Formation of Pyrrolidines and Piperidines on Heating L-Proline with Reducing Sugars

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In a series of model experiments L-proline was heated with monosaccharides for 1.5 h at 150 °C as well as pyrrolidine with D-glucose at 100 °C. Nineteen pyrrolidines and four piperidines were characterized and quantified by capillary GC–MS and nitrogen selective detector. Individual components were isolated by preparative GC and investigated by MS, IR, and ^{1}H NMR spectroscopy. 3-(1-Pyrrolidinyl)-2-butanone, 1-furfurylpyrrolidine, 2-acetyl-3-(1-pyrrolidinyl)furan, 3-(1-pyrrolidinyl)tetrahydro-2-furanone, 2-hydroxy-1-(1-pyrrolidinyl)-1-buten-3-one, 2-acetyl-, 2-propionyl-, 2-(2-furyl)-, and 2-(5-methyl-2-furyl)piperidine were identified for the first time as proline specific Maillard products.

INTRODUCTION

Among the reactions of amino acids with reducing sugars L-proline plays an outstanding role. During this Maillard reaction more than 120 proline specific compounds are formed depending on the reducing sugars and the reaction conditions. Some of the components possess bready aromas and extremely bitter taste qualities. Among the components we characterized pyrrolidines, pyrrolines, piperidines, tetrahydropyridines (pyridines), 2,3-dihydro-1*H*-pyrrolizines, 5,6-dihydroindolizines, di- and tetrahydro-1*H*-azepines, and 1,3-oxazines by MS, IR, and ^{1}H and ^{13}C NMR spectroscopy. Glucose, maltose, and lactose form maltoxazine (8-oxo-1,2,3,3a,5,6,7,8-octahydrocyclopenta(*d*)pyrrolo(2,1-*b*)-2.3-oxazine) on heating with proline (Tressl et al., 1982) which was also characterized in barley malt and beer (Tressl et al., 1981a,b). Pabst et al. (1984) characterized 5-hydroxy-5-methyl-2,3-bis(1-pyrrolidinyl)-2-cyclopenten-1-one on roasting saccharose with L-proline which possessed extremely bitter taste qualities. Mills and Hodge (1976) and Doornbos et al. (1981) identified pyrrolidines by the thermal degradation of 1-deoxy-1-L-prolino-D-fructose and 1,6-dideoxy-1-L-prolino-L-arabino-hexulose, respectively.

EXPERIMENTAL SECTION

L-Proline and sugars were high-grade commercial materials. Solvents for extraction and column chromatography were freshly distilled before use. Infrared spectra were obtained from $CDCl_3$ or CCl_4 liquid film samples with a Perkin Elmer Model 357 instrument. ^{1}H NMR spectra were taken in $CDCl_3$ solution by using a Bruker WH 270 instrument operating at 270 MHz and employing tetramethylsilane (Me_4Si) as internal reference standard. Chemical shifts are reported in ppm downfield from Me_4Si, and coupling constants J in hertz.

Sample Preparation. The reaction mixture of equimolar amounts of L-proline and reducing sugars (0.017–0.058 mol) dissolved in 50 mL water were autoclaved for 1.5 h at 150 °C in a stainless steel laboratory autoclave (Roth, I series) equipped with a 100-mL duran glass tube and heated by an electric heater with a magnetic stirrer. After the mixtures were cooled to room temperature, the pH was adjusted to 10 with 0.1 N NaOH and the solution extracted 3 times with 50 mL of diethyl ether. The combined ether extracts were dried over anhydrous sodium sulfate and concentrated to 1 mL on a 20-cm Vigreux column.

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Reaction of D-Glucose and Pyrrolidine. An equimolar mixture of 5 g (0.028 mol) of D-glucose and 1.98 g (0.028 mol) of pyrrolidine in 10 mL of ethanol was refluxed for 1.5 h. After the mixture was cooled to room temperature, the solvent was removed at reduced pressure and the dark brown residue was dissolved in 5 mL of ether.

Reaction of Maltol (3-Hydroxy-2-methyl-4-(4*H*)-pyranone) and Pyrrolidine. Maltol (10 g, 0.08 mol) and 5.65 g (0.08 mol) of pyrrolidine in 100 mL of water were refluxed for 15 min and cooled to room temperature. The mixture was extracted four times with ether and the combined extracts were dried over Na_2SO_4 and concentrated to 10 mL on a 20-cm Vigreux column.

Column Chromatography. Aliquot portions of the ether extracts were fractionated on 8 g of Al_2O_3 90 basic (Merck, deactivated with 10% water, 0.063–0.200 mm) in a 20-cm water cooled glass column with 1 cm i.d. The column was eluated successively with pentane–methylene chloride 9:1 (F1), pentane–methylene chloride 3:1 (F2), pentane–ether 9:1 (F3), pentane–ether 1:1 (F4), and ether (F5) in 40-mL portions. The eluates were concentrated to 1 mL on a Vigreux column.

Gas Chromatography (GC)–Mass Spectrometry (MS). The basic Al_2O_3 fractions were analyzed qualitatively and quantitatively by gas chromatography–mass spectrometry (GC–MS) by using a 25 m × 0.32 mm i.d. glass capillary column coated with Carbowax 20M + KOH (deactivated for basic compounds) coupled with a Finnigan MAT 4500 quadrupole instrument. The same capillary column was used on a Carlo Erba fractovap 2900 gas chromatograph equipped with a nitrogen selective detector (NSD) for detecting the nitrogen containing reaction products. In both cases the column temperature was elevated from 70 to 180 °C at a rate of 2 °C/min and kept at 180 °C. Higher boiling and more polar compounds were separated on a 50-m 0.32 i.d. glass capillary column coated with CP SIL 5 CB (Chrompack) coupled with a Varian MAT CH5 instrument by using on-column injection. The column temperature was programmed from 100 to 260 °C at a rate of 4 °C/min and kept at 260 °C. Mass spectra were recorded at an ionization energy of 70 eV and reported in m/e with relative intensities in brackets.

Preparative Gas Chromatography. For the isolation of the pyrrolidines from Al_2O_3 fractions preparative GC was used. On a Varian Aerograph 2700 equipped with a 3 m × 0.25 in. glass column the pyrrolidines were separated on either 15% Carbowax 20M on 80–90 mesh Chromosorb WAW/DMCS or on 5% SP 2401 DB on 100–120 mesh Supelcoport. Nitrogen was used and a glass split to a ratio of 1:10 (FID, split outlet). The column temperature was elevated from 60 to 230 °C at a rate of 4 °C/min and kept

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Table I. Mass Spectra and ^1H NMR Spectroscopic Data of Pyrrolidines and Piperidines, Characterized in Proline/Sugar Model Experiments

component	I_K CW 20M	mass spectral data, m/e (relative intensity)
(1) 1-pyrroline[b]		41 (100), 69 (95), 42 (47), 68 (45), 44 (15), 54 (6)
(2) pyrrolidine[b]	1100	43 (100), 28 (52), 70 (33), 71 (26), 42 (23), 41 (21), 39 (16), 68 (6)
(3) 1-formylpyrrolidine[b]	1691	99 (100), 71 (61), 43 (51), 42 (49), 70 (27), 98 (16), 56 (17), 68 (6)
(4) 1-acetylpyrrolidine[b]	1766	43 (100), 70 (92), 113 (71), 85 (21), 42 (19), 71 (9), 55 (8), 56 (7), 68 (6)
(5) 1-(1-pyrrolidinyl)-2-propanone[c]	1410	84 (100), 42 (31), 55 (18), 43 (7), 56 (6), 85 (6), 41 (4), 54 (3), 127 (2), 82 (2)
(6) 1-(1-pyrrolidinyl)-2-butanone[c]	1481	84 (100), 42 (17), 55 (11), 95 (8), 55 (5), 110 (3), 56 (3), 41 (3), 57 (1), 141 (1)
(7) 3-(1-pyrrolidinyl)-2-butanone[a]	1420	98 (100), 56 (23), 55 (8), 43 (7), 99 (7), 42 (6), 69 (5), 70 (3), 141
(8) 2-(1-pyrrolidinyl)-3-pentanone[a]	1490	98 (100), 84 (29), 56 (14), 42 (9), 55 (7), 99 (4), 70 (2), 57 (2), 155 (1)
(9) 3-(1-pyrrolidinyl)-2-pentanone[a]	1477	112 (100), 70 (11), 43 (8), 55 (7), 41 (6), 42 (6), 96 (4), 97 (3), 54 (3), 155 (1)
(10) 1-furfurylpyrrolidine[a]	1522	81 (100), 151 (34), 150 (27), 53 (21), 42 (17), 70 (12), 108 (10), 82 (7)
(11) 1-(5-methylfurfuryl)pyrrolidine[b]	1600	95 (100), 165 (20), 164 (9), 43 (8), 96 (8), 70 (7), 122 (6), 83 (6)
(12) 1-(5-(hydroxymethyl)furfuryl)pyrrolidine[b]	2375	111 (100), 83 (48), 181 (46), 150 (42), 180 (28), 70 (21), 84 (20), 42 (19), 81 (14)
(13) 2,5-dimethyl-4-(1-pyrrolidinyl)-3(2*H*)-furanone[a]	1842	181 (100), 124 (84), 43 (76), 110 (69), 138 (49), 55 (47), 82 (30), 54 (23), 70 (19), 166 (17)
(14) 2-acetylpiperidine[a]	1571	84 (100), 56 (35), 43 (17)8 42 (12), 55 (12), 41 (9), 127
(15) 2-propionylpiperidine[a]	1638	84 (100), 56 (22), 55 (5), 43 (3), 57 (4), 30 (1), 141
(16) 2-(2-furyl)piperidine[a]	1660	122 (100), 95 (89), 94 (81), 151 (47), 150 (25), 123 (25), 81 (25), 109 (23), 80 (17), 108 (16), 67 (14), 65 (12), 53 (11), 77 (10)
(17) 2-(5-methyl-2-furyl)piperidine[a]		109 (100), 122 (61), 136 (60), 165 (52), 108 (57), 150 (30), 95 (25), 164 (23), 43 (21), 94 (20), 123 (20), 148 (18), 107 (17), 53 (14), 81 (13)

IR and NMR Data

10: ^1H NMR (270 MHz, $CDCl_3$) δ 1.79 (mc, 4 H, β-CH_2), 2.56 (mc, 4 H, α-CH_2), 3.64 (br, 2 H, NCH_2), 6.20 (ddt, 1 H, J = 3.25, 1.0, 0.9 Hz, furan-H3), 6.32 (dd, J = 3.25, 2.0 Hz, furan-H4), 7.38 (dd, 1 H, J = 2, 0, 1.0 Hz, furan-H5)

13: ^1H NMR (270 MHz, $CDCl_3$) δ 1.35 (d, 3 H, J = 7.5 Hz, $CHCH_3$), 1.80 (mc, 4 H, β-CH_2), 2.17 (s, 3 H, CH_3), 3.06 (mc, 4 H, α-CH_2), 4.25 (q, 1 H, J = 7.5 Hz, $CHCH_3$; IR cm^{-1} 2970 m, 2930 w, 2870 w, 1695 s, 1610 s, 1410 w, 1330 s, 1300 m, 1260 s, 1155 m, 1020 m

16: ^1H NMR (270 MHz, $CDCl_3$) δ 1.50, 1.62 (each mc, 2 H), 1.90 (m, 3 H), 2.78 (mc, 1 H, H-6), 3.15 (mc, 1 H, H-6), 3.76 (br, d, 1 H, J = 10.5, 2.8 Hz, H-2), 6.16 (dt, 1 H, J = 3.25, 0.9 Hz, H-3'), 6.31 (dd, 1 H, 1 H, J = 3.25, 1.9 Hz, H-4'), 7.34 (dd, 1 H, J = 1.9, 0.9 Hz, H-5'); IR cm^{-1} 3260 m, 3120 m, 2940 s, 2860 s, 2800 m, 2705 w, 1740 w, 1635 s, 1600 w, 1575 w, 1510 m, 1490 s, 1450 s, 1355 s, 1260 m, 1220 m, 1185 m, 1155 s, 1110 m, 1075 m, 1010 s, 940 m, 920 s, 885 s

[a] Components identified for the first time. [b] Mills and Hodge (1976). [c] Doornbos et al. (1981).

at 230 °C. For higher boiling and more polar pyrrolidines the column temperature was adapted to the special separation problem. Repeated collections of the effluent fractions in glass capillary tubes were made until sufficient quantities were obtained.

Compounds **10** and **16** were isolated from Al_2O_3 fraction 1 and 2 of the proline/arabinose model system, **13** from the corresponding rhamnose experiment (F2), and 18 from the maltol/pyrrolidine system (F2); compounds **19–22** were isolated from the glucose/pyrrolidine model system (**19** from F3, **20** from F5, and **22** from F4). The isolated pyrrolidines were dissolved in $CDCl_3$ and characterized by IR and ^1H NMR spectroscopy.

RESULTS AND DISCUSSION

Equimolar amounts of L-proline and reducing sugars were heated at 150 °C for 1.5 h (pH 5–6); the volatiles were extracted with ether and investigated by capillary GC–MS and capillary GC–NSD. Some of the results are summarized in Tables I and II. This reaction is comparable to pressure cooking which may be applied to produce reaction type flavors. During the Maillard reaction proline catalyzes the transformation of reducing sugars via 1,2- and 2,3-enolization into reactive 3- and 1-deoxyosones, which undergo further reactions, forming furans, furanones, and γ-pyranones or are degraded via retroaldol cleavage into reactive α-dicarbonyls. Figure 1 presents a reaction scheme which may explain the formation of 1-pyrroline and pyrrolidine from proline with α-dicarbonyls. Pyruvaldehyde (which is produced form glyceraldehyde or by retroaldol cleavage from C_4 to C_6 sugars) and L-proline form an iminium carboxylate intermediate, which is transformed by decarboxylation into a reactive ylide or iminium ion (after protonation), which act as key intermediates in the formation of proline specific components. It can be seen that 1-pyrroline and acetol or pyrrolidine and pyruvaldehyde are formed by hydration and 1-acetonylpyrrolidine by reduction. 1-Acetonyl-2-pyrroline is produced as a minor constituent during roasting conditions.

The mass spectra of the characterized pyrrolidines and piperidines are summarized in Table I. Compounds **1–4** were identified by Mills and Hodge (1976) by vacuum thermolysis of 1-deoxy-1-L-prolino-D-fructose and compounds **5** and **6** by Doornbos et al. (1981). Components **7**, **8**, and **9** (which are formed as main constituents on heating proline with 2,3-butandione and 2,3-pentandione) were identified for the first time. The mass spectrum of compound **10** suggested a furfurylpyrrolidine. The ^1H NMR spectrum of the isolated constituent confirmed a pyrrolidine system [δ 1.79 (mc, 4 H, β-CH_2), 2.56 (mc, 4 H, α-CH_2)] and a furfuryl group [δ 3.64 (br s, 2 H, NCH_2), 6.20 (ddt, 1 H, J = 3.25 Hz, 1.0, 0.9 Hz, furan-H-3), 6.32 (dd, 1 H, J = 3.25, 2.0 Hz, furan-H-4), 7.38 (dd, J = 2.0, 1.0 Hz, furan-H5)]. Therefore, the spectroscopic data are consistent with 1-furfurylpyrrolidine. All spectroscopic data of components **11** (1-(5-methylfurfuryl)pyrrolidine) and **12** (1-(5-(hydroxymethyl)furfuryl)pyrrolidine) were in coincidence with the published data of Mills and Hodge (1976).

Compound **13** was determined by Doornbos et al. (1981) (without publishing spectroscopic data) on heating L-proline with 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone. **13** was isolated from the proline/rhamnose experiment by preparative GC and the MS, IR, and ^1H NMR spectroscopic data are consistent with the proposed structure. 2-Acetylpiperidine was identified by chemical reactions. **14** was tranformed into 2-acetyl-3,4,5,6-tetrahydropyridine by oxidation. **14** was synthesized according to Büchi and Wuest (1971) from 2-acetylpyridine. In addition **14** and 2-acetyl-3,4,5,6-tetrahydropyridine were reduced to 2-(1-hydroxyethyl)piperidine with $NaBH_4$. By analogous reactions **15** was identified as 2-propionylpiperidine. The mass spectrum of **16** suggested an isomer of compound **10** like 2-methyl-3-(1-pyrrolidinyl)furan or 2-(5-methyl-2,3-

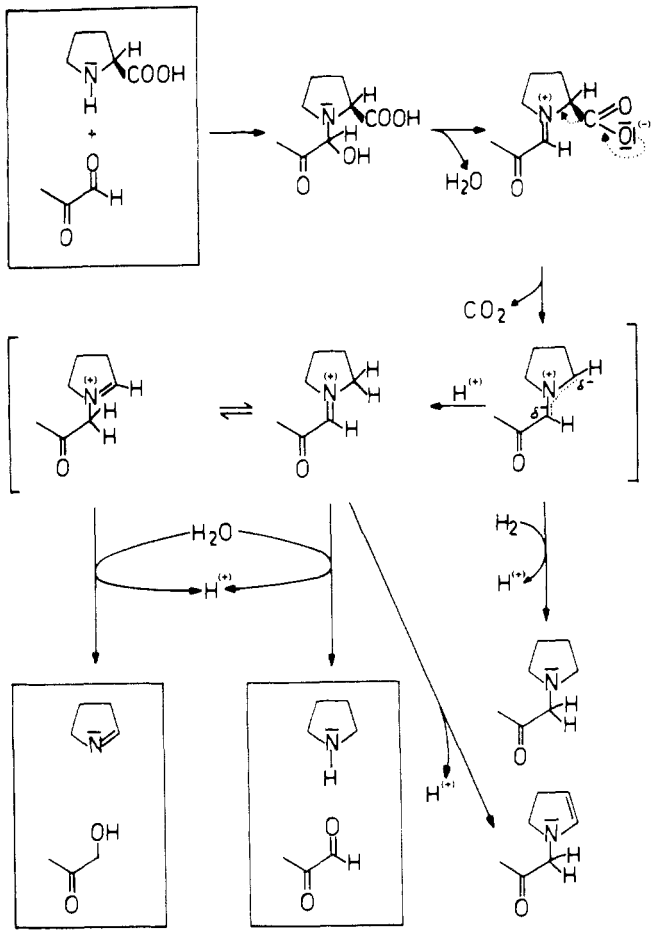
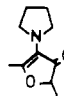


Figure 1. Reaction scheme to explain the formation of 1-pyrroline, pyrrolidine, 1-acetonylpyrrolidine, and 1-acetonyl-2-pyrroline.

dihydrofuran-3-ylidene)pyrrolidine. 2-Methyl-3-(1-pyrrolidinyl)furan was formed on heating pyrrolidine with 2-methyltetrahydro-3-furanone but the mass spectrum was not consistent. Therefore, **16** was isolated from the proline/arabinose experiment by preparative GC and investigated by ^1H NMR spectroscopy. The signals at δ 6.16 (dt, 1 H, J = 3.258, 0.9 Hz), 6.31 (dd, 1 H, J = 3.25, 1.9 Hz), and 7.34 (dd, 1 H, J = 1.9, 0.9 Hz) showed a 2-furyl group and the other data especially the broadened dd signal of the 2-proton with typical vicinal coupling constants to the methylene protons in 3-position exhibited a 2-piperidyl system. In addition **16** was synthesized according to LaLonde et al. (1977) by reduction of 2-(2-furyl)-3,4,5,6-tetrahydropyridine with $NaBH_4$. By an anologous reaction **17** was identified as 2-(5-methyl-2-furyl)-piperidine. As far as we know 2-acetyl- and 2-(2-furyl)-piperidines were characterized for the first time as Maillard products.

Pyrrolidines are formed depending on the reducing sugars. As shown in Table II 1-pyrroline, pyrrolidine, and 1-acetonylpyrrolidine were characterized in all model systems. Compound **6** possesses erythrose and glucose as precursor. Components **7–9** may be explained by the reaction of pyrrolidine with the corresponding α-hydroxy ketones which are formed during Strecker degradation. Compounds **10–12** are obviously derived from the corresponding 3-deoxyosones of arabinose, rhamnose, and glucose, respectively, and **13** from the 1-deoxyosone of rhamnose. The piperidines may be formed by a similar ring elongation of the proline system as discussed for tetrahydropyridines (Tressl et al., 1981a,b) which may also act as precursors. The pyrrolidines possess cereal aromas and

Table II. Pyrrolidines and Piperidines Characterized in Proline/Monosaccharide Model Experiments[a]

		GLY	ERY	ARA	GLU	RHAM
1		5	10	35	35	70
2		10	100	45	35	100
3		3	5	9	1	80
4		4	12	2	1	45
5		300	50	90	70	170
6		-	60	5	10	250
7		7	25	1	10	130
8		1	2	-	+	5
9		-	+	-	+	-
10		16	20	120	2	1
11		-	1	1	1	270
12		-	8	-	55	-
13		-	-	-	-	40
14		70	15	10	20	32
15		2	30	2	5	65
16		15	1	150	2	15
17		-	•	-	-	240

[a] Figures represent concentrations in ppm.

10 and **11** were perceived with sesame-like notes. The piperidines possess no characteristic odors but they are easily transformed into the corresponding tetrahydropyridines with bready, cracker-like odor qualities and thresholds in the ppb range.

Formation of Pyrrolidines on Roasting L-Proline with Glucose or Heating Pyrrolidine with Glucose. Pabst et al. (1984) identified 5-methyl-2,3-bis(1-pyrrolidinyl)-2-cyclopenten-1-one and 5-hydroxy-5-

Table III. Mass Spectra and ^1H NMR Spectroscopic Data of Pyrrolidines, Identified in Glucose/Pyrrolidine Model Experiments

(18) 2-Acetyl-3-(1-pyrrolidinyl)furan: MS 179 (100), 151 (65), 150 (29), 136 (87), 110 (58), 109 (36), 108 (39), 95 (55), 70 (23), 43 (50); ^1H NMR (270 MHz, $CDCl_3$) δ 1.96 (mc, 4 H, β'-CH_2), 2.41 (s, 3 H, $COCH_3$), 3.45 (mc, 4 H, α'-CH_2), 6.13 (d, 1 H, J = 2.5 Hz, H-4), 7.25 (d, 1 H, J = 2.5 Hz, H-5); I_K CP Wax 2347

(19) 3-(1-Pyrrolidinyl)tetrahydro-2-furanone: MS 155 (6), 111 (13), 110 (17), 97 (63), 96 (100), 70 (25), 69 (29), 55 (12), 42 (10), 41 (15); ^1H NMR (270 MHz, $CDCl_3$) δ 1.84 (mc, 4 H, β'-CH_2), 2.38 (mc, 2 H, H-4), 2.48 (t, 1 H, H-3), 2.66, 2.94 (each mc, 2 H, α'-CH_2), 4.22 (mc, 1 H, H-5), 4.38 (mc 1 H, H-5); IR cm^{-1} 2960 s, 2930 m, 2880 m, 2810 m, 1770 s, 1720 m, 1450 m, 1370 m, 1340 m, 1310 m, 1260 s, 1210 s, 1140 m, 1070 m, 1025 m; I_K CW 20M 2135

(20) 2-Hydroxy-1-(1-pyrrolidinyl)-1-buten-3-one: MS 155 (67), 138 (9), 137 (11), 126 (7), 112 (97), 84 (100), 83 (26), 70 (64), 57 (11), 56 (24), 55 (36), 43 (52), 42 (55); ^1H NMR, (270 MHz, $CDCl_3$) δ 1.90 (mc, 4 H, β'-CH_2), 2.0 (br s, 1 H, OH), 2.18 (s, 1 H, $COCH_3$), 3.62 (mc, 4 H, α-CH_2), 6.64 (s, 1 H, =CH); IR cm^{-1} 3400 s, 2980 s, 2870 s, 1660 s, 1480 m, 1360 s, 1340 m, 1310 s, 1265 s, 1070 m, 970 m; I_K CP Wax 2459

(21) 1,2-Bis(1-pyrrolidinyl)-1-buten-3-one: MS 208 (79), 193 (14), 192 (13), 165 (16), 139 (33), 124 (100), 122 (31), 110 (16), 109 (15), 96 (97), 84 (15), 82 (13), 70 (41), 55 (25), 43 (32), 41 (25); I_K CP Wax 2726

(22) 5-Hydroxy-5-methyl-2,3-bis(1-pyrrolidinyl)-2-cyclopenten-1-one: MS 250 (100), 234 (14), 232 (16), 207 (33), 191 (15), 189 (14), 181 (24), 179 (55), 163 (15), 161 (25), 151 (25), 138 (95), 123 (14), 110 (21), 108 (21), 96 (17), 95 (18), 93 (19), 81 (17), 70 (49), 55 (34); ^1H NMR (270 MHz, $CDCl_3$) δ 1.34 (s, 3 H, CH_3), 1.84 (mc, 4 H, β'-CH_2), 1.92 (mc, 4 H, β''-CH_2), 2.56 (d, 1 H, J = 16.5 Hz, H-4), 2.68 (d, 1 H, J = 16.5 Hz, H'-4), 2.74 (s, 1 H, OH), 3.02 (mc, 4 H, α''-CH_2), 3.67 (mc, 4 H, α'-CH_2); IR cm^{-1} 3560 w, 3300 w, 2980 m, 2930 m, 2880 m, 1665 w, 1555 s, 1450 s, 1370 w, 1320 w, 1290 m, 1270 w, 1220 w, 1160 w, 1130 w, 1005 w; I_K CP Wax 3000

(23) 5-Methyl-2,3-bis(1-pyrrolidinyl)-2-cyclopenten-1-one: MS 234 (100), 222 (22), 219 (7), 206 (59), 205 (21), 193 (43), 191 (82), 179 (16), 177 (17), 163 (32), 150 (14), 136 (55), 122 (23), 110 (13), 109 (16), 108 (14), 96 (33), 94 (13), 82 (13), 81 (14), 70 (69), 68 (29), 55 (37), 44 (31), 41 (55); I_K CP Wax 2975

methyl-2,3-bis(1-pyrrolidinyl)-2-cyclopenten-1-one on roasting proline with saccharose. Both components were formed as main constituents and possessed very bitter taste qualities. The thresholds were determined at 5–10 ppm, respectively. We could confirm these results and characterized four pyrrolidines which were also formed on heating pyrrolidine with glucose. The spectroscopic data are presented in Table III.

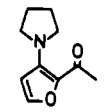
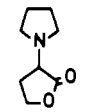
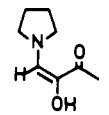
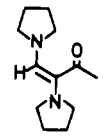
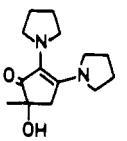
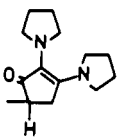
Compound **18** is formed on roasting maltose with proline or heating pyrrolidine with maltol. The mass spectrum showed a parent peak at m/e 179 and major fragments at m/e 136 (M – 43), 161 (M –28), and 70, the characteristic fragment of pyrrolidines. The ^1H NMR spectrum indicates a pyrrolidine system (δ 1.96 (m, 4 H) and 3.45 (m, 4 H)), a CH_3CO group (δ 2.41 (s, 3 H)) and two furan protons (δ 6.13 (d) and 7.25 (d)) with a coupling constant of 2.5 Hz characteristic for vicinal coupling between protons in the α- and β-position. Therefore, the proposed structure is in coincidence with the spectroscopic data.

Compound **19** was isolated from the pyrrolidine/glucose system. The mass spectrum (parent peak m/e 155, major fragments m/e 96 (M – 59), 97 (M – 58), 111 (M – 44), 110 (M – 45), and 70), the IR, and ^1H NMR spectroscopic data are consistent with 3-(1-pyrrolidinyl)tetrahydro-2-furanone. The ^1H NMR data given in Table III were established by decoupling experiments.

Compound **20** was isolated from the proline/glucose system. The mass spectrum showed a parent peak at m/e 155 and major fragments at m/e 112 (M – 43), 84, and 70, indicating a pyrrolidine system. The ^1H NMR spectroscopic data confirmed a pyrrolidine group (δ 1.90 (m, 4 H) and 3.62 (m, 4 H)) and the signals δ 6.64 (s, 1 H) and 2.18 (s, 3 H) are in coincidence with 3-hydroxy-4-(1-pyrrolidinyl)-3-buten-2-one. The IR spectrum showed a hydroxy group 3400, 1070 cm^{-1}) and a conjugated carbonyl group (1660 cm^{-1}).

Compound **21** is formed as main component on heating saccharose with proline or glucose with pyrrolidine. **22** was isolated by preparative GC from the pyrrolidine/glucose model system. The mass spectrum is similar to the published data (Pabst et al., 1984). The only difference is the fragment m/e 181 instead of m/e 183. The IR and ^1H NMR spectorscopic data are consistent with the proposed structure. The spectroscopic data of **23** were comparable to the published data (Pabst et al., 1984). **23** is formed on roasting L-proline with saccharose or heating pyrrolidine with glucose.

Table IV. Pyrrolidines Characterized in the Pyrrolidine/Glucose Model System and a Pyrrolidine/Maltol Model System[a]

18		0.5
19		1.5
20		3.5
21		0.7
22		3.8
23		0.1

[a] Figures represent concentrations in mg/g.

As shown in Table IV components **19–23** are formed during heating pyrrolidine with glucose. The corresponding piperidine derivatives of **18**, **19**, and **20** were characterized by Mills et al. (1970) by thermal degradation of 1-deoxy-1-piperidino-D-fructose. On the other hand components **18**, **19**, and **20** were not detected during vacuum thermolysis of 1-deoxy-1-L-prolino-D-fructose (Mills et al., 1970). Pabst et al. (1984) isolated **22** and **23** by roasting saccharose with proline (which possessed extremely bitter taste qualities) by HPLC. These constituents are also formed by pyrrolidine and glucose and were separated by capillary GC by using on-column injection. The furylpiperidines and some of the pyrrolidines possess

extremely bitter taste qualities and may contribute to the bitterness of roasted products like roasted malt and coffee.

Registry No. 1, 5724-81-2; 2, 123-75-1; 3, 3760-54-1; 4, 4030-18-6; 5, 54151-38-1; 6, 97073-14-8; 7, 97073-15-9; 8, 97073-16-0; 9, 97073-17-1; 10, 61893-12-7; 11, 61480-99-7; 12, 61481-02-5; 13, 80873-59-2; 14, 97073-22-8; 15, 97073-23-9; 16, 97073-24-0; 17, 97073-18-2; 18, 97073-19-3; 19, 6103-92-0; 20, 97073-20-6; 21, 97073-21-7; 22, 91999-33-6; 23, 91999-34-7; L-proline, 147-85-3; rhamnose, 3615-41-6; arabinose, 147-81-9; 2,3-butanedione, 431-03-8; 2,3-pentanedione, 600-14-6; D-glucose, 50-99-7; maltol, 118-71-8.

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A Viscometric Method for Determining Rubber Content in Guayule (*Parthenium argentatum* Gray)

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The viscosity of cyclohexane solutions of guayule rubber increased with rubber concentration. The relationship allows measurements to be made of the rubber content of guayule material by a viscometric technique that is simple and inexpensive. This viscometric method gave results comparable to other methods currently in use to determine rubber content, such as the gravimetric and infrared spectrophotometric methods. Oxidative degradation of the rubber solutions can affect viscosity but these effects can be minimized by avoiding light, by use of appropriate antioxidants, and by storage of samples under refrigeration.

There have been several techniques developed for determining the rubber content of guayule (Spence and Caldwell, 1933; Traub, 1946; Mehta et al., 1979; Willits et al., 1946; Tysdal and Finlayson, 1981; Visintainer et al., 1981; Banigan et al., 1982). Similarly, there are several laboratories engaged in guayule research each employing one, or a variation of one of the techniques, to evaluate the progress of their breeding efforts, to assess the effectiveness of bioregulators, and to measure the rubber content of plants under various physiological and environmental stimuli. Because of the disparity of methods employed by these various laboratories, comparison of data and interpretation of results are made difficult and open to question.

What is clearly needed then, is a relatively inexpensive and simple technique that allows consistent and accurate measurements of rubber content to be made. Also a method is required, that if adopted by the laboratories involved in this research, could standardize the procedure used to analyze for rubber content.

The viscometric method, described in this paper, promises to be a useful method for determining the rubber content of guayule plant material and one which meets the criteria of ease, cost effectiveness and consistency, while being relatively accurate. A comparison is made with other forms of analysis and some of the factors that can affect the viscosity of guayule rubber solutions are presented.

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EXPERIMENTAL SECTION

Plant Material. Material for rubber extraction and viscometric work was collected from three-year-old mixed variety plants grown at Berrenda Mesa Farms near Lost Hills, CA.

Extraction of a Rubber Sample. Shrubs were coppiced at varying heights and shredded with a hammermill at fieldside. The coarsely ground material was then sealed in plastic bags and stored in the dark over ice for transportation to the laboratory.

The material was then frozen in liquid nitrogen and passed through a Wiley mill twice, first with a 4 mm screen and subsequently a 2 mm screen. The finely ground samples were stored at −20 °C until they could be processed further.

Approximately 100 g fresh weight of material was placed in a 5-L Erlenmeyer flask, wrapped with aluminum foil to exclude light, and 2 L of cyclohexane added. The flask was placed on an orbital shaker for 2 h at 150 rpm. The rubber solution was then sieved through a 90-μm stainless steel screen into 1-L centrifuge buckets, and centrifuged at 5000 rpm for 30 min. Finally, the solution was vacuum filtered through Whatman no. 2 filter paper. By this stage, particulate matter had been removed and the green, viscous rubber solution was transparent.

A 2-fold volume of methanol was then added and the contents shaken. The rubber immediately precipitated and could be collected and used as a sample in subsequent studies. The cream colored rubber sample was stored in

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