosubstituted penta-*O*-methyl derivative (R_F 0.42). Although t.l.c. suggested only one compound the 1H n.m.r. spectrum clearly showed a mixture of two isomeric C-6 and C-8 substituted products; τ ($CDCl_3$) 2.68—3.35 (7 H, m, B-ring and benzyl ArH), 3.64 (0.3 H, s, 8-H), 3.80 (0.7 H, s, 6-H), 5.24—5.40 (1 H, d, 2-H), 5.70—6.00 (17 H, m, ArOCH₃ and benzyl CH₂), and 7.00—7.35 (2 H, m, 4-H).

Reaction of (+)-Catechin with *p*-Hydroxybenzyl Alcohol.—Catechin hydrate (2 g, 5.5 mmol) and *p*-hydroxybenzyl alcohol (0.68 g, 5.5 mmol), were dissolved in dioxan-

water (1 : 2; 25 ml). The pH was adjusted to 7.0 with a small amount of sodium hydroxide and the solution heated under reflux for 7.5 h. A portion of product was freeze-dried, dissolved in aqueous methanol and filtered through a Metrical 0.45 μ membrane. The solution was separated on a Licosorb RP-18 column with methanol-water as eluant (25 : 75 v/v) flowing at 1.5 ml/min; the retention volume of the 6-(*p*-hydroxybenzyl)-(+)-catechin was 20.2 ml and that of the 8-(*p*-hydroxybenzyl)-(+)-catechin was 41.9 ml. The relative peak areas for the 8- and 6-substituted catechins were 1.2 : 1 (u.v. detector, 280 nm).

6-(*p*-Hydroxybenzyl)-(+)-catechin was isolated as an off-white amorphous powder, yield 9.30 mg (Found: C, 61.0; H, 5.58. $C_{22}H_{20}O_7 \cdot 2H_2O$ requires C, 61.1; H, 5.56%); $[\alpha]_{578}^{20} + 58.2^\circ$ (c 0.11 in MeOH). Acetylation gave a product identical with 6-(*p*-acetoxybenzyl)-penta-*O*-acetyl-(+)-catechin in its t.l.c. chromatographic properties (see below).

8-(*p*-Hydroxybenzyl)-(+)-catechin was isolated as an off-white amorphous powder, yield 9.48 mg (Found: C, 60.7; H, 5.68. $C_{22}H_{20}O_7 \cdot 2H_2O$ requires C, 61.1; H, 5.56%); $[\alpha]_{578}^{20} - 17.5^\circ$ (c 0.08 in MeOH). Acetylation gave a product identical with 8-(*p*-acetoxybenzyl)-penta-*O*-acetyl-(+)-catechin in its t.l.c. chromatographic properties (see below).

The freeze-dried phenolic product was acetylated and separated by preparative t.l.c. [solvent system (B), developed twice]. A compound with R_F 0.92 was shown to be the diacetate of *p*-hydroxybenzyl alcohol, and a compound with R_F 0.74 to be the penta-*O*-acetate of catechin, on the basis of their 1H n.m.r. spectra.

6-(*p*-Acetoxybenzyl)-3,3',4',5,7-penta-*O*-acetyl-(+)-catechin.—A product from the acetylated reaction mixture which occurred at R_F 0.68 was isolated as an amorphous solid (Found: C, 62.9; H, 5.1. $C_{34}H_{32}O_{13}$ requires C, 63.0; H, 4.9%); m/e (175 °C) 648 (M^+ , 13%), 504 (100), 420 (48), 243 (20), 152 (74), and 123 (59); τ ($CDCl_3$) 2.82 (4 H, m, benzyl ArH), 2.98—3.03 (3 H, d, B-ring), 3.34 (1 H, s, A-ring), 4.80—4.95 (2 H, m, 2-H and 3-H), 6.26 (2 H, s, benzyl CH₂), 7.04—7.40 (2 H, m, 4-H), 7.75, 7.87, 7.90 (9 H, 3 H, 3 H, each s, ArOAc), and 8.03 (3 H, s, aliphatic OAc). Comparison of ArH and benzyl CH₂ shifts with those of other compounds showed the 6-(*p*-acetoxybenzyl) derivative (Table).

8-(*p*-Acetoxybenzyl)-3,3',4',5,7-penta-*O*-acetyl-(+)-catechin.—A further amorphous solid was isolated (R_F 0.64) (Found: C, 62.6; H, 5.1. $C_{34}H_{32}O_{13}$ requires C, 63.0; H, 4.9%); $[\alpha]_{578}^{20} + 7.9^\circ$ (c 1.02 in $CHCl_3$); τ ($CDCl_3$) 2.75—3.25 (7 H, m, B-ring and benzyl ArH), 3.39 (1 H, s, A-ring), 4.92 (2 H, m, 2-H and 3-H), 6.13 (2 H, s, benzyl CH₂), 7.05—7.45 (2 H, m, 4-H), 7.76 (12 H, s, catechin ArOAc), 7.88—7.92 (3 H, m, benzyl OAc), and 8.06 (3 H, s, aliphatic OAc). Comparison of ArH and benzyl CH₂ shifts with other compounds showed it to be the 8-(*p*-acetoxybenzyl) derivative (Table).

6,8-Bis(*p*-acetoxybenzyl)-3,3',4',5,7-penta-*O*-acetyl-(+)-catechin.—The third product isolated by preparative t.l.c. (R_F 0.56) was the amorphous, dibenzylated derivative (Found: C, 64.9; H, 5.2. $C_{43}H_{40}O_{15}$ requires C, 64.8; H, 5.0%); $[\alpha]_{578}^{20} + 13.2^\circ$ (c 1.97 in $CHCl_3$); m/e (175 °C) 796 (M^+ , 0.3%), 648 (5), 356 (42), 272 (37), and 149 (100); τ ($CDCl_3$) 2.70—3.15 (11 H, m, B-ring and 2 benzyl ArH, no A-ring ArH), 4.85—4.97 (2 H, m, 2-H and 3-H), 6.13—6.23 (4 H, m, benzyl CH₂), 7.00—7.20 (2 H, m, 4-H), 7.68 (12 H, s, catechin ArOAc), 7.83 (6 H, s, benzyl OAc), and 7.99 (3 H, s, aliphatic OAc).

Reaction of (+)-Catechin with *o*-Hydroxybenzyl Alcohol.—Catechin hydrate (2 g, 5.5 mmol) and *o*-hydroxybenzyl

alcohol (0.68 g, 5.5 mmol) were dissolved in dioxan-water (1 : 2; 25 ml) and the pH adjusted to 7.0 with a small amount of sodium hydroxide. The reaction mixture was then heated under reflux for 7 h. Three major products were observed, in addition to unchanged catechin and *o*-hydroxybenzyl alcohol, by t.l.c. [solvent system (A)]. These compounds were isolated by multiple separations on silica gel columns by elution with 15% methanol in chloroform, and purified further by reverse-phase h.p.l.c. (Lichrosorb RP-18) with methanol-water as eluant (30 : 70 v/v) flowing at 2.0 ml/min; the retention volume of 6-(*o*-hydroxybenzyl)-(+)-catechin was 60 ml and that of 8-(*o*-hydroxybenzyl)-(+)-catechin was 75.6 ml. The relative peak areas for the 6- and 8-substituted catechin derivatives were 1 : 2.6 (u.v. detector, 280 nm).

6-(*o*-Hydroxybenzyl)-(+)-catechin was isolated as an off-white amorphous powder, yield 6.2 mg (Found: C, 62.5; H, 5.2. $C_{22}H_{20}O_7 \cdot 1.5H_2O$ requires C, 62.4; H, 5.44%; $[\alpha]_{578} + 7.7^\circ$ (*c* 0.98 in MeOH); τ [(CD₃)₂CO] 2.56–3.30 (7 H, m, B-ring and benzyl ArH), 3.97 (1 H, s, 8-H), 5.43–5.53 (1 H, d, 2-H), 6.0–6.20 (1 H, m, 3-H), 6.11 (2 H, s, 6-benzyl CH₂), and 7.13–7.42 (2 H, m, 4-H).

8-(*o*-Hydroxybenzyl)-(+)-catechin was crystallized from trichloromethane-methanol as needles, m.p. 239–240 °C (Found: C, 65.7; H, 5.16. $C_{22}H_{20}O_7 \cdot 1/3H_2O$ requires C, 65.6; H, 5.14%; $[\alpha]_{578} - 18.4^\circ$ (*c* 0.32 in MeOH); τ [(CD₃)₂CO] 2.76–3.40 (7 H, m, B-ring and benzyl ArH), 3.90 (1 H, s, 6-H), 5.36–5.48 (1 H, d, 2-H), 5.95–6.28 (1 H, m, 3-H), 6.23 (2 H, s, 8-benzyl CH₂), and 7.05–7.35 (2 H, m, 4-H). When isolated by preparative h.p.l.c. it was recovered as an off-white amorphous solid (yield 20 mg).

6,8-Bis(*o*-methoxybenzyl)-3',4',5,7-tetra-*O*-methyl-(+)-catechin.—The first compound eluted from the silica-gel column had an R_F 0.50 [solvent system (A)]. After methylation (1 week in an excess of diazomethane), preparative t.l.c. gave an amorphous product with R_F 0.51 [solvent system (C)] (Found: C, 71.8; H, 6.5. $C_{35}H_{38}O_8$ requires C, 71.7; H, 6.5%; $[\alpha]_{578}^{20} - 28.3^\circ$ (*c* 0.82 in CHCl₃); m/e (175 °C) 586 (M^+ , 15%), 407 (29), 287 (19), 180 (29), and 121 (100); τ (CDCl₃) 2.8–3.4 (11 H, m, 2 benzyl and B-ring ArH, no A-ring ArH), 5.05–5.65 (2 H, m, 2-H and 3-H), 6.05–6.44 (22 H, m, 2 × benzyl CH₂ and ArOMe), 6.7–7.1 (2 H, m, 4-H), and 8.15 (1 H, br, OH).

6,8-Bis(*o*-acetoxybenzyl)-3',4',5,7-penta-*O*-acetyl-(+)-catechin.—The phenolic product with R_F 0.50 was also acetylated and further purified by t.l.c. [solvent system (B), R_F 0.60] to give an amorphous product (Found: C, 64.6; H, 5.1. $C_{43}H_{40}O_{15}$ requires C, 64.8; H, 5.0%; $[\alpha]_{578} - 5.7^\circ$ (*c* 2.03 in CHCl₃); m/e (220 °C) 796 (M^+ , 9%), 693 (27), 609 (43), 545 (54), 331 (25), 149 (68), and 107 (100); τ (CDCl₃) 2.70–3.20 (11 H, m, benzyl and B-ring ArH, no A-ring ArH), 4.85–5.05 (2 H, m, 2-H and 3-H), 6.20–6.55 (4 H, m, benzyl CH₂), 7.10–7.30 (2 H, m, 4-H), 7.73, 7.87, 7.95 (18 H, each s, ArOAc), and 8.05 (3 H, s, aliphatic OAc).

6-(*o*-Methoxybenzyl)-3',4',5,7-tetra-*O*-methyl-(+)-catechin.—A further compound obtained from the silica-gel column had an R_F value of 0.32 but was eluted very close to another major product which had an R_F value of 0.24; several column separations were therefore required to obtain pure fractions. Methylation of the former compound (R_F 0.32) gave a comparatively low yield of a product with R_F 0.4 [solvent system (C)] together with a series of lower R_F spots suggesting incomplete methylation. The partially methylated product was methylated further with dimethyl sulphate in acetone over potassium carbonate to obtain

higher yields of the product with R_F 0.45 (Found: C, 69.2; H, 6.5. $C_{27}H_{30}O_7$ requires C, 69.5; H, 6.4%; $[\alpha]_{578} + 7.7^\circ$ (*c* 1.1 in CHCl₃); m/e (175 °C) 466 (M^+ , 26%), 287 (91), 180 (30), 167 (17), 165 (17), 152 (17), 151 (30), and 121 (100); τ (CDCl₃) 2.8–3.3 (7 H, m, benzyl and B-ring ArH), 3.62 (1 H, s, A-ring ArH), 5.2–5.9 (2 H, m, 2-H and 3-H), 6.1, 6.32, 6.43 (11 H, 3 H, 3 H, each s, benzyl CH₂ and ArOMe) 6.85–7.22 (2 H, m, 4-H), and 8.22 (1 H, br, OH). The A-ring proton shift to τ 3.62 shows that this compound is the 6-substituted isomer.⁹

6-(*o*-Acetoxybenzyl)-3',4',5,7-penta-*O*-acetyl-(+)-catechin.—Acetylation of the phenol with R_F 0.32 followed by preparative t.l.c. [solvent system (B), R_F 0.62] gave the acetylated 6-substituted isomer as an amorphous product (Found: C, 62.8; H, 5.0. $C_{34}H_{32}O_{13}$ requires C, 63.0; H, 4.9%; $[\alpha]_{578}^{20} + 46.3^\circ$ (*c* 0.74 in CHCl₃); m/e (200 °C) 648 (M^+ , 1%), 588 (4), 546 (19), 504 (26), 462 (17), 355 (9), 152 (21), and 127 (100); τ (CDCl₃) 2.70–3.10 (7 H, m, B-ring and benzyl ArH), 3.32 (1 H, s, A-ring), 4.75–5.05 (2 H, m, 2-H and 3-H), 6.35 (2 H, s, benzyl CH₂), 7.20–7.40 (2 H, m, 4-H), 7.73, 7.78 (9 H, 6 H, both s, ArOAc), and 8.02 (3 H, s, aliphatic OAc).

8-(*o*-Methoxybenzyl)-3',4',5,7-tetra-*O*-methyl-(+)-catechin.—The compound with an R_F value of 0.23 [solvent system (A)] was also isolated by column chromatography and methylated for 1 week in an excess of diazomethane. T.l.c. [solvent system (C)] showed two major products at R_F 0.54 and 0.46. Preparative t.l.c. gave the compound with R_F 0.54 which crystallized from methanol, m.p. 204 °C (Found: C, 69.3; H, 6.6. $C_{27}H_{30}O_7$ requires C, 69.5; H, 6.4%; $[\alpha]_{578} - 51.7^\circ$ (*c* 1.47 in CHCl₃); m/e (175 °C) 466 (M^+ , 33%), 287 (79), 180 (38), 179 (88), 165 (25), 151 (38), and 121 (100); τ (CDCl₃) 3.07–3.40 (7 H, m, benzyl and B-ring ArH), 3.85 (1 H, s, A-ring ArH), 5.35–5.57 (2 H, m, 2-H and 3-H), 6.07–6.42 (17 H, m, benzyl CH₂ and ArOMe), 7.0–7.5 (2 H, m, 4-H), and 8.25 (1 H, br, OH). The A-ring proton shift to τ 3.85 shows that this compound is the 8-substituted isomer.⁹ The ¹H n.m.r. spectrum of the compound with R_F 0.46 [solvent system (C)] showed that it was an incompletely methylated derivative with one less methoxy-group than expected for the fully methylated derivative.

8-(*o*-Acetoxybenzyl)-3',4',5,7-penta-*O*-acetyl-(+)-catechin.—The phenolic product with R_F 0.23 [solvent system (A)] was acetylated and purified by preparative t.l.c. [solvent system (B), R_F 0.60] to give an amorphous product (Found: C, 62.6; H, 5.0. $C_{34}H_{32}O_{13}$ requires C, 63.0; H, 4.9%; $[\alpha]_{578} - 25.1^\circ$ (*c* 1.88 in CHCl₃); m/e (200 °C) 648 (M^+ , 4%), 545 (100), 503 (78), 462 (43), 420 (17), 356 (43), 314 (43), 152 (65), 151 (37), 123 (48), and 107 (35); τ (CDCl₃) 2.75–3.10 (7 H, m, benzyl and B-ring ArH), 3.38 (1 H, s, A-ring), 4.75–4.95 (2 H, m, 2-H and 3-H), 6.20 (2 H, s, benzyl CH₂), 7.10–7.30 (2 H, m, 4-H), 7.69, 7.71, 7.79 (15 H, each s, ArOAc), and 7.98 (3 H, s, aliphatic OAc).

6-*p*-Acetoxybenzyl-8-bromo-3',4',5,7-penta-*O*-acetyl-(+)-catechin.—8-Bromocatechin penta-*O*-acetate (250 mg) and *p*-hydroxybenzyl alcohol (75 mg) were dissolved in *p*-dioxan (20 ml) containing concentrated hydrochloric acid (0.25 ml). The reaction mixture was refluxed for 3 h, freeze-dried, and then acetylated (acetic anhydride-pyridine, 1 : 1) at room temperature for 5 h. The acetylation mixture was poured into water and extracted into dichloromethane. The solvent was evaporated off and the residue dried *in vacuo* for 48 h. Preparative t.l.c. [solvent system (B), developed twice] yielded a band with R_F 0.36 (35 mg)

that corresponded to the product (Found: C, 56.0; H, 4.3. $C_{34}H_{31}BrO_{13}$ requires C, 56.1; H, 4.26%); $[\alpha]_{D}^{25} -16.4^{\circ}$ (*c* 0.40 in $CHCl_3$); *m/e* (175 °C) 726, 728 (M^+ , $M^+ + 2$, 0.5%), 578, 580 (4), 349, 351 (19), 321, 323 (4), 217, 219 (7), and 149 (100); τ ($CDCl_3$) 2.78 (4 H, m, benzyl ArH), 2.95—3.03 (3 H, d, *v*-ring), 4.60—4.85 (2 H, m, 2-H and 3-H), 6.23 (2 H, m, benzyl CH_2), 7.10—7.40 (2 H, m, 4-H), 7.73, 7.90 (12 H, 3 H, each s, ArOAc), and 8.00 (3 H, s, aliphatic OAc). Bands corresponding to *p*-acetoxybenzyl acetate (R_F 0.92) and 8-bromocatechin penta-*O*-acetate (R_F 0.46) were also recovered.

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