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New Compounds Derived from Dihydropyrazines Having DNA Strand-Breakage Activity

Tadatoshi Yamaguchi*

Department of Hygiene, Miyazaki Medical College, Kiyotake-cho, Miyazaki 889-1601, Japan

Masashi Eto, Kazunobu Harano*

Faculty of Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan

Nobuhiro Kashige, Kenji Watanabe

Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka 814-0180, Japan

Shigeru Ito

Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101-0016, Japan

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Abstract

Dihydropyrazine derivatives such as 2,3-dihydro-5,6-dimethylpyrazine (**1**), 2,3-dihydro-2,5,6-trimethylpyrazine (**2**) and 2,3-dihydro-2,2,5,6-teramethylpyrazine (**3**) were found to be transformed into (2R*, 3S*, 5R*)-1,2 ethylene-imino-1,7,10-triaza-2,3,6-trimethyl-3-hydroxy-spiro [4,5] decan-6-ene (**4**), the stereoisomeric mixtures of 2,4aR*,7,9aS* -tetramethylcyclohexano [1,2-e: 4,5-e']-dipiperazin-6-ene (**5**) and (4aR*, 9aS*)-2,2,4a,7,7,9a-hexamethylcyclohexano [1,2-e: 4,5-e']-dipiperazin-6-ene (**6**), respectively. These dimerized compounds (**4**, **5** and **6**), whose structures were determined by X-ray and nmr spectral analyses, showed almost the same DNA strand-breakage activity as their parent dihydropyrazines. The dimerization pathway is discussed on the basis of the PM3 calculation data.

Keywords: dimerization; ene reactions; pyrazines; X-ray crystallography

Introduction

In a previous paper [1], we showed that some dihydropyrazine derivatives such as 2,3-dihydro-5,6-dimethylpyrazine (**1**) caused single strand-breakage of the ccc-DNA of

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plasmid pBR322. However,these dihydropyrazine derivatives are unstable as monomers and gradually transformed into brown-colored, strong-smelling compounds when they were allowed to stand at room temperature. At first, we presumed that the intermediary substances produced during the chemical transformation might have shown the DNA strand-breakage activity.

In this paper, we show the isolation of some new dimeric products, which have DNA strand-breakage activity, from dihydropyrazines, and their structures. The pathways of the formation of the dimeric products are also discussed, using the newly obtained data to clarify the overall character of the reactions.

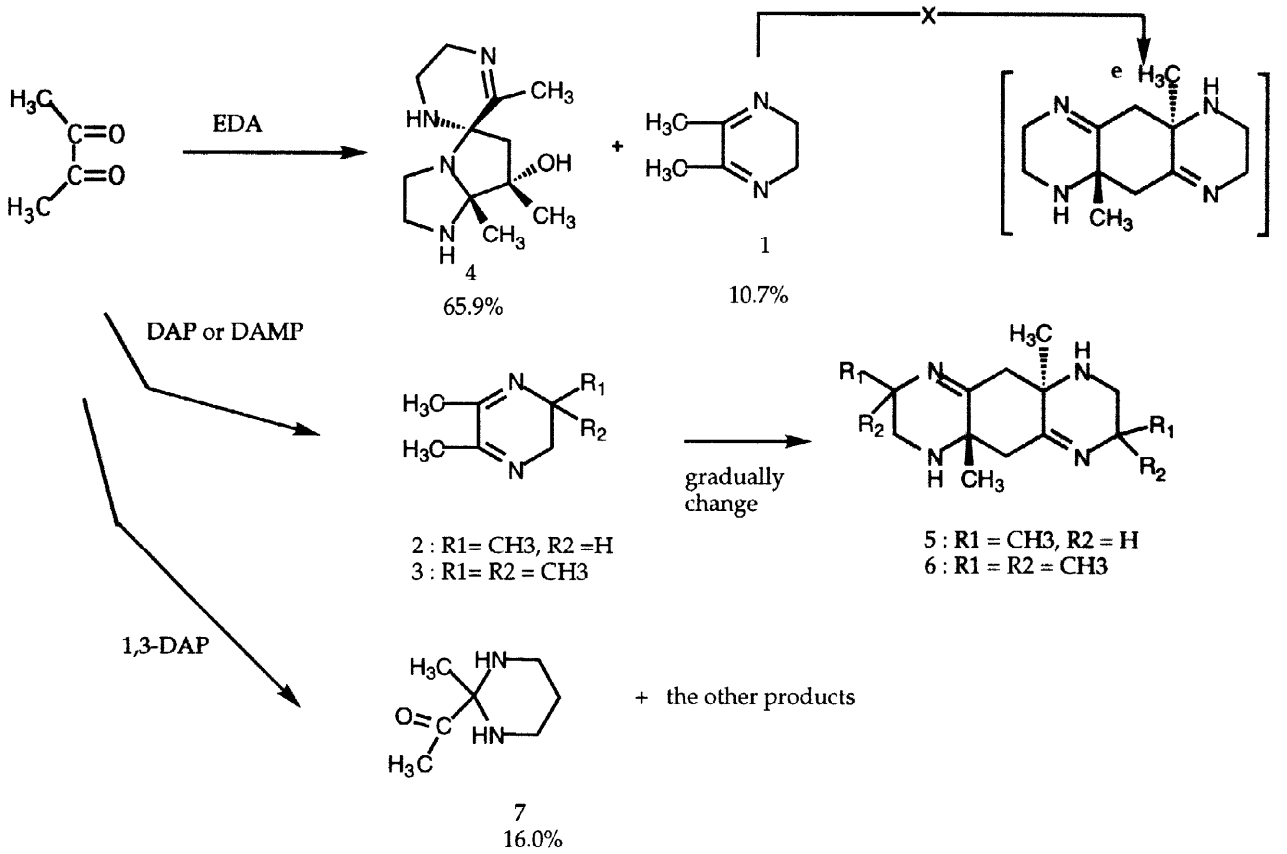


Chart 1

Results

As shown in Chart 1, the reactions [2] of a diketone (diacetyl) with diamines [ethylene-diamine (EDA), 1,2-diaminopropane (DAP), 1,2-diamino-2-methylpropane (DAMP) and 1,3-diaminopropane (1,3-DAP)] are well known. However, the reaction of diacetyl with EDA in ether gave a new product: (2R*, 3S*, 5R*)-1,2-ethyleneimino-1,7,10-triaza-2,3,6-trimethyl-3-hydroxy-spiro [4,5] decan-6-ene (**4**) as the main product using a slightly modified procedure, in which the reaction solution was merely stood at 4°C overnight in the course of the synthesis. The structure of **4** was fully clarified by single-crystal X-ray analysis (Fig.1) [3]. In contrast to the conventional reaction of diacetyl with diamine, **4** was

obtained as the main product. Compound **1** was a minor product. However, the reaction of diacetyl with diamines substituted by an alkyl group did not give a product such as **4** under similar reaction conditions. Dihydropyrazines (**2** and **3**), which are also unstable at room temperature, although it has not yet been clarified whether light, air and (or) temperature, etc., affect the subsequent reactions, gave crystalline compounds as stereoisomeric mixtures of 2,4aR*,7,9aS*-tetramethylcyclohexano [1,2-e: 4,5-e']-dipiperazin-6-ene (**5**) and (4aR*, 9aS*)-2,2,4a,7,7,9a-hexamethylcyclohexano [1,2-e: 4,5-e']-dipiperazin-6-ene (**6**), even after storage in a freezer at -30°C. Compound **1** did not give a dimer like **5** or **6**. The structure of **6** was clarified by single-crystal X-ray analysis (see Fig 2) [3]. Although the molecule has two asymmetric carbons at C4a and C9a, **6** was the sole product whose nmr (^{1}H &^{13}C) spectra were assigned on the basis of the structure clarified by the X-ray analysis. However, the structure of **5** was determined by comparison of the nmr signal pattern with that of **6**, because a single crystal for the X-ray analysis could not be obtained. Compound **5** showed a more complex signal pattern than that of **6** because the structure of **5** has two asymmetric carbons at C2 and C7 in addition to C4a and C9a. Compound **5** has four isomers [2R*,7R*-(**5**-1), 2R*,7S*-(**5**-2), 2S*,7S*-(**5**-3) and 2S*,7R*-isomer(**5**-4)] at C2 and C7 on the basis of C4aR* and C9aS* configurations. The relative signal areas of Ha at C5 indicated that the product ratio (**5**-2:**5**-1+**5**-3:**5**-4) was 6:8:1. This conclusion was supported by inspection of the heats of formation calculated by PM3 [**5**-1:(ΔHf = 327.039 Kcal/mol), **5**-2:(ΔHf = 326.929 Kcal/mol), **5**-3:(ΔHf = 327.039 Kcal/mol), **5**-4:(ΔHf = 327.171 Kcal/mol)]. The relative stability from the heats of formation is **5**-2 > **5**-1 = **5**-3 > **5**-4 (**5**-1 is a mirror image of **5**-3), in accordance with the 6:8:1 ratio (**5**-2 : **5**-1+**5**-3 : **5**-4).

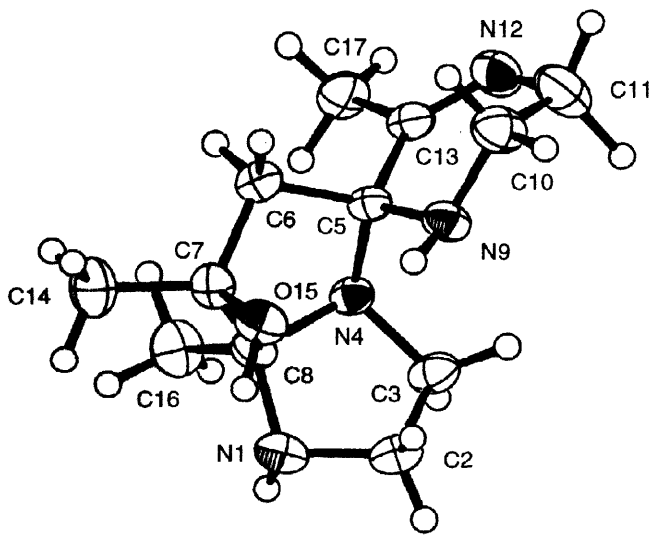


Fig. 1. ORTEP Drawing of **4**

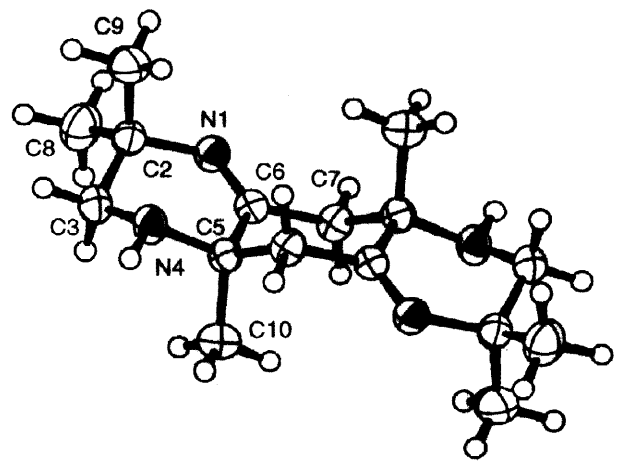


Fig. 2. ORTEP Drawing of **6**

Table 1. DNA Single-Strand Breakage by Dihydropyrazines in the Absence or Presence of Cu^{2+}

Compound[a)]	Concn. (mM)	Rel. amts. of remaining ccc-DNA (%)	
		Without Cu^{2+}, incubation for 3hr	With Cu^{2+}(1mM), incubation for 1hr
Control	-	100	100
1	1	94	76
	10	84	6
4	1	76	6
	10	40	15
2	1	91	44
	10	63	0
5	1	91	31
	10	85	0
3	0.001	99	100
	0.01	99	98
	0.1	97	64
	1.0	91	0
	10.0	71	0
6	0.001	100	100
	0.01	100	100
	0.1	99	61
	1.0	86	0
	10.0	76	0

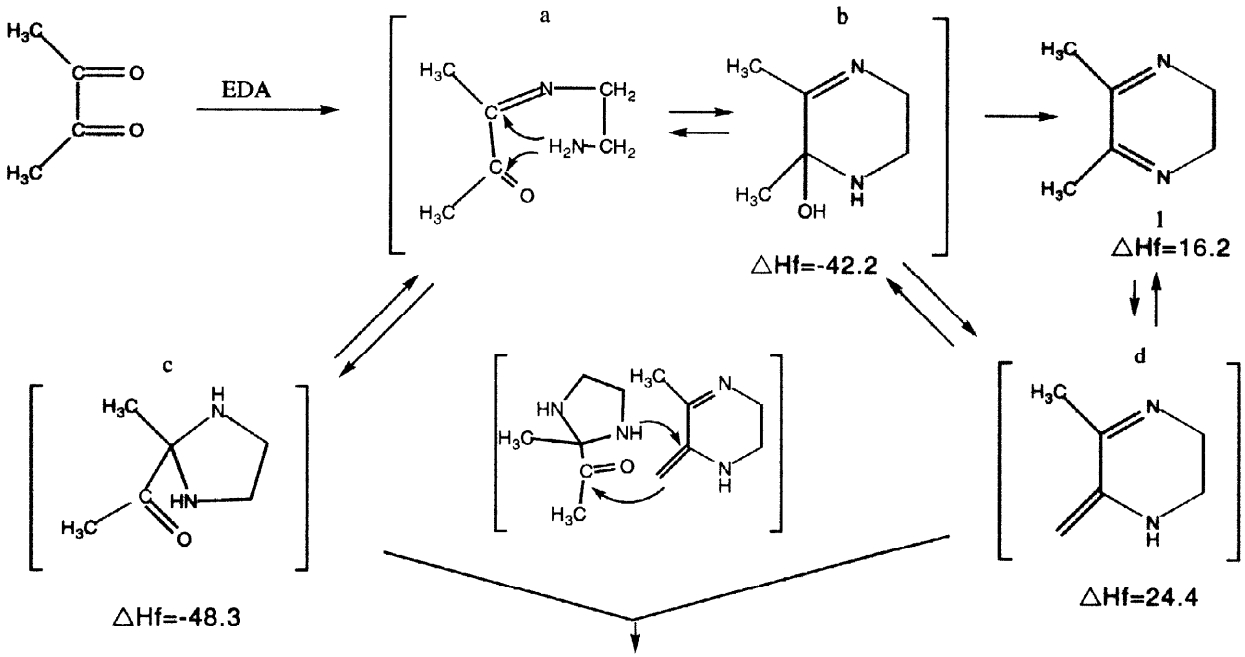
a) The numbers of compounds tested. **1** : 2,3-dihydro-5,6-dimethylpyrazine, **2** : 2,3-dihydro-2,5,6-trimethylpyrazine, **3** : 2,3-dihydro-2,2,5,6-teramethylpyrazine, **4** : (2R*, 3S*, 5R*)-1,2-ethyleneimino-1,7,10-triaza-2,3,6-trimethyl-3-hydroxy-spiro [4,5] decan-6-ene, **5** : the mixture of four isomers of 2,4aR*,7,9aR* -tetramethyl-cyclohexano [1,2-e: 4,5-e']-dipiperazin-6-ene, **6** : (4aR*, 9aS*)-2,2,4a,7,7,9a-hexamethyl-cyclohexano [1,2-e: 4,5-e']-dipiperazin-6-ene. Plasmid pBR322 ccc-DNA was incubated with the compound listed at 37°C in 50mM Tris-HCl buffer(pH 7.2).

The DNA strand-breakage activity data are shown in Table 1. As the indicator of the activity was the remaining amounts of cccDNA, the smaller value in Table 1 represented higher activity. Although the products (**5** and **6**) indicated the same level of activity as the parent compounds (**2** and **3**), only **4** showed lower activity in increasing the quantity. This phenomenon will be discussed elsewhere in connection with the relationship between the structure and the activity.

Discussion

The formation process of **4** is assumed as shown in Chart 2. First, an intermediate (**a**) is produced from diacetyl with EDA and an intramolecular attack of the remaining amino group to another carbonyl group of **a** takes place to give **1** via an intermediate (**b**) or to a

carbon atom of the >C=N- moiety to give an intermediate (**c**). The intermediate (**c**) then reacts with an intermediate (**d**) derived from **1** or **b** to give the final product (**4**). According to the literature, the reaction pathways of **a** --> **b** --> **1** are known processes.



4

Chart 2

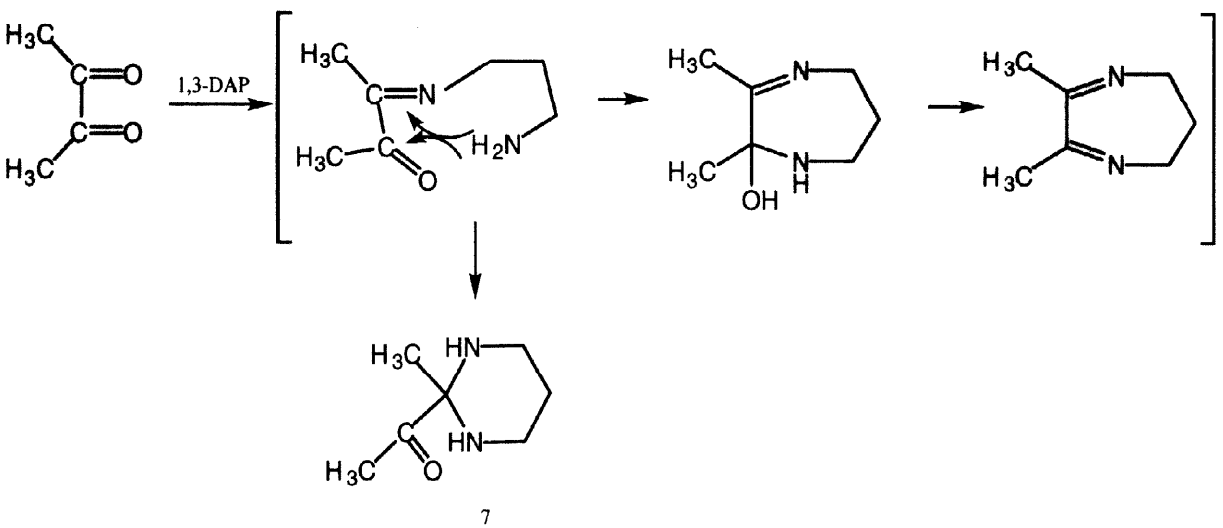


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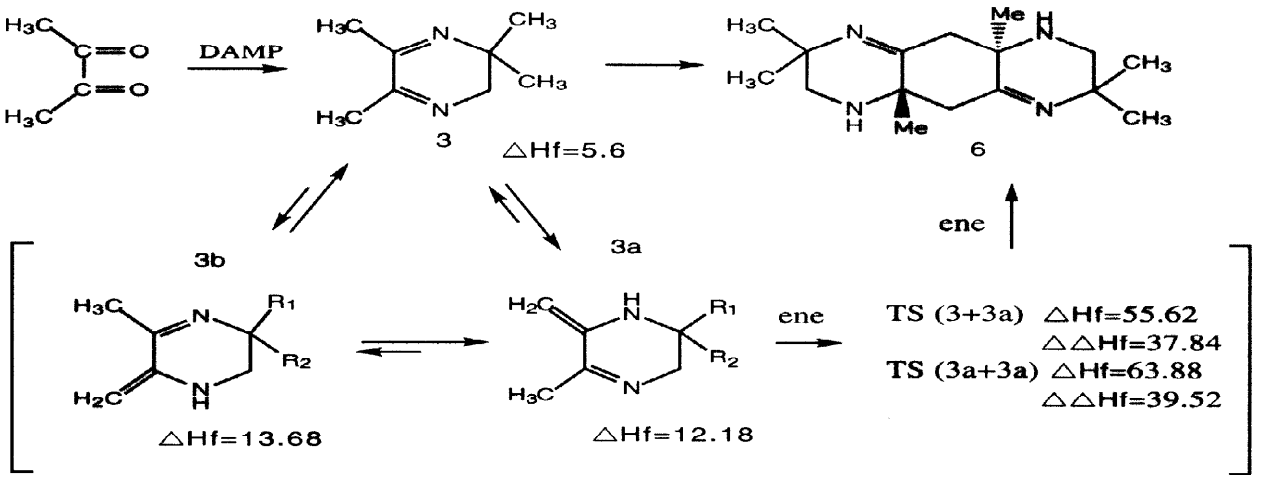
Chart 3

The present study clarified new reaction processes of **a** --> **b** --> **d** , **a** --> **c** and **c** + **d** --> **4**. The presence of the intermediate (**c**), even though **c** could not be isolated in the reaction mixture, was supported by some experimental evidence that similar imidazolidine products were obtained in previous work [4] and in our studies. As shown in Chart 3, the reaction of

diacetyl with 1,3-DAP gave 2-acetyl-2-methyl-hexahydropyrimidine (**7**) as a crystalline state having a free carbonyl group (16.0% yields).[1]

Inspection of the PM3 [5,6] heats of formation (ΔHf) indicates that **c** (ΔHf = -48.3 Kcal/mol) is more stable than b (ΔHf = -42.2 kcal/mol) and **1** + H_2O (ΔHf = -37.3 kcal/mol), being predominant in an equilibrium mixture of the initial condensation products. The intermediate (**c**) probably reacted with **1** to give **4**. The PM3 calculation indicates that the



$R_1 = R_2 = CH_3$ or $R_1 = CH_3$, $R_2 = H$

Chart 4

formation of **d** is energetically unfavorable. However, the 1,3-shift is considered to be promoted under a basic condition, indicating that the possible formation mechanism of **4** via an enamine (**d**) cannot be ruled out.

The plausible formation pathway of **5** and **6** is shown in Chart 4. It is predictable that **3** might be in equilibrium with exocyclic isomers **3a** and **3b**. However, **3** is assumed to be converted to **6** via **3a**, not via **3b**, because the PM3 heat of formation of **3a** (ΔHf = 12.18 kcal/mol) is smaller than that of **3b** (ΔHf = 13.18 kcal / mol). The fact that **6** is the sole product indicates that **6** is formed via **3a**.

To obtain additional information about the mechanism of these pathways, transition structure (TS) calculations were performed. The retro-synthesis approach gave us a clue to clarification of the formation mechanism of **6**, indicating that the dimer might have been derived from the **3** + **3a** or **3a** + **3a** reaction. Both TSs for the intermolecular ene reaction were successfully located using the TS keyword implemented in MOPAC-V6 [7]. The TS structures and the first-step product with the heats of formation (ΔHf) are depicted in Chart 4 . The reaction barriers (ΔΔHf) of **3** + **3a** and **3a** + **3a** reactions in the gas phase were

1. As the other products except for **7** were also present in the reaction mixture, this study is now under way.

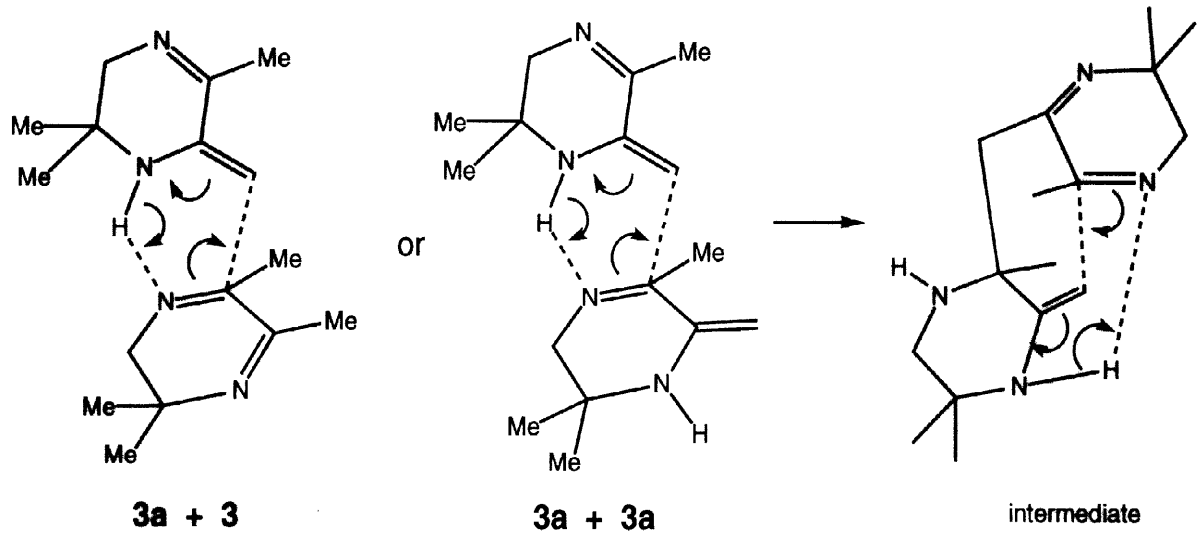
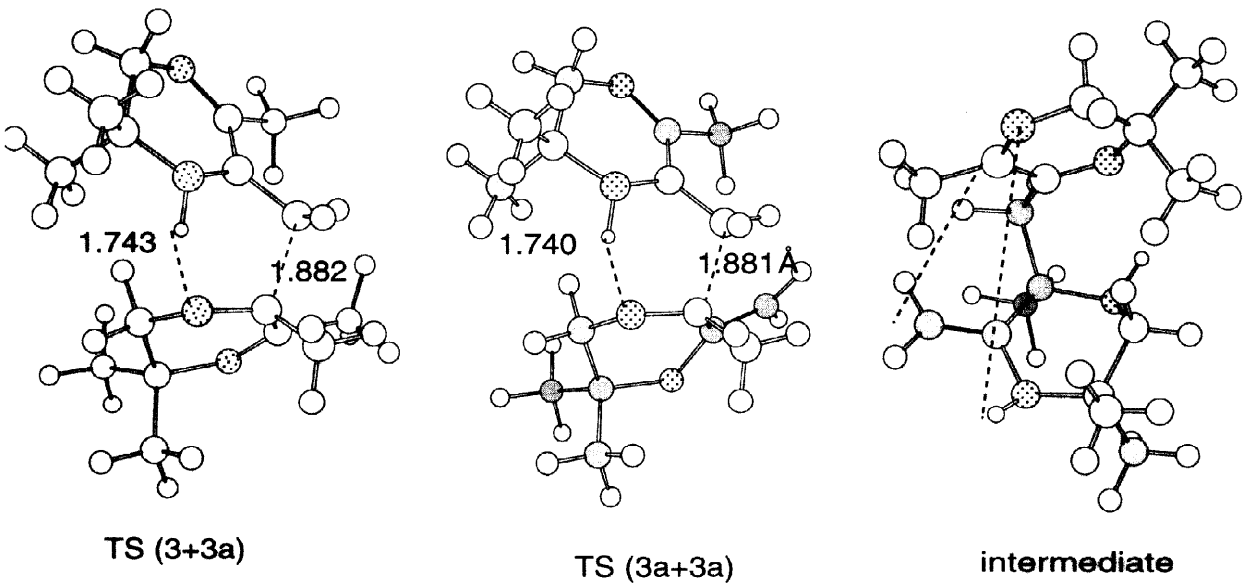


Fig. 3 Possible Reaction Mechanism and PM3-Calculated Transition States for the Ene Reaction.



calculated to be 37.84 and 39.52 kcal /mol, respectively.[2] The second-step intramolecular ene reaction is considered to proceed through a stepwise mechanism because the concerted six-membered transition structure would be strained considerably. The ene reaction in solution involving a movable N-H hydrogen may allow the mechanistic modification from a concerted to a stepwise reaction.

From our results, the reaction of diacetyl with diamines (EDA, DAP and DAMP) can be summarized as shown in Chart 5. The reaction with DAP or DMPA did not give the spiro-compound in the same way as **4**, whereas the reaction with EDA did not give a tricyclic

2. The heat of formation of a model compound simplified on ene-reaction is 33.807 Kcal/mol, which approximates that of TS.

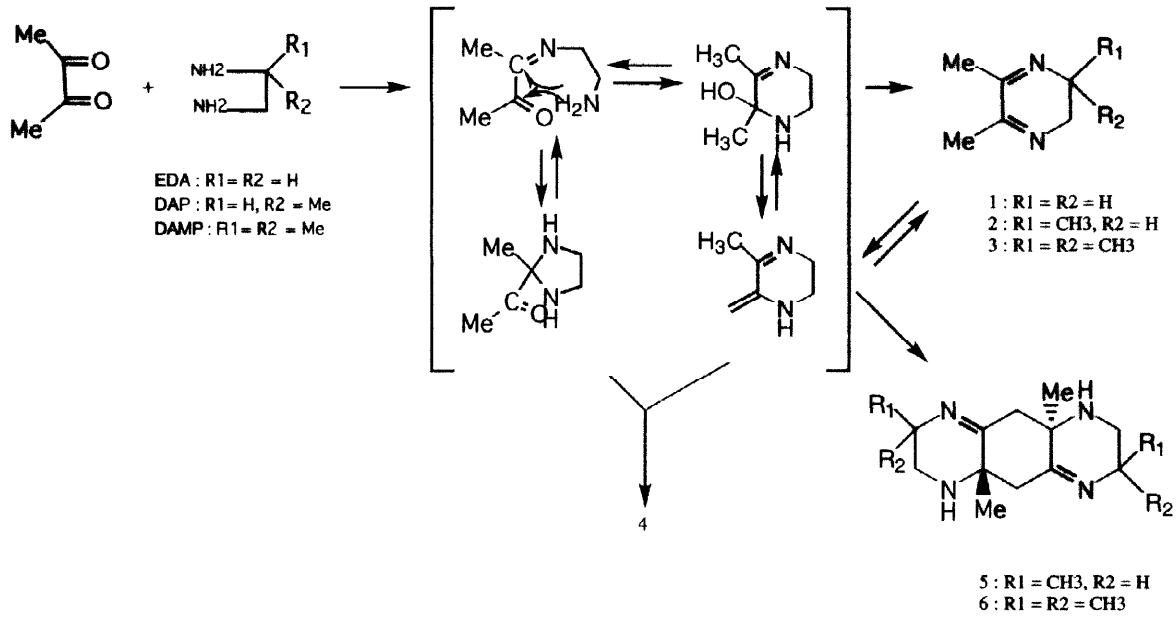


Chart 5

compound as same as **5** and **6**. The reasons for this result are presumed as mentioned below.The absence of the spiro-compounds in the cases of **2** and **3** may be accounted for by the difficulty of the cyclization of a' to c' (a' and c' are a and c with additional methyl groups, respectively). This may be due to the steric repulsion between the methyl and acetyl groups. The instability of c' is supported by the elongation of the C-N bond in the calculated structure of c'. In the formation reaction of the tricyclic compound, the pathway (3a +3a --> **6**) is considered to be predominant since the HOMO energy level (-8.958 eV) of 3a is considerably higher than that of **3** (-9.786 eV). However, although compound (e) has not yet been isolated, the possibility of the reaction (d + d --> e) can not be rule out.

The relationship between the structure and the DNA strand-breakage activity is clarified in term of the amounts of radicals generated in the DNA scission reaction system. In a preliminary experiment [8], we found that **1** generated certain radicals in water , and the dihydropyrazines, containing their converted products (**4**, **5** and **6**), were revealed to generate hydroxyl and carbon-centered radicals [9] in the DNA strand scission reaction. Consequently, the conversion of unstable dihydropyrazine affected by air, light, temperature or humidity, might involve the carbon radicals as the reactive species.

Experimental

^{1}H-(500MHz) and ^{13}C-(125MHz) NMR spectra were obtained on a JEOL JNM-a500 spectrometer equipped with 5 mm tunable or inverse field gradient II probes and operating at 26°C in $CDCl_3$. Chemical shifts values were expressed as δ ppm downfield from an internal tetramethyl silane(TMS) signal. The J values are given in Hz. A distortionless enhancement by polarization transfer(DEPT) technique was performed using three

different decoupler pulse angles(45°, 90° and 135°) to determine the multiplicity of the 13C-NMR signals. Inverse proton detected HMBC spectra was carried out under a field gradient mode applying a delay optimized for long-range coupling constants of 8 Hz under a direct coupling constant of 145Hz. The two-dimensional NOE experiment was performed using the field gradient NOE pulse sequence using a mixing time of 500 msec per acquire. The mass spectrometer employed was a JEOL(Tokyo,Japan),JMS-HX110 double-focussing model equipped with a FAB ion source and was interfaced with a JEOL JMA-DA7000 data system. A methanol solution of the sample was mixed with m-nitrobenzylalcohol and subjected to FAB mass spectrometry.The elemental composition of the ion was measured by high-resolution mass spectrometry using the same mass spectrometer.

Dihydropyrazine derivatives such as 2,3-dihydro-5,6-dimethylpyrazine (**1**), 2,3-dihydro-2,5,6-trimethylpyrazine (**2**), 2,3-dihydro-2,2,5,6-teramethylpyrazine (**3**) were synthesized according to the previously reported method [1]. The oily compounds (**2** and **3**) were stored in a deep freezer at -30°C. During the storage, the oily compounds were transformed into crystalline compounds. Compounds **2** and **3** changed into the four isomers of 2,4aR*,7,9aS*-tetramethylcyclohexano [1,2-e: 4,5-e']-dipiperazin-6-ene (**5**) due to the difference in configuration of the methyl groups at C-2 and C-7,and (4aR*, 9aS*)-2,2,4a, 7,7,9a-hexamethylcyclohexano[1,2-e: 4,5-e']-dipiperazin-6-ene (**6**), respectively.

2,4aR,7,9aS*-Tetramethyl-cyclohexano [1,2-e: 4,5-e']-dipiperazin-6-ene (5)* which was obtained by recrystallization from a solid mass grown in the oil (**2**), was colorless amorphous (acetonitrile). mp 176-180°C. The ^{1}H-NMR signal assignments of **5** were based on ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, DEPT and HMBC spectra. ^{1}H-nmr (δ , 500 MHz, $CDCl_3$) : 1.088 (d, J_{gem} = 6.4Hz, C2 and C7-CH_3), 1.263 (s, C4a & C9a-CH_3), 1.268 (d, J_{gem} = 4.8Hz, C2 & C7-CH_3), 1.274 (s, C4a & C9a-CH_3), 2.0315 and 2.0415 (d, J= 13.5 Hz, C5 & C10-methylene-Ha), 2.121 and 2.124 (d, J = 12.5Hz, C5 & C10-methylene-Ha), 2.4775 and 2.4915 (d, J_{gem} = 13.0Hz, C3 & C8-methylene-Ha), 2.793~2.833 (m, C5 & C10 -methylene-Hb), 2.793 (m, C2 & C7-methine-H), 2.947~3.052 (m, C5 & C10-methylene-Hb, C3 & C8-methylene-Hb and C3 & C8-methylene-Ha), 3.464 (m, C2 & C7-methine-H) and 3.716~3754 (m, C3 & C8-methylene-Hb). ^{13}C-nmr (δ , 500MHz, $CDCl_3$): 19.355, 20.605, 25.064, 25.130, 26.034 and 26.111 (CH_3), 44.805, 48.227, 48.638 and 57.341 (CH_2), 43.341, 43.423, 53.310 and 53.343 (C2 & C7), 54.774, 54.939, 56.633 and 56.732 (C4a & C9a) and 170.525, 170.739, 171.315 and 171.594 (C5a & C10a). FAB-Mass: m/z(%) : 249(100) [M+H$^+$], 125(48.6) [1/2M+H$^+$]. Observed m/z: 249.2079 (err: mmu=+0.0), [M+H$^+$]: $C_{14}H_{25}N_4$.

As a single crystal suitable for X-ray analysis could not be obtained from **5**, the structure was determined by comparison of the spectra of H-NMR and C-NMR with those

of **6**, whose structure was established by X-ray analysis as mentioned below. The ^{1}H- and ^{13}C-NMR spectra of **5** were similar to those of **6** mentioned below, showing a more complex pattern than that of **6**. In ^{13}C-NMR spectrum, **6** showed the sole signals at 168.52 for C5a & C10a, 49.21 for C5 & C10, 49.44 for C3 & C8, 53.44 for C2 & C7 and 54.40 for C4a & C9a, respectively. On the other hand, **5** showed four signals at 170.5, 170.7, 171.3 and 171.5 for C5a & C10a, and C4a & C9a and C2 & C7 individually showed each signal, and then, methylene carbons for C5 & C10 and C3 & C8 individually showed two kinds of signals as described above, indicating that **5** was a mixture of four isomers. Also, Ha-signals of methylene at C5 and C10 clearly showed four kinds of signals, supporting the structure of **5** to be a mixture of four isomers. The proportion of the amounts of the four isomers was 6:4:4:1.

(4aR, 9aS*)-2,2,4a,7,7,9a-Hexamethyl-cyclohexano [1,2-e: 4,5-e']-dipiperazin-6-ene (**6**)* which was obtained by recrystallization from a solid mass grown in the oil (**3**), was colorless granular crystal (ether). mp 223°C. The structure was determined by X-ray analysis [3].The assignment of signals was based on ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, HMBC, decoupling and D_2O addition spectra. The NMR spectral data for **6** are as follows: ^{1}H-nmr (δ, 500MHz, $CDCl_3$) : 1.18 (s, 6H, C2 & C7-CH_3), 1.24 (s, 6H, C2 & C7-CH_3), 1.26 (s, 6H, C4a & 9a-CH_3), 1.42 (broad, 2H, NH), 2.18 (d, J_{gem} = 13.2Hz, 2H, C5 & C10-Ha), 2.65 (d, J_{gem} = 13.2Hz, 2H, C5 & C10-Hb), 2.67 (d, J_{gem} = 13.2Hz, C3 & C8-Ha), 2.73 (d, J_{gem} = 13.2Hz, C3 & C8-Hb). ^{13}C-nmr (500MHz, $CDCl_3$) : δ = 24.82 (C4a & C9a-CH_3), 27.42 (C2 & C7-CH_3), 27.84 (C2 & C7-CH_3), 49.21(C5 & C10), 49.44 (C3 & C8), 53.44 (C2 & C7), 54.40 (C4a & C9a), 168.52 (C5a & C10a). FAB-Mass : m/z(%) : 277 (100) [M+ H+], 139 (67) [1/2M+H+]. Observed m/z: 277.2391 (Err:mmu=-0.1) [M+H+]: $C_{16}H_{29}N_4$.

Preparation of (2R, 3R*, 5R*)-1,2-ethyleneimino-1,7,10-triaza-2,3,6-trimethyl-3-hydroxy-spiro [4,5] decan-6-ene (**4**).* According to the previous procedure [2], 2,3-dihydro-5,6-dimethylpyrazine (**1**) was prepared by the reaction of diacetyl with ethylenediamine. On a partial modification of the procedure, an unexpected unique product **4** was obtained. To a solution of ethylenediamine (1.8 g, 31 mmole) in ether (20 ml), which was mechanically stirred in an ice bath, a solution of 2,3-butanedione (2 g, 23 mmole) in ether (16 ml) was slowly added dropwise. The reaction mixture was stirred at room temperature until the mixture become a clear solution. The mixture was refluxed for 30 min. Potassium hydroxid was added to the mixture for dehydration. The resulted clear ether solution was condensed to about half volume and allowed to stand at 4°C overnight. The product precipitated was filtered off, washed with cold ether and dried under reduced pressure. Clear colorless columned crystal (**4**, 1.805 g, 65.9% yields) was obtained. The filtrate was distilled under reduced pressure in a stream of nitrogen gas to give a colorless oil (**1**, 272.1 mg, 10.7%

yields). Recrystallization of **4** from ether gave a single crystal for X-ray analysis, though **4** was very unstable at room temperature. The structure was determined by X-ray analysis [3].The assignment of signals was based on ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, HMBC, decoupling and D_2O addition spectra. The NMR spectral data for **4**: ^{1}H-nmr (δ , 500MHz, $CDCl_3$): 1.22 (s, 3H, C3-CH_3), 1.29 (s, 3H, C2-CH_3), 1.60 (broad, 1H, OH or NH), 2.00 (dd, $J_{C8\text{-}Ha,C6\text{-}CH3}$ and $J_{C8\text{-}Hb,C6\text{-}CH3}$ = 1.2 and 1.8 Hz, 3H, C6-CH_3), 2.00 (d, J_{gem}= 13.4 Hz, 1H, C4-Ha), 2.24 (d, J_{gem}= 13.4 Hz, 1H, C4-Hb), 2.54~2.64 (m, 1H, C1'-Ha), 2.65~2.73 (m, 1H, C2'-Ha), 2.79~2.83 (m, 1H, C9-Ha), 2.90 (broad, 1H, NH or OH), 2.92~2.96 (m, 1H, C9-Hb), 3.08~3.11(m, 1H, C2'-Hb), 3.46~3.49 (m, 3H, C8-CH2 and C1'-Hb) and 4.90(broad, 1H, NH or OH). ^{13}C-nmr (δ , 500MHz, $CDCl_3$) : 21.89 (C6-CH_3), 24.27 (C3-CH_3), 25.72 (C2-CH_3), 40.45 (C9), 44.36 (C1'), 45.61 (C4), 49.07 (C8), 49.25 (C2'), 76.82 (C3), 78.35 (C5), 90.67 (C2), 171.58 (C6). FAB-Mass : m/z (%) : 239 (100) [M+H$^+$], 221 (52), 197 (44.5), 179 (87), 111 (94) [1/2M+H$^+$], 85 (70). Observed m/z: 239.1872 (Err:mmu=+0.0) [M+H$^+$]: $C_{12}H_{23}N_4O_1$.

Preparation of 2-acetyl-2-methyl-hexahydropyrimidine(7). To a solution of 1,3-diaminopropane (1.7 g, 22.9 mmole) in ether (10 ml) which was mechanically stirred in an ice bath, a solution of 2,3-butanedione (2 g, 23.2 mmole) in ether (20 ml) was slowly added dropwise. The reaction mixture was stirred at room temperature for 2 h and then refluxed for 30 min. An adequate amount of potassium hydroxide was added to the mixture for dehydration. The resulted clear ether solution was condensed to about half-volume and allowed to stand at 4°C overnight. In sharp contrast to the case of **4**, crystallization at this stage could not be observed. The ether solution was distilled under reduced pressure in a stream of nitrogen gas to give a colorless oil which immediately changed into a crystal (**7**, 548.7 mg, 16.0 % yields), (bp 41°C, 5 mmHg). Compound **7** was unstable at room temperature. The NMR spectral data of **7** : ^{1}H- nmr (δ , 500MHz, $CDCl_3$) : 1.19(s, 3H, CH_3), 1.40~1.43 (m, 1H, C5- Ha), 1.51~1.58 (m, 1H, C5- Hb), 1.95 (broad, 2H, NH), 2.31 (s, 3H, acetyl-CH_3), 2.58~2.64 (m, 2H, C4 & C6-Ha), 2.94~2.99 (m, 2H, C4 & C6-Hb). ^{13}C-nmr (δ , 500MHz, $CDCl_3$) : 25.12 (acetyl-CH_3), 26.14 (C5), 27.54 (CH_3), 42.49 (C4 & C6), 75.75 (C2), 213.70 (>C=O). FAB-Mass:m/z (%): 143 (100) [M+H$^+$], observed m/z: 143.1183 (Err:mmu=-0.1) [M+H$^+$]: $C_7H_{15}O_1N_2$.

Assay of DNA Strand-Breakage Activity . The methods of assaying the DNA strand-breaking activity of the compounds, using a covalently closed circular duplex DNA of plasmid pBR322(ccc-DNA) as a substrate, were described in a previous paper [1].

X-Ray Crystallography. Single crystals of **4** and **6** suitable for X-ray analysis were

prepared by slow evaporation of ether solutions at room temperature. Crystal Data; **4**: $C_{12}H_{22}N_4O$; monoclinic (Space group $P2_1/c$); a=7.156 (2), b=15.314 (2), c=11.689 (2)Å, β=94.70 (2)°; V=1276.5 (2)Å^3, Dc=1.240 gcm^{-3}, Z=4; Num of RD=3045, Num of F_{obs}=2000, R_f=0.058, Rw=0.052. **6**: $C_{16}H_{28}N_4$; tetragonal (Space group $I4_1/a$); a=12.078 (4), c=21.995 (7)Å; V=3208(1)Å^3, Dc=1.144 gcm^{-3}, Z=16; Num of RD=1906, Num of F_{obs}= 1076, R=0.041, Rw=0.045. Intensity data were collected on a RIGAKU AFC7R four-circle automated diffractometer with a graphite monochromated Mo-Kα (λ=0.7107 Å) radiation (50 kV-150 mA) using θ-2θ scan tecnique to a maximum 2θ value of 55°. The intensities of three representative reflections were measured after every 150 reflections. The structures were solved by the direct method (SAPI91) [10]. The non-hydrogen atoms were refined anisotropically with full-matrix, least-squares calculations. At this stage, all hydrogen atoms were placed in calculated position and refined isotropically. The final cycle of full-matrix, least-squares refinement was based on the observed reflections (I> 3.00σ(I)). All calculations were performed using the *teXsan* [11,12,13] crystallographic software package on a Silicone Graphics IRIS Indigo EWS.

The ORTEP drawings of **4**,**6** are shown in Fig.1 and 2, respectively. The final coordinates of **4**, and **6** are deposited in the Cambridge Crystallographic Data Center.

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References

[1] Yamaguchi T, Kashige N, Mishiro N, Miake F, Watanabe K.Biol.Pharm.Bull. 1996;19:1261-1265.
[2] Ishiguro, T, Matsumura M.Yakugaku Zasshi, 1958;78:229-231.
[3] Yamaguchi T, Eto M, Watanabe K, Kashige N, Harano K.Chem.Pharm.Bull. 1996;44:1977-1979.
[4] Willer RL, Moore W, Vanderah DJ.J.Org.Chem., 1985; 50:2365-2368.
[5] Stewart JJP. J. Comput. Chem. 1989;10:209-220.
[6] Stewart JJP. J. Comput. Chem. 1989;10: 221-264.
[7] Stewart JJP. QCPE Bull. 1989;9:10.
[8] Yamaguchi T, Kashige N, Matsumoto S, Satoh K,Yasuda M, Watanabe K.Biol.Pharm.Bull. 1998;21:205-209.
[9] Yamaguchi T, Matsumoto S, Watanabe K. Tetrahedron Lett. 1998 (Accepted: TL-9807-452).
[10] Hai-Fu F. "Structure Analysys Programs with Intelligent Control", Rigaku Cooporation, Tokyo, 1991.
[11] Crystal Structure Analysis Package, Molecular Structure Corporation, 1985 and 1992.
[12] Johnson CK. ORTEP, Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, TN, 1965.
[13] teXsan: Crystal Structure Analysis Package, molecular Structure Corporation (1985 and 1992).