

CONDENSED TANNINS: THE FORMATION OF A
DIARYLPROPANOL-CATECHINIC ACID DIMER FROM
BASE-CATALYZED REACTIONS OF (+)-CATECHIN

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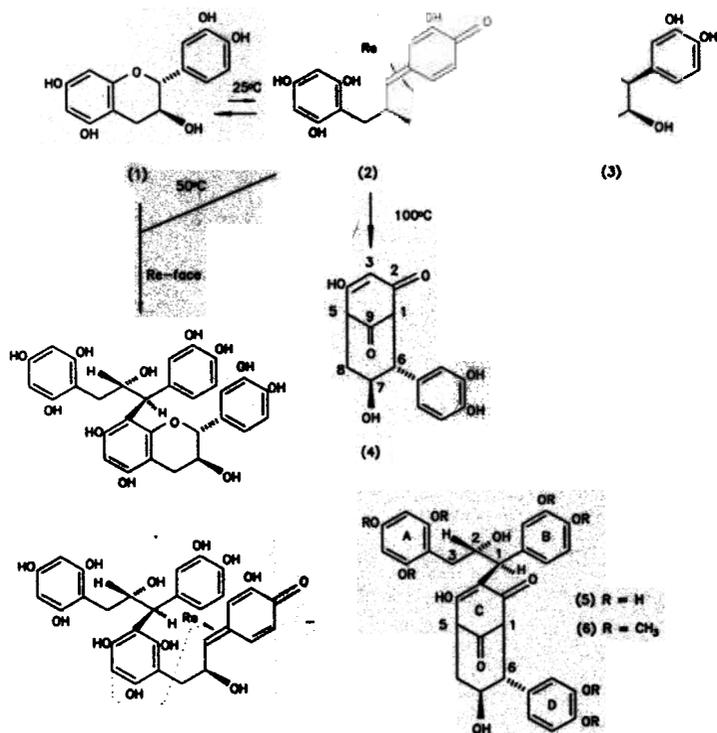
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ABSTRACT

Reaction of (+)-catechin at pH 12 and 40 °C results in the stereoselective (if not stereospecific) formation of an enolic form of 1-[6-(3',4'-dihydroxyphenyl)-7-hydroxybicyclo[3.3.1]nonane-2,4,9-trione-3-yl] -1-(3,4-dihydroxyphenyl)-3-(2,4,6-trihydroxyphenyl)-propan-2-ol. The n.m.r. chemical shift assignments determined by a variety of two-dimensional experiments permit a conclusion that the compound is one of four diastereoisomers possible from reaction of the two quinone methide intermediates. Stereoselectivity in the formation of this compound can be accounted for by preference for *Re*-face attack on both 2-C's of the quinone methides involved in the formation of the molecule.

INTRODUCTION

As part of a series of studies on base-catalyzed reactions of proanthocyanidins,¹⁻³ the reactions of (+)-catechin (1) at pH 12 were examined. In reactions at 25 °C, the principal reaction (Scheme 1) is opening of the pyran



Scheme 1

ring to give the quinone methide (2) and reclosure of the pyran ring to give (+)-catechin (*Re*-face) and (+)-epicatechin (*Si*-face) (3) with the former dominating in a ratio of about 3 to 1.^{4,5} When the same reaction is carried out at a temperature of 100°C , catechinic acid (4) is obtained in high yield.⁶ The absolute stereochemistry of the major isomer formed has been defined by x-ray crystallography⁶ and is consistent with stereoselectivity in the *Re*-face attack on the 2-C of the quinone methide in this rearrangement as well (Scheme 1).

At intermediate temperatures (40°C) a different compound is produced in about 20% yield together with catechinic acid and much smaller amounts of at least 4 other compounds. ¹³C n.m.r. spectra of the phenol as well as ¹H and ¹³C n.m.r. spectra of the methyl ether derivative (Figures 1 and 2) suggest that this compound is a dimeric product consisting of a 1-(3,4-dihydroxyphenyl)-3-

(2,4,6-trihydroxyphenyl)propan-2-ol substituted at C-1 with a catechinic acid moiety (5). A series of two-dimensional n.m.r. experiments was made on the methyl ether derivative (6) in an attempt to assign the ^1H - and ^{13}C n.m.r. spectra and particularly to determine if the product is one stereoisomer that would be consistent with a stereospecific or at least stereoselective *Re*-face attack on the quinone methide intermediates in both instances (Scheme 1). The yields of the unidentified minor products were small relative to that of (5) so, even if those compounds were found to be diastereoisomers, the reaction would have to be highly stereoselective.

In earlier work on the reactions of polymeric proanthocyanidins and (+)-catechin with toluene- α -thiol¹ at alkaline pH, 1-benzylthio-1-(3,4-dihydroxyphenyl)-3-(2,4,6-trihydroxyphenyl)propan-2-ol was obtained from the terminal (+)-catechin unit. ^1H and ^{13}C n.m.r. chemical shifts and coupling constants suggested that this compound was obtained as a mixture of two isomers but one isomer predominated, implying the 1R isomer (ie. *Re*-face attack). However, reaction of (+)-catechin with phloroglucinol under similar conditions² gave the corresponding 1-(3,4-dihydroxyphenyl)-1,3-bis-(2,4,6-trihydroxyphenyl)propan-2-ol which, after acetylation, gave a ^1H n.m.r. spectrum that suggested both 1R and 1S substitution in equal proportion because the propyl H-1 appeared to be two sets of doublets at 3.02 and 3.06 ppm (0.5 H each, both with $J_{1,2} = 5.0$ Hz). In contrast, Steynberg⁷⁻⁹ has demonstrated stereospecific (*Re*-face) attack of (+)-catechin on an analogous quinone methide intermediate generated in a flavanyl migration that occurs in base-catalyzed rearrangement of profisetinidins to phlobatannins. Steynberg's results cast doubt on the interpretation of the ^1H n.m.r. spectra made in earlier work² and emphasize the importance of establishing if the compound (5) is consistent with stereoselective *Re*-face attack of (+)-catechin on the quinone methide in the formation of the dimer as well as in the rearrangement of the flavanyl unit to catechinic acid (Scheme 1).

RESULTS AND DISCUSSION

The FAB mass spectrum of the phenol (5) showed $M + 1 = 581$ which was consistent with the proposed structure. In addition to ^1H (Figure 1) and ^{13}C (Figure 2) n.m.r. spectra of the methyl ether derivative (6), the following two-

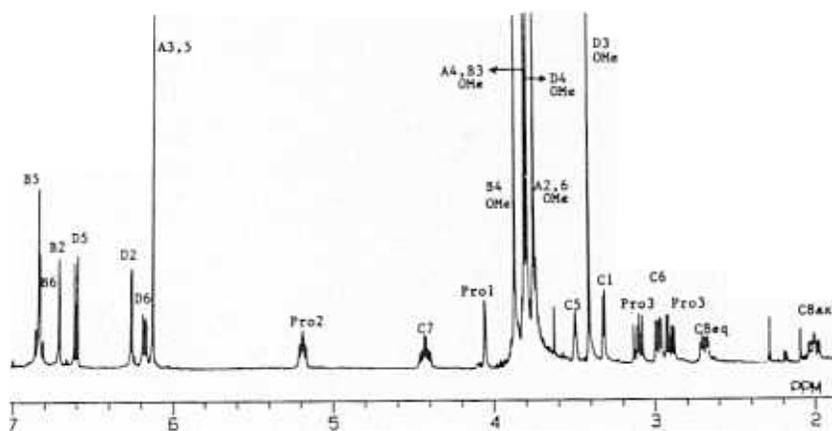


Figure 1. 400 MHz ¹H n.m.r. spectrum of (6).

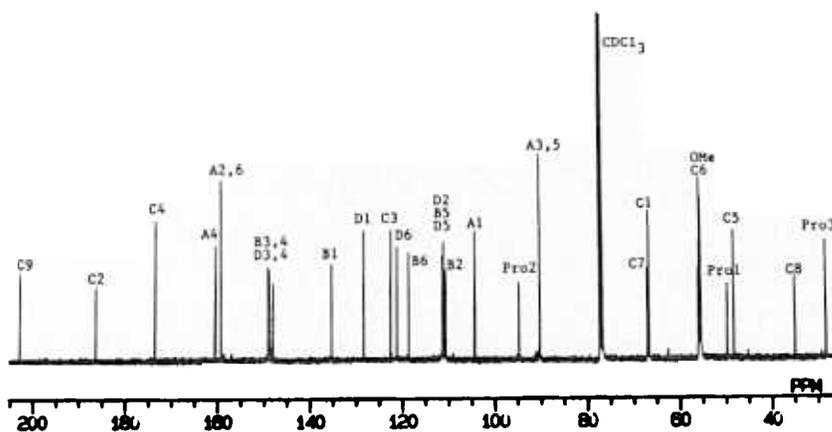


Figure 2. ¹³C n.m.r. spectrum of (6)

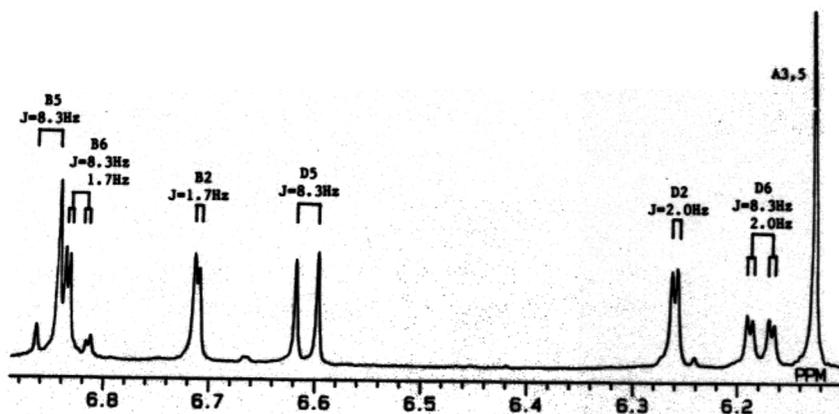
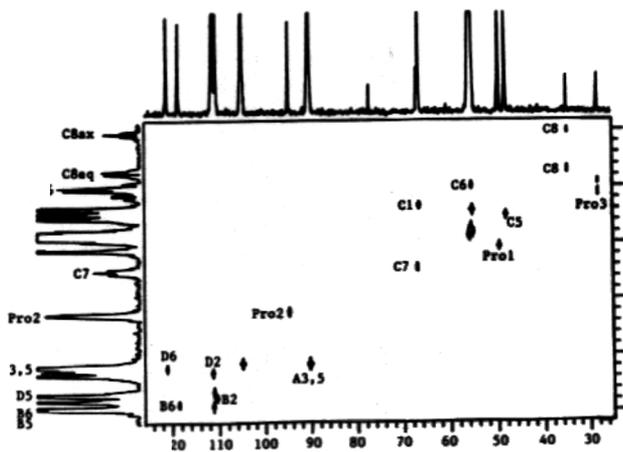


Figure 3. Expanded Aromatic Proton Region of ^1H n.m.r. Spectrum.

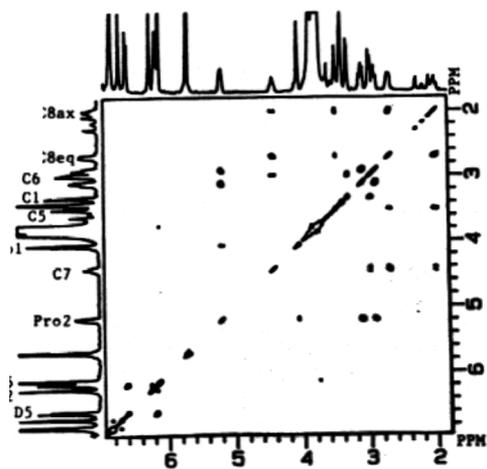
dimensional experiments were made to assist in assigning the spectra: a) ^1H - ^1H COSY, b) ^1H - ^1H NOESY, c) long-range ^1H - ^1H COSY, d) C-H HETCORR, and e) long-range C-H HETCORR as described in the Experimental Section. To simplify the discussion of the rationale used for these assignments, each ring system and the propyl chain will be discussed individually.

Phloroglucinol Ring A

The aromatic portion of the ^1H n.m.r. spectrum (Figure 3) showed a two proton singlet at 6.13 ppm that could be assigned to the H-3 and H-5 of the phloroglucinol ring (A) by a number of ways but, most obviously, by connectivity to the 2 carbon singlet at 90.27 ppm in the C-H HETCORR (Figure 4) experiment. Assignment of the methoxyl protons in this ring system was accomplished by consideration of the ^1H - ^1H COSY (Figure 5) and particularly the long-range ^1H - ^1H COSY (Figure 6) as well as the ^1H - ^1H NOESY (Figure 7) spectra, both of which clearly showed association of the C-2, C-4 and C-6 methoxyl signals at 3.75 and 3.81 ppm with the aromatic proton signal at 6.13 ppm. The signal at 159.24 ppm was assigned to C-2 and C-6 by connectivity to the H-3 of the propyl chain at 2.90 ppm in the long-range C-H HETCORR (Figure 8) experiment. Consequently, the signal at 3.75 ppm could be assigned to C-2 and C-6 methoxyl protons and at 3.81

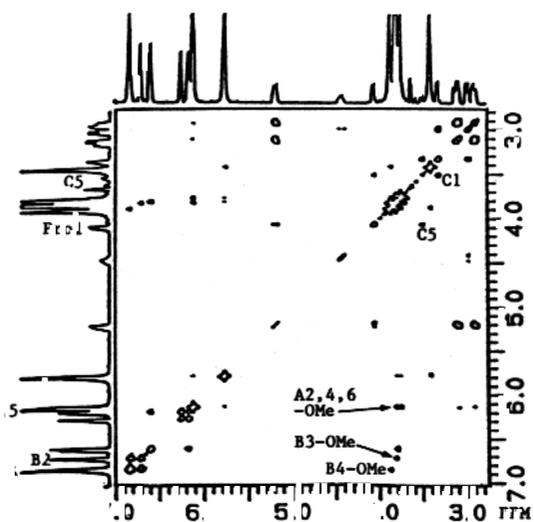


CO

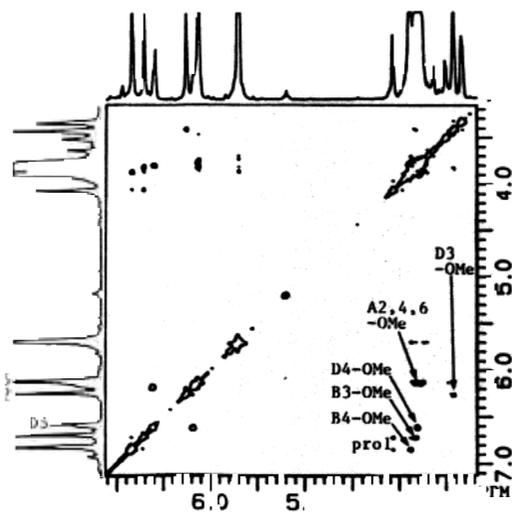


CO

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1



2

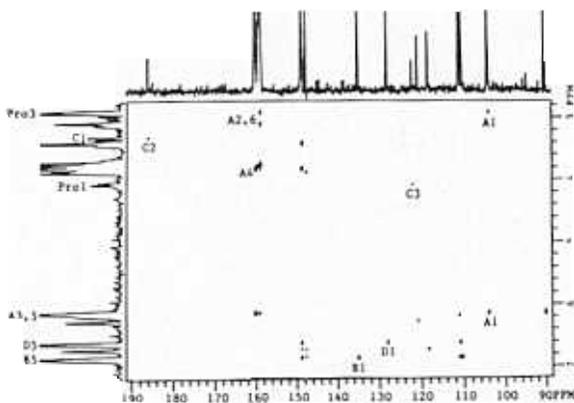


Figure 8. Long-Range C-H HETCORR Spectrum of (6)

ppm to the C-4 methoxyl protons by consideration of the long-range C-H HETCORR spectrum. This experiment also permitted assignment of the quaternary carbons through association of the aromatic ring protons at 6.13 ppm with the signals at 104.29 (C-1), 159.24 (C-2 and C-6) and 160.35 ppm (C-4).

Catechol Ring B

Two catechol rings are evident in the aromatic portion of the ^1H n.m.r. spectrum (Figure 3). Assignment of the signal at 6.71 ppm (d, $J = 1.7$ Hz) to H-2 because of the association of this proton with the H-1 of the propyl chain as seen in the ^1H - ^1H -NOESY spectrum (Figure 7). Accurate chemical shifts of H-6 and H-5 are 6.8537 and 6.8250 ppm, respectively. The distorted doublet ($J = 8.3$ Hz) at 6.83 ppm is, therefore, due to the H-5 of this ring. The difference of resonance due to H-6 and H-5 is calculated to be 11.48 Hz so it is reasonable for both protons not to form a tight coupling. The C-H HETCORR experiment (Figure 4) then permits assignment of the carbon signals at 110.54 (C-2), 111.21 (C-5), and 118.55 ppm (C-6) of ring B. The ^1H - ^1H -NOESY (Figure 7) and long-range ^1H - ^1H COSY (Figure 6) spectra both provided the evidence needed for assignment of the methoxyl protons. Resonance at 3.87 ppm is due to the protons of the methoxyl of C-4 and signals at about 3.80 ppm include the protons of the C-3 methoxyl. Assignment of the quaternary

C-1 was possible because of correlation between the H-5 doublet at 6.85 ppm and the resonance at 135.22 ppm shown in the long-range C-H HETCORR spectrum (Figure 8). The C-3 and C-4 carbons cannot be resolved from the closely spaced group of 4 carbon signals between 147.9 and 149.0 ppm.

Catechol Ring D

Assignment of the aromatic proton signals of the catechol ring attached to the nonatrione ring is straightforward after identification of those attached to ring B. These signals are shifted far upfield compared to those of the ring B with the doublet at 6.61 ppm with $J = 8.3$ Hz assigned to H-5, the doublet at 6.26 ppm with $J = 2.0$ Hz assigned to H-2, and the double doublet centered at 6.18 ppm assigned to H-6 (Figure 3). The C-H HETCORR experiment (Figure 4) then permits assignment of the carbon resonances as 110.10 (C-5), 111.33 (C-2), and 121.05 ppm (C-6) of ring D. The ^1H - ^1H NOESY (Figure 7) showed that the upfield methoxyl signal at 3.41 ppm is the methoxyl at C-3 and that the C-4 methoxyl is in the group of methoxyl signals at about 3.80 ppm. As in the assignment of the quaternary C-1 in ring B, consideration of the long-range C-H HETCORR spectrum (Figure 8) permitted assignment of the C-1 of ring D to the resonance at 128.30 ppm while the C-3 and C-4 signals are in the group of 4 carbons at 147.9 to 149.0 ppm.

Propyl Chain

The proton signals for the propyl chain are shown in expanded form in Figure 9. The 8.6 and 6.2 Hz coupling of the H-3 protons at 2.90 and 3.10 ppm to the H-2 proton at 5.20 ppm and the 3.2 Hz coupling of the H-1 at 4.06 ppm to the H-2 proton are all consistent with the proposed structure (6). However, the H-1 proton signal appears as a double doublet with 3.2 and 1.1 Hz coupling or perhaps as two doublets arising from an equal population of two isomers as was proposed in the analogous phloroglucinol adduct.² The long-range ^1H - ^1H COSY spectrum (Figure 6) solved this problem as three-bond coupling seen between H-1 of the propyl chain and the H-5 of the nonatrione ring system. The multiplicity seen in the H-1 proton signal observed in the corresponding phloroglucinol adduct could probably be explained similarly. Therefore, (6) is obtained as a single isomer and, on the basis of the results obtained by Steynberg,⁷⁻⁹ it is reasonable to assume that it is the *trans* isomer resulting

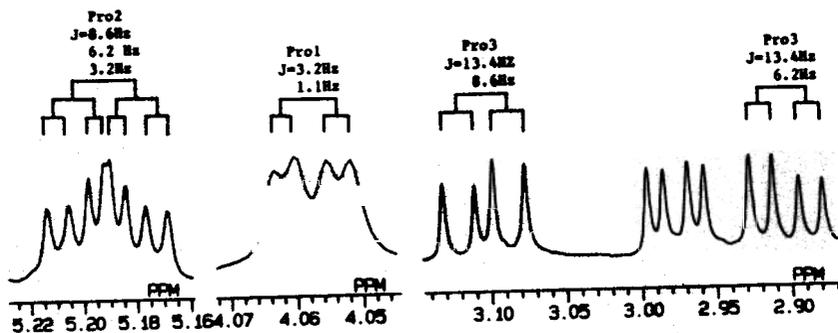


Figure 9. Expanded Proton Spectrum for the Propyl Chain.

from attack of catechin at the *Re*-face of the quinone methide (Scheme 1). Assignment of the carbon signals was then clear from the C-H HETCOR experiment with the C-1 at 49.63 ppm connected to the proton signal at 4.06, the C-2 shifted far downfield at 94.71 ppm clearly connected to the proton multiplet at 5.20 ppm, and the C-3 at 28.14 ppm connected to the pairs of double doublets at 2.90 and 3.10 ppm.

Nonatrione Ring C

The proton spectrum of trimethylcatechinic acid (*Re*-face attack) together with proof of its absolute stereochemistry has been reported earlier by Sears et al.⁶ In agreement with their results, the H-7 is split by one equatorial and two axial protons appearing as a triplet with $J = 11$ Hz that is split further at 5.5 Hz. The proton α to the catechol ring D (H-6) appears as a double doublet at 2.98 ppm with coupling constants of 10.7 and 4.4 Hz very similar to the coupling observed by Sears⁶ for trimethylcatechinic acid (Figure 10). The large $J_{6,7}$ coupling requires *trans* stereochemistry. In the spectrum of (6), H-1 appears as a double doublet with $J = 4.4$ Hz from coupling to H-6 and a small long-range coupling of 1.5 Hz due to connectivity to H-5 at 3.50 ppm as seen in the long-range ^1H - ^1H COSY (Figure 6) spectrum.

The rearrangement to the nonatrione ring system in (6), like the formation of catechinic acid, results from stereospecific attack at the *Re*-face of the quinone methide. If this rearrangement had occurred by attack at the *Si*-face,

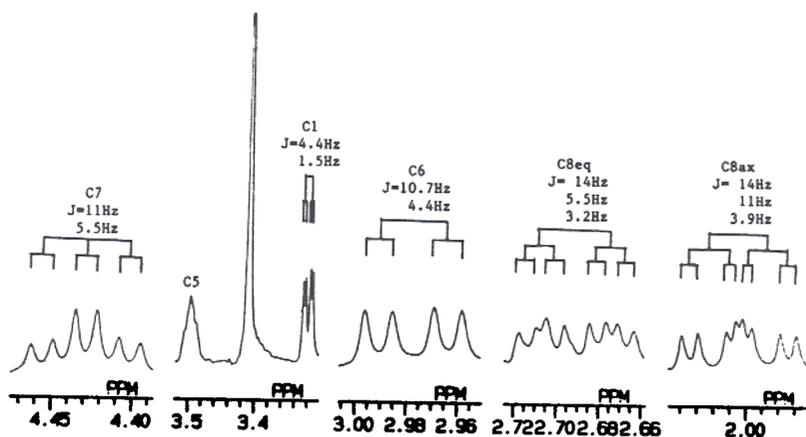


Figure 10. Expanded Proton Spectrum for the Nonatrione Ring C.

the coupling constants would be very different: in the conformer with a axial catechol ring, $J_{1,6} \sim 9$ and $J_{6,7} \sim 4$ Hz, or in the conformer with the D-ring equatorial and the 7-hydroxyl axial, $J_{1,6} \sim 4$ and $J_{6,7} \sim 4$ Hz.

The chemical shifts for the carbons in the nonatrione ring system of (6) differ substantially from those recorded for catechic acid in the phenolic form.² Here the C-H HETCORR experiment (Figure 4) was essential to making assignments. For example, the proton signal at 3.32 ppm assigned to H-1 was correlated with the carbon signal at 66.63 ppm and the multiplet that is clearly H-7 is correlated with the close signal at 67.03 ppm. The broad signal at 3.50 ppm assigned to H-5 is correlated with the carbon signal at 48.21 ppm, suggesting that the previous assignments for C-1 and C-5 of catechic acid² may need to be inverted. The H-6 proton α to the catechol ring centered at 2.98 ppm is correlated with the carbon signal at 55.45 ppm, and the connectivity of the H-8 equatorial protons with the carbon signal at 34.90 ppm is evident. This leaves the assignment of the two carbonyl carbons, the vinyl alcohol and the C-3 carbon. The long-range C-H HETCORR experiment (Figure 8) showed correlation between the H-1 proton signal at 3.32 ppm and the carbonyl signal at 186.34 ppm. In another long-range C-H HETCORR experiment, in which a value of J_{CH} of 15 Hz was used, correlation between H-5 at 3.50 ppm, H-8

at 2.0 ppm and the signal at 173.48 ppm was observed. Therefore, the signal at 186.34 ppm was assigned to C-2 and the signal at 173.48 ppm to C-4. The remaining carbonyl signal at 202.87 ppm must then be assigned to C-9. The signal at 122.43 ppm was assigned to C-3 by consideration of the long-range C-H HETCORR spectrum that showed correlation between the H-1 of the propyl chain at 4.06 ppm and the resonance at 122.43 ppm.

CONCLUSIONS

The stereochemistry of (5) is consistent with the *Re*-face attack of both the quinone methide intermediates involved in its formation from base-catalyzed reactions of (+)-catechin. Although other compounds are produced in low yield and have not yet been characterized, the structure of (5) is consistent with Steynberg's observation of stereospecific flavanyl migration in the formation of phlobatannins.

EXPERIMENTAL

The n.m.r. spectra were recorded on a JNM-GSX 400 spectrometer.* Acetone- d_6 - D_2O (1:1, v/v) was used as the solvent for the phenol (5) and chloroform- d_1 was used for the methyl ether derivative (6). FAB-mass spectra were obtained using a JEOL DX-303 spectrometer.

Separations of the reaction product were made with Sephadex LH-20 using 95% ethanol as the eluting solvent. Cellulose t.l.c. (Schleicher and Schull F1440), developed with A: t-butyl alcohol-acetic acid-water (3:1:1, v/v/v) and/or B: 6% acetic acid, was used to monitor separations. The compounds were visualized by spraying the plates with vanillin-HCl and heating the plates under a hair drier. The compound (5) was finally purified by h.p.l.c. on a JASCO TRI ROTAR-V chromatograph fitted with an Inertsil ODS-2 column eluted with MeOH- H_2O (1:4, v/v).

Reaction of (+)-Catechin at pH 12 and 40 °C

(+)-Catechin (1.0 g) was combined with 40 mL of water. The pH was adjusted to 12.0 by adding solid NaOH with constant stirring while the surface was continually flushed with a stream of N_2 . The resulting dark-red solution

*Use of trade names does not constitute endorsement by the U.S. Department of Agriculture.

was sealed in a 100 mL reaction vial under N_2 and kept at 40 °C in a thermostatically controlled water bath for 24 hours. After neutralization of the reaction solution by passing it through an Amberlite IR-120 resin (H^+ form, 25 g), the eluate was freeze-dried. This crude product (753 mg) was applied to a Sephadex LH-20 column (1.5 x 80 cm) eluted with 95% ethanol. Fractions 48-65 were combined and evaporated to give 139 mg of solid. This fraction was further purified by reverse phase h.p.l.c. to give compound (5).

Reaction of (+)-Catechin at pH 12 and 100 °C.

(+)-Catechin (1.0 g) was treated in an NaOH solution as described above except that the sample was heated at 100 °C for 45 minutes. Neutralization on an Amberlite column as described above gave a crude product (800 mg) that was applied to a Sephadex LH-20 column that was eluted with 95% ethanol. Fractions 1-17 were combined and evaporated to give 616 mg of one compound that was identical with authentic catechinic acid (4) on cellulose t.l.c. and by the ^{13}C n.m.r. spectrum.

Reaction of (+)-Catechin at pH 12 and 25 °C.

(+)-Catechin (1.0 g) was treated in NaOH solution at pH 12 and 25 °C in a sealed reaction vial for 67 hours using the same procedures as above to obtain a crude product (776 mg) after neutralization on an Amberlite column. Cellulose t.l.c. showed only two products that co-chromatographed with (+)-catechin and (-)-epicatechin.

Methylation of (5).

Compound (5) (120 mg), acetone (30 mL), dimethyl sulfate (0.5 mL), and K_2CO_3 (2.9 g) were refluxed for 17 hours. Two spots were detected on Si-gel t.l.c. (upper phase of benzene-ethanol-water-acetic acid; 200:47:15:1; v/v/v/v). The main product was collected by preparative t.l.c. to give 23 mg of (6).

NMR Pulse Sequences.

The 1H - 1H COSY and 1H - 1H NOESY spectra were recorded at the same time using the CONOESY experiment,^{10,11} in which a mixing time of 1025 ms was used. The long-range 1H - 1H COSY spectrum was recorded using a 45° mixing pulse and a delay time of 200 ms. The C-H HETCORR experiment¹² used a $J_{CH} = 140$ Hz. Two long-range C-H HETCORR experiments, one with $J_{CH} = 5.5$ and another with $J_{CH} = 15$ Hz were made.

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