(*p*-nitroanisole) was added. The samples and controls were analyzed by HPLC. Some **2** was always detected, but no more than was contained in the controls. A new product, however, was obtained. On the basis of its high-resolution mass spectrum, it was identified as 1-nitroso-4-hydroxy-2,3-dehydropiperidine (**6**): mass spectrum, *m/z* (relative intensity) 128 (M^+, 98), 111 (3), 98 (4), 81 (25), 80 (30), 71 (92), 63 (15), 53 (100); exact mass (*m/z* 128), 128.0566 ($C_5H_8O_2N_2$). This spectrum was virtually identical with that of authentic **6**, prepared by the method of Saavedra.

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Registry No. **1**, 70501-82-5; **2**, 81971-40-6; **3**, 74719-26-9; **4**, 81971-41-7; **5**, 81971-42-8; **6**, 71785-88-1; 3-hydroxypiperidine, 6859-99-0; 1-nitropiperid-3-yl nitrate, 81971-43-9.

Kinetics of Epimerization of (+)-Catechin and Its Rearrangement to Catechinic Acid[1]

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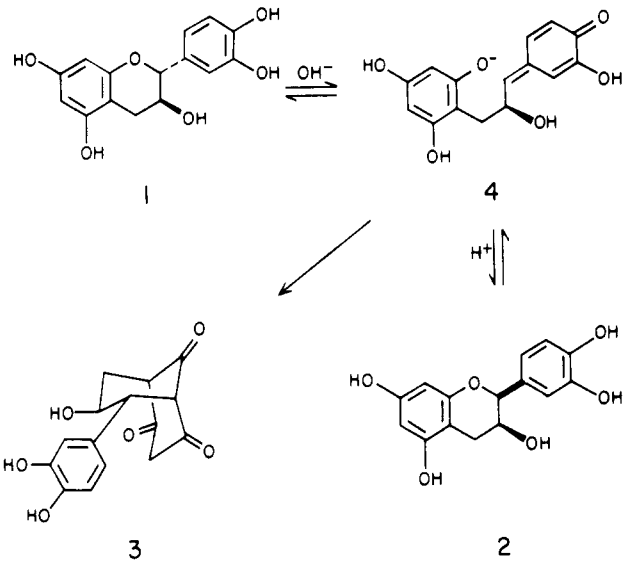
The rates of epimerization of (+)-catechin (**1**) to (+)-epicatechin (**2**) and of (−)-epicatechin to (−)-catechin in aqueous solution were measured over the pH range 5.4–11.0 and the temperature range 34–100 °C. The rate of conversion of (+)-catechin to catechinic acid (**3**) also was measured under these conditions. First-order kinetics were observed for all three processes. At low pH, k(epimerization) ≫ k(rearrangement), and epimerization approached an equilibrium in which (+)-catechin predominated over (+)-epicatechin. Near pH 11 and at elevated temperatures, k(epimerization) was only slightly greater than k(rearrangement), and the rapid, irreversible formation of catechinic acid under these conditions determined product composition. Both the epimerization of catechin and its rearrangement to catechinic acid can be rationalized in terms of a quinone methide intermediate (**4**).

Condensed tannins from conifer bark are polyflavanoids containing flavan-3-ol repeat units such as (+)-catechin [**1**; (2*R*,3*S*)-3,3′,4′,5,7-pentahydroxyflavan] and (−)-epicatechin [(2*R*,3*R*)-3,3′,4′,5,7-pentahydroxyflavan] linked between C-4 of one unit and either C-6 or C-8 of the next unit.[3] The possibility of using these subunits in polymers cross-linked with formaldehyde or methylolated phenol has been considered as a means of improving the adhesive properties of phenol–formaldehyde resins, but the results have been generally disappointing.[4]

A primary concern in the design of polymeric systems derived from catechins is the instability of these flavanoids toward epimerization and rearrangement. Epimerization of **1** in hot water or dilute caustic solution to (+)-epicatechin (**2**) is well-known,[5] and, more recently, it was shown by Sears et al.[6] that **1** undergoes rearrangement to catechinic acid (**3**) in hot alkaline solution. The quinone methide **4** suggested by Mehta and Whalley[7] is a logical intermediate in both processes (see Scheme I). Because these competitive reactions could potentially interfere with tannin extraction from plant material and with resin synthesis based on flavanoids, it was important to determine their relative rates over a range of pH and temperature. We report the results of kinetic studies (a) on the epimerization of **1** to **2** and of (−)-epicatechin to (−)-catechin and (b) on the rearrangement of **1** and **2** to **3**. These studies were conducted in aqueous solution over the pH range 5.4–11.0 and at temperatures from 34 to 100 °C.

Scheme I



Results

Catechin, epicatechin, and catechinic acid can readily be resolved by high-pressure liquid chromatography (HPLC); this permits an accurate assay by measurement of peak area. A preliminary survey with **1** and **2** revealed

(1) (a) Taken in part from the Ph.D. thesis of P.K., Oregon State University, 1980. (b) Paper 1575, Forest Research Laboratory, School of Forestry, Oregon State University.

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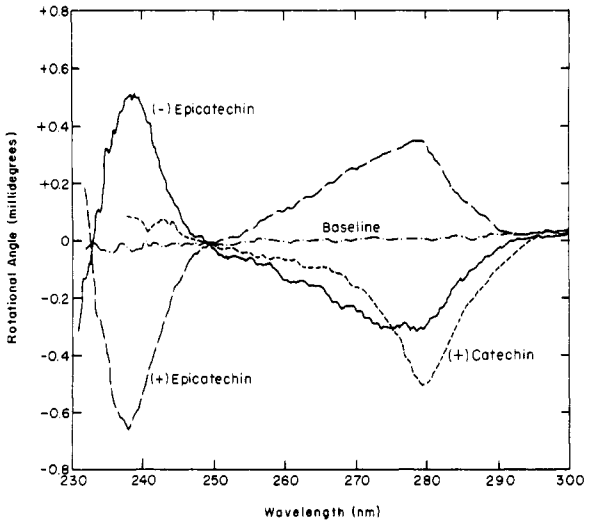

Figure 1. ORD-CD spectra of (+)-catechin, (+)-epicatechin, and (−)-epicatechin (in aqueous methanol).

Table I. Percentage of (+ or −)-Catechin in an Equilibrium Mixture with (+ or −)-Epicatechin

temp, °C	pH				
	11.0	10.0	9.0	8.0	5.4
34	65	78	77		
47	75	80	80	69	
57	76	84	81	82	
67				71	
77					43
87					54
94					62
100					75

that, at low pH, epimerization gave an equilibrium mixture in which catechin predominated over its epimer. Virtually no catechinic acid was present. At pH >8, rearrangement of 1 and 2 to 3 became significant, but epimerization was still ca. 10 times more rapid than rearrangement at pH 9 and ca. 5 times more rapid at pH 11. Thus, the kinetic parameters could be determined separately for epimerization and rearrangement.

Epimerization of (+)-Catechin and (−)-Epicatechin. In the pH range 9–11, solutions of 1 or (−)-epicatechin reached equilibrium with the corresponding epimers at room temperature in several hours or less. Birch et al.[8] showed that epimerization involved only a change in configuration at C-2 of the flavanoid nucleus (as required by the mechanistic postulate in Scheme I), and comparison of the circular dichroism spectra of 1 and 2 (which is antipodal to the spectrum of (−)-epicatechin) agrees with this (Figure 1). At pH 5.4, epimerization was much slower than at higher pH, and elevated temperatures were necessary to bring the mixture to equilibrium within 2 h. The percentage of (+ or −)-catechin in an equilibrium mixture with (+ or −)-epicatechin was measured under various conditions of pH and temperature (Table I). In general, higher temperatures favored an increased proportion of 1. Epimerization of 1 and (−)-epicatechin obeyed first-order kinetics, with correlation coefficients >0.95 for plots of log concentration vs. reaction time. First-order rate constants for the epimerization of 1 and (−)-epicatechin (Figure 2) varied with pH. Epimerization of (−)-epicatechin was 2–3 times more rapid than that of 1, and the activation energies calculated for epimerization ranged

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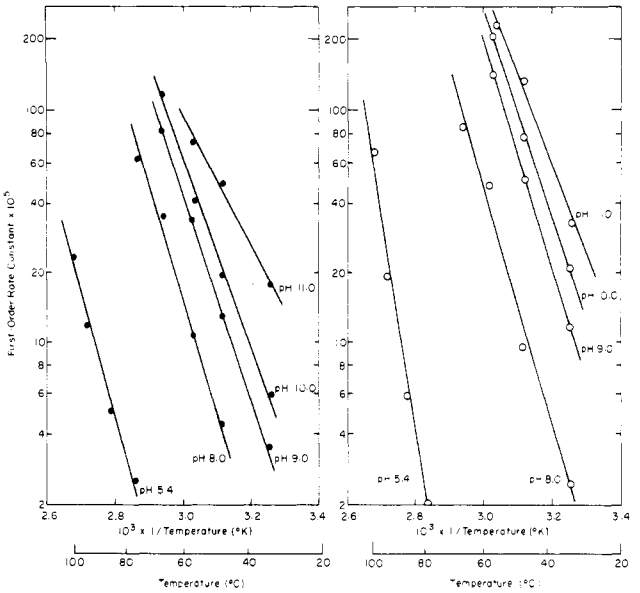

Figure 2. First-order rate constants for (+)-catechin (left) and (−)-epicatechin (right) epimerization.

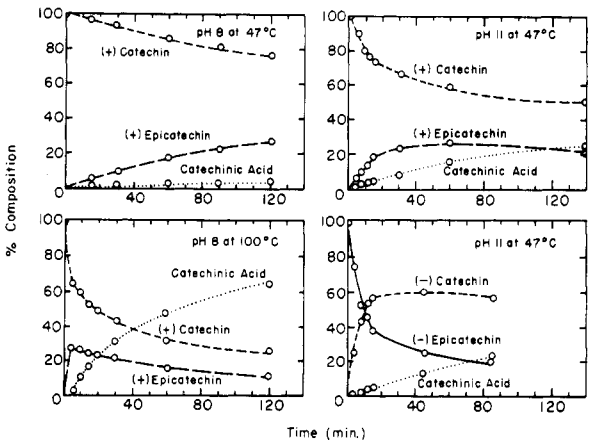

Figure 3. Composition of products from (+)-catechin and (−)-epicatechin rearrangement as a percentage of the mass of the starting material. (−)-Catechin was not verified but had the same HPLC elution volume as (+)-catechin.

from 3 to 6 kcal/mol, with the higher values corresponding to lower pH. The forward and backward rate constants for epimerization of 1 and (−)-epicatechin were also used to verify the measured equilibrium concentrations of these substances in a mixture with 2 and (−)-catechin, respectively.

Rearrangement of (+)-Catechin and (+)-Epicatechin to Catechinic Acid. Although neither 1 nor its epimer rearranged to 3 under acidic conditions even at 94 °C, the rearrangement became competitive with epimerization at pH >8. At pH 10 and greater, catechinic acid was formed rapidly and irreversibly from both catechin and epicatechin and was apparently a single stereoisomer (of undetermined configuration). As the reaction proceeded from 1 to 2 or 3, more than 95% of the starting material was accounted for by the liquid chromatography. The conversion to 3 ultimately went to >90% completion, which indicates negligible side reactions other than epimerization of catechin and epicatechin. Typically, the distribution of (+)-catechin, (+)-epicatechin, and catechinic acid with respect to time and as a function of pH and temperature was as shown in Figure 3.

As expected, the rate of appearance of 3 equaled the sum of the rates of disappearance of catechin and epicatechin,

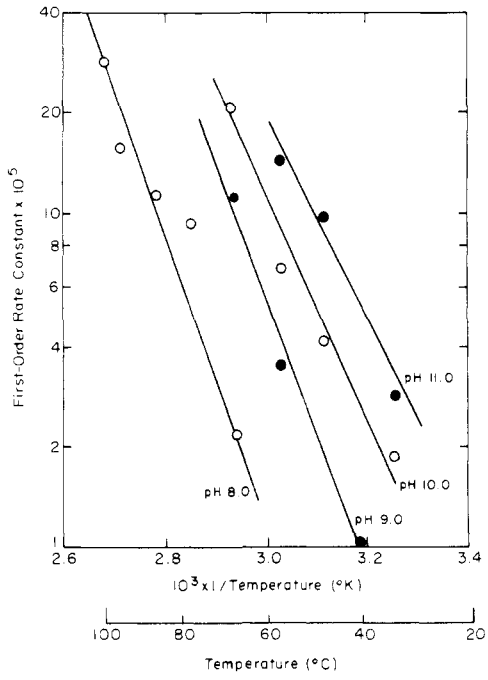

Figure 4. First-order rate constants of combined (+)-catechin/(+)-epicatechin disappearance.

and the reaction obeyed first-order kinetics with a correlation coefficient of >0.98. The combined first-order rate constant depended on temperature and pH (Figure 4), and the slopes of these plots were similar to those determined for the epimerization of (+)-catechin and (−)-epicatechin (Figure 2), which suggests that these processes have similar activation energies.

Discussion

Kinetic measurements of the epimerization of (+)-catechin and (−)-epicatechin and of the rearrangement of 1 to catechinic acid (3) suggest a common intermediate for both processes. This intermediate could be the proposed quinone methide 4[6,7] although no direct evidence was obtained by this study. Assuming 4 is that intermediate, our results indicate that under acidic conditions, closure of the quinone methide occurs principally at the phenolic oxygen to yield catechin epimers, whereas a basic medium leads to irreversible carbon alkylation on the phenolate ring and, hence, to 3. The relatively rapid formation of catechinic acid at temperatures near 100 °C and at pH >10 implies that catechin residues in tannin polymers similarly rearrange during conventional extraction in hot caustic solution. In a separate study, however, (+)-catechin condensed with formaldehyde much faster than it either epimerized or rearranged to catechinic acid.[9]

Experimental Section

Melting points were determined on a Fisher-Johns apparatus. HPLC was performed with a Waters Associates ALC/GPC 244 instrument by using a μ-Bondapak-CN column (4 mm × 300 mm, ~3000 theoretical plates) with 2-propanol–water–acetic acid (1.5/95.4/3.1 v/v/v) flowing at 1.0 mL/min. The eluent was monitored at 280 nm, the peak area was determined by using a Hewlett-Packard 3370B integrator, and the mass was calculated from response factors for known compounds.

IR spectra were recorded on a Beckman IR-20A spectrophotometer, NMR spectra were measured on a Varian FT-80A instrument, and CD spectra were obtained on a JASCO J-41A spectropolarimeter. Gas chromatography–mass spectrometry was conducted by using helium (30 mL/min) as the carrier on a Varian Aerograph 1200 gas chromatograph with a stainless steel column (3 mm × 152 cm), packed with Gas Chrom Q (80/100 mesh) coated with 3% OV-17 (injection port 245 °C, column 235 °C, detector 265 °C), coupled to a Varian MAT CH7 mass spectrometer.

Materials. (+)-Catechin (1, U.S. Biochemical Corp.) was recrystallized twice from distilled, deionized water and then freeze–dried and vacuum dried over P_2O_5 (1 torr) at 60 °C: mp 158 °C (lit.[10] sinters at 150 °C, melts at 176–177 °C); $[\alpha]^{22}_D$ +15.4° (acetone/H_2O, 1/1 v/v) (lit.[10] +16.0°); UV λ_{max} (H_2O/CH_3OH, 1/1 v/v) 279 nm (ε 3840).

Because the melting point of (+)-catechin did not agree with the published value, (+)-tetra-*O*-methylcatechin [(2*R*,3*S*)-3′,4′,5,7-tetra-*O*-methylflavan-3-ol] was synthesized. A solution of (+)-catechin in methanol was methylated three times with excess ethereal diazomethane at 5 °C for 36 h. The crystalline material obtained from ether upon evaporation was dried over P_2O_5 in vacuo (1 torr) at 60 °C: mp 144–146 °C (lit.[10] mp 143–144 °C); mass spectrum, *m/e* (relative intensity) 346 (30), 180 (30), 167 (100), 165 (8), 152 (19).

(+)-Epicatechin was obtained by epimerization of (+)-catechin. Thus, 0.5 g of (+)-catechin was dissolved in 50 mL of H_2O, the pH was adjusted to 7.0 with 0.1 N NaOH, and the mixture was refluxed for 45 min under a nitrogen atmosphere. The excess (+)-catechin was removed by crystallization at 5 °C. The supernatant liquid was subjected to HPLC, and a solution containing (+)-epicatechin was collected. (+)-Epicatechin (0.03 g) was isolated by freeze–drying the eluent and then vacuum drying over P_2O_5 (1 torr) at 60 °C; mp 240 °C dec. This sample was used for CD spectra and to verify that its IR spectrum was identical with that of (−)-epicatechin. (−)-Epicatechin (mp 240 °C) was purchased from Aldrich Chemical Co. Catechinic acid (3) was synthesized by the method of Sears et al.[6] Free catechinic acid was isolated by repeatedly dissolving the complex in acetone and precipitating it with ether. The precipitate was isolated by filtration and dried over P_2O_5 in vacuo (1 torr) at 60 °C to produce 3 as a white amorphous powder which charred at 200 °C. For characterization, 3 was converted to tetra-*O*-methylcatechinic acid by treated a solution of catechinic acid in CH_3OH with excess ethereal diazomethane at 5 °C for 36 h.[6]

Kinetic Measurement. A solution of 5.5 mg of (+)-catechin or (−)-epicatechin in 25 mL of distilled water was purged continually with N_2 and heated to the desired temperature in a 100-mL three-necked boiling flask in a water bath. A predetermined amount of 0.1 N NaOH was added at time zero to adjust the pH, and the reaction was allowed to proceed for 5 h. Samples (15 μL) of the reaction mixture were analyzed by HPLC at various reaction times. The elution times and response factors of (+)-catechin, (−)-epicatechin, and catechinic acid were determined by using the purified products prepared as described above.

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Registry No. 1, 154-23-4; 1 tetra-*O*-methyl, 51079-25-5; 2, 35323-91-2; 3, 52484-79-4; 3 tetra-*O*-methyl, 52358-33-5; (-)-catechin, 18829-70-4; (-)-epicatechin, 490-46-0.

Supplementary Material Available: Kinetic data from which Figures 2 and 4 were constructed (3 pages). Ordering information is given on any current masthead page.

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