A Theoretical Study on the *N*-Alkylation of a Pyrimidine with a Cyclopropa[c]inden-5-one; A Model Pharmacophore of Duocarmycins and CC-1065

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Received October 7, 2003

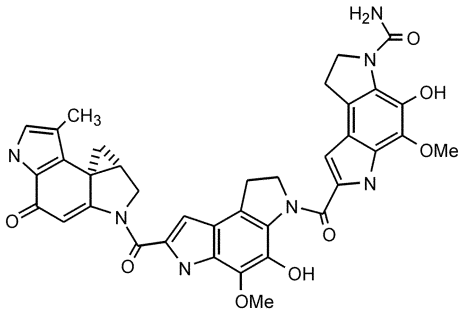
The *N*-alkylation of 4-aminopyrimidine with a tetrahydro-3-aza-cyclopropa[c]inden-5-one, which is a model reaction of the pharmacophore of duocarmycins, was studied with a quantum chemical method. We consider two factors for the acceleration of the *N*-alkylation; distortion and protonation of the model pharmacophores. The distortion of the spirocyclopropyl moiety in the model spirocyclopropylcyclohexadienone could induce an intrinsic energy of 3-4 kcal/mol, but the protonation on the carbonyl oxygen of the model cyclohexadienone lowers the transition energy of the *N*-alkylation of 4-aminopyrimidine dramatically (~46 kcal/mol) and is considered to play a major role in the enzyme reaction. The distorted and protonated spirocyclohexadienone is exothermally relieved to a phenol with the heat of reaction of -37 kcal/mol. The protonation process is proposed to be the mode of action of duocarmycins in the DNA alkylation.

Key Words : Simulation, Pharmacophore, Cyclopropa[c]inden-5-one, Duocarmycins

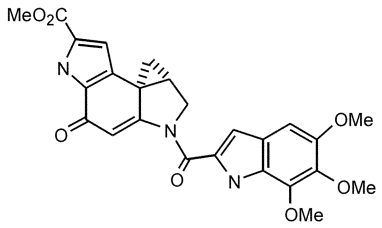
Introduction

Doucarmycins and CC-1065 which are originally isolated from cultures of Streptomyces,[1] shows high potency *in vitro* cytotoxic activity, *in vivo* antitumor activity, and broad spectrum antimicrobial activity.[2] In extensive studies, the agents have shown to bind within the double-strand B-DNA minor groove in an initial high-affinity, nonintercalative manner and subsequently forms irreversible covalent adducts by 3-adenine N-3 alkylation of the electrophilic spirocyclopropylcyclohexadienone segment of the agents.[3,4] The agents are stable toward conventional nucleophiles at pH 7, but they undergo the DNA alkylation reaction efficiently. The cytotoxic potency and antitumor activity have been correlated with their sequence-selective minor groove binding properties.[3b] The sequence selective DNA alkylation is thought to be originated from the noncovalent binding selectivity of the agents (shape-selective recognition).[5,6] There is also a report that a water molecule is fixed toward the oxygen of the dienone during the DNA alkylation of the pharmacophore,[7] which suggest the presence of catalytic proton during the alkylation. However the detail of the mode of action of the agents is yet to be studied.

The pharmacophore of the agents is the spirocyclopropyl-cyclohexadienone which has a highly strained cyclopropyl moiety and a cyclodienone moiety. The adenine N-3 alkylation gives the stable form of the pharmocophore, *i.e.*, an ethylphenol derivative. The strain energy of the spirocyclopropyl moiety is relieved and the aromatic resonance stabilization is acquired through the N-alkylation. At the final stage of the alkylation, one proton is necessary for the complete conversion of the dienone to the phenol and the adenine moiety will possess a positive charge at either the ring nitrogen or the amino group. The alkylation product was observed,[3] however, the full mechanism is yet to be studied with a theoretical method. Here we have selected several model molecules and studied the model reaction of the DNA alkylation of the model pharmacophore theoretically and tried to figure out the mode of action of the pharmacophore.[8]



(+)-CC-1065



(+)-Duocarmycin SA

Calculation

We simulated the model reaction of the DNA alkylation with Duocarmycins. The model pharmacophore is the spirocyclopropylcyclohexadienone (**Quin1**; spiro[2.5]octa-4,7-dien-6-one) and the model for an adenine or a guanine of DNA is 4-aminopyrimidine (**Pyrim**). The mode of action of a pharmacophore is the followings; a model cyclohexadienone is converted to a 4-ethylphenol after the covalent bond formation between the cyclopropyl moiety and the ring

nitrogen at the 1-position of **Pyrim**, and the same cyclohaxadienone protonated at the carbonyl oxygen undergoes the alkylation. We also calculated the distorted dienone (**Quin18**) by fixing a spirocyclopropyl C-C bond on the same plane with the cyclohexadieneone (the torsional angle is 180 degree) to estimate the intrinsic energy in the distortion, which appears in the structure of the tricyclic antibiotic agents. We have calculated a tricyclohexadienone (**Quin2**; 3-acetyl-1,1a2,3-tetrahydro-3-aza-cyclopropa[c]inden-5-one) and its protonated form (**Quin2_H**) which have another pyrrolidine moiety and are more likely model for the antibiotic agents than **Quin1**. The transition state (**QuinTS**) for the alkylation from **Quin2** and **Pyrim** was located. For the protonated alkylation, the transition state (**QuinTS_H**) from the **Quin2_H** and **Pyrim** was also calculated. Both transition structures have one and only one imaginary frequency in the frequency calculations. In the transition structures, the **Pyrim** rings are near vertical to the cyclopropyl rings of the hexadieneones. The end products (**QuinPD** and **QuinPD_H**) of the *N*-alkylation were calculated. The whole calculation was performed with GAUSSIAN98W at the basis set of RHF/6-31G (d,p) and B3LYP/6-31G (d,p).[9]

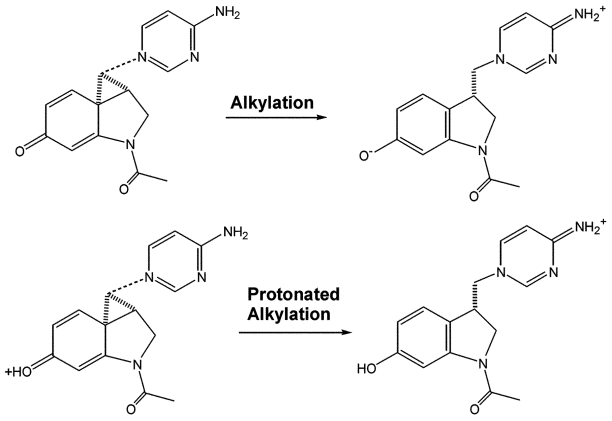


Table 1. Total energies and the reaction energies of the models of the pharmacophore

Basis set Model	RHF/6-31G (d,p)		B3LYP/6-31G (d,p)	
	Total energy (hartree)	ΔE (relative) (kcal/mol)	Total energy (hartree)	ΔE (relative) (kcal/mol)
Quin1	-382.429816	0.00 (relative)	-384.847497	0.00
Quin1_H	-382.807682	0.00 (relative)	-385.225845	0.00
Quin18	-382.422675	4.43 *vs* **Quin1**	-384.841316	3.88 *vs* **Quin1**
Quin18_H	-382.802354	3.34 *vs* **Quin1_H**	-385.221356	2.82 *vs* **Quin1_H**
Pyrim	-317.751782	–	-319.703238	–
Quin2	-627.118098	(-944.869880)[a]	-630.970344	(-950.673582)[a]
Quin2_H	-627.507807	(-945.259589)[a]	-631.360221	(-951.077688)[b]
QuinTS	-944.790612	49.74[c]	-950.626681	29.43[c]
QuinTS_H	-945.254657	3.09[d]	-951.072441	3.30[d]
QuinPD	-944.809830	37.68[c]	-950.644712	18.12[c]
QuinPD_H	-945.314150	-34.23[d]	-951.121986	-27.80[d]

[a]Total energy sum of **Pyrim** and **Quin2** or **Quin2_H**. [b]Total energy sum of a complex of **Pyrim** and **Quin2_H** separated by 5.0 Å. [c]Relative energy vs (**Pyrim** and **Quin2**). [d]Relative energy vs (**Pyrim** and **Quin2_H**).

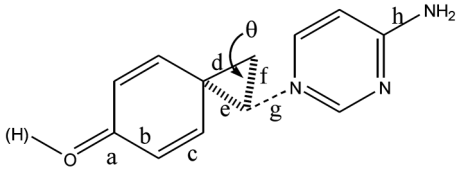


Table 2. Geometric parameters of the models of the pharmacophore at RHF/6-31G (d,p) (bond lengthes in Å, bond angles in degree)

Model	a	b	c	d	e	f	g	h	*θ*
Quin	1.2280	1.4692	1.3313	1.5324	1.5324	1.4855	–	–	60.9
Quin18	1.1998	1.4820	1.3260	1.5084	1.5408	1.4726	–	–	62.7
Quin_H	1.2940	1.4186	1.3506	1.5712	1.5716	1.4422	–	–	62.7
Quin18_H	1.2958	1.4170	1.3526	1.5384	1.6239	1.4406	–	–	66.0
Quin2	1.2000	1.4763	1.3303	1.5064	1.5184	1.4823	–	–	61.1
Quin2_H	1.2996	1.3940	1.3746	1.5272	1.5548	1.4590	–	–	62.7
QuinTS	1.2200	1.4609	1.3528	1.4982	2.1421	1.4782	1.9102	1.3327	92.1
QuinTS_H	1.3225	1.3972	1.3751	1.5259	1.9650	1.4444	2.2880	1.3315	82.8
QuinPD	1.2285	1.4534	1.3613	1.4950	2.4508	1.5380	1.5036	1.3230	107.8
QuinPD_H	1.3399	1.3950	1.3775	1.5111	2.4734	1.5362	1.4874	1.3143	108.6

Results and Discussion

Distortion of the cyclopropyl ring. The cyclohexadienone (**Quin1**) and the distorted cyclohexadienone (**Quin18**) are slightly different in geometry and energy (Table 1 and 2). The energy difference is 4.43 kcal/mol at RHF/6-31G(d,p). When we compressed the cyclopropyl ring from **Quin1** to **Quin18**, the C=O bond **a** is shortened, and the C-C bonds **b** becomes longer, and the C=C bond **c** becomes shorter; the geometric parameters show they moved in the reverse direction toward the aromatic phenol. The spirocyclopropyl moieties have longer C-C bondlengthes compared to the ordinary cyclopropyl groups (1.5324 vs 1.4971 Å), which indicated the conjugation between the cyclohexadiene and the spirocyclopropyl group. The C-C bond **d** of the distorted cyclopropyl moiety of **Quin18** which is placed on the same plane with the cyclohaxadienone ring has even shorter C-C length (1.5084 Å) and the other spiro C-C bond **e** has a longer bondlength of 1.5408 Å which is near the same of the acyclic C_{sp^3}-C_{sp^3} bond. The C-C bonds in cyclopropane are, so called, a banana bond, whose orbital angles of C-C-C are larger than 60 degree. The longer spirobond **e** in the model structure **Quin18** seems, therefore, to have better conjugation with two olefins of the cyclohexadienone. This elongation will lower the transition energy and make the structure more suitable to the transition state.

The comparison between the protonated cyclohexadienone (**Quin1_H**) and the distorted protonated cyclohexadienone (**Quin18_H**) shows that the energy difference is 3.34 kcal/mol, but their geometries are similar (Table 1). However the protonation makes the ring structure have more aromatic characters; longer carbonyl bond **a**, shorter C-C bond **b**, and longer C=C bond **c** than those of **Quin1** and **Quin18**. The major changes in **Quin18_H** are the elongation of the bond **e** from 1.5716 Å to 1.6239 Å and the bond angle of θ (66.0 degree), which is assumed to have better conjugation with the cyclohexadieone after the ring compression and is more suitable to the transition states of the alkylation.

Unprotonated alkylaton. The geometric parameters in **Quin18** and **Quin2** are similar. These geometric parameters are well correlated with the parameters from the experimental crystallography works (X-ray crystallography).[5] The model aminopyrimidine ring in the **QuinTS** approaches vertically to the cyclohexadienenone ring. The cyclopropyl ring angle **θ** in **QuinTS**, the index of the ring opening, is 92.1 degree and the length of the bond **e** in **QuinTS** is 2.1421 Å (those of **Quin2** and **QuinPD** are 1.5184 Å and 2.4508 Å, respectively), which indicates almost 70% ring opening. Therefore the transition state **QuinTS** is product-like. The product model, **QuinPD**, has a cyclohexadienone-like structure rather than an aromatic phenol-like structure; *i.e.*, the bond **b** and **c** are 1.4534 and 1.3613 Å and still are similar to a single and double bond, respectively. In the unprotonated process the charge delocalization seems to be not achieved.

The transition state energy of the alkylation was calculated from the **QuinTS** and the reactants of **Quin2** and **Pyrim** which are assumed to be separated infinitely. The energy difference between **Quin1** and **Quin18** is 4.43 kcal/mol and about 10% of the transition energy (49.7 kcal/mol). The distorted energy of 4.43 kcal/mol seems to play a minor role in the unprotonated alkylation; although there will be some catalytic environmental surroundings in the real system, they are ignored in this model calculation for simplicity. In the unprotonated alkylation, the negative and positive charge generated during the alkylation is not well delocalized; the

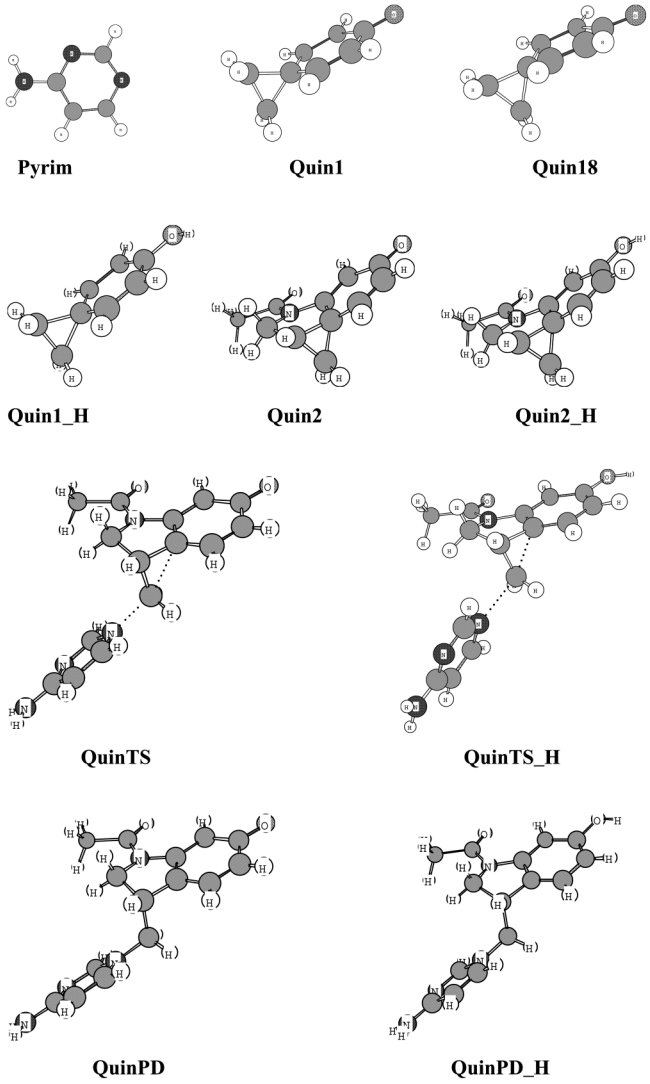


Figure 1. The various model structures of the reactants, the transition structures, and the products.

Table 3. Mullikan charge distribution of the models at RHF/6-31G (d,p)

Model	O/OH	N_1	4-NH_2
Pyrim	–	-0.5762	-0.1320
Quin2	-0.5797	–	–
QuinTS	-0.6921	-0.7166	-0.0728
QuinPD	-0.7341	-0.6599	-0.0340
Quin2_H	-0.1747	–	–
QuinTS_H	-0.2338	-0.7142	-0.0652
QuinPD_H	-0.2758	-0.6729	+0.0056

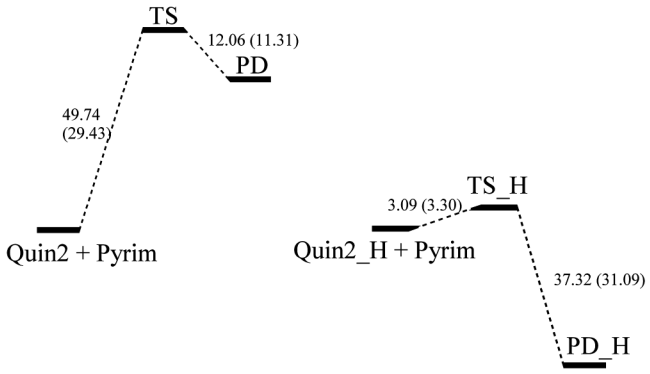


Figure 2. Energy profiles of the protonated alkylation and unprotonated alkylation (RHF/6-31G (d,p) in kcal/mol; the values in parenthesis are from B3LYP/6-31G (d,p)).

atomic charge of the carbonyl oxygen of **QuinPD** is increased only by 0.1544 and its atomic charge of 4-NH_2 is decreased by 0.0980 compared to **Pyrim** (Table 3). As a result, the energy of **QuinPD** is high compared to the reactants (**Quin** and **Pyrim**) by 37.7 kcal/mol. At B3LYP/6-31G (d,p) level, the energy gaps are reduced, but still the transition energy is 29.43 kcal/mol and the product is above the reactants by 18.12 kcal/mol.

Protonated alkylation. The bond lengthes of the carbonyl **a** increase from 1.2996 Å of **Quin2_H** to 1.3225 Å of **QuinTS_H** to 1.3399 Å of **QuinPD_H**, and those of the C-C bond **b** change from 1.3940 Å to 1.3972 Å to 1.3950 Å, respectively, and those of the C=C bond **c** change from 1.3746 Å to 1.3751 Å to 1.3775 Å, respectively. Unlike the previous unprotonated alklyation, the geometry of the protonated cyclohexadienone moiety of **Quin2_H** is already quite similar to the structure of the product phenyl, which surely is a factor lowering the transition state energy. The transition state **QuinTS_H** is above the separated reactants by 3.09 kcal/mol at RHF/6-31G (d,p). Its cyclopropyl moiety has a bond angle (**θ**) of 82.8 degree and the length of the bond **e** in **QuinTS_H** is 1.9650 Å (those of **Quin2_H** and **QuinPD_H** are 1.5548 Å and 2.4734 Å, respectively), which indicates about 40% ring opening. Therefore the protonated transition state **QuinTS_H** is an early transition state compared to that of the unprotonated **QuinTS** (70% ring opening). The bond length of the C-NH_2 of the product **QuinPD_H** is 1.3143 Å, well shorter than that of **Pyrim** (1.3494 Å), which indicates that the non-bonding electron pair on the NH_2 group participates in the conjugation[10] in **QuinPD_H**. And the ring structure of **QuinPD_H** shows the positive charge delocalized over the pyrimidine ring well in the protonated process; the atomic charge of 4-NH_2 is decreased by 0.1376.

The transition energies of **QuinTS** (49.72 kcal/mol) and **QuinTS_H** (3.09 kcal/mol) are quite different and the protonation seems to lower the transition energy by ~46 kcal/mol at RHF/6-31G (d, p) and ~26 kcal/mol at B3LYP/6-31G (d,p). When we consider that the energy lowering by the distorted spirocyclopropyl moiety is only 3-4 kcal/mol and the protonation process on the carbonyl of the cyclohexadienone lowers again the transition state energy by 46 kcal/mol (RHF/6-31G (d,p)) or 26 kcal/mol (B3LYP/6-31G (d,p)), we have to note that the major factor for the transition state is the protonation.

In conclusion, the spirocycloproyl moieties of the spirocyclopropylcyclohexadienone have a minor contribution of 3-4 kcal/mol to the transition of the pyrimidine alkylation, but the major contribution to the alkylation comes from the protonation on the keto-oxygen of the pharmacophore. Either the direct protonation on the keto-oxygen or the strong hydrogen binding of the water molecule toward the keto-oxygen will be necessary in the real DNA alkylation of the spirocyclopropylcyclohexadienone of Duocarmycins and CC-1065.

Acknowledgement. This work was supported by the Korea Research Foundation (1999-015-DP0220) and by Korean Council for University Education (2001 Domestic Faculty Exchange) and the Yeungnam University research grants.

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