Chemistry of Muconaldehydes of Possible Relevance to the Toxicology of Benzene

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(*Z,Z*)-Muconaldehyde reacted with primary amines to give *N*-substituted-2-(2'-oxoethyl)-pyrroles, which were reduced to *N*-substituted-2-(2'-hydroxyethyl)-pyrroles by sodium borohydride. The pyrrole-forming reaction is exhibited by valine and its methyl ester, and is being developed with terminal valine in hemoglobin as a means of dose monitoring (*Z,Z*)-muconaldehyde, a putative metabolite of benzene. Reactions in aqueous solution between (*Z,Z*)-muconaldehyde and adenosine, deoxyadenosine, guanosine, or deoxyguanosine leading to pyrrole-containing adducts are described. The elucidation of the structures of the adducts was assisted by the study of reactions between (*Z,Z*)-muconaldehyde and both nucleoside derivatives and a model compound for guanosine. Reactions of (*Z,Z*)-muconaldehyde are complicated by its isomerization to (*E,Z*)- and (*E,E*)-muconaldehyde. The kinetics of this process have been studied in benzene, acetonitrile, and dimethylsulfoxide. — Environ Health Perspect 104(Suppl 6):1201–1209 (1996)

Key words: muconaldehyde, muconaldehyde (isomers), muconaldehyde (stability), pyrrole adducts (amino acid), pyrrole adducts (nucleoside), benzene

Introduction

Following the discovery that (*E,E*)-muconaldehyde is a metabolite of benzene (*1*), it was of interest and importance to determine the contribution of the muconaldehyde isomers 1a to 1c (see Appendix for all structures) to the toxic effects of benzene on humans. (*E,E*)-Muconaldehyde 1c may be derived from the (*E,Z*)-isomer 1b, which is formed by isomerization of the (*Z,Z*)-isomer 1a, the putative primary product of the oxidative cleavage of benzene. Scheme 1 shows a possible route (*2,3*) from benzene to 1a, in which the oxidant is either cytochrome P-4502E$_1$ in metabolism or a powerful chemical oxidant [e.g., 2-methyl-2-trifluoromethyldioxirane, which converts benzene into a mixture of 1b and 1c, presumably via 1a (*4*)]. Muconaldehydes are obviously α,β-unsaturated aldehydes and may therefore be expected to exhibit properties analogous to those of acrolein, for example, which is known to react with DNA to form cyclic adducts (*5,6*). However, muconaldehydes duplicate acrolein functionality and have the potential to cross-link biomolecules. We wish to determine the nature of the interactions of muconaldehydes with nucleic acids and proteins because these interactions may be relevant to the toxicity of benzene (*7*). Adducts of muconaldehydes with DNA or hemoglobin might be found in animals and humans exposed to benzene. Adducts with hemoglobin might be useful for dose monitoring, while adducts with DNA might be indicative of an oncogenic pathway.

This article describes a detailed study of the behavior of muconaldehyde isomers in solution, their reactions with amine nucleophiles including amino acids and nucleosides, and a preliminary study of their reactions with DNA and a terminal heptapeptide of hemoglobin.

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Abbreviations used: AB system, in ^{1}H nuclear magnetic resonance spectroscopy, protons (A and B, in different environments) that are interacting, with each appearing as a doublet resonance; DMF, dimethylformamide; HOMO, highest occupied molecular orbital; KBr, potassium bromide; LUMO, lowest unoccupied molecular orbital; nb, nota bene = note; nOe, nuclear Overhauser effect.

Materials and Methods

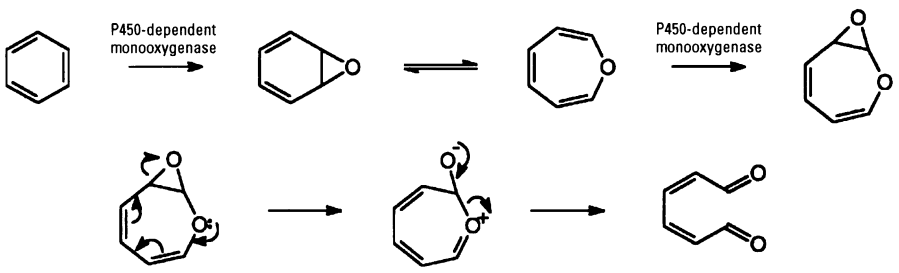
Chemicals, Reagents, and Solvents

All chemicals and reagents used were obtained commercially and were purified where necessary by standard procedures (*8*). *N*-(Benzyloxycarbonyl)-L-lysine benzyl ester benzenesulfonate was purchased from Bachem (Bachem Feinchemikalien AG, CH-4416 Bubendorf, Switzerland). Sodium methoxide in methanol solutions were prepared by dissolving sodium metal in methanol under nitrogen. The resultant sodium methoxide solution was assayed by titration with standard hydrochloric acid using bromothymol blue as indicator.

Solvents used for reactions of muconaldehyde were of high performance liquid chromatography (HPLC) grade or were purified by standard procedures (*8*). Dimethylformamide was purified by standing over molecular sieves (3 Å) for 24 hr, distilling from phosphorus pentoxide onto molecular sieves (3 Å), and storing under nitrogen. Dichloromethane was passed through basic alumina immediately prior to use.

Methods

Solvents were removed on a rotary evaporator under reduced pressure at ambient temperature, except for dimethylformamide, which was removed by short path distillation under high vacuum at < 50°C.



Scheme 1. Proposed route (2,3) for the conversion of benzene into (*Z,Z*)-muconaldehyde.

Residual volatile solvents were also removed from samples (e.g., for preparation of samples for submission for analysis) under high vacuum. Molecular sieves were activated prior to use by heating with a Bunsen burner and cooling in a desiccator. Thin-layer chromatography (TLC) was performed on aluminum-backed Kieselgel 60 F_{254} (Merck Ltd., Poole BH15 1BR, UK) or Merck 5550 (Merck Ltd., Poole BH15 1BR, UK) neutral alumina plates. Developed plates were visualized by examination under an ultraviolet light source or by contact with molybdophosphoric acid in ethanol solution followed by heating. Column chromatography was performed on Kieselgel 60 or neutral alumina under pressure. Solvents for column chromatography were HPLC grade or redistilled laboratory grade. HPLC analyses were performed using a Jones chromatography reverse phase [Spherisorb ODS (5 μ)] column (25 × 0.46 cm) (Jones Chromatography Ltd., Hengoed CF8 8AU, Wales) with gradient elution in which solvent A was water and solvent B was methanol. All analyses were performed using a pump program that delivered the same gradient and differed only in total time for the analysis. The gradient was 0.67% B min^{-1}.

Instrumentation

HPLC analyses were performed on a Merck-Hitachi L-6200A intelligent pump fitted with a Merck-Hitachi L-4500 ultra violet diode array detector (Merck Ltd., Poole BH15 1BR, UK) and a Rheodyne injector (Rheodyne Inc., Cotati, CA 94928).

Infrared spectra were recorded on either a Nicolet 20-SXB FTIR spectrophotometer, or on a Nicolet 20-PCIR spectrophotometer (Nicolet Instruments Ltd., Warwick CV34 5XH, UK). Samples were run using KBr disks or as films on NaCl plates. Peaks are designated by their wavenumber (cm^{-1}).

Ultraviolet spectra were recorded on a Kontron Uvikon-810 spectrophotometer (Kontron AG, CH-8010 Zurich, Switzerland). Samples were run in solution in HPLC grade methanol or acetonitrile. All absorbances are quoted by their λ_{max} (nm) values followed in brackets by the extinction coefficient. When appropriate, the absorption may be described as a "shoulder" designated "sh."

Mass spectra were recorded on a Kratos MS80 RF spectrometer (ElectroMed Ltd., Trafford Park, Manchester M17 1QS, UK). Samples were analyzed by either electron impact (EI) or fast atom bombardment (FAB) mass spectrometry as indicated. All peaks are quoted as *m/z* followed in brackets by their percent intensity and, where possible, the fragment ion to which they correspond. The molecular ion (where present) is designated by M^+.

^{1}H NMR (nuclear magnetic resonance) spectra were recorded on a Bruker WP-200 (200 MHz) or a Bruker WM-300 (300 MHz) spectrometer (Bruker Spectrospin Ltd., Coventry CV4 9GH, UK). Peaks are assigned by their chemical shift in parts per million followed in brackets by their multiplicity (*s*, singlet; *d*, doublet; *t*, triplet *q*, quartet; *dd*, double doublet; and *m*, multiplet), their spin–spin coupling constants (*J*) in Hertz (where appropritate), and their relative integral values in number of protons to which it equates. All samples were run as solutions in an appropriate deuteriated solvent (commonly MeCN-d_3, Me_2SO-d_6, or dimethylformamide-d_7) using the residual nondeuteriated solvent peak as an internal standard.

^{13}C NMR were recorded on a Bruker WP-200 (50 MHz) or a Bruker WM-300 (75 MHz) spectrometer operated in a broad-band decoupling mode. Peaks are assigned by their chemical shift in parts per million.

Synthetic Chemistry

***Improved Preparation of* N-*[(S)-1′-(Methoxycarbonyl)-2′-methyl-n-propyl]*-2-*(2′-hydroxyethyl)*pyrrole 2c.** A suspension of *L*-valine methyl ester hydrochloride (23 mg, 1.4×10^{-4} mol) and sodium carbonate (15 mg, 1.4×10^{-4} mol) in acetonitrile (2 ml) was stirred for 10 min at 20°C. A solution of (*Z,Z*)-muconaldehyde (*9*) (15 mg, 1.4×10^{-4} mol) in acetonitrile (2 ml) was added subsequently. After stirring for 15 min the solvent was removed under reduced pressure and the reaction mixture redissolved in methanol (4 ml). Sodium borohydride (16 mg, 4.2×10^{-4} mol) was added and the mixture stirred for 15 min. The solvent was removed *in vacuo*, the residue taken up in diethyl ether and dried ($MgSO_4$). Removal of the ether afforded the crude product, which was purified by column chromatography (elution with 1:1 ethyl acetate–petrol ether) to give *N*-[(*S*)-1′-(methoxycarbonyl)-2′-methyl-*n*-propyl]-2-(2′-hydroxyethyl)pyrrole 2c, (20 mg, 8.9×10^{-5} mol, 64%).

The material obtained was identical with previously prepared as by ^{1}H NMR (*10*).

***Reaction of (Z,Z)-Muconaldehyde with 2-Amino-4-hydroxy-6-methylpyrimidine 3a to Afford 4,5-Dihydro-5-hydroxy-8-methylpyrrolo[1′,2′:3,4]pyrimido[2,1-b]pyrimidine-6-one 3b.** 2-Amino-4-hydroxy-6-methylpyrimidine 3a (671 mg, 5.36×10^{-3} mol) was dissolved in dimethylformamide (110 ml) by heating to near reflux under nitrogen and allowing the solution to cool to room temperature. To the resulting solution was added (*Z,Z*)-muconaldehyde (648 mg, 5.89×10^{-3} mol) in dimethylformamide (20 ml) in one portion, and pyridinium *p*-toluenesulfonate (148 mg, 5.89×10^{-4} mol) in dimethylformamide (10 ml) (nb total volume of dimethylformamide used = 160 ml, including that used on each transfer). The reaction mixture was stirred under nitrogen in the dark at room temperature for 21 days. The solvent was removed *in vacuo*. The residue was partially dissolved in methanol (200–250 ml) and the resultant suspension filtered. The methanolic filtrate was evaporated onto silica gel 60 *in vacuo*, and subjected to medium pressure chromatography on silica gel 60 (elution with ethyl acetate) to give the crude product. This was rechromatographed on silica gel 60 (elution with 7:3 ethyl acetate–dichloromethane). The partially purified product was washed with acetone to afford purified 4,5-dihydro-5-hydroxy-8-methylpyrrolo[1′,2′:3,4]pyrimido[2,1-*b*]pyrimidine-6-one 3b as a white solid (61 mg, 2.81×10^{-4} mol, 5.2%). A further small quantity of the product was recovered from the acetone washings. After removal of the acetone *in vacuo*, the residue was dissolved in methanol and passed through activated charcoal to afford 4,5-dihydro-5-hydroxy-8-methylpyrrolo[1′,2′:3,4]pyrimido[2,1-*b*]pyrimidine-6-one 3b (16.0 mg, 7.37×10^{-5} mol, 1.4%).

^{1}H NMR (200 MHz, Me_2SO-d_6): δ = 2.30 (*d*, *J* 0.6 Hz, 3H, Me), 3.23 (br, 2H, CH_2), 6.21 (*q*, *J* 0.8 Hz, 1H, 7-H), 6.24 (*dd*, $J_{2\text{-H},3\text{-H}}$ 3.0 and $J_{1\text{-H},3\text{-H}}$ 1.4 Hz, 1H, 3-H), 6.40 (*t*, $J_{2\text{-H},3\text{-H}}$ 3.2 and $J_{1\text{-H},2\text{-H}}$ 3.2 Hz, 1H, 2-H), 6.52 (*br*, 1H, C*H*OH), 7.16* (*br*, 1H, CHOH), 7.56 (*dd*, $J_{1\text{-H},3\text{-H}}$ 1.5 and $J_{1\text{-H},2\text{-H}}$ 3.2 Hz, 1H,1-H). *This signal disappears on shaking with D_2O. ^{13}C NMR (126 MHz, Me_2SO-d_6): δ = 23.59 (*q*, Me), 28.56 (*t*, CH_2), 70.77 (*d*, CHOH), 107.55, 109.73, 112.59, 116.72 (all *d*, 1-C, 2-C, 3-C, and 7-C), 125.99, 144.80 (both *s*, 3a-C and 8-C), 160.22, 163.53 (both *s*, 9a-C and 6-C). EI-MS: *m/z* (%) = 217 (6, M^+), 199 (100, M^+-H_2O), 170 (41), 143 (25), 118 (15), 109 (30), 80.(40), 69 (17); found 217.0854 (calcd for $C_{11}H_{11}N_3O_2$: 217.0851). λ_{max} (MeOH) 231 nm (log ε = 3.99), 247 (log ε = 4.00), 298 (log ε = 4.21). ν_{max} (KBr disk) 471, 532, 610, 637, 698, 739, 808, 833, 868, 912, 1070, 1111, 1138, 1190, 1281,

1314, 1337, 1372, 1393, 1428, 1491, 1528, 1653, 3137, 3450 (*br*) cm^{-1}.

Reaction of (Z,Z)-Muconaldehyde with 2,3,5-Tri-O-acetylguanosine to Afford 4,5-Dihydro-5-hydroxy-9-β-D-tri-O-acetylribosylpyrrolo[1',2':3,4]-pyrimido[1,2-a]purin-6(9H)-one 4a and 9-β-D-Tri-O-acetylribosylpyrrolo[1',2':3,4]-pyrimido[1,2-a]purin-6(9H)-one 5a. To a solution of 2,3,5,-tri-*O*-acetylguanosine (562 mg, 1.37×10^{-3} mol) in dimethylformamide was added a solution of (*Z,Z*)-muconaldehyde (151 mg, 1.37×10^{-3} mol) in dimethylformamide in one portion and a solution of pyridinium *p*-toluenesulfonate (35.2 mg, 1.40×10^{-4} mol) in dimethylformamide in one portion (total volume of dimethylformamide used, 33 ml). The reaction mixture was stirred under nitrogen, in the dark at 76°C for 5 days. The solvent was removed *in vacuo*. The residue was partially dissolved in methanol (125 ml) and filtered. The methanolic filtrate was evaporated onto silica gel 60 *in vacuo*, and subjected to medium pressure chromatography on silica gel 60 (elution with 1:1 ethyl acetate-acetone) to give the crude product mixture. This was rechromatographed on silica gel 60 (elution with 4:1 ethyl acetate–acetone), and the partially purified, separated products each passed through activated charcoal to afford 4,5-dihydro-5-hydroxy-9-β-D-tri-*O*-acetylribosylpyrrolo[1',2':3,4]-pyrimido[1,2-*a*]purin-6(9*H*)-one 4a (31.8 mg, 6.35×10^{-5} mol, 5%) and a small quantity of impure 9-β-D-tri-*O*-acetylribosylpyrrolo[1',2':3,4]-pyrimido[1,2-*a*]purin-6(9*H*)-one 5a.

4,5-Dihydro-5-hydroxy-9-β-D-tri-O-acetylribosylpyrrolo[1',2':3,4]-pyrimido[1,2-a]purin-6(9H)-one 4a.

^{1}H NMR (200 MHz, dimethylformamide-d_7): = 2.12 (*s*, 3H, 5'-$OCOCH_3$), 2.147 (s, 1.5H, 3'-OCOCH3), 2.152 (*s*, 1.5H, 3'-$OCOCH_3$), 2.176 (*s*, 1.5H, 2'-$OCOCH_3$), 2.186 (*s*, 1.5H, 2'-$OCOCH_3$), 3.32 (*br m*, 2H, 4-CH_2), 4.25–4.59 (complex multiplet, 3H, 5'-CH_2 and 4'-H), 5.85-5.97 (complex multiplet, 1H, 3'-H), 6.09–6.16 (complex miltiplet, 1H, 2'-H), 6.22 (*br m*, 1H, 3-H), 6.33–6.38 (*m*, 2H, 2-H and 1'-H), 6.79 (*m*, 1H, 5-H), 7.21–7.25 (*m*, 1H, OH), 7.80 (*dd*, $J_{1\text{-H},2\text{-H}}$ 3.1 and $J_{1\text{-H},3\text{-H}}$ 1.3 Hz, 0.5H, 1-H), 7.85 (*dd*, $J_{1\text{-H},2\text{-H}}$ 3.2 and $J_{1\text{-H},3\text{-H}}$ 1.3 Hz, 0.5H, 1-H), 8.30 (*br s*, 1H, 8-H). EI-MS: *m/z* (%) = 501 (1.4, M^+), 483 (6.4, M^+-H_2O), 441 (0.7, M^+–AcOH), 259 (13, [tri-*O*-AcRib]$^+$), 225 (20, [M-H_2O-tri-*O*-AcRib+H)]$^+$), 139 (35), 115 (10), 97 (20), 80 (100), 62 (13), 50 (98); found 501.1541 (calculated for $C_{22}H_{23}N_5O_9$: 501.1496). TLC [silica gel 60 (4:1 ethyl acetate–acetone)]: R_f = 0.30.

9-β-D-Tri-O-acetylribosylpyrrolo[1',2':3,4]-pyrimido[1,2-a]purin-6(9H)-one 5a.

^{1}H NMR (200 MHz, dimethylformamide-d_7): δ = 1.90 (*s*, 3H, 5'-$OCOCH_3$), 2.09 (*s*, 3H, 3'-$OCOCH_3$), 2.14 (*s*, 3H, 2'-$OCOCH_3$), 4.27 (*m*, 1H, 4'-H), 4.40–4.50 (*m*, 2H, 5'-CH_2), 5.86 (*t*, *J* 5.9 Hz, 1H, 3'-H), 6.05 (*dd*, *J* 3.6 and *J* 5.9 Hz, 1H, 2'-H), 6.16 (*d*, *J* 3.8 Hz, 1H, 1'-H), 6.64 (*dd*, $J_{2\text{-H},3\text{-H}}$ 3.6 Hz, 1H, 3-H), 6.87 (*t*, $J_{1\text{-H},2\text{-H}}$ 3.2 and $J_{2\text{-H},3\text{-H}}$ 3.2 Hz, 1H, 2-H), 7.10 (*d*, $J_{4\text{-H},5\text{-H}}$ 8.0 Hz 1H, 4-H), 7.96 (*s*, 1H, 8-H), 8.18 (*d*, $J_{4\text{-H},5\text{-H}}$ 8.0 Hz, 1H, 5-H), 8.28 (*dd*, $J_{1\text{-H},2\text{-H}}$ 3.2 Hz, 1H, 1-H). EI-MS: *m/z* (%) = 483 (12, M^+), 279 (40), 225 (23, [M-tri-*O*-AcRib + H]$^+$), 139 (35), 113 (18), 97 (20), 83 (35), 77 (18), 69 (60), 55 (75), 43 (100), 32 (43); found 483.1444 (calculated for $C_{22}H_{21}N_5O_8$: 483.1390). TLC [silica gel 60 (4:1 ethyl acetate–acetone)]: R_f = 0.60.

Preparation of 4,5-Dihydro-5-hydroxy-9-β-D-ribosylpyrrolo[1',2':3,4]-pyrimido[1,2-a]purin-6(9H)-one 4c from 4,5-Dihydro-5-hydroxy-9-β-D-tri-O-acetylribosylpyrrolo[1',2':3,4]-pyrimido[1,2-a]purin-6(9H)-one 4a. A solution of 4,5-dihydro-5-hydroxy-9-β-D-tri-*O*-acetylribosylpyrrolo[1',2':3,4]-pyrimido[1,2-*a*]purin-6(9*H*)-one 4a (15.0 mg, 2.99×10^{-5} mol) in methanol (2.5 ml) was treated with a catalytic quantity of sodium methoxide (1.96 µl of a 2.29-M solution in methanol, 5 mol% per acetate function), and the mixture was stirred for 22 hr at room temperature. A further quantity of sodium methoxide (1.00 µl of a 2.29-M solution in methanol) was added, and the mixture stirred for 4 hr at room temperature. Excess of Dowex-50 X8 (H^+ form) was added and the mixture was stirred for 30 min at room temperature. The solvent was removed *in vacuo* from the filtered mixture. The residue was subjected to medium pressure chromatography on silica gel 60 (elution with 7:1 acetone–methanol) to give a fraction that was washed with a small volume of acetone to afford 4,5-dihydro-5-hydroxy-9-β-D-ribosylpyrrolo[1',2':3,4]-pyrimido[1,2-*a*]purin-6(9*H*)-one 4c as a white solid (9.8 mg, 2.61×10^{-5} mol, 87%).

^{1}H NMR (200 MHz, dimethylformamide-d_7): δ = 3.33 (*br m*, 2H, 4-CH_2), 3.81 (*br m*, 2H, 5'-CH_2), 4.12(*m*, 1H, 4'-H), 4.42 (*br*, 1H, 3'-H), 4.78 (*m*, *J* 5.5 Hz, 1H, 2'-H), 5.20 (*m*, 1H, 5'-OH), 5.37 (*br d*, 1H, 3'-OH), 5.74 (*br d*, 1H, 2'-OH), 6.06 (*d*, *J* 5.7 Hz, 1H, 1'-H), 6.22 (*m*, 1H, 3-H), 6.37 (*t*, $J_{2\text{-H},3\text{-H}}$ and $J_{1\text{-H},2\text{-H}}$ 3.3Hz, 1H, 2-H), 6.81 (*m*, 1H, 5-H), 7.21 (*d*, 1H, 5-OH), 7.64 (*m*, 1H, 1-H), 8.38 (*s*, 1H, 8-H). ^{13}C NMR (50 MHz, dimethylformamide-d_7): δ = 60.69 (5'-C), 69.73, 70.52, and 73.69 (2', 3', and 4'-C), 84.95 and 86.96 (1' and 5'-C), 108.09, 11.04, and 115.76 (1, 2, and 3-C), 124.89 (3a-C), 137.46 (8-C). FAB–MS: *m/z* (%) = 376 (30, MH^+), 361 (45), 307 (20), 289 (18), 244 (35, MH^+-Rib + H), 226 (20, MH^+-H_2O-Rib + H), 117 (17), 89 (22). λ_{max} (MeOH) 265 (log ε = 3.86), 298 (log ε = 3.94).

Preparation of 9-β-D-Ribosylpyrrolo[1',2':3,4]-pyrimido[1,2-a]purin-6(9H)-one 5b from 9-β-D-Tri-O-acetylribosylpyrrolo[1',2':3,4]-pyrimido[1,2-a]purin-6(9H)-one 5a. A solution of the 9-β-D-tri-*O*-acetylribosylpyrrolo[1',2':3,4]-pyrimido[1,2-*a*]purin-6(9*H*)-one 5a (1.5 mg, 3.1×10^{-6} mol) in methanol (0.50 ml) was treated with a catalytic quantity of sodium methoxide (21 µl of a 0.023-M solution in methanol, 5 mol% per acetate function), and the mixture was stirred for 48 hr at room temperature. A further quantity of sodium methoxide (21 µl of a 0.023-M solution in methanol) was added, and the mixture stirred for 4 hr at room temperature. The solvent was removed *in vacuo* and the residue subjected to medium pressure chromatography on silica gel 60 (elution with 7:1 acetone–methanol) to give the crude product. This was subjected to reverse-phase HPLC on 5 µ ODS (isocratic elution with 2:5 methanol–water) to afford 9-β-D-ribosylpyrrolo[1',2':3,4]-pyrimido-[1,2-*a*]purin-6(9*H*)-one 5b as a white solid (0.8 mg, 2.2×10^{-6} mol, 72%).

^{1}H NMR (300 MHz, dimethylformamide-d_7): δ = 3.84 (*dd*, J_{gem} 12.0 and $J_{4'\text{-H},5'\text{-H}}$ 3.8 Hz, 1H, 5'-CH_2), 3.92 (*dd*, J_{gem} 12.2 and $J_{4'\text{-H},5'\text{-H}}$ 3.6 Hz, 1H, 5'-CH_2), 4.18 (*m*, 1H, 4'-H), 4.50 (*t*, *J* 4.4 Hz, 1H, 3'-H), 4.81 (*t*, *J* 5.2 Hz, 1H, 2'-H), 4.96 (*m*, 1H, 5'-OH), 5.41 (*br*, 1H, 3'-OH), 5.67 (*br*, 1H, 2'-OH), 6.20 (*d*, $J_{1'\text{-H},2'\text{-H}}$ 5.5 Hz, 1H, 1'-H), 6.75 (*dd*, $J_{2\text{-H},3\text{-H}}$ 3.6 and $J_{1\text{-H},3\text{-H}}$ 1.4Hz, 1H, 3-H), 6.97 (*t*, $J_{2\text{-H},3\text{-H}}$ and $J_{1\text{-H},2\text{-H}}$ 3.4Hz, 1H, 2-H), 7.31 (*d*, $J_{4\text{-H},5\text{-H}}$ 8.0 Hz, 1H, 4-H), 8.22 (*dd*, $J_{1\text{-H},2\text{-H}}$ 3.0 and $J_{1\text{-H},3\text{-H}}$ 1.3 Hz, 1H, 1-H), 8.28 (*d*, $J_{4\text{-H},5\text{-H}}$ 8.0 Hz, 1H, 5-H), 8.54 (*s*, 1H, 8-H). EI-MS: *m/z* (%) = 225 (15, M^+-rib+H), 143 (20), 91 (23), 80 (52), 69 (20), 50 (45), 44 (100), 32 (58, CH_3OH^+).

Reaction of (Z,Z)-Muconaldehyde with 3,5-Di-O-acetyl-2'-deoxyguanosine to Afford 4,5-Dihydro-5-hydroxy-9-β-D-di-O-acetyl-2-deoxyribosylpyrrolo-[1',2':3,4]-pyrimido[1,2-a]

purin-6(9H)-one 4b. To a solution of 3,5-di-*O*-acetyl-2′-deoxyguanosine (390 mg, 1.11×10^{-3} mol) in dimethylformamide was added a solution of (*Z,Z*)-muconaldehyde (123. mg 1.12×10^{-3} mol) in dimethylformamide in one portion and a solution of pyridinium *p*-toluenesulfonate (28.0 mg, 1.11×10^{-4} mol) in dimethylformamide in one portion (total volume dimethylformamide used = 27 ml). The reaction mixture was stirred under nitrogen in the dark at 80°C for 5 days. The solvent was removed *in vacuo*, the residue partially dissolved in methanol (100 ml), and filtered. The methanolic filtrate was evaporated onto silica gel 60 *in vacuo*, and subjected to medium pressure chromatography on silica gel 60 (elution with acetone) to yield the crude product. This was rechromatographed on silica gel 60 (elution with 1:1 ethyl acetate–acetone) to afford 4,5-dihydro-5-hydroxy-9-β-D-di-*O*-acetyl-2′-deoxyribosylpyrrolo[1′,2′:3,4]-pyrimido[1,2-*a*]purin-6(9*H*)-one 4b (15.6 mg, 3.52×10^{-5} mol, 3%).

^{1}H NMR (200 MHz, dimethylformamide-d_7): δ = 2.03 (s, 1.5H, 5′-$OCOCH_3$), 2.07 (*s*, 1.5H, 5′-$OCOCH_3$), 2.13 (*m*,2H, 2′-CH_2), 2.15 (*s*, 1.5H, 3′-$OCOCH_3$), 2.18 (*s*,1.5H, 3′-$OCOCH_3$), 3.33 (*br m*, 2H, 4-CH_2), 4.08–4.27 (complex multiplet, 3H, 5′-CH2 and 4′-H), 5.61–5.69 (complex multiplet, 1H, 3′-H), 6.24 (*br m*, 1H, 3-H), 6.34–6.42 (complex multiplet, 1H, 2-H), 6.52 (*br t*, *J* 7.0 Hz, 1H, 1′-H), 6.82 (*br m*, 1H, 5-H), 7.25 (*br d*, *J* 4.9 Hz, 1H, OH), 7.72 (*dd*, $J_{1\text{-H,2-H}}$ 3.1 and $J_{1\text{-H,3-H}}$ 1.5 Hz, 0.5H, 1-H), 7.76 (*dd*, $J_{1\text{-H,2-H}}$ 2.8 and $J_{1\text{-H,3-H}}$ 1.6 Hz, 0.5H, 1-H), 8.12 (*s*, 0.5H, 8-H), 8.14 (*s*, 0.5H, 8-H).

Preparation of 4,5-Dihydro-5-hydroxy-9-β-D-2′-deoxyribosylpyrrolo-[1′,2′:3,4]-pyrimido[1,2-a]purine-6(9H)-one 4d from 4,5-Dihydro-5-hydroxy-9-β-D-di-O-acetyl-2′-deoxyribosylpyrrolo[1′,2′:3,4]-pyrimido[1,2-a]purine-6(9H)-one 4b. A solution of 4,5-dihydro-5-hydroxy-9-β-D-di-*O*-acetyl-2′-deoxyribosylpyrrolo-[1′,2′:3,4]-pyrimido[1,2-*a*]purin-6(9*H*)-one 4b (7.0 mg, 1.58×10^{-5} mol) in methanol (1.25 ml) was treated with a catalytic quantity of sodium methoxide (1.00 μl of a 2.29-M solution in methanol, 7 mol% per acetate function) and the mixture was stirred for 24 hr at room temperature. A further quantity of sodium methoxide (0.50 μl of a 2.29-M solution in methanol) was added and the mixture stirred for 1 hr at room temperature. The methanolic solution was passed through a column of Dowex-50 X8 (H^+ form). The solvent was removed from the eluate *in vacuo* and the residue was subjected to medium pressure chromatography on silica gel 60 (elution with 12:1 acetone–methanol) to afford 4,5-dihydro-5-hydroxy-9-β-D-2′-deoxyribosylpyrrolo[1′,2′:3,4]-pyrimido[1,2-*a*]purin-6(9*H*)-one 4d as an off-white solid (3.6 mg, 1.00×10^{-5} mol, 63%).

^{1}H NMR (300 MHz, dimethylformamide-d_7): δ = 3.36 (*br m*,2H, 4-CH_2), 3.77 (*br d*, J 11.7 Hz, 1H, 5′-CH_2), 3.84 (*dd*, *J* 11.6 and 4.5 Hz, 1H, 5′-CH_2), 4.07 (*br m*, 1H, 4′-H), 4.70 (*br m*, 1H, 3′-H), 6.25 (*dd*, *J* 3.0 and 1.4 Hz, 1H, 3-H), 6.41 (*t*, $J_{2\text{-H,3-H}}$ 3.2 and $J_{1\text{-H,2-H}}$ 3.2 Hz, 1H, 2-H), 6.50 (*br t*, *J* 6.8 Hz, 1H, 1′-H), 6.84 (*br t*, *J* 2.7 Hz, 1H, 5-H), 7.10–7.50 (*br*, 1H, 5-OH), 7.68 (*br m*, 1H, 1-H), 8.39 (*s*, 1H, 8-H). FAB-MS: *m/z* (%) = 360 (8, MH^+), 329 (4, MH^+-CH_2OH), 244 (14, MH^+-dRib+H), 226 (6, MH^+-H_2O-dRib+H). λ_{max} (MeOH) 265 (log ε = 3.72), 297 (log ε = 3.78).

Reaction of (Z,Z)-Muconaldehyde with Adenine to Afford 7a/7b. To a solution of adenine (189 mg, 1.39×10^{-3} mol) in dimethylformamide (adenine was dissolved by warming in dimethylformamide) was added a solution of pyridinium *p*-toluenesulfonate (41.0 mg, 1.63×10^{-4} mol) in dimethylformamide in one portion, and a solution of (*Z,Z*)-muconaldehyde (168 mg, 1.53×10^{-3} mol) in dimethylformamide (total volume of dimethylformamide used, 33 ml). The reaction mixture was stirred under nitrogen, in the dark at 80°C for 3 days. The solvent was removed *in vacuo*, the residue was partially dissolved in methanol (80 ml) and filtered. The methanolic filtrate was evaporated onto silica gel 60 *in vacuo*, and subjected to medium-pressure chromatography on silica gel 60 (elution with 47:3 acetone–methanol) to yield the crude product. This was rechromatographed on silica gel 60 (elution with 100% acetone), and finally on silica gel 60 (elution with 4:1 diethyl ether–methanol) to afford 7a or 7b (7.2 mg, 3.17×10^{-5} mol, 2.3%).

^{1}H NMR (200 MHz, Me_2SO-d_6): δ = 3.48 (*br d*, *J* 15.1 Hz, 1H, CH_2), 3.64 (*dd*, *J* 5.3 and 15.1 Hz, 1H, CH_2), 6.27 (*br d*, *J* 4.0 Hz, 1H, 5-H), 6.42–6.46 (complex miltiplet, *J* 3.2 Hz, 2H, 3-H and 2-H), 7.35 (*br*, 1H, CHOH), 7.93 (*dd*, *J* 2.0 and *J* 3.1 Hz, 1H,1-H), 8.81 and 8.89 (both *s*, both 1H, 6-H and 9-H). FAB-MS: *m/z* (%) = 288 (100, MH^+). λ_{max} (MeOH) 295 (log ε = 3.93).

Reaction of Guanosine with (Z,Z)-Muconaldehyde in pH 7 Buffer. To a partial solution of guanosine monohydrate (27.3 mg, 9.06×10^{-5} mol) in sodium phosphate buffer (0.1 M, pH 7) (2.17 ml) was added (*Z,Z*)-muconaldehyde (9.9 mg, 9.00×10^{-5} mol). The mixture was stirred at 35 to 40°C in the dark, and the reaction was analyzed periodically by HPLC for 8 days.

Reaction of 2′-Deoxyguanosine with (Z,Z)-Muconaldehyde in pH 7 Buffer. To a partial solution of 2′-deoxyguanosine monohydrate (28.4 mg, 1.00×10^{-4} mol) in sodium phosphate buffer (0.1 M, pH 7) (2.43 ml) was added (*Z,Z*)-muconaldehyde (11.1 mg, 1.01×10^{-4} mol). The mixture was stirred at 35 to 40°C in the dark, and the reaction was analyzed periodically by HPLC for 7 days.

Reaction of Adenosine with (Z,Z)-Muconaldehyde in pH 5.9 Buffer. To a partial solution of adenosine (27.0 mg, 1.01×10^{-4} mol) in sodium phosphate buffer (0.1 M, pH 5.9) (2.45 ml) was added (*Z,Z*)-muconaldehyde (11.2 mg, 1.02×10^{-4} mol). The mixture was stirred at 35 to 40°C in the dark for 11 days. A sample of the reaction mixture was then heated at 70°C for 3 days. The reaction was analyzed periodically by HPLC throughout the 14 days.

Reaction of 2′-Deoxyadenosine with (Z,Z)-Muconaldehyde in pH 5.9 Buffer. To a partial solution of 2′-deoxygadenosine (23.8 mg, 8.84×10^{-5} mol) in sodium phosphate buffer (0.1 M, pH 5.9) (2.12 ml) was added (*Z,Z*)-muconaldehyde (9.7 mg, 8.81×10^{-5} mol). The mixture was stirred at 35 to 40°C in the dark, and the reaction was analyzed periodically by HPLC for 8 days.

Reaction of Guanosine with (Z,Z)-Muconaldehyde in Aqueous Solution Containing 10 mol% Pyridinium p-Toluenesulfonate. To a partial solution of guanosine monohydrate (55.7 mg, 1.85×10^{-4} mol) in a solution of pyridinium *p*-toluenesulfonate (4.36 ml of a 4.22-mM solution in water, 1.84×10^{-5} mol) was added (*Z,Z*)-muconaldehyde (20.2 mg, 1.84×10^{-4} mol). The mixture was stirred at 35 to 40°C in the dark, and the reaction was analyzed periodically by HPLC for 9 days.

Reaction of Guanosine with (Z,Z)-Muconaldehyde in Water. To a partial solution of guanosine monohydrate (52.5 mg, 1.74×10^{-4} mol) in water (4.10 ml) was added (*Z,Z*)-muconaldehyde (19.0 mg, 1.73×10^{-4} mol). The mixture was stirred at 35 to 40°C in the dark, and the reaction was analyzed periodically by HPLC for 9 days.

Reaction of 2′-Deoxyadenosine with (Z,Z)-Muconaldehyde in Aqueous Solution Containing 10 mol% Pyridinium p-Toluenesulfonate. To a partial solution of 2′-deoxyadenosine monohydrate (36.2 mg, 1.34×10^{-4} mol) in a solution of pyridinium *p*-toluenesulfonate (3.24 ml of a 4.22-mM solution in water, 1.36×10^{-5} mol) was added (*Z,Z*)-muconaldehyde (14.8 mg, 1.35×10^{-4} mol). The mixture was stirred at 35 to 40°C in the dark, and the reaction was analyzed periodically by HPLC for 5 days.

Results

Stability of (*Z,Z*)-Muconaldehyde in Solution and Conversion into (*E,Z*)- and (*E,E*)-Muconaldehyde

In variety of solvents, (*Z,Z*)-muconaldehyde undergoes a remarkable, highly diastereoselective isomerization to (*E,Z*)-muconaldehyde, which suffers either acid- or base-catalyzed conversion into the thermodynamically stable end product, (*E,E*)-muconaldehyde (*9*). Kinetic data for isomerizations of (*Z,Z*)-muconaldehyde into (*E,Z*)-muconaldehyde in benzene, acetonitrile, and dimethylsulfoxide were obtained by monitoring reactions by ^{1}H NMR (Table 1).

The similarity in rate of isomerization for a range of solvent polarity suggests a thermally allowed electrocyclic process, as shown in Scheme 2, in which 2-formyl-2*H*-pyran interconnects (*Z,Z*)- and (*E,Z*)-muconaldehyde (*3,9*). Analogous reactions have been described, for example, the ring-opening of 2-alkenyl-2*H*-pyrans at room temperature (11). The reaction in the direction 2-formyl-2*H*-pyran → (*Z,Z*)- or (*E,Z*)-muconaldehyde can be analyzed by considering the interaction of HOMO diene with LUMO sigma (O/C-2 bond). Hence, a disrotatory opening of this *sigma* bond must occur, which gives (*Z,Z*)-muconaldehyde if the hydrogen at C-2 moves outward (and the formyl group moves inwards), or (*E,Z*)-muconaldehyde if the formyl group moves outward. Using the data in Table 1, the following activation parameters were calculated for the reaction in acetonitrile-d_3: E_a 89 ± 5 *kJ* mol^{-1} and ΔS‡ −99 ± 5 *J* mol^{-1} k^{-1}. The relatively large negative entropy of activation is consistent with the conversion of an acyclic precursor into a cyclic transition state.

Studies of the isomerization of (*Z,Z*)- and (*E,Z*)-muconaldehyde in water and methanol were complicated by the formation of hydrates in water and acetals in methanol. The major species formed could be diagnosed by ^{1}H NMR, e.g., 3 hr after dissolution of (*Z,Z*)-muconaldehyde in methanol-d_4, the major species were (*E,Z*)-muconaldehyde and an acetal (or hemiacetal) of (*E,Z*)-muconaldehyde. In protic solvents an alternative mechanism of isomerization of (*Z,Z*)- into (*E,Z*)-muconaldehyde needs to be considered (Scheme 3). This may explain the observation that conversion of (*Z,Z*)- into (*E,Z*)-muconaldehyde is faster at higher pH. The cyclization process shown certainly occurs in basic methanol because the the *cis*- and *trans*-isomers of 2-(2′-hydroxyethyl)-5-methoxy-2,5-dihydrofuran were isolated after borohydride reduction (*10*). Further information on the isomerizations of (*Z,Z*)- into (*E,Z*)- and (*E,E*)-muconaldehyde in water and aqueous buffers is given in the sections on reactions of (*Z,Z*)-muconaldehyde with nucleosides.

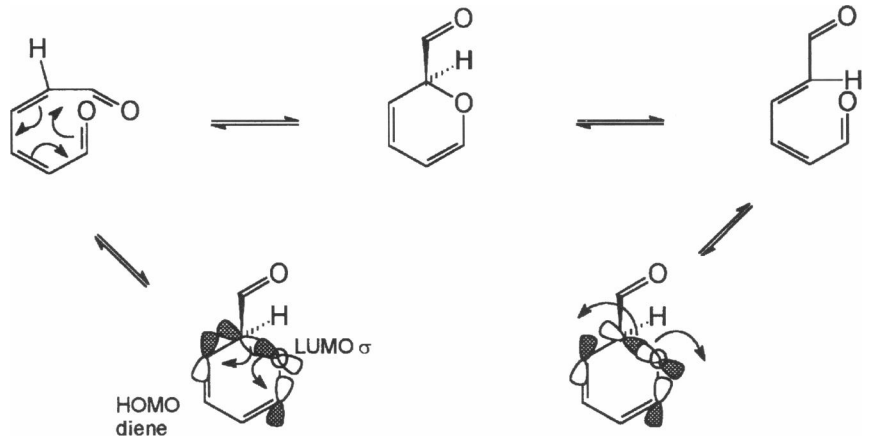
Reactions of (*Z,Z*)-Muconaldehyde with Simple Amines

We have previously reported (*10*) selected reactions of (*Z,Z*)-muconaldehyde with primary aliphatic and aromatic amines leading to pyrrole–aldehydes 2a, probably by the mechanism shown in Scheme 4. These reactions proceed within minutes at 20°C for a muconaldehyde concentration of ca. 0.04 M with 1 mol equivalent of amine. For amines of high nucleophilicity (e.g., propylamine), the yield of product is essentially quantitative in a variety of solvents (e.g., water, methanol, dichloromethane or acetonitrile). The pyrrole-aldehydes 2a are relatively unstable, showing a tendency to polymerize, probably by an intermolecular condensation between the aldehyde of one molecule and the free pyrrole α-position of another. They are best handled after reduction to the corresponding pyrrole-alcohol 2b with sodium borohydride. The reaction leading to a pyrrole-aldehyde also occurs, albeit less cleanly, with (*E,Z*)-muconaldehyde, but not with the (*E,E*)-isomer because the mechanism of pyrrole formation (Scheme 4) requires

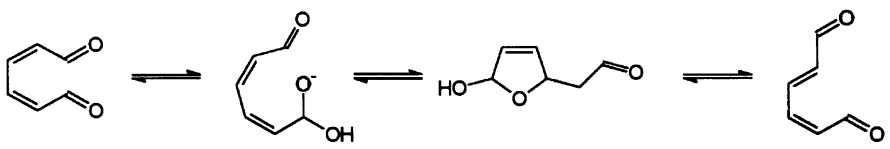
Table 1. Rate constants[a] for the isomerization of (*Z,Z*)-muconaldehyde into (*E,Z*)-muconaldehyde.

T/K	$k \times 10^7/s^{-1}$ (C_6D_6)	$k \times 10^7/s^{-1}$ (CD_3CN)	$k \times 10^7/s^{-1}$ [$(CD_3)_2SO$]
281	—	2.0 ± 0.05	—
289	—	7.9 ± 0.2	—
309	150 ± 4	73 ± 1.9	54 ± 1.4
328	1100 ± 28	630 ± 16	400 ± 10
342	2900 ± 73	1800 ± 45	1600 ± 40

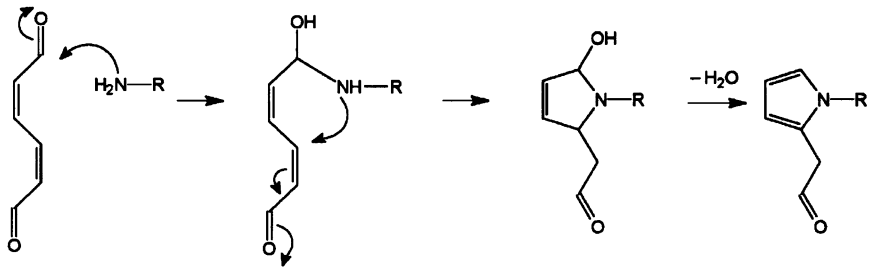
[a]Determined from the integral of the aldehyde resonances observed in ^{1}H NMR spectra. Typically, 30 to 35 mg of (*Z,Z*)-muconaldehyde was dissolved in 0.6 ml of the chosen solvent (except for benzene, in which the muconaldehyde was less soluble).



Scheme 2. Mechanism proposed for the conversion of (*Z,Z*)- into (*E,Z*)-muconaldehyde.



Scheme 3. Alternative mode (base-catalysed) of isomerization of (*Z,Z*)- to (*E,Z*)-muconaldehyde.



Scheme 4. Mechanism of formation of pyrrole–aldehydes from (*Z,Z*)-muconaldehyde and primary amines.

at least one Z double bond. (*E,E*)-Muconaldehyde yields a mono-imine and bis-imine product (with an excess of amine) (*10*).

Reactions of (*Z,Z*)-Muconaldehyde with Amino Acids and Peptides

We reported (*10*) that L-valine methyl ester and *N*-(benzyloxycarbonyl)-L-lysine methyl ester both react with (*Z,Z*)-muconaldehyde to afford pyrrole–alcohols 2c and 2d, respectively, after borohydride reduction of the initially formed pyrrole-aldehydes. In an improved procedure, L-valine methyl ester hydrochloride was treated with (*Z,Z*)-muconaldehyde in acetonitrile containing sodium carbonate for 15 min at 20°C. After addition of sodium borohydride in methanol and strirring for 15 min, a 64% yield of *N*-[(*S*)-1′-(methoxycarbonyl)-2′-methyl-*n*-propyl]-2-(2′-hydroxyethyl)pyrrole 2c was obtained. This procedure was not satisfactory for unprotected valine, but we found that the tetraethylammonium salt of L-valine, which is soluble in acetonitrile, gave 44% of (*S*)-2-[2-(2′-hydroxyethyl)pyrrol-1-yl]-3-methylpropanoic acid 2e on reaction with (*Z,Z*)-muconaldehyde (10 min at 20°C), followed by addition of sodium borohydride in methanol.

It is well established that N terminal valines in hemoglobin are a common site of modification by reactive electrophiles [e.g., epoxides (*12*)]. We propose to develop immunochemical procedures based on hemoglobin valine adducts (*13*) for dose-monitoring muconaldehyde. It should be possible to raise monoclonal antibodies specifically to a pyrrole adduct derived from (*Z,Z*)-muconaldehyde and the N terminal heptapeptide. The antibodies obtained can be used in immunoaffinity enrichment procedures (*14*) to concentrate a valine–pyrrole adduct from enzymic digests of hemoglobin, thus permitting quantification of the adduct by mass spectrometry. By analogy with the reaction we observed for (*Z,Z*)-muconaldehyde with valine (above), it is expected that (*Z,Z*)-muconaldehyde [and (*E,Z*)-muconaldehyde] will convert the amino groups of N terminal valines into pyrrole units. To help define this reaction, the α-chain N terminal heptapeptide NH_2-Val-Leu-Ser-Pro-Ala-Asp-Lys-OH (a product of the digestion of hemoglobin by trypsin) has been synthesized by automated synthesis. Reaction of this heptapeptide with (*Z,Z*)-muconaldehyde in methanol containing sodium carbonate (10 min at 20°C), followed by addition of sodium borohydride in methanol, gave an 85% yield of a 1:1 pyrrole adduct, which is subject to further investigation.

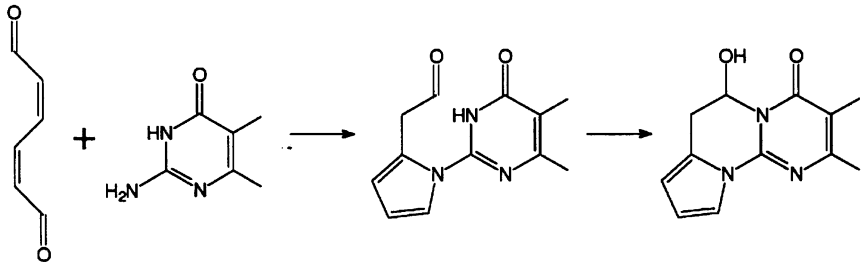
Reactions of (*Z,Z*)-Muconaldehyde with Nucleosides and Calf Thymus DNA

We have found that cyclic adducts are formed from the reaction of (*Z,Z*)-muconaldehyde with nucleosides and model compounds for nucleosides. With such compounds, the reaction is probably initiated by an interaction between an exocyclic amino group and (*Z,Z*)-muconaldehyde leading to a pyrrole-aldehyde, the aldehyde function of which is intramolecularly trapped by a neighboring nucleophilic nitrogen function (Scheme 5).

2-Amino-4-hydroxy-6-methylpyrimidine 3a was chosen as a model compound for the pyrimidine systems of guanosine and deoxyguanosine in a reaction with (*Z,Z*)-muconaldehyde. This reaction was performed in dimethylformamide using pyridinium *p*-toluenesulfonate as acid catalyst (10 mol%) and gave the adduct 4,5-dihydro-5-hydroxy-8-methylpyrrolo[1′,2′:3,4]pyrimido[2,1-b]pyrimidine-6-one 3b in 7% yield. The structure of 3b was rigorously established by X-ray analysis (Bleasdale et al., unpublished results). In connection with the acidic catalysis of the formation of 3b, we also observed that reactions of anilines with (*Z,Z*)-muconaldehyde are promoted by silica gel, and reactions of aliphatic amines with (*Z,Z*)-muconaldehyde are much faster in dichloromethane that has not been passed through basic alumina prior to use.

The protected nucleosides tri-*O*-acetylguanosine and di-*O*-acetyl-2′-deoxyguanosine were allowed to react at 80°C with (*Z,Z*)-muconaldehyde under the dimethylformamide/pyridinium *p*-toluenesulfonate conditions. The adducts 4,5-dihydro-5-hydroxy-9-β-D-tri-*O*-acetylribosylpyrrolo[1′,2′:3,4]-pyrimido[1,2-*a*]purin-6(9*H*)-one 4a and 4,5-dihydro-5-hydroxy-9-β-D-di-*O*-acetyl-2′-deoxyribosylpyrrolo-[1′,2′:3,4]-pyrimido[1,2-*a*]purin-6(9*H*)-on e 4b, respectively, were isolated from these reactions and purified by medium pressure chromatography. A small quantity of the dehydrated adduct 9-β-D-tri-*O*-acetylribosylpyrrolo[1′,2′:3,4]-pyrimido[1,2-*a*]purine-6(9*H*)-one 5a was isolated as a by-product from the tri-*O*-acetylguanosine reaction. Base-catalyzed methanolysis of adducts 4a and 4b gave the adducts 4,5-dihydro-5-hydroxy-9-β-D-ribosylpyrrolo-[1′,2′:3,4]-pyrimido[1,2-*a*]purin-6(9*H*)-on e 4c and 4,5-dihydro-5-hydroxy-9-β-D-2′-deoxyribosylpyrrolo[1′,2′:3,4]-pyrimido-[1,2-*a*]purin-6(9*H*)-one 4d, respectively, which were used as standards for monitoring reactions of unprotected nucleosides with (*Z,Z*)-muconaldehyde under aqueous conditions (below).

The assignment of the structures of the guanosine and deoxyguanosine adducts (4c



Scheme 5. Mechanism of formation of cyclic adducts from (*Z,Z*)-muconaldehyde and guanosine derivatives.

and 4d, respectively) was based primarily on comparison of their ^{1}H NMR spectra with the ^{1}H NMR of the 2-amino-4-hydroxy-6-methylpyrimidine adduct 3b. The ^{1}H NMR spectra of adducts 4a and 4b displayed duplication of proton resonances indicative of a mixture of diastereoisomers.

The structure of adduct 5a was determined by noting that the resonances for the 4-CH_2, 5-CHOH, and 5-CHOH of 4a were absent from its ^{1}H NMR, which exhibited an AB system at δ 7.10 and 8.18 (J 8.0 Hz). Furthermore, its electron impact mass spectrum showed a molecular ion at m/z 483. Adduct 5a is obviously derived by dehydration of 4a. It was deprotected to afford adduct 5b. The pyrimidine adduct 3b was dehydrated by heating in dimethylformamide and afforded a mixture of two compounds, one of which is believed to be the dehydrated species 6 by comparison of its ^{1}H NMR with 5a.

Adenine was reacted with (*Z,Z*)-muconaldehyde under similar conditions to those described for di-*O*-acetyl-2′-deoxyguanosine to give an adduct for which two alternative structures, 7a and 7b are possible. Structure 7b is preferred because an nOe was observed between the C*H*OH and the resonance at higher δ value (assumed to be 8-H by analogy with adenine). The adenine adduct 7a/7b gave a retention time of 53 to 54 min under the standard HPLC conditions.

The identification of adducts 4d and 7a/7b makes available reference standards for a study of reactions of (*Z,Z*)-muconaldehyde with oligonucleotides and DNA. It is expected that adduct 7a/7b will be spontaneously released from DNA as a consequence of reaction with (*Z,Z*)-muconaldehyde, whereas adduct 4d will require enzymic digestion of modified DNA to effect its release. Preliminary reactions of (*Z,Z*)-muconaldehyde with calf thymus DNA have been carried out. These involved the addition of a 10 × molar excess of (*Z,Z*)-muconaldehyde to a solution of DNA in Tris buffer at pH 7.0, with heating at 37°C for 8 hr. As yet no adducts have been isolated from these reactions.

Reaction of (*Z,Z*)-Muconaldehyde with Nucleosides under Aqueous Conditions

Reactions of (*Z,Z*)-muconaldehyde with guanosine, 2-deoxyguanosine, adenosine and 2′-deoxyadenosine under aqueous conditions were studied using adducts prepared by the dimethylformamide/pyridinium *p*-toluenesulfonate and base-catalyzed methanolysis chemistry (above) as HPLC standards. The reactions were carried out under both aqueous acidic and neutral conditions at 35 to 40°C. Reverse-phase HPLC of the reaction mixtures was performed with ultraviolet diode array detection of the eluted compounds, allowing "on-line" identification by comparison with the standards. The adducts were formed readily under acidic conditions for all nucleosides and near neutral pH for guanosine and 2′-deoxyguanosine. Deoxyadenosine showed ready formation of the adenine adduct 7a/7b at pH 6 and temperature of 35 to 40°C. However, adenosine required heating to give 7a/7b, presumably to induce depurination of an intact adduct, as expected on the basis of the relative depurination rates of adenosine and 2′-deoxyadenosine.

Reactions of (*Z,Z*)-Muconaldehyde with Guanosine and Deoxyguanosine under Aqueous Conditions

The reaction of guanosine with (*Z,Z*)-muconaldehyde was studied in pure water, in aqueous solution containing 10 mol% pyridinium *p*-toluenesulfonate, pH 3.5, and in 0.1 M, pH 7.0, sodium phosphate buffer. The guanosine adduct 4c appeared within 3 hr under the pH 3.5 conditions as judged by the retention time of the peak (a reference standard for the guanosine adduct 4c gave a retention time of 47–48 min under the HPLC conditions used) and its ultraviolet spectrum. After 24 hr there was no (*Z,Z*)-muconaldehyde remaining, with the most prevalent muconaldehyde isomer being the (*E,Z*) form, while after 5 days the (*E,E*)-isomer predominated. In pure water, in which the pH of the reaction mixture varied within the range 4.0 to 5.5, adduct 4c was also apparent within 3 hr. The predominant muconaldehyde isomer at 45 hr was (*E,Z*)-muconaldehyde. In pH 7 sodium phosphate buffer, adduct 4c was also apparent in the first few hours. The predominant muconaldehyde isomer after 48 hr was the (*E,E*)-isomer.

The reaction of 2′-deoxyguanosine with (*Z,Z*)-muconaldehyde in 0.1 M, pH 7.0, sodium phosphate buffer gave adduct 4d, behaving in a similar manner to the guanosine reaction under these conditions (adduct 4d gave a retention time of 52–53 min under the HPLC conditions used).

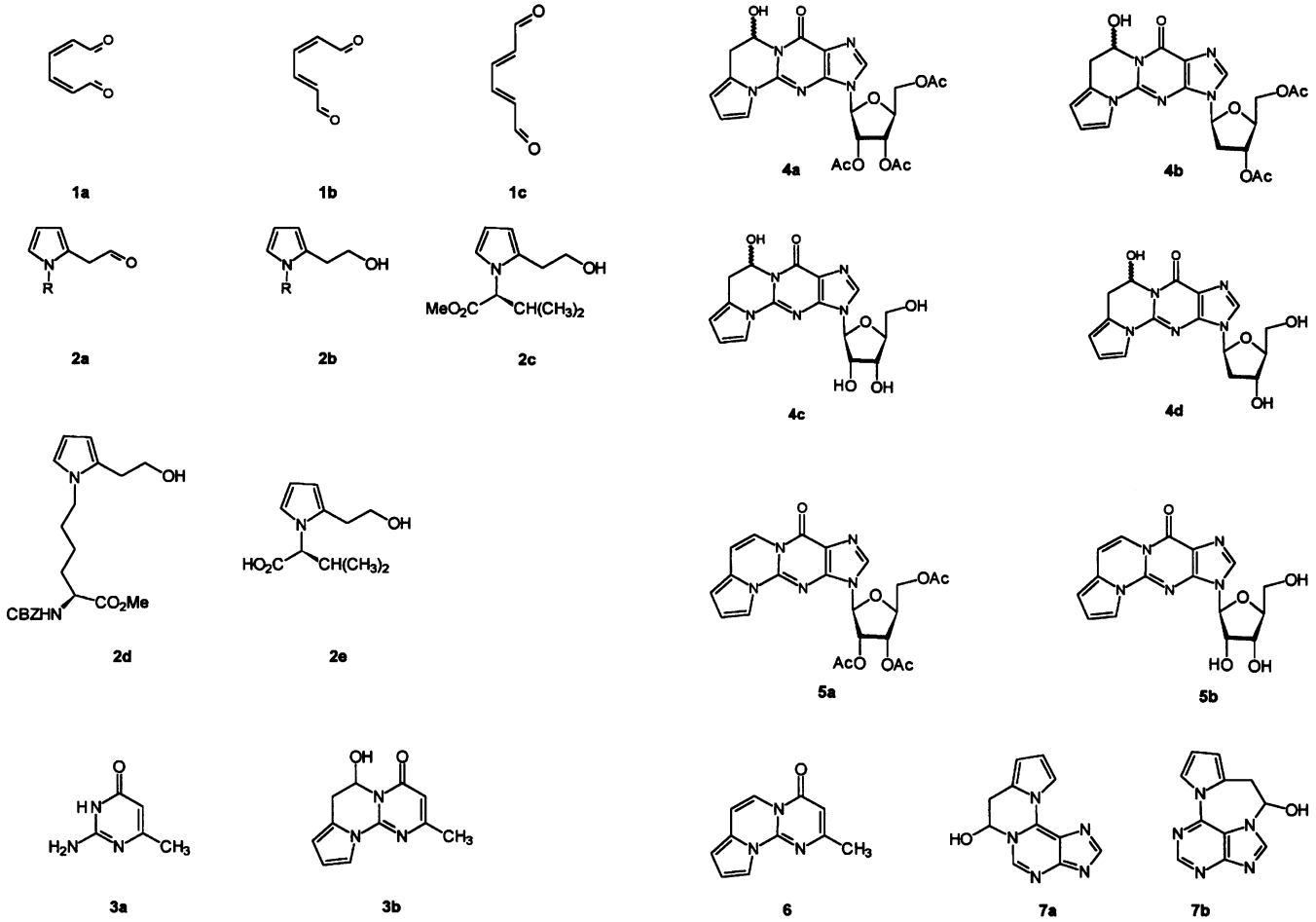
Reactions of (*Z,Z*)-Muconaldehyde with Adenosine and 2-Deoxyadenosine under Aqueous Conditions

The reaction of 2′-deoxyadenosine with (*Z,Z*)-muconaldehyde was studied in aqueous solution containing 10 mol% pyridinium *p*-toluenesulfonate, in 0.1 M, pH 6.0, sodium phosphate buffer, and in 0.1 M, pH 7.0, sodium phosphate buffer, while the reaction of adenosine with (*Z,Z*)-muconaldehyde was only performed in 0.1 M, pH 6.0, sodium phosphate buffer. 2′-Deoxyadenosine with (*Z,Z*)-muconaldehyde in aqueous solution containing 10 mol% pyridinium *p*-toluene sulfonate gave the adenine adduct 7a/7b (which was detected after 2.5 hr, and clearly evident after 24 hr). Another product (retention time of 43–44 min) was observed in this reaction mixture, but was not identified. When 2′-deoxyadenosine and (*Z,Z*)-muconaldehyde were incubated in 0.1 M, pH 6.0, sodium phosphate buffer, the adduct 7a/7b was apparent after 18 hr. The unknown product at 43 to 44 min, observed for the reaction with 10 mol% pyridinium *p*-toluenesulfonate, was also apparent. Incubation of 2-deoxyadenosine with (*Z,Z*)-muconaldehyde in 0.1 M, pH 7.0, sodium phosphate buffer did not give the adduct 7a/7b. Adenosine with (*Z,Z*)-muconaldehyde at pH 6 did not appear to give the adenine adduct 7a/7b after 1 week at 35 to 40°C. A sample of the reaction mixture was then heated to 70°C and produced adduct 7a/7b.

Discussion

In aqueous solution, muconaldehydes exhibit a complex behavior in which isomerization of the isomers competes with hydration reactions. In the presence of a purine nucleoside, (*Z,Z*)-muconaldehyde affords a tetracyclic adduct containing a pyrrole ring derived from the muconaldehyde (4d from 2-deoxyguanosine; 7a/7b from 2′-deoxyadenosine). The structures of these adducts and the mechanisms of their formation (Schemes 4,5) have been inferred from studies of reactions of (*Z,Z*)-muconaldehyde with simple amines and model compounds for nucleosides. If these kinds of adduct are formed in DNA, Watson-Crick hydrogen bonding sites would be blocked. The pyrrole adducts 4d and 7a/7b are structurally analogous to known mutagenic cyclic adducts, e.g., the etheno adducts implicated in cancer caused by exposure to vinyl chloride (*15,16*) and ethyl carbamate (*17*). However, assessment of the cancer risks from muconaldehydes and the relevance to benzene toxicology must await the results of further experiments.

Appendix



Structures 1a–3b. Structures of muconaldehyde isomers (1a–1c), 2-amino-4-hydroxy-6-methylpyrimidine (3a) and pyrrole adducts (2a–2e, and 3b) derived from (*Z,Z*)-muconaldehyde with an amine or aminoacid.

Structures 4a–7b. Structures of pyrrole adducts (4a–4d, 5a, 5b, 6, 7a, and 7b) derived from (*Z,Z*)-muconaldehyde with a nucleoside or related compound.

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