Research Resource facility (RR-01317) in this department.

**Registry No.** (±)-1, 26057-70-5; (±)-3, 94859-58-2; 4, 40365-61-5; 5-ene, 94889-76-6; 5-yne, 94859-59-3; 6, 94859-60-6; (±)-7 (isomer 1), 94859-61-7; (±)-7 (isomer 2), 94942-24-2; (±)-7 (acid), 94859-62-8; (±)-8, 94889-64-2; 8 (selenolactone), 94859-63-9; (±)-9 (isomer 1), 94859-64-0; (±)-9 (isomer 2), 94942-25-3; (±)-10, 39949-88-7; octanal, 124-13-0; benzyloxyacetyl chloride, 19810-31-2.

## 2-Acetyl-4(5)-(1,2,3,4-tetrahydroxybutyl)imidazole: Detection in Commercial Caramel Color III and Preparation by a Model Browning Reaction

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Commercial caramel colors are divided into four classes on the basis of the ingredients used in their manufacture.[1] A number of studies have shown that caramel colors produce no toxicologically significant effects in mammals.[2] However, it was recently demonstrated that Caramel Color III was associated with a reduction in circulating lymphocyte counts, when fed at high concentrations to rats receiving a diet deficient or marginal in vitamin $B_6$.[3-5] The work described here concerns the isolation and characterization of a Caramel Color III component that reduces circulating lymphocytes in the rat.

### Results and Discussion

In order to allow procedures for isolating the active component from ammonia caramel to be evaluated, a modified bioassay was developed using pyridoxine-deficient rats.[5] The bioassay is based on the relative reduction of blood lymphocyte counts induced by test material given in drinking water, with respect to the counts of control rats.

Using this bioassay, it was ascertained that the activity was associated with a water soluble, dialyzable, highly hydrophilic, weak base. Its weakly basic nature was indicated from its behavior toward ion-exchange resins; the activity was removed from caramel solutions at pH 5 by sulfonic resins but not by carboxylic ones. This behavior provided the basis for a simple isolation procedure in which Caramel Color III was treated first with a weakly acidic resin (to remove strong bases like 4-methylimidazole) followed by the exchange of the active component with a sulfonic-type cation-exchange resin, from which it was eluted with 0.5 M HCl. At this point, the active material constituted 0.6% of the dry matter of caramel, an enrichment of 150-fold over the level found in the original caramel.

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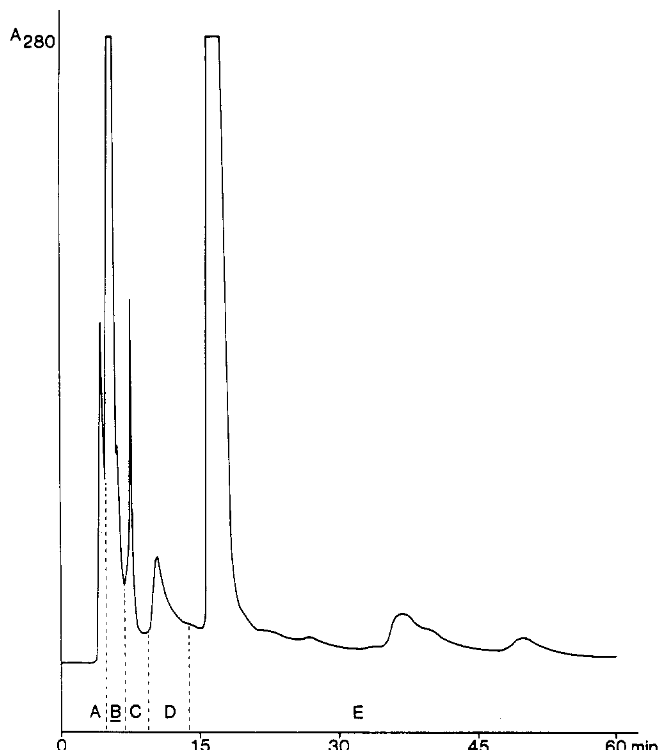
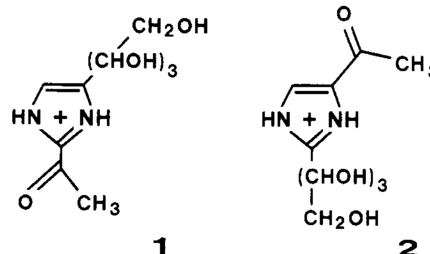



**Figure 1.**

Further purification was achieved on a LiChroprep RP-18 reverse-phase chromatographic column, using 0.01 M HCl (pH 2) as eluant. The activity was eluted in a single fraction (fraction B, Figure 1), representing 0.06% of the original caramel solids. Field-desorption mass spectrometry showed this fraction to contain 80–85% of a compound of molecular weight 230 and molecular formula $C_9H_{14}N_2O_5$. Acetylation gave a derivative whose field-desorption spectrum contained an $(M + H)^+$ at $m/z$ 399, suggesting that a tetraacetate had been produced and that the original material contained four OH groups. The remaining oxygen was assigned to a carbonyl on the basis of the IR spectrum of the hydrochloride ($\lambda = 1705\ cm^{-1}$) and the formation of a 2,4-dinitrophenylhydrazone.

The nature of the heterocyclic ring was ascertained from the $^{13}C$ NMR spectrum. In addition to the hydroxylated side-chain carbons, CHOH (three doublets at 73.6, 71.5, and 65.8 ppm) and $CH_2OH$ (triplet at 63.6 ppm), and the acetyl group ($CH_3$ quartet at 27.0 ppm and C=O singlet at 186.4 ppm), three aromatic carbons were found. Two at 140.5 and 139.0 ppm were singlets and hence substituted; the third at 120.3 ppm was a doublet and hence bore a hydrogen. This hydrogen appeared at 7.4 ppm in the $^1H$ NMR spectrum.

From the $^{13}C$ and $^1H$ NMR and MS data, the active material was either 2-acetyl-4-(1,2,3,4-tetrahydroxybutyl)imidazole (1) or 2-(1,2,3,4-tetrahydroxybutyl)-4-acetylimidazole (2). The former structure was established




from the UV spectrum which showed $\lambda_{max}$ (pH 1) 275 nm,

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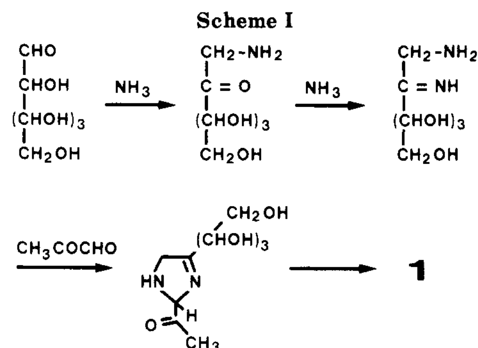
Scheme I



(pH 7) 289 nm, and (pH 13) 311 nm. These values are in keeping with those reported by Grimmett[6] for 2-acetylimidazoles and are not consistent with the 4-acetyl isomer. Additional support for 1 was derived from the electron-impact mass spectrum, which contained abundant ions corresponding to the fragmentations given in the Experimental Section. As final proof of structure, the isolated material was shown to be identical with a synthetic sample of 1 prepared by an unambiguous route.[7]

One possible mode for the formation of 1 during the caramelization of glucose in the presence of ammonia involves the condensation of fructosamine and pyruvaldehyde, two products known to form via Amadori rearrangement and alkaline degradation of glucose, respectively (Scheme I).

In an attempt to reproduce such a reaction, fructosamine acetate was boiled with an excess of pyruvaldehyde in 28% aqueous ammonia. The resulting brown solution was worked up as described above to give 1 (isolated as the hydrochloride) in 0.5–1% yield on the basis of fructosamine. The low yield of imidazole 1 in this model reaction is understandable, considering the tendency of pyruvaldehyde to degrade under alkaline conditions and also to react at both carbonyls to afford pyrazines instead of imidazoles.

## Experimental Section

Melting points (uncorrected) were measured on a SM-LUX (Leitz, Wetzlar, FRG) melting point apparatus. UV spectra were recorded on a Graphicord UV-240 (Shimadzu, Kyoto, Japan) spectrometer. IR spectra were determined on a PE 398 spectrometer (Perkin-Elmer, Uberlingen, FRG). Caramel Color III was an experimental product supplied by the International Technical Caramel Association, Washington, DC. Pyruvaldehyde (40% aqueous solution) was supplied by Sigma-Chemie, Taufkirchen, FRG.

**Preparative HPLC.** Columns (55 × 5.4 cm) were made by F. Schluter, Essen, FRG.[8] A rotating-piston pump RP-D-1 CKC (Fluid Metering Inc., Oyster Bay, NY) was used and effluents were monitored with a type UV-2 detector with 254- and/or 280-nm filters (Deutsche Pharmacia, Freiburg, FRG).

Weakly acidic cation-exchange Amberlite AG CG-50 I, $H^+$ (100–200 mesh) was supplied by Serva, Heidelberg, FRG, and strongly acidic cation-exchange Dowex AG 50 W × 12, $H^+$ (minus 400 mesh) by Bio-Rad, Munich, FRG. Reverse-phase material LiChroprep RP-18 (25–40 μm) was purchased from E. Merck, Darmstadt, FRG.

**Analytical HPLC.** The equipment consisted of a double-piston pump 6000 A (Waters, Eschborn, FRG), injector U6K (Waters), and a variable-wavelength detector 87.00 (Knauer, Bad Homburg, FRG) or a diode-array detector HP 1040 A with an HP 85 computer (Hewlett-Packard, Düsseldorf, FRG). Stainless steel columns (250 × 4.6 mm) were packed with reverse-phase material LiChrosorb RP-18, 10 μm (Merck) by the balanced-density slurry technique using a Haskel pump, type 286 46 (Ammann-Technik, Weil, FRG).

**Ammonia Caramel Fractionation.** (Ammonia caramel (250 g, dry matter 175 g) was dissolved in water (625 mL); the pH of the resulting solution was 4.3. The solution was adjusted to pH 5.0 by addition of 9.4 mL 2.5 M NaOH, loaded onto the first (Amberlite) of the sequentially linked cation-exchange columns, and eluted with water (4 L). Then the columns were disconnected and elution of the second (Dowex) column was continued with water (70 L) at a flow rate of 24 mL/min. Effluents were monitored at 254 nm; when transmission exceeded 90%, the eluent was changed to 0.5 M HCl, and subsequent elution was monitored by absorbance at 280 nm. Sixteen fractions of 500-mL each were collected. Fractions 9 and 10, carrying bioactivity, had a dry weight of 1040 mg (0.6% of caramel dm).

One-half of this material was redissolved in water (20 mL) and chromatographed on a LiChroprep RP-18 column with 0.01 M HCl at a flow rate of 36 mL/min. Absorption at 280 nm was monitored. Bioactivity was present in one peak solely (B in Figure 1) with a dry weight of 50 mg, corresponding to an overall 0.06% of the original caramel solids.

In the same manner, in a subsequent fractionation the center part of the bioactive peak was collected and freeze-dried. Dry weight: 30 mg (0.02% of caramel dm). Spectral data were the same as those given below for 1 prepared from a model browning reaction.

**Model Browning Reaction.** Fructosamine acetate[9] (23.9 g, 0.1 mol) was dissolved in water (125 mL), and ammonia (28%, 1 L) was added, followed by pyruvaldehyde (20 mL, ca. 20% excess). The mixture was refluxed for 1 h with a slow stream of gaseous ammonia passing through the flask. After cooling, the light brown solution was concentrated in vacuo at 40 °C to a syrup. Degassed water (ca. 150 mL) was added under nitrogen, and then the pH was adjusted to 5.0 with hydrochloric acid.

The sample was processed further in the same manner as described with caramel. When 1 was recovered from the Dowex column, aliquots (5 mL) of the 500-mL fractions were taken to dryness in vacuo, redissolved in water (0.5 mL), acidified with $H_3PO_4$ to pH 2, and subjected to HPLC on a LiChrosorb RP-18 column with the same solvent. Absorption at 214, 254, and 280 nm was recorded and compared with an authentic sample; usually $t_R$ 2.0 ± 0.1 min is found at a flow rate of 2 mL/min.

Upon evaporation and dissolution in a small volume of 95% ethanol, 1 crystallized spontaneously as the hydrochloride. It was filtered by suction, washed with ice-cold ethanol and ether, and then dried. The yield of colorless crystals (mp 198–203 °C dec) was 0.5–1%, based on fructosamine.

Anal. Calcd for $C_9H_{15}N_2O_5Cl$: C, 40.53; H, 5.63; N, 10.51. Found: C, 40.37; H, 5.60; N, 10.42. IR (KBr) 3360, 2920, 1705, 1625, 1490, 1400, 1365, 1215, 1075, 1035, 880, 625, 540 $cm^{-1}$; UV ($H_2O$) 275 nm, log $\epsilon$ = 3.91 (pH 1), 289, 3.98 (pH 7), 311, 4.10 (pH 13); $^{13}C$ NMR ($D_2O$, 75.46 MHz) 186.4 (s), 140.5 (s), 139.0 (s), 120.3 (d), 73.6 (d), 71.5 (d), 65.9 (d), 63.7 (t), 27.0 (q) ppm (q) ($D_2O$, 306 K, pH 2, internal standard, dioxane 67.4 ppm); high-resolution MS, $m/z$ 230 ($C_9H_{14}N_2O_5$), 215 ($C_8H_{11}N_2O_5$), 212 ($C_9H_{12}N_2O_4$), 199 ($C_8H_{11}N_2O_4$), 169 ($C_7H_9N_2O_3$), 139 ($C_6H_7N_2O_2$), 97 ($C_4H_5N_2O$) and 43 ($CH_3CO$); $^1H$ NMR ($CH_3OD$, 400 MHz) by dissolving the hydrochloride in $CD_3OD$ the dimethyl ketal is formed, $\delta$ 7.4 (d, $J$ = 0.8 Hz, 1 H), 5.1 (m, $J$ = 0.8, 2.0 Hz, 1 H), 3.80 (m, $J$ = 3.4, 11.0 Hz, 1 H), 3.74 (m, $J$ = 3.4, 5.0, 8.4 Hz, 1 H), 3.66 (m, $J$ = 5.0, 11.0 Hz, 1 H), 3.62 (m, $J$ = 2.0, 8.4 Hz, 1 H), 1.71 (s, 3 H). All couplings are absolute values obtained from first-order analysis at 400 MHz.

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## Synthesis of 2-Acetyl-4-(1,2,3,4-tetrahydroxybutyl)imidazole

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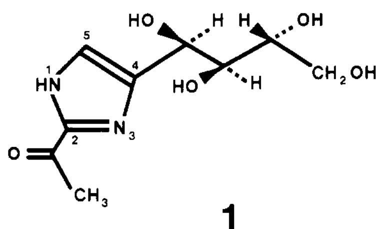
Recent studies on Caramel Color III, aimed at the isolation of the component responsible for reducing circulating lymphocyte counts in rats, maintained on a vitamin $B_6$ deficient diet, has led to the characterization of 2-acetyl-4-(1,2,3,4-tetrahydroxybutyl)imidazole (1) as the bioactive factor.[1]



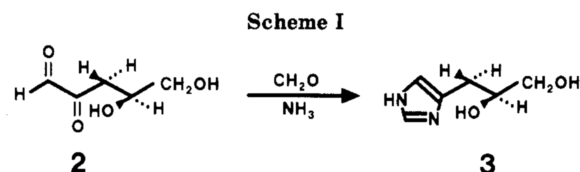

In this paper we describe an efficient synthesis of 1 from D-glucose that confirms the proposed structure and proves the stereochemistry of the side chain as written.

The strategy adopted for the synthesis of 1 was patterned after the prior work of Komoto,[2] who found that 3-deoxyxylosone (2) condensed with ammonia and formaldehyde to give the corresponding imidazole 3 in 80% yield (Scheme I).

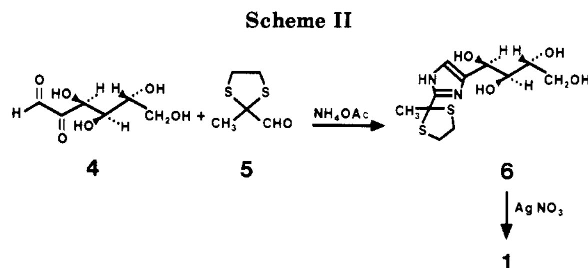
The extension of this reaction to the synthesis of 1, however, would require the condensation of D-glucosone (4) with pyruvaldehyde and ammonia. However, it was known that $\alpha$-keto aldehydes suffer considerable fragmentation in the presence of ammonia. For example, the condensation of pyruvaldehyde with ammonia gave 4-methyl- and 2,4-dimethylimidazoles as major products; the expected 4-methyl-2-acetylimidazole was formed in only 0.2% yield.[3] Similarly, the reaction of 3-deoxyglucosone with ammonia afforded 4-(2,3,4-trihydroxybutyl)imidazole as the major basic product.[4]

Scheme II depicts the sequence we have adopted to synthesize 1, which involved the condensation of D-glucosone (4)—readily available from D-glucose phenylosazone via acid-catalyzed exchange with benzaldehyde[5]—with an excess of pyruvaldehyde ethylene dithioketal 5[6] and ammonium acetate. The use of pyruvaldehyde with the keto group protected prevented the cleavage of the $\alpha$-keto aldehyde function and allowed the condensation to proceed exclusively on the aldehyde group.

Scheme I




Scheme II




When D-glucosone (4) was reacted with a threefold excess of pyruvaldehyde ethylene dithioketal (5) and ninefold excess of $NH_4OAc$ for 18 h at room temperature in methanol, the imidazole 6 was obtained in 30% yield. Purification of the product was readily accomplished by diluting the reaction mixture with water, extracting with ether to remove the excess 5, and then isolating the imidazole fraction via ion-exchange chromatography on a poly(styrenesulfonic acid) resin.

Removal of the dithioethane group proved to be more difficult than expected. Acid-catalyzed procedures[7,8] were unsuccessful, while $HgCl_2$[9] gave only a low yield of 1 mixed with starting material even after 18 h at reflux. The best method found involved stirring 6 with aqueous $AgNO_3$[10,11] for 18 h in the dark. The precipitation of excess $Ag^+$ with HCl was followed by chromatography on an open $C_{18}$ $\mu$-Bondapak column. The eluant was passed through a Bio-Rad AG1X2 anion exchange resin column in the acetate form to afford 1 in 39% yield (12% overall from D-glucosone). Synthetic 1 was found to be identical with an authentic sample[1] by $^{13}C$ NMR, UV, and HPLC comparison.[14]

The 300-MHz proton and 75.46-MHz $^{13}C$ NMR spectra of the free base 1 in $Me_2SO$-$d_6$ indicated the existence of two tautomers. The nature of this tautomerism is currently under investigation, but it clearly results from the presence of a carbonyl at C-2, conjugated to an unprotonated imidazole ring. In aqueous acid and acidified $Me_2SO$-$d_6$ solutions, the protonated form of 1 was observed as a single species. In acidified $CD_3OD$, the initial carbonyl compound solvated to the mono- and finally the dimethyl

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