Reaction of Malonaldehyde with Nucleic Acid. IV.[1,2] Formation of Pyrimido[1,2-*a*]purin-10(3*H*)-one Nucleoside by Thermal Decomposition of Diastereomers Containing Oxadiazabicyclononene Residues Linked to Guanosine

Hiroshi SETO,* Takako SETO, Tomoko OHKUBO and Ikue SAITOH

Tokyo Metropolitan Research Laboratory of Public Health, Hyakunincho 3-24-1, Shinjuku-ku, Tokyo 169, Japan. *Received May 11, 1990*

The reaction of 1,1,3,3-tetraethoxypropane with guanosine under strongly acidic conditions resulted in the formation of diastereomeric oxadiazabicyclononene residues linked to guanine base. The diastereomers were decomposed by heat, giving changed pyrimido[1,2-*a*]purin-10(3*H*)-one nucleoside (3) in a good yield. A convenient method for the preparation of 3, which includes the thermal decomposition process of the diastereomers, was developed.

Keywords malonaldehyde; guanosine; pyrimidopurine; 1,1,3,3-tetraethoxypropane; lipid peroxidation; diastereomeric adduct; thermal decomposition

Malonaldehyde (**1**), a product of lipid peroxidation and prostaglandin biosynthesis,[3] is not only mutagenic[4] but also carcinogenic.[5] Exposure of humans to **1** may be considerable because of the abundance of **1** as a product of normal metabolism and in a variety of foodstuffs. Malonaldehyde is reactive toward amino groups of proteins[6] and nucleic acids,[7] even *in vivo*. Fluorescent adducts, pyrimido[1,2-*a*]purin-10(3*H*)-one homologues, have been separated from the reaction mixture of **1** with guanosine (**2**)[8] or RNA[9] under mild conditions (pH 4.5). The adduct, pyrimido[1,2-*a*]purin-10(3*H*)-one nucleoside (**3**), may have some biological activities. The yield of this adduct was very low (1.4%)[8]; however, the amount of the compound preparable was insufficient for pharmacological and biological tests. The reaction conditions were investigated in order to improve the yield, and the diastereomeric products (**4a**, **4b**) by the reaction of **2** with 1,1,3,3-tetraethoxypropane as a generator of **1** were obtained. Each adduct, **4a** and **4b** was identical with 5,6,7,8-tetrahydro-11-formyl-3(β-D-ribofuranosyl)-6,8-epoxyethenopyrimido[1,2-*a*]purin-10(3*H*)-one which was reported by Marnett *et al.*[10] Both **4a** and **4b** are decomposed by heat, yielding **3** in a good yield. This is a simple and effective method for the preparation of **3**.

This paper describes the preparation of the diastereomers (**4a**, **4b**) and the straightforward preparation of **3** through the thermal decomposition process of **4a** and **4b**.

Experimental

Apparatus Melting points are uncorrected. Optical rotations were measured with a JASCO DIP 181 polarimeter. IR, UV and CD spectra were recorded on Perkin-Elmer 1640, Shimadzu UV 240 and JASCO J-40A spectrophotometers, respectively. NMR spectra were obtained on a JEOL FX-270 spectrometer with 1,4-dioxane as an internal standard in D_2O or with TMS in DMSO-d_6 at room temperature. FAB mass spectra (glycerol matrix) were recorded on a JEOL DX 303 mass spectrometer. Analytical HPLC was carried out with a JASCO 880PU equipped with a three-dimensional detector (Hewlett-Packard HP 1040M) on a Nucleosil 7C18 (Nagel, 4.6 i.d. × 250 mm) column (the mobile phase was 7% (v/v) acetonitrile/water). Preparative HPLC was carried out with a Milton Roy Constametric III pump equipped with a UV detector (Oyobunko Kiki Uvilog II, cell length 1 mm).

Synthesis and Isolation of 3, 4a and 4b All reagents were purchased from commercial sources. Guanosine (**2**) (5.56 g, 0.02 mol) was dissolved in 100 ml of 0.05 M HCl at 50 °C. 1,1,3,3-Tetraethoxypropane (11.0 g, 0.05 mol) was added to the guanosine solution. The reaction mixture was kept at 50 °C with stirring for 2 h, then concentrated to about 50 ml at 35 °C. It was subjected to polyamide column chromatography (Wako Pure Chemicals C-200, 50 i.d. × 350 mm) and eluted with 500 ml of water. The eluate was concentrated to about 50 ml. The solution was injected little by little to a Lichroprep RP-18 (Merck, 22 i.d. × 300 mm) HPLC column and chromatographed with 7% (v/v) acetonitrile/water as the eluent. The fractions of **3**, **4a** and **4b** were collected and concentrated to about 50 ml. The solution was applied little by little to an Inertsil ODS-2 column (Gasukuro Kogyo, 5 μm, 7.6 i.d. × 250 mm, 3% (v/v) acetonitrile/water). The final chromatography was carried out repeatedly more than 500 times. Each product solution was evaporated to dryness *in vacuo*.

Compound **3** (p3): mp > 195 °C (dec.). UV $\lambda_{max}^{H_2O}$ nm (ε): 250 (13000), 308 (2770), 319 (3100), 348 (2700). EI MS *m/z*: 535 ($C_{13}H_{10}N_5O_5(TMS)_3$ M^+). ^{1}H-NMR (D_2O) δ: 3.89 (2H), 4.24 (1H), 4.45 (1H), 4.76 (1H), 6.02 (1H, d, *J* = 5.3 Hz), 7.34 (1H, dd, *J* = 4.0, 7.30 Hz), 8.31 (1H), 8.98 (1H, dd, *J* = 2.1, 3.8 Hz), 9.22 (1H, dd, *J* = 2.0, 7.3 Hz). Yield, 35 mg.

Compound **4a** (p1): mp > 160 °C (dec.); $[\alpha]_D$ −140° (*c* = 0.4, water). UV $\lambda_{max}^{H_2O}$ nm (ε): 249 (20100). IR (KBr): 3329, 1700, 1620, 1560 cm^{-1}. High-resolution FAB MS: Calcd for $C_{16}H_{18}N_5O_7$ ($M+H^+$) *m/z*: 392.1206. Found *m/z*: 392.1185. CD (*c* = 0.11 mM, water) $[\theta]^{22}$ (nm): 0 (310), −45500 (282), 0 (269), +116000 (256), 0 (246), −102000 (237), 0 (219). ^{1}H-NMR (D_2O) δ: 2.14 (1H, d, *J* = 14.5 Hz), 2.28 (1H, d, *J* = 14.5 Hz), 3.81 (2H, m), 4.17 (1H, m), 4.39 (1H, m), 4.70 (1H, m), 5.87 (1H, d, *J* = 5.9 Hz), 5.97 (1H, m), 6.15 (1H, m), 7.78 (1H, s), 7.97 (1H, s), 9.15 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 23.5 (t), 33.6 (d), 61.2 (t), 70.3 (d), 73.4 (d), 76.2 (d), 85.1 (d), 86.2 (d), 117.0 (s), 119.8 (s), 136.3 (d), 148.5 (s), 148.6 (s), 153.8 (s), 163.7 (d), 188.2 (d). Yield, 200 mg.

Compound **4b** (p2): mp > 160 °C (dec.); $[\alpha]_D$ +111° (*c* = 0.4, water). UV $\lambda_{max}^{H_2O}$ nm (ε): 249 (19800). IR (KBr): 3287, 1701, 1618, 1561 cm^{-1}. High-resolution FAB MS Calcd for $C_{16}H_{18}N_5O_7$ ($M+H^+$) *m/z*: 392.1206. Found *m/z*: 392.1190. CD (*c* = 0.11 mM, water) $[\theta]^{22}$ (nm): 0 (310), +43200 (282), 0 (269), −118000 (256), 0 (246), +114000 (237), 0 (219). ^{1}H-NMR (D_2O) δ: 2.14 (1H, d, *J* = 14.5 Hz), 2.27 (1H, d, *J* = 14.5 Hz), 3.80 (2H, m), 4.18 (1H, m), 4.37 (1H, m), 4.67 (1H, m), 5.85 (1H, d, *J* = 5.6 Hz), 5.97 (1H, m), 6.14 (1H, m), 7.76 (1H, s), 7.97 (1H, s), 9.14 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 23.6 (t), 33.6 (d), 61.3 (t), 70.3 (d), 73.8 (d), 76.2 (d), 85.2 (d), 86.3 (d), 116.9 (s), 119.8 (s), 136.1 (d), 148.5 (s), 148.7 (s), 153.8 (s), 163.7 (d), 188.2 (d). Yield, 200 mg.

Reaction of Malonaldehyde with Pyrimido[1,2-*a*]purin-10(3*H*)-one Nucleoside (3) Malonaldehyde sodium salt[11] (1 mg) and **3** (1 mg) were dissolved in 0.2 ml of 0.05 M HCl. The reaction mixture was maintained for 1 h at 50 °C. The mixture was neutralized by 0.05 M NaOH. Product analysis of the mixture was carried out by the HPLC method. The product (**4a**, **4b**) was identified by both the UV spectrum and the retention time.

Thermal Decomposition of 4a and 4b A mixture of the diastereomers (**4a**, 5 mg and **4b**, 5 mg) in a glass test tube was heated at 160 °C in an oil bath for 1 h. The product was dissolved in water (1 ml). An aliquot of the solution was chromatographed repeatedly on the Inertsil ODS-2 column with 5% (v/v) acetonitrile/water as the eluent. The fractions including **3** were collected and dried *in vacuo*. Yield, 5 mg (60%). The UV spectrum obtained was identical to that of authentic **3**.

Simplified Method for Preparation of 3 from Guanosine The mixture of guanosine (**2**) (56 mg) and 1,1,3,3-tetraethoxypropane (110 mg) in 5 ml of 0.05 M HCl was stirred at 50 °C for 3 h. The solution was neutralized with 1 M NaOH. The neutral solution was applied to a polyamide gel column (20 i.d. × 300 mm, water as the eluent). The eluate (20—150 ml) was evaporated to dryness *in vacuo*. The mixture of dry materials in a flask was heated at 160 °C in an oil bath for 1 h. The product (**3**) was extracted

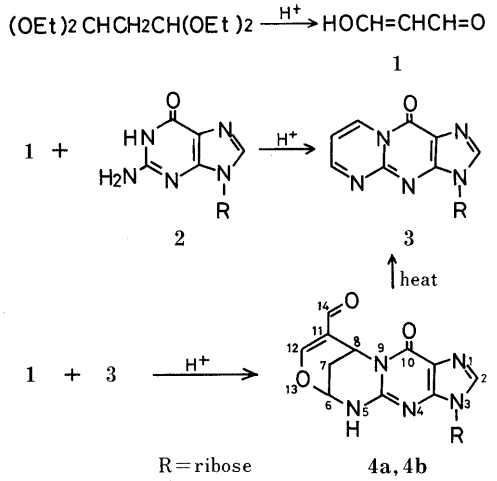

Chart 1

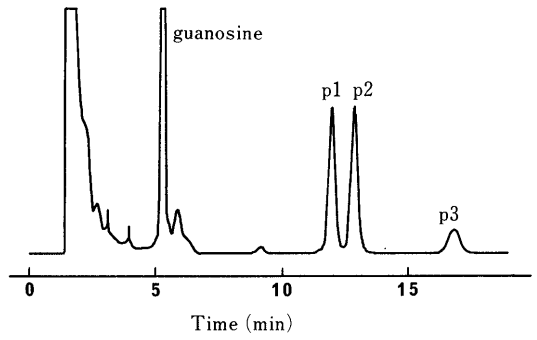

Fig. 1. HPLC Chromatogram of Guanosine–malonaldehyde Reaction Mixture

Column, Nucleosil 7C18 (4.6 i.d. ×250 mm); mobile phase, 7% (v/v) acetonitrile/water 1 ml/min; detector, 254 nm.

from the residue with 5 ml of hot water (60 °C). The extract was cleaned on a polyamide column (13 i.d. × 60 mm, water as the eluent). The eluate (0—20 ml) was concentrated to 5 ml. The solution was subjected to a Lichroprep RP-18 column and chromatographed with 7% (v/v) acetonitrile/water as the eluent (Fig. 2). The fractions of **3** (50—70 min) were collected and evaporated to dryness *in vacuo*. Yield 16 mg.

Results and Discussion

Preparation of the Diastereomers (4a and 4b) Figure 1 shows an HPLC chromatogram of a reaction mixture of guanosine with 1,1,3,3-tetraethoxypropane as a generator of malonaldehyde. The least polar adduct (p3) was identified as **3** by spectroscopic analyses. p1 and p2 showed a UV maximum at 249 nm. These adducts were separated by repeated chromatography. Though the formation of the compounds (p1, p2) proceeded only in strongly acidic media, the adduct (**3**) seems most likely to be formed *in vivo*. In fact, detection and identification of pyrimido[1,2-*a*]purin-10(3*H*)-one (aglycone of the adduct (**3**)) actually formed *in vivo* has been carried out by Draper's group.[12]

Adducts p1 and p2 were isolated as a white solid and 200 mg of each (purity 99%, HPLC) were obtained. They were not fluorescent and possessed UV, NMR, MS and CD spectral properties indicating the presence of oxadiazabicyclononene residues linked to guanosine, as reported by Marnett *et al.*[10] Each adduct, p1 (**4a**) and p2 (**4b**) was deduced to 5,6,7,8-tetrahydro-11-formyl-3(β-D-ribofuranosyl)-6,8-epoxyethenopyrimido[1,2-*a*]purin-10(3*H*)-one

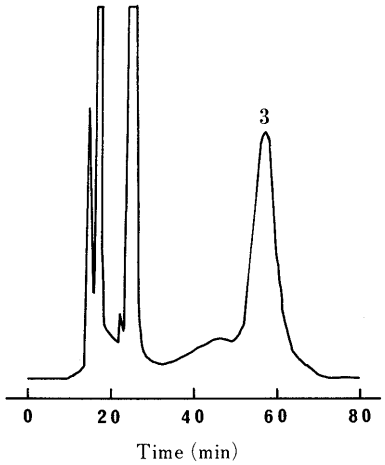

Fig. 2. Preparative HPLC Chromatogram of the Solution Containing **3**

Column, Lichroprep RP-18 (22 i.d. ×300 mm); mobile phase, 7% (v/v) acetonitrile/water 5 ml/min; detector, 254 nm. Details about the sample are described in the text.

(Chart 1). There were two isomers, namely (6*R*,8*R*)- and (6*S*,8*S*)-. Since spectroscopic details about p1 and p2 were not exhibited in a distinguishable form,[10] we listed the data in the experimental section. As shown in Chart 1, the isomers were formed by the addition of a second molecule of **1** to **3** and were easily cleaved by alkali (0.01 M NaOH) into **1** and **2** (data not shown).

Formation of Pyrimido[1,2-*a*]purin-10(3*H*)-one Nucleoside (3) by Thermal Decomposition of 4a and 4b Both **4a** and **4b** were decomposed by heat, yielding **3**. Heating of 10 mg of the isomers at 160 °C for 1 h gave **3** in a 60% yield. The product (**3**) was isolated (5 mg) by HPLC and identified by means of UV spectral comparison.

Based on these results, we proposed a convenient method which includes a thermal decomposition process of **4a** and **4b** for the preparation of **3**.

Preparation of 3 from 2 by the Proposed Method Including the Thermal Decomposition Process of 4a and 4b The product (**3**) was obtained in a 25% yield (16 mg of **3** from 56 mg of **2**) by use of the proposed method in this work (Fig. 2). When the preparation of **3** was carried out without the heating process, only 4 mg (6%) of product (**3**) was obtained. The heating process for the decomposition of **4a** and **4b** is necessary to improve the yield of **3**. In contrast, the reaction of **1** with **2** under a higher pH condition (pH 4.5) produced **3** in only a 1.4% yield.[8]

The rate of formation of 7-alkyl pyrimido[1,2-*a*]purin-10(3*H*)-one on the reaction of 2-alkyl malonaldehyde with guanine was high in a low pH medium.[13] However, as the pH lowered (0.05 M HCl) in the reaction of non-substituted malonaldehyde (**1**) with guanosine (**2**), formation of **4a** and **4b** seemed to be dominant compared to that of **3** (Fig. 1). Our results showed that **3** was the intermediate, and **4a** and **4b** the ultimate, products in the successive reaction. Fortunately, it is possible to obtain **3** in a good yield by use of the thermal decomposition process of **4a** and **4b**. In addition, it took 10 d for a reaction in the previous work.[8] Our proposed method requires only 3 h for the liquid phase reaction and 1 h for the heating process.

The proposed method, which includes the thermal decomposition process of **4a** and **4b**, is rapid and effective for the preparation of **3**.

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References and Notes

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