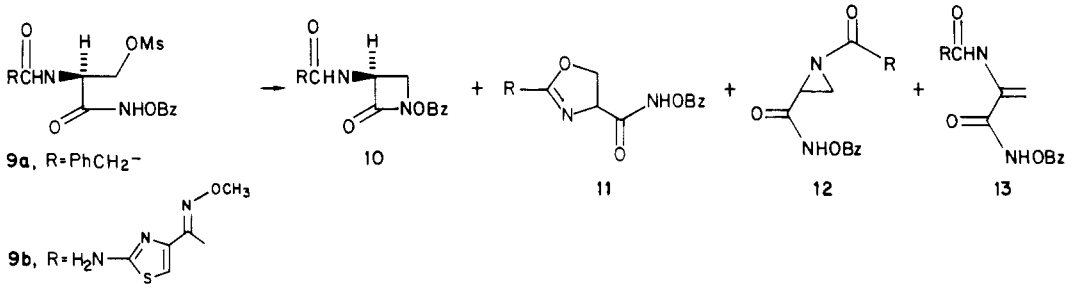
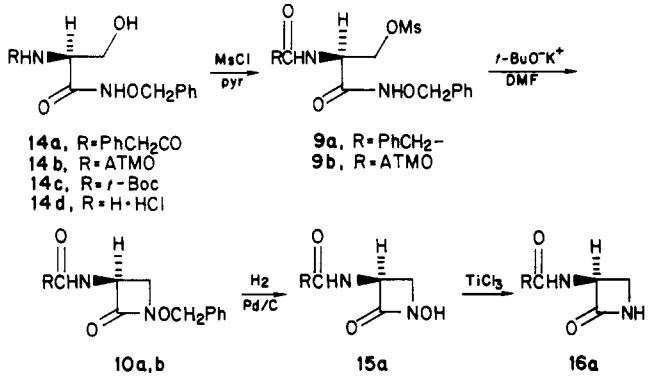
Table I



reactant	reaction conditions	product ratio, % 10	11	12	13
9a	$KHCO_3/CH_3CHCl_2$, Δ		94		
9a	*t*-BuO⁻K⁺/THF, Δ	26		47	9
9a	*t*-BuO⁻K⁺/THF, 0 °C	34		43	
9a	*t*-BuO⁻K⁺/DMF, 0 °C	55		21	
9a	*t*-BuO⁻K⁺/DMF, −23 °C	75			
9b	*t*-BuO⁻K⁺/DMF, −23 °C	40			

Scheme I



While stirring, methanesulfonyl chloride (0.044 mL, 0.56 mmol) was added, neat, by syringe. After 3 h the reaction mixture was poured into EtOAc (50 mL), washed with 1.2 N HCl until acidic and then with brine, and dried ($MgSO_4$), and the solvent was evaporated. The residue was redissolved in CH_2Cl_2 and eluted through a plug of silica gel. Solvent evaporation gave **9a** as a white solid in 95% yield (182 mg). Compound **9a** was once carefully recrystallized from EtOAc/hexanes in 82% yield, otherwise purification by recrystallization was not necessary: mp 94–96 °C; NMR ($CDCl_3$) δ 7.32 (s, 5 H), 7.22 (s, 5 H), 4.77 (s, 2 H superimposed on m, 1 H), 4.3 (m, 2 H), 3.38 (s, 2 H), 2.84 (s, 3 H); IR (KBr) 1620 br, 1160 cm^{-1}.

***N*-[*syn*-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetyl]-*O*-mesyl-L-serine *O*-benzylhydroxamate (9b)** was prepared by the above mesylation procedure from **14b** in 62% yield (recrystallized from EtOAc/hexanes) except that the pyridine was removed by washing with a KH_2PO_4/H_3PO_4 buffer, pH 3.5: mp 96 °C sinter and dec; NMR ($CDCl_3$ + acetone-d_6) δ 8.5 (d, NH), 7.37 (s, 5 H), 6.90 (s, 1 H), 4.93 (s, 2 H), 4.3–5.0 (m, 3 H), 3.81 (s, 3 H), 3.06 (s, 3 H); IR (KBr) 1760, 1165, 1030 cm^{-1}.

1-(Benzyloxy)-3-(phenylacetamido)-2-azetidinone (10a). Mesylate **9a** (924 mg, 2.27 mmol) was dissolved in DMF (20 mL, shaken with KOH and freshly distilled over CaO) and cooled to −23 °C (CCl_4/CO_2) under nitrogen. While stirring, *t*-BuO⁻K⁺/*t*-BuOH solution (2.10 mL, 1.08 M, 2.27 mmol) was added, and the reaction was allowed to slowly warm to room temperature overnight. After 10 h the mixture was poured into EtOAc (100 mL) and washed twice with 5% $NaHCO_3$ (25 mL) and then several times with water to remove the DMF. The organic layer was dried ($MgSO_4$) and the residue was recrystallized from EtOAc/hexanes to give **10a** in 75% yield (529 mg): mp 124–126 °C (note: depending on the method of preparation, differing melting points have been reported for this compound, both in our laboratories (127.5–129 °C)[4] and those of Squibb (126–128 °C, 130–131 °C)[7]); NMR ($CDCl_3$) δ 7.41 (s, 5 H), 7.30 (s, 5 H), 6.5 (br s, 1 H), 4.94 (s, 2 H), 4.65 (m, 1 H), 3.54 (s, 2 H superimposed on t, 1 H), 3.15 (dd, 1 H); IR (KBr) 1760, 1650 cm^{-1}; IR ($CHCl_3$) 1780, 1680 cm^{-1}; this material was identical with previously reported material.[4]

1-(Benzyloxy)-3-[*syn*-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-azetidinone (10b) was prepared by the above cyclization procedure from **9b** and recrystallized from EtOAc/hexanes in 40% yield as a mixture of isomers: NMR (acetone-d_6) δ 8.63 (d, NH), 7.44 (s, 5 H), 6.88 and 6.73 (s, 1 H, isomeric), 5.9 (br s, 2 H), 5.00 (s, 2 H), 4.67 (m, 1 H), 4.02 and 3.90 (s, 3 H, isomeric), 3.70 (apparent t, 1 H), 4.46 (dd, 1 H); IR (KBr) 1760, 1650 cm^{-1}.

3-(Phenylacetamido)-2-azetidinone (16a) was prepared form **15a** according to previously published reduction procedures[8] with the following modification: Prior to basification and workup, tartaric acid (250 mol % relative to $TiCl_3$) was added to facilitate Ti^{IV} removal. The pH was then adjusted to 8 with 10% Na_2CO_3 and worked up as reported. Recrystallization from EtOAc/hexanes gave **16a** in 35% yield: mp 166–168 °C dec; NMR ($CDCl_3$ + Me_2SO-d_6) δ 8.25 (br s, 1 H), 7.26 (s, 5 H), 4.98 (m, 1 H), 3.52 (s, 2 H superimposed on t, 1 H), 3.2 (dd, 1 H); IR (KBr) 1770 cm^{-1}. Anal. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.44; H, 5.77; N, 13.47.

Acknowledgment. We are grateful for the support of our research by the NIH, Eli Lilly and Company, and a Reilly Fellowship for M.A.K. The 300-MHz NMR system used was obtained by grants from the NIH and the University of Notre Dame. Ms. Therese Debiak and Mrs. Kathleen Peterson recorded the 300-MHz NMR spectra.

Registry No. 9a, 95070-21-6; **9b**, 95070-22-7; **10a**, 75624-37-2; **10b** (isomer 1), 95070-23-8; **10b** (isomer 2), 95070-24-9; **11a**, 95070-25-0; **12a**, 95070-26-1; **13a**, 95070-27-2; **14a**, 75624-33-8; **14b**, 95070-28-3; **14c**, 26048-92-0; **14d**, 26191-98-0; **15a** (R = $PhCH_2$), 82933-25-3; **16a** (R = $PhCH_2$), 80543-45-9; 1-hydroxybenzotrizaole *syn*-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetate, 71445-20-0.

A Short, Stereocontrolled Synthesis of Avenaciolide

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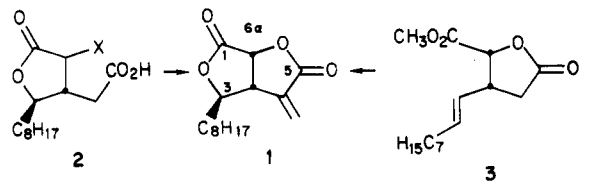
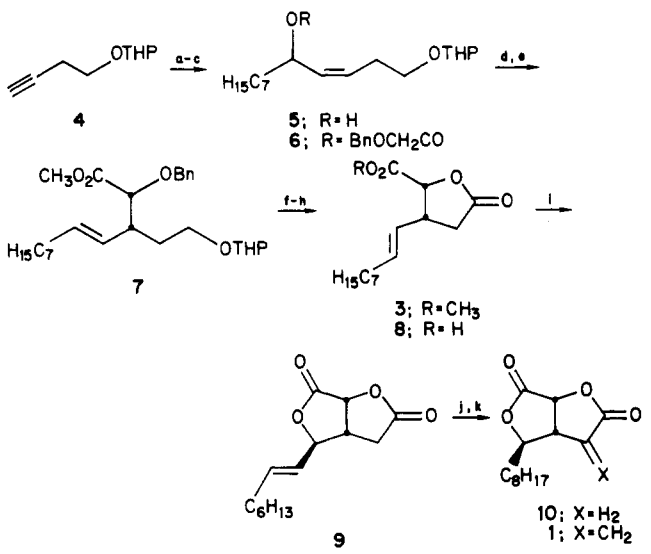
The antifungal metabolite avenaciolide (1) is representative of a group of bislactone fungicides[1] which have

0022-3263/85/1950-1128$01.50/0

Scheme I



Scheme II[a]



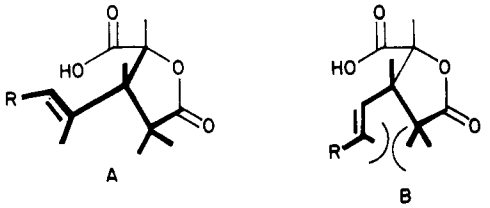
[a] (a) EtMgBr, then $C_7H_{15}CHO$; (b) H_2, Pd-$CaCO_3$, EtOAc; (c) $BnOCH_2COCl$, pyridine; (d) LDA, Me_3SiCl, −78 °C; then NH_4Cl, H_2O; (e) CH_2N_2; (f) Jones reagent; (g) Na, NH_3; (h) KOH, H_2O; (i) PhSeCl, then MCPBA; (j) H_2, Pd-C, CH_2Cl_2; (k) ref 2a.

been the focus of recent synthetic attention. Syntheses of avenaciolide have been reported,[2] including an enantiospecific route by Fraser-Reid.[2e] These schemes share a common entry to the bislactonic skeleton of 1 in which the α-methylene-containing lactone ring is formed in a penultimate step, either by nucleophilic displacement at C_{6a} or by direct lactonization of a C_{6a} hydroxyl group (Scheme I, 2 → 1). We have investigated an alternative approach (i.e., 3 → 1) to the bislactone fungicides wherein the bislactonic system is generated by a stereocontrolled seleno lactonization[3] which introduces the correct stereochemistry of the octyl side chain. We now report the successful application of this strategy to the synthesis of avenaciolide (1).

A retrosynthetic consideration of 1 suggests that the structural and stereochemical elements of the key intermediate 3 would be rapidly derived from the Claisen rearrangement of a suitably functionalized allylic glycolate.[4]

As shown in Scheme II, the required (*Z*)-glycolate 6 was prepared in three steps from the protected butynol 4[5] by Grignard formation and treatment with octyl aldehyde to give alcohol 5, followed by Lindlar hydrogenation and acylation with *O*-benzylglycolyl chloride. Enolate Claisen rearrangement[6] of 6 using our modified procedure[4a] gave the anti ester 7 as the only product.[7] Treatment of 7 with Jones reagent afforded a carboxylic acid which upon debenzylation was transformed to the desired lactone 3.

Attempts to form the bislactonic system from 3 by iodo lactonization[8] were unsuccessful. However, hydrolysis of 3 afforded the corresponding acid 8, which upon treatment with phenylselenyl chloride and subsequent oxidative elimination gave a 7:1 mixture of stereoisomeric bislactones. Examination of these isomers by ^1H NMR indicated that they were epimeric with respect to side-chain orientation. The structure of the major isomer[9] was established as bislactone 9 by hydrogenation to give the normethylene compound 10; introduction of the α-methylene group as reported previously[2a] affords avenaciolide (1).[10] We suggest that the stereoselectivity of the selenium-mediated cyclization results from preferential lactonization via conformer A which minimizes steric interactions of the side chain with the existing lactone ring.



In conclusion, we have described a new stereocontrolled route to the bislactone fungicide avenaciolide which should be applicable to other members of this series. Our synthesis further demonstrates the utility of the Claisen rearrangement of glycolate esters as an efficient and highly stereoselective entry to advanced, oxygenated intermediates. The potential for enantioselective synthesis of 1 via the chiral ester 6[11] and the further application of the glycolate Claisen protocol to natural products synthesis will be the subject of future reports from these laboratories.

Experimental Section

1-[(Tetrahydro-2*H*-pyran-2-yl)oxy]dodec-3-yn-5-ol (5). To a stirred solution of EtMgBr (from ethyl bromide (6.1 mL, 82

(1) Avenaciolide: (a) Brookes, D.; Tidd, B. K.; Turner, W. B. *J. Chem. Soc.* **1963**, 5385. (b) Ellis, J. J.; Stodola, F. H.; Vesonder, R. F.; Glass, C. A. *Nature (London)* **1964**, *203*, 1382. (c) Brookes, D.; Sternhell, S.; Tidd, B. K.; Turner, W. B. *Aust. J. Chem.* **1965**, *18*, 373. Isoavenaciolide, ethisolide: Aldridge, D. C.; Turner, W. B. *J. Chem. Soc. C* **1971**, 2431. Canadensolide: McCorkindale, N. J.; Wright, J. L. C.; Brian, P. W.; Clarke, S. M.; Hutchinson, S. A. *Tetrahedron Lett.* **1968**, 727.

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(6) (a) Ireland, R. E.; Mueller, R. H. *J. Am. Chem. Soc.* **1972**, *94*, 5897. (b) Ireland, R. E.; Mueller, R. H.; Willard, A. K. *J. Am. Chem. Soc.* **1976**, *98*, 2868.

(7) Ester 7 was obtained as a mixture of diastereomers at the tetrahydropyranyl center. Removal of the THP group affords a single diastereomeric ester, which was determined to be stereochemically homogeneous by HPLC and ^{13}C NMR. Limits of detection in these analyses for related systems is >100:1.

(8) Bartlett, P. A.; Myerson, J. *J. Am. Chem. Soc.* **1978**, *100*, 3950. Iodo lactonization of acid 8 afforded the expected iodo lactones; however, we were unable to dehydrohalogenate this intermediate under conditions which left the bislactonic system intact.

(9) Olefin geometry in 9 has not been unequivocally established. The ^{13}C NMR spectrum of 9 shows 14 signals indicating the presence of a single isomer, which we have tentatively assigned the *E* stereochemistry.

(10) Analytical data for our synthetic avenaciolide was consistent with that reported for natural[1c] and synthetic[2] material, including comparison with an authentic proton NMR kindly provided by Professor Fraser-Reid.

(11) The requisite chiral allylic alcohol is readily available from the corresponding acetylenic ketone. See: Midland, M. M.; McLoughlin, J. I. *J. Org. Chem.* **1984**, *49*, 1316 and references therein.

mmol) and Mg (2.3 g, 93 mmol)) in THF (120 mL) at 0 °C was added butyne 4[5] (12.0 g, 78 mmol) in 20 mL of THF. The reaction mixture was stirred for $^1/_2$ h at 0 °C, and a solution of octyl aldehyde (10.5 g, 82 mmol) in 15 mL of THF was added. After 1 h, the reaction was quenched with H_2O (50 mL) and extracted (3 × 50 mL) with ether. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Flash chromatography[12] on silica gel (12:1 hexane/ethyl acetate) gave alcohol **5** as a pale oil (13.6 g, 62%): IR (neat) 3415, 2220 (w), 1120, 1030 cm^{-1}; ^1H NMR (250 MHz) δ 4.66 (br s, 1 H), 4.31 (t, J = 6.6 Hz, 1 H), 3.91–3.76 (m, 2 H), 3.59–3.50 (m, 2 H), 3.16 (br s, 1 H), 2.51 (t, J = 6.9 Hz, 2 H), 1.85–1.10 (m, 18 H), 0.88 (t, J = 7.3 Hz, 3 H).

An analytical sample was prepared by bulb-to-bulb distillation (oven temperature 200 °C, 0.1 mm). Anal. Calcd for $C_{17}H_{30}O_3$: C, 72.30; H, 10.71. Found: C, 72.40; H, 10.94.

(*Z*)-1-[(Tetrahydro-2*H*-pyran-2-yl)oxy]dodec-3-en-5-ol (Benzyloxy)acetate (6). To a stirred suspension of Lindlar catalyst (0.78 g) in ethyl acetate (50 mL) was added by syringe a solution of alcohol **5** (6.40 g, 22.7 mmol) in ethyl acetate (30 mL). The mixture was stirred under H_2 gas for $1^1/_2$ h in which time it absorbed 553 mL of gas (109% theory). The mixture was filtered through a bed of Celite and concentrated to give a pale oil. This material was dissolved in THF (60 mL) with pyridine (5.50 mL, 3.0 equiv). The resulting solution was cooled to 0 °C and stirred while a solution of (benzyloxy)acetyl chloride[13] (5.90 g, 1.4 equiv) in THF (20 mL) was added. The reaction mixture was stirred overnight and then quenched by addition of saturated aqueous $NaHCO_3$ (25 mL). The mixture was extracted with ether (3 × 75 mL), and the combined extracts were dried over Na_2SO_4, filtered, and concentrated. The residual oil was flash chromatographed on silica gel (15:1 hexane/ethyl acetate) to give an oil which was further purified by medium-pressure liquid chromatography (12:1 hexane/ethyl acetate) to yield ester **6** as a pale oil (8.15 g, 83%): IR (neat) 1755, 1450, 1200 cm^{-1}; ^1H NMR (250 MHz) δ 7.33 (s, 5 H), 5.63 (m, 2 H), 5.38 (t, J = 9.8 Hz, 1 H), 4.61 (s, 2 H), 4.56 (br s, 1 H), 4.06 (s, 2 H), 3.81 (m, 2 H), 3.47 (m, 2 H), 2.49 (br q, J = 6.9, Hz, 2 H), 1.82–1.40 (m, 8 H), 1.27 (br s, 10 H), 0.88 (t, J = 7.0 Hz, 3 H).

An analytical sample was prepared by bulb-to-bulb distillation (oven temperature 220 °C, 0.1 mm). Anal. Calcd for $C_{26}H_{40}O_5$: C, 72.19; H, 9.32. Found: C, 72.01; H, 9.34.

Methyl (2*RS*,3*SR*)-(*E*)-2-(Benzyloxy)-3-[2-[(tetrahydro-2*H*-pyran-2-yl)oxy]ethyl]dodec-4-enoate (7). To a stirred solution of **6** (0.87 g, 2.01 mmol) in THF (10 mL) at −78 °C was added lithium diisopropylamine (6.06 mL, 0.5 M solution) in THF by syringe. After 1 min, chlorotrimethylsilane (0.57 g, 2.6 equiv) was added quickly by syringe. After 10 min the solution was allowed to warm to room temperature and was stirred for 6 h. Saturated aqueous NH_4Cl (5 mL) was added and the mixture was stirred overnight. The reaction mixture was extracted with ether (3 × 10 mL); the combined extracts were treated with diazomethane (excess) and concentrated under reduced pressure. The residual yellow oil was flash chromatographed on silica gel (20:1 pentane/ether) to give ester **7** as a yellow oil (0.67 g, 75%): IR (neat oil) 1745, 1450, 1200 cm^{-1}; ^1H NMR (360 MHz) δ 7.33 (s, 5 H), 5.38 (m, 2 H), 4.77 (d, J = 11.7 Hz, 1 H), 4.51 (m, 1 H), 4.36 (d, J = 11.7 Hz, 1 H), 3.97 (d, J = 3.7 Hz, 1 H), 3.82 (m, 1 H), 3.71 (s, 3 H), 3.68 (m, 1 H), 3.48 (m, 1 H), 3.29 (m, 1 H), 2.64 (m, 1 H), 1.96 (q, J = 6.6 Hz, 2 H), 1.95–1.68 (m, 4 H), 1.57–1.45 (m, 4 H), 1.25 (br s, 10 H), 0.87 (t, J = 7.0 Hz, 3 H).

An analytical sample was prepared by bulb-to-bulb distillation (oven temperature 240 °C, 0.1 mm). Anal. Calcd for $C_{27}H_{42}O_5$: C, 72.62, H, 9.48. Found: C, 72.64, H, 9.67.

Methyl (4*SR*,5*RS*)-Tetrahydro-4(*E*)-nonen-1-yl-2-oxofuran-5-carboxylate (3). To a stirred solution of ester **7** (229 mg, 0.5 mmol) in acetone (10 mL) at 0 °C was added Jones reagent (1 mL, 8 N). The mixture was stirred for 1 h at 0 °C and additional Jones reagent (0.5 mL) was added. The reaction mixture was allowed to warm to room temperature and stirred an additional $3^1/_3$ h. The reaction was quenched with ethanol and extracted with ether (100 mL). The organic layer was washed with water (2 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The residual oil was flash chromatographed on silica gel (10:1 hexane/ethyl acetate) to give the carboxylic acid as a pale oil (161 mg). A solution of this oil (149 mg, 0.4 mmol) in THF (2 mL) was added to a solution of sodium metal (20 mg, 0.9 mmol) in liquid ammonia (30 mL) at −78 °C. Additional sodium metal (5 mg) was added and the reaction was quenched by addition of solid NH_4Cl. The ammonia was evaporated, and THF (10 mL) and 5% aqueous HCl solution (10 mL) were added to the residue. The resulting mixture was extracted with ether (4 × 20 mL), and the combined extracts were dried (Na_2SO_4), stripped of solvent, and taken up in toluene (25 mL). *p*-Toluenesulfonic acid (10 mg) was added and the mixture was refluxed for $1^1/_2$ h. After removal of solvent under reduced pressure, the residual oil was chromatographed on silica gel (20:1 hexane/ethyl acetate) to give lactone **3** as an oil (67 mg, 63%): IR (neat oil) 1800, 1750 cm^{-1}; ^1H NMR (360 MHz) δ 5.66 (dt, 15.3, 7.0 Hz, 1 H), 5.21 (dd, J = 15.3, 8.1 Hz, 1 H), 4.93 (d, J = 8.2 Hz, 1 H), 3.76 (s, 3 H), 3.43 (m, 1 H), 2.60 (d, J = 9.3, 2 H), 2.01 (br q, J = 6.8 Hz, 2 H), 1.26 (m, 10 H), 0.88 (t, J = 6.7 Hz, 3 H); ^{13}C NMR (62.9 MHz) ppm 175.3, 168.7, 136.2, 123.5, 79.2, 52.0, 41.6, 32.4, 32.3, 32.0, 31.6, 29.0, 28.9, 22.5, 14.0. An analytical sample was prepared by bulb-to-bulb distillation (oven temperature 200 °C, 0.1 mm). Anal. Calcd for $C_{15}H_{24}O_4$: C, 67.14, H, 9.01. Found: C, 67.02, H, 9.00.

(3aα,4α,6aα)-Dihydro-4-((*E*)-octen-1-yl)furo[3,4-*b*]furan-2,6[3*H*,4*H*]dione (9). To a stirred solution of lactone **3** (63 mg, 0.23 mmol) in THF (5 mL) was added 15% NaOH solution (1.5 mL). The mixture was stirred overnight. The solution was then acidified with 5% aqueous HCl solution (5.5 mL) and extracted with ether (4 × 15 mL). The combined extracts were dried over Na_2SO_4 and filtered, and solvent was removed by rotary evaporation. The residue was taken up in toluene (25 mL) containing *p*-toluenesulfonic acid (2 mg) and heated to reflux for 90 min. The solvent was removed to leave a light brown oil which was taken up in ethyl acetate (5 mL) and cooled to −78 °C, and a solution of phenyl selenylchloride (56 mg, 0.3 mmol) in ethyl acetate (5 mL) was added. After 1 h the cold bath was removed, and the reaction mixture was warmed to room temperature and stirred for 2 days. The resulting mixture was filtered, stripped of solvent, and chromatographed on silica gel (10:1 hexane/ethyl acetate) to give the crude selenolactone (77 mg). This material was taken up in CH_2Cl_2 (5 mL), *m*-chloroperbenzoic acid (114 mg, 3 equiv) was added, and the reaction was stirred 15 min. A solution of saturated $NaHCO_3$ (10 mL) was added followed by aqueous $Na_2S_2O_3$ (1 mL, 1 M). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 7 mL). The combined organic fractions were washed with saturated $NaHCO_3$, dried over Na_2SO_4, filtered, concentrated, and chromatographed on silica gel (8:1 hexane/ethyl acetate) to give the olefin (30 mg, 50% overall) as a pale oil which solidified on standing. Further elution afforded the side-chain epimer as a pale oil (4 mg, 7% overall). Recrystallization of the major isomer from ether/pentane gave bislactone **9** (mp 45.2–46.1 °C): IR (KBr) 1795, 1780 cm^{-1}; ^1H NMR (250 MHz) δ 5.90 (dt, J = 15.3, 6.6 Hz, 1 H), 5.48 (dd, J = 15.3, 7.3 Hz, 1 H), 5.01 (d, J = 7.3 Hz, 1 H), 4.72 (t, J = 6.6, 5.8 Hz, 1 H), 3.14 (m, 1 H), 2.93 (dd, J = 8.8, 18.3 Hz, 1 H), 2.58 (dd, J = 3.7, 18.3 Hz, 1 H), 2.09 (br q, J = 6.6 Hz, 2 H), 1.42–1.20 (m, 8 H), 0.89 (t, J = 6.7 Hz, 3 H); ^{13}C NMR (62.9 MHz) ppm 173.4, 169.6, 138.3, 125.1, 84.7, 76.9, 41.2, 32.2, 32.1, 31.6, 28.7, 28.5, 22.5, 14.0. Anal. Calcd for $C_{14}H_{20}O_4$: C, 66.65, H, 7.99. Found: C, 66.75, H, 7.95.

Avenaciolide (1). To a stirred solution of compound **9** (29.2 mg, 0.116 mmol) in CH_2Cl_2 (6 mL) was added 10% Pd on carbon (10 mg). A balloon of H_2 gas was attached and the mixture was stirred overnight. The mixture was filtered, stripped of solvent, and chromatographed on silica gel (8:1 hexane/ethyl acetate) to give normethyleneavenaciolide (**10**) (11.4 mg, 38%) with spectral and physical characteristics in agreement with those reported.[2a]

The normethylene compound **10** was converted to avenaciolide (1) by the literature procedure.[2a,10]

Acknowledgment. We gratefully acknowledge the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the National Institutes of Health (AI-19632-01) for their support of this work. High-field NMR spectra were obtained at the NIH

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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.

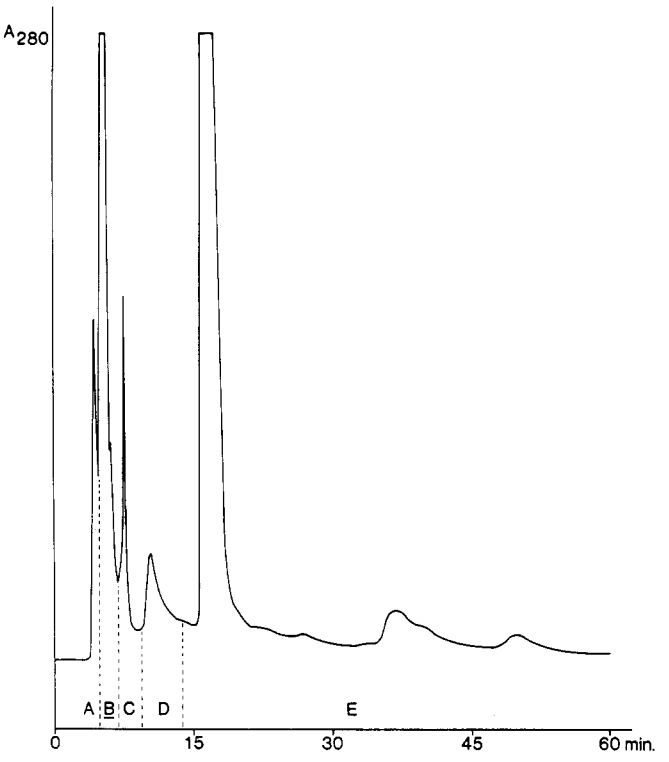
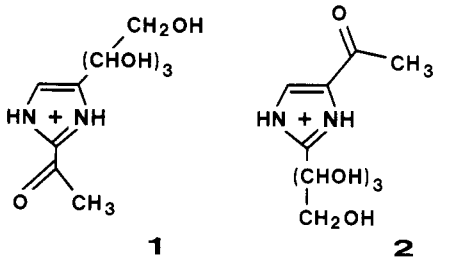


Figure 1.

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 (λ = 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 λ_{max} (pH 1) 275 nm,

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