Characterization of the Condensation Product of Malvidin 3,5-Diglucoside and Catechin

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Malvidin 3,5-diglucoside and (+)-catechin react in aqueous 10 mM HCl to form a colorless, C_{44}, bicyclic condensation product. Chromatographic, chemical, and NMR data support assignment of structure IV for this condensation product. The relevance of this condensation reaction to the phenolic polymerizations that occur in red wines during aging is discussed.

During the aging of red wines anthocyanin pigments undergo condensation reactions producing polymeric pigments. These polymers are responsible for the characteristic spectral changes observed in aged wines and are associated with reduced astringency (Singleton and Noble, 1976). Two main reaction pathways are considered to account for the formation of these polymeric pigments: (1) a direct condensation between the electrophilic pyrilium ring of anthocyanins and the aromatic ring of other phenolics such as catechin; (2) a Baeyer condensation involving addition of acetaldehyde to a flavanol followed by electrophilic substitution of this intermediate onto another flavanol or anthocyanin (Jurd, 1967). In the latter the resonance of the anthocyanin would not be directly affected by substitution. A complex alternating sequence of anthocyanin and flavanol could result in a highly pigmented polymer having spectral properties similar to those of monomeric anthocyanins. The direct condensation mechanism is complex but presumably must involve the formation of colorless flavenyl–flavanol intermediates that then are oxidized to xanthylium or pyrillium salts (Jurd, 1967, 1972; Jurd and Somers, 1970).

Timberlake and Bridle (1976) have studied these reactions in model systems and have presented evidence for the formation of acetaldehyde-bridged intermediates and xanthylium salts. They did not isolate a direct condensation dimer (a flavenyl glycoside—flavanol). A more complete understanding of the properties of acetaldehyde-bridged and direct-condensed polymers might make it possible to define the relative importance of these reactions in aging of red wines. The production and characterization of a bicyclic malvidin 3,5-diglucoside–catechin condensation product are reported in this paper.

MATERIALS AND METHODS

The equipment used for HPLC consisted of two Waters Associates (Milford, MA) Model 6000 A pumps and a Model 660 solvent programmer. The detector was a Perkin-Elmer (Norwalk, CT) LC 75 variable-wavelength detector. Peak areas and retention times were measured with a Spectra-Physics minigrator (Santa Clara, CA). Both the analytical and preparative columns were packed with reversed-phase (C_{18}) silica. The preparative column (9.4 mm i.d. × 25 cm) has been previously described (Wulf and Nagel, 1978). The 4.6 mm i.d. × 25 cm analytical column was purchased from Supelco, Inc. (Bellefonte, PA). The following conditions were used for HPLC separation of the compounds unless otherwise stated: Solvent A, 0.01 M trifluoroacetic acid (TFA) in water; solvent B, 0.01 M TFA and 40% (v/v) acetonitrile in water; flow rate, 2.0 mL/min; initial conditions, 34% solvent B for 4 min; gradient, 34% B–60% B in 10 min by the linear solvent program, curve 6 of the programmer; detector 520 or 280 nm, 0.02 AUFS; between injections the column was flushed with redistilled methanol.

Malvidin 3,5-diglucoside (M-3,5-G) was purified from Royalty grapes by extracting with ethanol (1:2 v/v), filtering, evaporating off the ethanol under vacuum, and separating the pigments on a Polyclar AT (GA Corp., New York, NY) column according to the procedure of Hrazdina (1970). Final purification was accomplished using preparative HPLC followed by removal of the acetonitrile from the M-3,5-G-containing fractions by rotory evaporation under reduced pressure. The resulting solution was then diluted into a large excess of 0.1 M aqueous HCl and the M-3,5-G recovered by lyophilization. This preparation of malvidin 3,5-diglucoside hydrochloride was found to be chromatographically pure by analytical HPLC monitored at both 280 and 520 nm. For preparation of large amounts of condensed product, commercially available M-3,5-G (Aldrich Chemical Co., Milwaukee, WI) was used. The (+)-catechin (Sigma Chemical Co., St. Louis, MO) was recrystalized 4 times from distilled H_2O after being decolorized with Darco KB carbon. The crystals were air-dried and stored under N_2 in a sealed desiccator over P_2O_5. The pure (+)-catechin thus obtained consisted of colorless needles having a $[\alpha]^{20}_D = +17.25$.

The reaction mixture used for the production of the direct condensation product consisted of 0.5 mM M-3,5-G and 2.0 mM (+)-catechin, in aqueous 10 mM HCl. The reaction mixture was purged with N_2, sealed in a 250-mL flask, and incubated for 7 days at 38 °C. When large amounts of condensation product were prepared, this protocol was correspondingly scaled up. The progress of the reaction was monitored by HPLC. Under these conditions the M-3,5-G–catechin compound (condensed dimer) had a retention time similar to that of M-3,5-G. Preparative HPLC was therefore performed using 13.6% acetonitrile in aqueous 0.05 M sodium acetate, pH 4.1. Under these conditions M-3,5-G was not retained and the condensed dimer could be isolated as a single peak. The fractions were pooled, the acetonitrile removed by rotary evaporation (30 °C), and the residual sodium acetate removed by preparative HPLC using 13.6% acetonitrile in aqueous 0.01 M TFA. Acetonitrile was removed as before and the condensed product recovered by lyophilization. The direct condensation product isolated by this procedure from reaction mixtures containing either the highly purified M-3,5-G or commercial M-3,5-G as the substitute were both found to be chromatographically pure by analytical HPLC and gave identical ^1H NMR.

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Table I. ^{1}H NMR[e] of (+)-Catechin (cat.), Malvidin 3,5-Diglucoside (M-3,5-G), and Condensation Product (CP)[f] of Aromatic Protons

		A rings				B rings			
		cat.		M-3,5-G		cat.			M-3,5-G
compd	solvent	6	8	6	8	2′	5′	6′	2′, 6′
cat.	$(CD_3)_2CO$	5.83[a]	5.99[a]			6.86[b]	6.77[b]	6.69[b]	
M-3,5-G	D_2O			6.49	6.62				7.18
	2 N DCl[g]			6.88	6.98				7.66
CP	$(CD_3)_2CO$[h]	6.04[c]		6.15	6.26	6.90[d]	6.79[d]	6.79[c]	7.09
	D_2O	6.09[c]		6.19	6.19	6.93[d]	6.82[d]	6.82[d]	6.91

[a] Part of an AB doublet centered at δ given. [b] Part of an ABC multiplet centered at δ given. [c] Exact assignment of this resonance to either the 6- or 8-position of the catechin portion of the molecule could not be made. [d] Part of an ABC multiplet with principal resonance given. [e] 200-MHz Fourier transformed spectra; chemical shifts relative to the acetone internal standard assigned to 2.04 ppm. [f] Reported as catechin and malvidin 3,5-diglucoside portions of dimer. [g] 2 N DCl in D_2O (used to avoid flavilium, quinoidal, and carbinol multiple equilibria present in D_2O). [h] A small amount of Me_2SO-d_6 added to enhance solubility.

Table II. ^{1}H NMR[a] of (+)-Catechin (cat.), Malvidin 3,5-Diglucoside (M-3,5-G), and the Condensation Product (CP)[b] Heterocyclic, Glucose, and Methoxy Protons

		(+)-catechin				malvidin 3,5-diglucoside				
compd	solvent	2	3	4S	4R	3[c]	4	anomeric CH's		$PhOCH_3$
cat.	$(CD_3)_2CO$	4.52 (d, J = 7.9)	3.94 (m)	2.85 (q, J_{AB} = 5.6, J_{AC} = 16)	2.48 (q, J_{AC} = 8.6, J_{BC} = 16)					
M-3,5-G	D_2O						8.40	5.14 (d, J = 7.6)	5.10 (d, J = 8.0)	3.60
	2 N DCl[d]						8.88[f]	5.39 (d, J = 7.3)	5.23[f] (d, J = 7.1)	3.81
CP	$(CD_3)_2CO$[e]	5.32 (d, J = 2.7)	4.18 (m)	2.58[g] (J = 15)	2.35[g] (J = 15)	5.12 (d, J = 3.5)	4.83 (d, J = 3.5)	5.07 (d, J = 7.0)	4.45 (d, J = 7.4)	3.87
	D_2O	[h]	4.18 (m)	2.75 (q, J_{AB} = 6.4, J_{BC} = 16)	2.39 (q, J_{AC} = 9.2, J_{BC} = 16)	4.98 (d, J = 2.9)	[h]	[h]	4.29 (d, J = 7.7)	3.72

[a] 200-MHz Fourier transformed spectra; chemical shifts relative to acetone internal standard assigned 2.04 ppm; coupling constants, J, in Hz. [b] Reported as catechin and malvidin 3,5-diglucoside portions of dimer. [c] Present in dimer only. [d] See footnote g, Table I. [e] See footnote h, Table I. [f] Part of a triplet centered at 5.12 ppm. [g] Appears as a broad doublet. [h] Completely or partially obscured by DHO resonance.

Ultraviolet and visible spectra were obtained by using a Beckman Model 35 scanning spectrophotometer (Beckman Instruments, Inc. Irvine, CA). The procedure of Pompei as described by Amerine and Ough (1980) was used for the procyanidin test.

The glucose content of the condensed dimer was determined by a modification of the glucose oxidase assay (Bergmeyer and Bernt, 1971). M-3,5-G was used as the control. The direct condensation product (1.055 mg) and M-3,5-G (1.174 mg) were dissolved in 2 M TFA in separate hydrolysis tubes, purged with N_2, and heated under vacuum for 2 h at 120 °C. The tubes were then cooled to room temperature and the hydrolysates diluted to 5 mL. Aliquots (1 mL) of each were passed over identical 4 mm × 3 cm PVP columns to remove the phenolic materials released during hydrolysis. The eluates plus approximately 5 mL of water wash were collected, evaporated to dryness, and dissolved in 0.05 mL of water, and each was analyzed for glucose.

^{1}H NMR spectra were recorded at 200 MHz with a Nicolet Model NT 200 wide-bore pulsed Fourier transform instrument (acquired with the assistance of the Boeing Co., Seattle, WA) (Nicolet Magnetics Corp., Fremont, CA) using a 2000-Hz sweep width. Samples (15 mg) were exchanged in D_2O (98 atom %), lyophilized, and then redissolved in 0.7 mL of 100 atom % acetone-d_6 D_2O, or 2 M DCl in D_2O in 5-mm tubes. Deuterated solvents were obtained from Aldrich Chemical Co. (Milwaukee, WI). A small amount of acetone was included as an internal standard by using the relationship $\delta(Me_4Si) = \delta(CH_3COCH_3) - 2.04$ ppm. ^{13}C NMR proton decoupled spectra were recorded at 22.5 mHz by using a JEOL FX 90 Q pulsed Fourier transform instrument (Cranford, NJ) using a 5000-Hz sweep width. Samples (30 mg) were dissolved in 0.5 mL of D_2O–CH_3CN in a 5-mm tube. Acetonitrile was used as the internal reference by the relationship $\delta(Me_4Si) = \delta(CH_3CN) - 0.5$ ppm.

RESULTS

Reaction mixtures containing M-3,5-G and catechin were observed to undergo a steady loss of color relative to that of the control solution of M-3,5-G alone. HPLC of the reaction mixture monitored at 520 nm indicated that this color loss paralleled the disappearance of M-3,5-G with no significant synthesis of other pigmented compounds (Figure 1B). However, a major newly synthesized compound was detected at 280 nm at a retention time similar to that of M-3,5-G (Figure 1A). This compound was absent in control solutions of catechin and M-3,5-G alone (Figure 1C). The reaction product was purified by preparative reversed-phase HPLC as a single symmetrical peak. Purity was confirmed by analytical HPLC and NMR spectroscopy.

The colorless reaction product gave an intense red color in hot butanol–HCl and likewise in hot dilute TFA in the absence of oxygen, a characteristic reaction of proanthocyanidins. This finding suggested that the compound resulted from the direct condensation between the electrophilic pyrylium ring of M-3,5-G and the A ring of (+)-catechin. Such a reaction scheme is depicted in Figure 2 though various possible positional and stereoisomers can also be envisioned. Similar types of condensation reactions have been proposed to account for the naturally occurring proanthocyanidins (Jacques et al., 1977) and have been demonstrated in model reaction systems (Jurd, 1967; Jurd and Waiss, 1965).

Table III. Chemical Shifts and Tentative Assignments[a] of the ^{13}C NMR Spectrum[b] of the Condensation Product (CP) As Compared to That of (+)-Catechin (cat.)

CP	cat.	assignment[c]	CP	cat.	assignment
155.7	155.3		100.1		4a, [4a]
154.4	155.3		100.0	100	
153.1	154.2	5, 7, 8a	98.5		6, 8
152.2		[5, 7, 8a]	98.4		
152.0			95.0	95.4, 94.7	[8 or 6]
150.3			80.4	80.7	[2]
147.0		3′, 5′	76.2		3 and the gluc C2's, C3's, and C5's
143.8	143.9	[3′, 4′]	75.2		
131.6		1′	72.6		
129.2	130.4	[1′]	71.6		
119.6		4′	71.4		
119.2	119.4	[6′]	69.2		gluc 4
116.8	115.5	[5′]	67.4	66.6	[3]
114.3	114.5	[2′]	60.9		gluc 6
109.7		2′6′	60.5		
104.8		8 or 6	56.2		$PhOCH_3$
102.7			26.5		4
102.4		2 and the gluc C1's	25.2	26.8	[4]
102.3					

[a] Tentative assignments based on comparisons with (+)-catechin, methyl β-D-glucoside, and substituent effects on ^{13}C chemical shifts (Maciel, 1974). [b] Proton-decoupled 22.5-MHz Fourier transformed spectra; chemical shifts relative to CH_3CN internal standard assigned 0.5 ppm. [c] Numbers in brackets refer to lower flavanol catechin portion of condensation product structure IV or V.

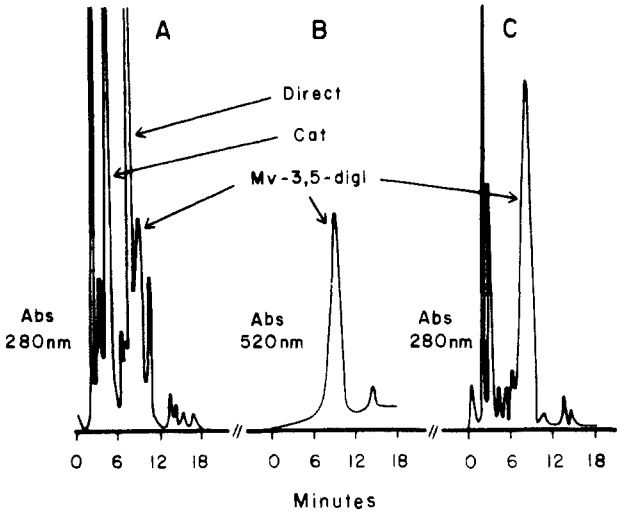


Figure 1. HPLC profiles at 280 nm of the reaction mixtures containing (A) 0.5 mM M-3,5-G, 2.0 mM catechin, and 10 mM HCl after 7 days at 38 °C, (B) the same as (A) except monitored at 520 nm, and (C) the same as (A) except catechin was excluded from the reaction mixture.

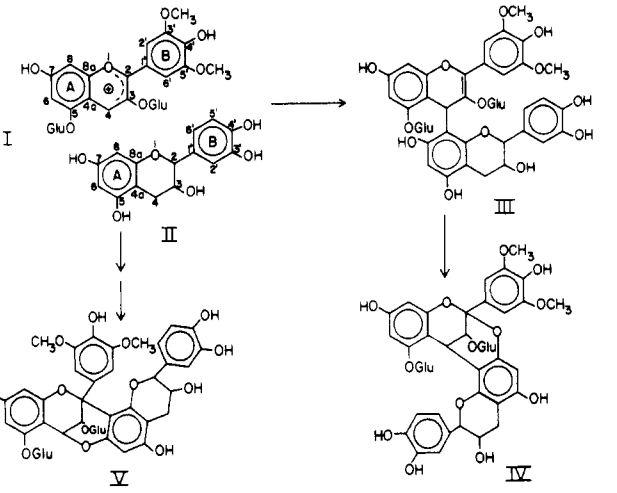


Figure 2. Proposed reaction pathways for production of dimer.

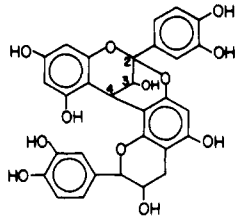
Proton NMR of this reaction product provided convincing evidence that structural elements of both M-3,5-G and (+)-catechin were present in the molecule (Tables I and II). All proton resonances present in the spectra were assigned except those associated with glucose C2 to C6 (generally these glucose protons are not sufficiently resolved by ^1H NMR methods). In addition, the integral values associated with the resonances were consistent with all the proposed assignments. A two proton aromatic singlet at 7.09 ppm and a six-proton methoxy resonance at 3.87 ppm indicate the presence of the symmetrical malvidin B ring. The aromatic ABC splitting pattern centered at 6.8 ppm is characteristic of the catechin B ring. Two nonidentical protons observed as two quartets in the D_2O spectrum centered at 2.75 and 2.39 ppm were assigned to the H-4 of the catechin portion of the molecule. These quartets were collapsed to two doublets by decoupling a multiplet at 4.18 ppm assigned to the H-3 of catechin. The H-2 of the catechin portion was identified in the acetone-d_6 spectrum as a doublet at 5.32 ppm (J = 2.7 Hz), which was also shown to be coupled to the catechin H-3 multiplet at 4.18 ppm (it may be noted that the H-4 protons appeared as two broad doublets in the acetone-d_6 spectrum but were noticeably sharpened in this decoupling experiment).

Hydrolysis of the condensation product in 2 N TFA for 2 h at 120 °C liberated 31.5% glucose. This result is in reasonable agreement with the theoretical 38.2% expected for diglucoside structures such as III, IV, and V. A stoichiometry of two β-linked glucoses per mole of condensation product was confirmed with the observation of two anomeric proton doublets at 5.07 ppm (J = 7.0 Hz) and 4.45 ppm (J = 7.4 Hz) in the acetone-d_6 ^1H NMR spectrum (Table II). These protons were also shown to be coupled to the broad nonanomeric glucose CH region (proton integral ca. 12 ± 1) at 3.0–3.8 ppm.

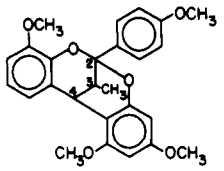
^{13}C NMR spectra (Table III) gave 37 separate resonances, 7 of which were degenerate: 3 owing to nuclear symmetries of the 2′- and 6′-, the 3′- and 5′, and the methoxy carbons in the B ring of M-3,5-G, 3 owing to symmetries of the 3-, 4-, and 5-carbons of the two glucose residues (Dorman and Roberts, 1970), and 1 due to the observed similarity in the chemical shifts of the 3′- and 4′-carbons in the B ring of catechin. Thus, the ^{13}C NMR data are consistent with 44 carbon structures such as III–V

(Figure 2) for our product. These data strongly support the contention that the major product isolated from the reaction mixture is a pure M-3,5-G–catechin adduct.

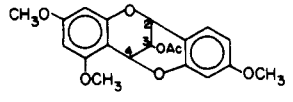
The nature of the linkage between the M-3,5-G and catechin portions of this dimer may be deduced in part from the following evidence: (1) The colorless condensation product had an UV spectrum (λ_{max} 275 nm; log ϵ 3.72 for M_r 944 in 1 M HCL) quite similar to (+)-catechin (λ_{max} 277 nm; log ϵ 3.61 in 1 M HCL). This also is consistent with the reaction scheme outlined in Figure 2 wherein the pyrilium resonance of M-3,5-G would be quenched upon condensation with catechin. (2) The proton NMR spectra of the condensation product gave three A-ring proton resonances (Table I), indicating that electrophilic substitution had occurred. It may be noted that one of these resonances at 6.15 ppm was observed to slowly exchange in the presence of added D_2O—a characteristic observed for catechin 6 and 8 aromatic protons. No coupling was observed between the A-ring protons either in the condensation product spectra or in the parent M-3,5-G spectrum. However, the spectrum of (+)-catechin gave a typical AB splitting pattern for the A-ring protons. Again this evidence is consistent with the scheme depicted in Figure 2. (3) Proton NMR spectra of M-3,5-G show a characteristic low-field singlet (8.8 ppm in 2 M DCl) that is assigned to the pyrylium H-4 (I). In the acetone-d_6 spectrum of the adduct, this resonance is conspicuously absent. However, a pair of doublets at 5.12 ppm (J = 3.5 Hz) and 4.83 ppm (J = 3.5 Hz) that were shown to be coupled by experimentation are most appropriately assigned to the H-3 and H-4 of the M-3,5-G portion of the adduct. Thus, structures IV and V in Figure 2 appear to be the most consistent with our experimental evidence. Structures similar to V although proposed (Clark-Lewis, 1968; Jurd and Waiss, 1965) have been rejected largely on the basis of ^{13}C NMR and proton NMR interpretations. Nonetheless, such a structure cannot be ignored, particularly in view of the relatively low field (5.12 ppm) doublet assigned to the bridgehead proton (H-4) of our product. For example, the bridgehead proton appears at 4.44 ppm in the spectrum of the proanthocyanidin A-2 (VI) obtained from horse chestnut (*Aesculus hippocastanum*; Jacques and Haslam, 1974) and as a 4.12-ppm doublet in the spectrum of the flavylium–phloroglucinol condensation product VII (Jurd and Waiss, 1965). However, the protons of cyanomaclurin VIII and its derivatives appear at 5.1–5.4 ppm (Nair and Venkataraman, 1963).



VI



VII



VIII

(4) ^{13}C NMR assignments of the pyran rings of the condensation product are compared with those of selected proanthocyanidins and flavanols in Table IV. In the condensed dimer spectrum the resonances of the C-4 of the M-3,5-G portion and the C-2 and C-4 of the catechin portion appeared well separated from the aromatic, methoxy, and glucose carbon resonances. Assignment of the C-3 resonances for both the M-3,5-G and the catechin portions of the adduct was made by comparisons with spectra obtained from D-glucose and methyl β-D-glucoside and published glycoside spectra (Dorman and Roberts, 1970). The C-2 of the M-3,5-G portion of the adduct was not accounted for in the 0–95-ppm region of the spectrum. The spectrum for our condensation product shows a clear similarity to that of the procyanidin A-2, again supporting an assignment involving a bicyclic structure such as IV.

Table IV. Comparison of ^{13}C NMR[a] Pyran Ring Chemical Shifts for the Condensation Product (CP), Procyanidins, and Flavanols

	pyran carbons[b]					
compd	2	[2]	3	[3]	4	[4]
CP[c]	95.0[g]	80.4	71.4–76.2[h]	67.4	26.5	25.2
A-2[d]	102.6	79.3	66.0	64.4	29.0	27.6
B-2[e]	76.9	79.3	72.9	66.4	36.9	29.6
B-3[e]	83.5	82.0	73.2	68.3	38.0	28.2
B-4[e]	83.6	79.5	73.2	66.9	38.0	29.7
(+)-catechin[f]	80.7		66.6		26.8	
(−)-epicatechin[f]	77.9		65.6		27.6	

[a] Proton-decoupled 22.5-MHz Fourier transformed spectra; chemical shifts relative to CH_3CN internal standard assigned 0.5 ppm. [b] Numbers in brackets refer to lower flavanol catechin portion. [c] In D_2O. [d] In Me_2SO-d_6 (Jacques et al., 1974); also see Weinges et al. (1973). [e] In acetone-d_6 (Fletcher et al., 1977). Similar values given by Hemingway et al. (1983). [f] In CH_3CN-D_2O (4:1). [g] The next resonance that appears downfield from the C2 of the catechin portion of the dimer; see also Table III. [h] The C2, C3, and C5 glucose resonances also appear in this region.

CONCLUSIONS

Our finding that M-3,5-G and (+)-catechin condense to form a bicyclic proanthocyanidin is suprising since bicyclic condensed tannins are rare in nature and none to our knowledge occur as glycosides. Even more interesting was our observation that only one major chromatographically and spectroscopically resolvable isomer was found under these reaction conditions—the yield being 29% of theoretical. Considering the many possible M-3,5-G–catechin condensation products, it appears that a highly stereospecific condensation reaction occurs. Glycosidation at C-5 of the malvidin A ring appears to be important in this condensation reaction since we were unable to demonstrate malvidin 3-glucoside condensation with (+)-catechin under similar conditions or even at elevated pH (5.25). Glycosylation of malvidin may affect its stereospecificity and its activity as an electrophile.

Higher order oligomeric and polymeric reaction products were not observed under our reaction conditions even after several weeks of incubation at 38 °C. Pure solutions of the condensation product (under N_2) were quite stable. No significant formation of pigment was observed in the presence of 0.25 M H_2O_2 after 2 weeks at room temperature. These observations, taken together with the failure of the malvidin 3-glucoside to react with (4)-catechin, suggest that the formation of directly condensed polymers is not a likely explanation of phenolic polymerizations in the aging of red wines and is certainly not directly responsible for the enhancement of color. It appears that acetaldehyde bridging of the anythocyanins and catechins is the major reaction occurring in aging of red wines (Ribereau-Gayon et al., 1983; Wildenradt and Singleton, 1974). Characterization of the acetaldehyde-bridged di-

mers of M-3,5-G and M-3-G will be reported in a separate publication.

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Registry No. IV, 91158-62-2; malvidin 3,5-diglucoside, 16727-30-3; (+)-catechin, 154-23-4.

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Effect of Debittering Treatment on the Composition and Protein Components of Lupin Seed (*Lupinus termis*) Flour

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In the traditional Egyptian practice, lupin seed is debittered by steeping in water overnight followed by boiling for 20 min, soaking in water for 4 days, and addition of NaCl. About 15% of total solids are leached out in this method. The protein, available lysine, and ash content increased due to debittering, but the sugar and tannin content decreased. The lupin seed or debittered lupin seed flour did not show trypsin and α-amylase inhibitor activity and hemagglutinin activity. Lupin seed flour showed caseinolytic activity, but the debittered flour did not have this activity. The nitrogen solubility of the debittered flour was lower in water and 1 M NaCl solution (12.6%) but nearly the same in 0.02 M NaOH (92.6%). The in vitro digestibility of debittered lupin seed proteins with pepsin/pancreatin was slightly higher. Debittering treatment denatured lupin seed proteins and also dissociated the high molecular weight protein component.

In spite of its high (36%) protein content, lupin seed (*Lupinus termis*) is not fully utilized in the Egyptian diets unlike faba bean (*Vicia faba*) or other legumes. This limitation is mainly due to the presence of alkaloids and bitter substances (Mogghaddam et al., 1976). Lupin seed is debittered by a process of prolonged steeping, boiling, and salting, for edible use to a limited extent.

There is considerable information available on the chemical composition and protein quality (Hove, 1974, Ballester et al., 1980), nutritive value (Mogghaddam et al., 1976), factors affecting protein solubility (Ruiz and Hove, 1976), and functional properties of several different species of lupinus (Sosulski et al., 1978; Malgarini and Hudson, 1980; Sathe et al., 1982). Recently, Morad et al. (1980) have reported that the natural amber color of lupin (*L. termis*) gives a desirable color to macaroni when blended with wheat flours at 2–6% levels. However, the changes in the chemical and physicochemical properties of the proteins that may occur due to the debittering treatment have not been studied, to the best of our knowledge. The method used in Egypt to remove the bitter substances and alkaloids from lupin seed was used in this study, and its effect on the chemical composition of the flour and the physicochemical properties of the proteins has been determined.

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EXPERIMENTAL SECTION

Materials. Lupin seed, *L. termis*, of the variety Giza-I was obtained from the Plant Breeding Department, Min-

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