Synthesis of New Dihydropyrazines with DNA Strand-Breakage Activity

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Received June 13, 2005; accepted August 6, 2005; published online August 9, 2005

Treatment of 1,2-cyclohexanedione with 1,2-diamines, *e.g.* ethylenediamine and *cis*-(and *trans*-)1,2-diaminocyclohexane, caused [4+2] cyclocondensation to give the corresponding dihydropyrazine derivatives (compounds 1—6). They exhibited stronger DNA strand-breakage activity than that of dihydropyrazines, which has already been reported in previous papers.

Key words dihydropyrazine; DNA strand-breakage; 1,2-diamine; α-diketone; cyclocondensation

The biological relevance of dihydropyrazines (DHPs) continues to foster immense interest in their design and synthesis. DHPs are produced primarily from sugar *in vitro*.[1)] Predictably, the formation of DHPs *in vitro* also occurs *in vivo* under non-enzymatic or enzymatic conditions. A number of pyrazine derivatives which are readily created from the DHP ring structure *via* oxidation[2)] have been detected in human urine[3)] and foods.[4—6)] Thus, it is thought that DHPs are formed and found *in vivo* as precursors of pyrazine derivatives. For these reasons, we are interested in the effects of DHPs *in vivo*.

In our previous papers,[7—9)] we discussed the synthesis and DNA strand-breakage activities of some DHPs, such as 2,3-dihydro-5,6-dimethylpyrazines (DHP-1, DHP-2 and DHP-3) (Fig. 1). Recently, we also reported that DHPs cause apoptosis[10)] and mutagenesis[11)] *in vivo*. In this context, the synthesis of new DHPs provides an interesting challenge. Despite considerable interest in the DHP core among the medicinal and synthetic chemistry communities, we are not aware of any available routes that give the various substituted patterns. Indeed, very few examples of the synthesis of substituted DHPs have been reported.[12—14)] In addition, few reports have been published on the biological and physiological roles of DHPs. In keeping with our interest in the synthetic chemistry and biological activity of DHPs, to investigate the hypothesis that intermediate (*exo*-type) DHPs might reveal higher DNA strand-breakage activity than other types,[15)] we attempted to synthesize new DHPs and to elucidate their biological activity, including DNA strand-breakage activity, *in vitro*.

Preparation of Dihydropyrazines 1—6 The DHPs used in our investigation were synthesized *via* cyclocondensation of α-diketone and 1,2-diamines, according to our earlier work.[9)] All synthesized products were too labile to exist at room temperature, but were fairly stable in the freezer. It is known that DHPs, such as DHP-1, DHP-2 and DHP-3 (Fig. 1), are unstable at room temperature. When an equimolar mixture of 1,2-cyclohexanedione and ethylenediamine (ED) in chloroform was stirred at room temperature for 24 h, the expected hexahydroquinoxaline derivative **1**[16)] was obtained in 63% yield (Chart 1). Compound **1** was purified by distillation and solidified in the freezer. The elemental analysis and spectral data of **1** are consistent with the proposed structures (see references and notes 16). Interestingly, the ^{1}H-NMR spectrum of **1** showed the spectral patterns of two isomers, **1A** and **1B**. In CD_3CN at room temperature, compound **1** existed almost exclusively as **1A**, with the following ratios observed: **1A** : **1B** = 38 : 1 in CD_3CN, 28 : 1 in C_5D_5N and 3 : 1 in $CDCl_3$. The ^{1}H-NMR spectrum of **1** in $CDCl_3$ at room temperature shows three signals at δ 3.03 assignable to the H-3 methylene protons of **1A**, near δ 3.3 due to the H-2 and -3 methylene protons of **1B** and near δ 3.7 due to the H-2 methylene protons of **1A**. In addition, the NH and H-8 protons of **1A** appear at δ 3.40 and 5.15, respectively. The ^{13}C-NMR spectrum of **1** exhibits seven signals at δ 40.2 assignable to C-3 of **1A**, at δ 44.9 due to the C-2 and -3 carbons of **1B**, at δ 49.5 due to the C-2 carbon of **1A**, at δ 109.4 due to the C-8 carbon of **1A**, at δ 134.8 due to the C-8a carbon of **1A**, at δ 161.1 due to the C-4a and -8a carbons of **1B** and at δ 162.1 due to the C-4a carbon of **1A**. On the basis of these observations, it seems likely that the ratio of the two isomers depends on the solvent. The above spectral studies confirmed the formation of the desired DHP derivative **1**, which was characterized as 1,2,3,5,6,7-hexahydroquinoxaline (**1A**) or 2,3,5,6,7,8-hexahydroquinoxaline (**1B**). The treatment of 1,2-cyclohexanedione (1.0 equiv.) with ED (2.0 equiv.) in chloroform at room temperature for 24 h afforded 4a,8a-butanodecahydropyrano[2,3-*b*]pyrazine (**2**)[17)] as a pale yellow solid in 54% yield (Chart 1). In ^{1}H-NMR solvents, *e.g.* $CDCl_3$ and CD_3CN, it was found that compound **2** dissociated into **1B** and ED at room temperature. In CD_3CN, however, reassociation to **2** was observed at certain temperatures, *i.e.* **2** : **1B** : ED = 1 : 2 : 2 at −40 °C. The ^{1}H-NMR spectrum of **2**

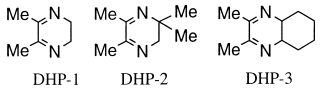

Fig. 1. Some DNA Strand-Breakage Dihydropyrazines

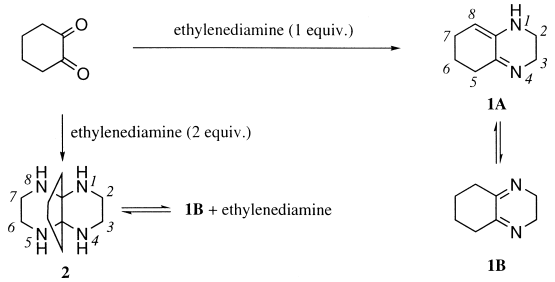

Chart 1

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Table 1. DNA Strand-Breakage by Dihydropyrazines **1**—**6** in the Absence or Presence of Cu^{2+}

Entry	Compound	DNA type	Relative amounts of DNA (%)	
			Without Cu^{2+}, incubation for 3 h[a)]	With Cu^{2+} (1 mM), incubation for 1 h[b)]
1	Control	ccc-	99	97
		oc-	1	3
		linear-	0	0
2	**1**	ccc-	54	38
		oc-	46	62
		linear-	0	0
3	**2**	ccc-	40	46
		oc-	60	54
		linear-	0	0
4	**3**	ccc-	59	54
		oc-	41	44
		linear-	0	2
5	**4**	ccc-	27	16
		oc-	70	84
		linear-	3	0
6	**5**	ccc-	1	24
		oc-	94	74
		linear-	5	2
7	**6**	ccc-	3	20
		oc-	89	80
		linear-	8	0
8	DHP-1	ccc-	41	67
		oc-	59	33
		linear-	0	0

a) Amount: 10 mM. *b*) Amount: 0.1 mM. Since activity was accelerated upon addition of Cu^{2+}, the quantity of DHPs and the incubation time were minimized until differences in activity could be observed.

in CD_3CN at room temperature shows only the sum of **1B** and ED, whereas that of **2** in CD_3CN at −40 °C shows peaks for the methylene protons (H-2, -3, -6 and -7) of **2** in the range 2.96—3.18 ppm. The ^{13}C-NMR spectrum of **2** in CD_3CN at −40 °C displays signals at δ 42.5 due to the C-2, -3, -6 and -7 carbons and at δ 66.9 due to the C-4a and -8a carbons (see references and notes 17). This implies that compound **2** can easily dissociate in the solvent, such as CD_3CN, $CDCl_3$ and D_2O, as is the case for DHPs reported in our earlier work.[18,19)] In this case, we did not observe **1A** at all, which suggests that when compound **2** dissociates in this solvent, **1B** is formed as a single isomer. Although the calculation of heat of formation by 6-31G* indicated that **1B** is more stable than **1A**, with a difference of 3.73 kcal/mol, it is not clear why **1A** is not formed. The experimental data suggests that the interconversion of **1B** to **1A** cannot proceed in the presence of ED. In a similar way, by reaction of 1,2-cyclohexanedione with *cis*-(and *trans*-)1,2-diaminocyclohexane, DHPs **3**—**6** were obtained in 38, 34, 41 and 20% yield, respectively (Fig. 2). Similarly to **1** and **2**, the isomerization of decahydrophenazines **3** and **5** and the dissociation of 5a,11a-butanooctadecahydroquinoxalino[2,3-*b*]quinoxalines **4** and **6** were observed in ^{1}H-NMR solvents. The structural assignments of **3**—**6** were made on the basis of spectral data.

Evaluation of DNA Strand-Breakage Activity by Dihydropyrazines 1—6 DNA strand-breakage activity data are summarized in Table 1, compared with the activity of DHP-1 as detailed in a previous paper. DNA strand-breakage activity is accelerated to a remarkable degree by the addition of cupric ions (Cu^{2+}), which may stimulate the production of active radicals,[20)] resulting in DNA strand-breakage. The

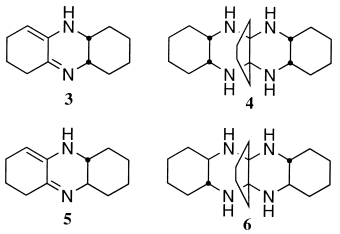

Fig. 2. Dihydropyrazines **3**—**6** Prepared from 1,2-Cyclohexanedione and *cis*-(and *trans*-)1,2-Diaminocyclohexane

generation of radicals as well as DHP-1 was detected (data not shown); the details will be published in a later paper. The values obtained for activity were based on the remaining amounts of covalently closed circular duplex DNA (ccc-DNA) of plasmid pBR322.[21,22)] In the absence of Cu^{2+}, **4**—**6** showed higher activity than DHP-1, and these activities were obviously accelerated by the addition of 1 mM Cu^{2+} (entries 5—7). Furthermore, in the presence of Cu^{2+}, it was found that all compounds **1**—**6** have higher activity than that of DHP-1.

In conclusion, we have prepared six new DHP compounds **1**—**6**, which show high DNA strand-breakage activity *in vitro* with or without Cu^{2+}. Our results suggest that these new DHPs may play a role *in vivo*. Further studies on the biological activity of DHPs are under way.

Acknowledgements We are grateful to H. Hanazono for obtaining the mass spectra and to Y. Iwase for her valuable help with NMR analyses.

References and Notes

1) Kashige N., Yamaguchi T., Ohtakara A., Mitsutomi M., Brimacombe J. S., Miake F., Watanabe K., *Carbohydr. Res.*, **257**, 285—291 (1994).
2) Manley C. H., Vallon P. P., Erickson R. E., *J. Food Sci.*, **39**, 73—76

(1974).
3) Zlatkis A., Bertsch W., Lichtenstein H. A., Tishbee A., Shunbo F., Liebich H. M., Coscia A. M., Fleischer N., *Anal. Chem.*, **45**, 763—767 (1973).
4) Maga J. A., Sizer C. E., *J. Agric. Food Chem.*, **21**, 22—30 (1973).
5) Koehler P. E., Odell G. V., *J. Agric. Food Chem.*, **18**, 895—898 (1970).
6) Koehler P. E., Mason M. E., Newell J. A., *J. Agric. Food Chem.*, **17**, 393—396 (1969).
7) Mibu N., Yukawa M., Kashige N., Iwase Y., Goto Y., Miake F., Yamaguchi T., Ito S., Sumoto K., *Chem. Pharm. Bull.*, **51**, 27—31 (2003).
8) Kashige N., Yamaguchi T., Miake F., Watanabe K., *Biol. Pharm. Bull.*, **23**, 1281—1286 (2000).
9) Yamaguchi T., Kashige N., Mishiro N., Miake F., Watanabe K., *Biol. Pharm. Bull.*, **19**, 1261—1265 (1996).
10) Yamaguchi T., Nomura H., Matsunaga K., Ito S., Takata J., Karube Y., *Biol. Pharm. Bull.*, **26**, 1523—1527 (2003).
11) Takechi S., Yamaguchi T., Nomura H., Minematsu T., Nakayama T., *Mutat. Res.*, **560**, 49—55 (2004).
12) Jones R. G., *J. Am. Chem. Soc.*, **71**, 78—81 (1949).
13) Felder E., Pitre D., Boveri S., Grabitz E. B., *Chem. Ber.*, **100**, 555—559 (1967).
14) Flament I., Sonnay P., Ohloff G., *Helv. Chim. Acta*, **56**, 610—619 (1973).
15) The relationship between the activity and the chemical structure of dihydropyrazines with DNA strand-breakage activity, The 14th European Symposium on Organic Chemistry, July 4—8, 2005 Helsinki-Finland.
16) Signal assignments were confirmed by ^{1}H–^{1}H COSY and HMQC spectra. ^{1}H-NMR (500 MHz, $CDCl_3$, 35 °C): δ 1.79—1.85 (2.5H, m), 2.18—2.21 (1.5H, m), 2.43—2.46 (1.5H, m), 2.49—2.50 (1H, m), 3.03 (1.5H, t, J=5.5 Hz), 3.35—3.36 (1H, m), 3.40 (0.75H, br), 3.69—3.72 (1.5H, m), 5.15 (0.75H, t, J=4.6 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$, 35 °C): δ 23.4, 24.2, 24.7, 35.0, 35.9, 40.2, 44.9, 49.5, 109.4, 134.8, 161.1, 162.1. IR (Neat) cm^{-1}: 3287. Positive FAB-MS *m*/*z*: 137 $(M+H)^+$. High-resolution positive FAB-MS *m*/*z*: 137.1076 (Calcd for $C_8H_{13}N_2$: 137.2046). *Anal.* Calcd for $C_8H_{12}N_2 \cdot 0.2H_2O$: C, 68.73; H, 8.94; N, 20.04. Found: C, 68.80; H, 8.72; N, 20.05. Boiling point: 101—110 °C/7 mmHg.
17) Signal assignments were confirmed by ^{1}H–^{1}H COSY, HMQC and HMBC spectra. ^{1}H-NMR (500 MHz, CD_3CN, 35 °C): δ 1.76—1.80 (4H, m), 1.86 (4H, br), 2.40—2.42 (4H, m), 2.57 (4H, s), 3.22 (4H, s). ^{1}H-NMR (500 MHz, CD_3CN, −40 °C): δ 0.81—0.83 (1.3H, m), 1.40—1.44 (1.3H, m), 1.59—1.62 (1.3H, m), 1.78—1.84 (2.7H, m), 2.42—2.44 (4H, m), 2.54 (2.7H, s), 2.61—2.68 (1.3H, m), 2.96—3.18 (2.7H, m), 3.22 (2.7H, s). ^{13}C-NMR (125 MHz, CD_3CN, 35 °C): δ 25.0, 36.6, 45.5, 46.0, 161.7. ^{13}C-NMR (125 MHz, CD_3CN, −40 °C): δ 22.8, 24.7, 33.0, 36.2, 39.7, 42.5, 44.8, 45.2, 66.9, 161.6. IR (Neat) cm^{-1}: 3321, 3220. Positive FAB-MS *m*/*z*: 137 (M−ethylenediamine+H)$^+$.
18) Yamaguchi T., Ito S., Iwase Y., Harano K., *Heterocycles*, **51**, 2305—2309 (1999).
19) Yamaguchi T., Ito S., Iwase Y., Watanabe K., Harano K., *Heterocycles*, **53**, 1677—1680 (2000).
20) Kashige N., Takeuchi T., Matsumoto S., Takechi S., Miake F., Yamaguchi T., *Biol. Pharm. Bull.*, **28**, 419—423 (2005).
21) Kashige N., Yamaguchi T., Mishiro N., Hanazono H., Miake F., Watanabe K., *Biol. Pharm. Bull.*, **18**, 653—658 (1995).
22) Watanabe K., Kashige N., Nakashima Y., Hayashida M., Sumoto K., *Agric. Biol. Chem.*, **50**, 1459—1465 (1986).