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Reaction of Malonaldehyde with Adenosine. Formation of a Novel Adduct Containing a Dioxazatricycloundecene Residue in the Base-pairing Region

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Reaction of malonaldehyde with adenosine at pH 4.5 gave three major adducts including a novel one containing diastereomeric dioxazatricycloundecene residue, formed by addition of three malonaldehyde units.

Malonaldehyde (1), a product of lipid peroxidation, is both mutagenic[1] and carcinogenic.[2] Studies of the reaction of 1 with nucleic acids are essential to elucidate the chemical basis for its biological activity. Our study indicates that guanine base is the most reactive to 1 among nucleic acid bases,[3] and the next most reactive base is adenine. Formation of several adducts as a result of the modification of adenine bases by 1 has been reported.[4-6] We describe here a new type of adduct formed by reaction of 1 with adenosine (2).

Malonaldehyde was prepared by hydrolysis of 1,1,3,3-tetraethoxypropane (40 g) with 0.1 M HCl (500 ml, 1 M = 1 mol dm^{-3}). The mixture was stirred at 37 °C for 30 min, then adjusted to pH 4.5. Adenosine (6 g) and potassium dihydrogenphosphate (12 g) were added to the solution of 1. The reaction mixture was kept at 37 °C for 48 h with stirring. Three major peaks of adducts in the reaction mixture were observed on an HPLC chromatogram (Fig. 1). The compounds were isolated by chromatographic technique (yield: 3; 88.2 mg, 4; 83.5 mg, and 5; 213 mg).

All the compounds were obtained as white powders (decomposition temp: 3 148 °C, 4 137 °C, and 5 144 °C) and their structures were determined by means of UV, IR, MS, and NMR examinations.

Compound 3 was identified as the adduct containing an enaminal moiety at the 6-position of the purine riboside (Fig. 2), as reported by Nair *et al.*[4] Compound 5 was identified as the adduct containing a diformyloxazabicyclononadiene residue at the 6-position of the purine riboside (Fig.2), as reported by Stone *et al.*[6] The previous identification[4,5] of this compound as the adduct containing a cyclopropyl ring was not supported.

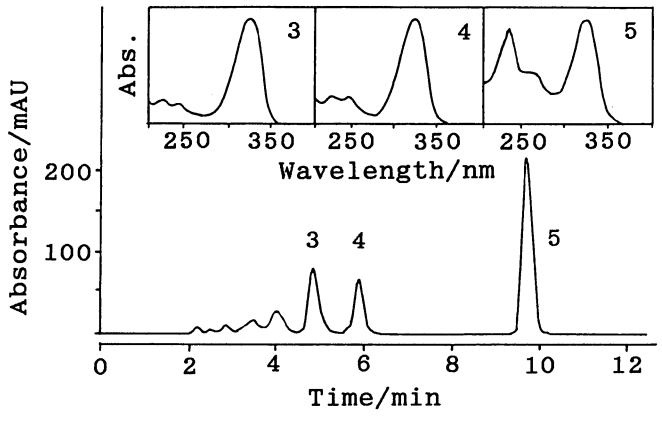

Fig. 1. HPLC profile and UV spectra.[9]

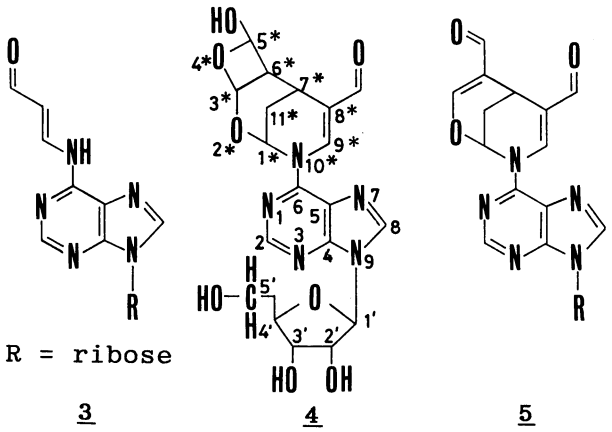

Fig. 2. Structures of the adducts.

The UV spectrum of peak 4 closely resembles that of peak 3, as shown in Fig. 1. The result suggested that 4 has an enaminal moiety in common with 3. The IR spectrum (KBr) of 4 showed absorption bands at 3386, 1673, 1632, 1573, and 1457 cm^{-1}. The FAB-MS spectrum of 4 showed $(M+H)^+$ at *m/z* 448. An important peak of the base was observed at *m/z* 316 (base + 2H)$^+$. The molecular formula of 4, $C_{19}H_{21}N_5O_8$, was established by high-resolution FAB-MS (found: m/z 448.1460. Calcd for $C_{19}H_{22}N_5O_8$: M+H, 448.1467). The EI mass spectrum was obtained after trimethylsilylation of 4 (M^+ *m/z* 735).

Some of the peaks in the ^{13}C NMR spectra of 4 were weakly split doublets (<0.3 ppm). Furthermore, the number of carbon signals was 27, whereas elemental analysis by high-resolution mass spectrometry indicated the presence of only 19 carbons in the molecule. The ^1H NMR spectrum showed 6 pairs of signals (3*-H, 6*-H, 7*-H, 9*-H, 11*-H, and 5*-OH) with very similar splitting patterns (Fig. 3) but no coupling between them was revealed by a ^1H-^1H COSY experiment. The ratios of integrated proton signals of the paired peaks were about 0.55:0.45 when ribose C_1'-H was taken as 1. These results suggested that the product 4 consisted of two major components (isomers) named 4a and 4b.

Two-dimensional (2D) NMR techniques were effective for the structural determination of 4. The C-H relation of the signal peaks was established by ^1H-^{13}C COSY spectroscopy. ^1H-^1H Coupling data of 4 were obtained from a ^1H-^1H COSY experiment. Remote atoms (H and C) from the determined proton were indicated as cross-peaks in HOHAHA or COLOC spectra. The NMR signals were assigned to each isomer (4a or 4b) by 2D NMR experiments. The multiplicities of carbon NMR signals of 4 were confirmed by a DEPT experiment.

The NMR data are summarized in Table 1. The spectroscopic evidence led us to conclude that compound 4 contains an 8-formyl-5-hydroxy-2,4-dioxa-10-azatricyclo[5.3.1.$0^{2,6}$]undeca-8-ene moiety at the 6-position of purine riboside (Fig. 2). An unusual chemical shift of the ^{13}C NMR signal

at 14.3 ppm for the 7* carbon could be explained by the γ -oxygen effect.[6] Compounds **4** and **5** have a common carbon skeleton. The most important

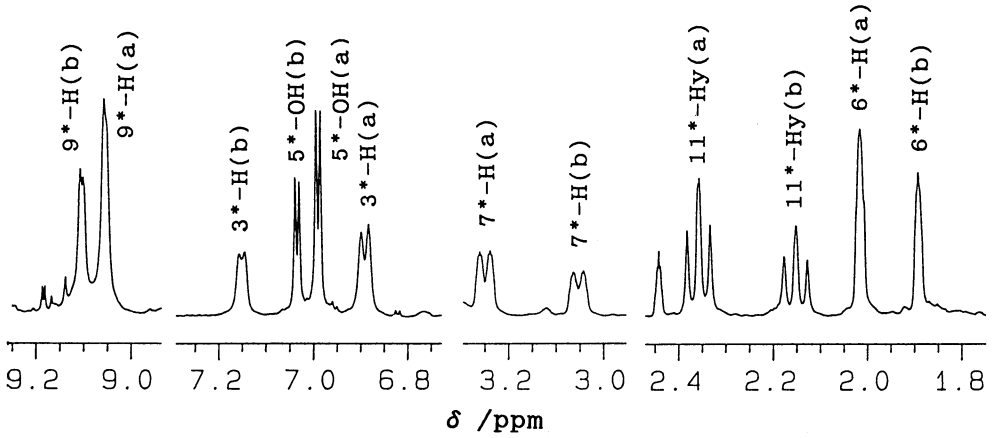

Fig. 3. Expansion of the ^{1}H NMR signals of **4**.

Table 1. NMR data for compound **4** in $(CD_3)_2SO$[8] (δ /ppm)

Site	Cm[a]	**4a** δ C	**4a** δ H	**4b** δ C	**4b** δ H
1*	CH	(86.69)	4.88	(86.81)	4.88
3*	CH	76.19	6.90	73.51	7.15
5*	CH	(90.32)	5.39	(91.02)	5.39
6*	CH	32.54	2.01	33.12	1.89
7*	CH	14.33	3.25	18.26	3.05
8*	C	(125.42)	-	(125.61)	-
9*	CH	142.77	9.06	142.77	9.11
11*	CH_2	35.73	1.23(x)	35.95	1.23(x)
			2.35(y)		2.15(y)
5*-O<u>H</u>	-	-	6.99	-	7.03
			7.00		7.04
8*-<u>CH</u>O	CH	(189.07)	9.33	(189.31)	9.33
2	CH	151.61	8.60	151.61	8.60
4	C	148.74	-	148.74	-
5	C	121.08	-	121.08	-
6	C	152.36	-	152.36	-
8	CH	142.77	8.75	142.77	8.75
1'	CH	88.05	6.00	88.05	6.00
2'	CH	74.02	4.53	70.02	4.53
3'	CH	70.19	4.15	70.19	4.15
4'	CH	85.69	3.95	85.69	3.95
5'	CH_2	61.17	3.53(x)	61.17	3.53(x)
			3.66(y)		3.66(y)
2'-O<u>H</u>	-	-	5.52	-	5.52
3'-O<u>H</u>	-	-	5.21	-	5.21
5'-O<u>H</u>	-	-	5.13	-	5.13

a) Cm, Carbon multiplicity determined by a DEPT experiment. ^{1}H-^{13}C relation was established by a ^{1}H-^{13}C COSY experiment. The δ C values in parentheses may be interchanged between corresponding signals of **4a** and **4b**.

feature of 4 is the oxygen linkage between the 3* and 5* carbons to form a four-membered ring. From the proposed structure of 4, its conversion to 5 by dehydration is expected. Formation of 5 was confirmed by HPLC-UV spectroscopic analysis after thermal decomposition of 4 at 140 °C . The observed conversion of 4 to 5 supports the proposed structure.

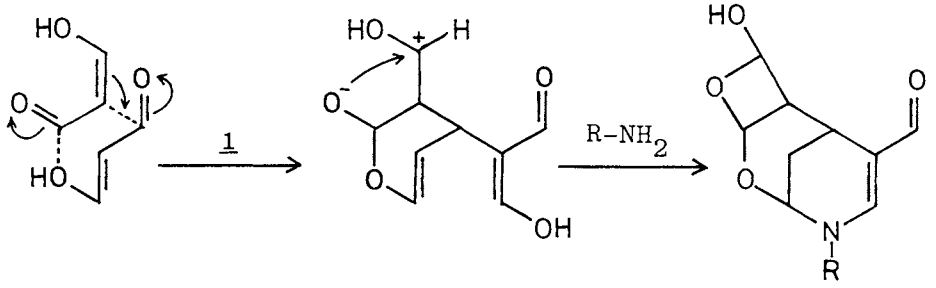

Fig. 4. Proposed mechanism for the formation of 4. R = purine riboside.

Compounds 4 and 5, the multimeric adducts, were only formed at high concentrations of 1. They were not formed by further addition of 1 to compound 3, whereas in the case of guanine nucleoside, oxadiazabicyclo[3.3.1]-nonene residue was formed by further addition of 1 to the pyrimidopurinone adduct.[7] The results imply that the formation of adenosine adducts 4 (see Fig. 4) and 5 requires the presence of sufficient multimers of 1.

References

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8) NMR spectra were recorded on a JEOL α 500 spectrometer with tetramethylsilane as an internal standard. Two-dimensional data for the ribose moiety were omitted.
^{1}H-^{1}H COSY 1*-H:11*-H(x,y) 3*-H:6*-H 5*-H:6*-H,5*-OH 6*-H:7*-H 7*-H:11*-H(y) 11*-H(x):11*-H(y)
HOHAHA 1*-H:7*-H,11*-H(x),11*-H(y) 3*H:5*-H,6*-H,7*-H 5*-H:3*-H,6*-H, 5*-OH 6*-H:3*-H,5*-H,7*-H,11*-H(x),11*-H(y),5*-OH 7*-H:1*-H,3*-H, 6*-H,11*-H(x),11*-H(y) 9*-H:8*-CHO 11*-H(x,y):1*-H,6*-H,7*-H,11*-H(y,x) 5*-OH:5*-H 8*-CHO:9*-H
COLOC 1*-H:3*,5*,7*,2 3*-H:1* 5*H:7* 6*-H:11* 7*-H:6*,8* 9*-H:7*, 8*-CHO 11*-H(x):1*,6*,8* 11*-H(y):1*,8* 5*-OH:5*,6* 8*-CHO:8*,7*
9) Column, Inertsil ODS-2 4.6 i.d. x 250 mm; oven temp, 35 °C ; carrier, 15% (V/V) acetonitrile/water; flow rate, 1 ml/min; detection, 325 nm.

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