NUCLEIC ACID BINDING DRUGS*. SOME CONFORMATIONAL PROPERTIES OF THE ANTI-CANCER DRUG DAUNOMYCIN AND SEVERAL OF ITS DERIVATIVES:

Implications for DNA-binding

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1. Introduction

The antibiotic daunomycin (fig.1) and its hydroxyl derivative adriamycin are highly active anti-cancer compounds that have found considerable clinical use [1–4]. Whereas both are potent antileukemic agents, adriamycin has an exceptionally broad spectrum of activity, particularly against a variety of solid tumours. The mechanisms of cytoxic activity of these drugs are complex, but it is generally accepted that they largely act by inhibiting nucleic acid synthesis via direct template binding [5–7]. In view of the pronounced cardiotoxicity of these drugs, there has been an extensive search for analogues possessing comparable neoplastic potency yet with reduced harmful side-effects (see, e.g. [2,4,8,9]). It has been suggested that the cardiotoxicity residues in the quinone part of the drug molecules, which could be activated to free radicals [10], or which could act as an inhibitor of the structurally-related Q_{10} co-enzymes [11].

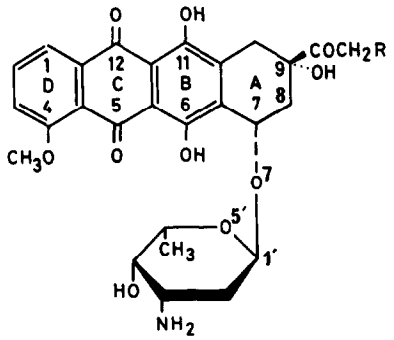

Fig.1. The structure of daunomycin (R=H). (Adriamycin has R=OH).

The interactions of these drugs with DNA at the molecular level are believed to involve:

(i) Intercalation of the planar daunomycinone chromophore in between adjacent base pairs of the double helix;

(ii) An electrostatic/hydrogen bond between the protonated daunosamine sugar amino nitrogen atom, and a phosphate oxygen atom of the polynucleotide backbone.

Various molecular models have been proposed for this binding, all of which involve these features [2,7,12,13].

The solid-state conformation of daunomycin has been determined in such environments as: pyridine [14] and butanol [15] adducts; the *N*-bromoacetyl derivatives [16]; and 4-hydroxydaunomycin (carminomycin) [17–19]. In all cases, the apparently flexible sugar–chromophore system adopts similar conformations. It has therefore been suggested that this preferred conformation is a minimum-energy conformation, and that it may also be the biologically active conformation [7,14]. It plausibly fits into a DNA-binding model with the two functional features of the drug most important for this (the chromophore and the

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charged nitrogen atom), being optimally spatially arranged with respect to each other.

Here we have explored, using semi-empirical energy calculations, the flexibility of daunomycin about the chromophore–sugar linkages. This technique has also been used to study the effects on this flexibility, of varying several atomic groupings attached to the drug molecule.

2. Methods

The total potential energy of a particular conformation was calculated by a standard semi-empirical approach, using classical in vacuo potential-energy functions [20–23]. Bond lengths and angles were constrained to their crystallographically-determined values [14]. All computations were performed on

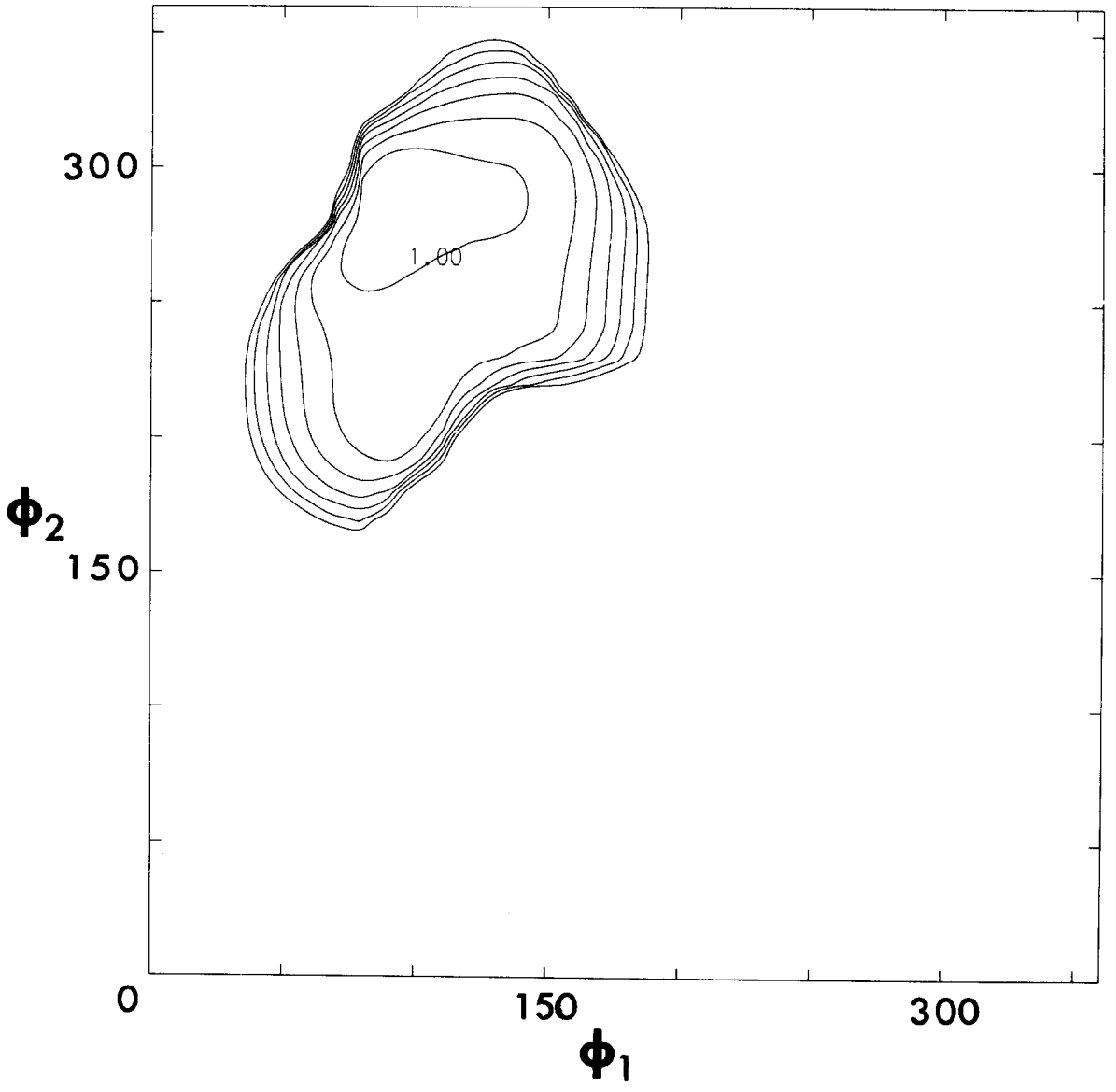

Fig.2. Energy map for daunomycin, the contours are at 1 kcal/mol intervals.

the University of London CDC7600 computer.

In all the calculations, the two torsion angles simultaneously varied were C8–C7–07–C1′ ($\phi(1)$) and C7–07–C1′–05′ ($\phi(2)$). Both were varied between 0° and 360° in 10° intervals, and potential energy surfaces were constructed. Relative populations of conformers were calculated by a standard Boltzmann distribution approach. Differences in conformer geometry were defined by a 'structural triangle' of the three atoms 011, 012 and N [24].

3. Results and discussion

Figure 2 shows the energy surface obtained for daunomycin itself. There is only one low-energy region which is relatively sharp and well-defined. The conformation corresponding to the minimum energy is close to, but not identical with, the experimentally-determined ones, as defined by the $\phi(1)$ and $\phi(2)$ values (table 1). The $\phi(1)$ values differ the most; it is significant that the calculated one is closest to that found for the *N*-bromoacetyl derivative, which does not have the intramolecular 07. . .09 hydrogen bond in its crystal structure (in contrast to daunomycin itself and carminomycin). Examination of the daunomycin molecular structure shows that the optimum geometry for this hydrogen bond is compatible with a $\phi(1)$ of 110–120°. Our calculations, which have not taken this additional interaction into account, show that the $\phi(1) = 124°$ conformation is only 0.7 kcal/mol less stable than the minimum-energy conformation. Computation of the hydrogen-bond stabilisation by standard methods [25] gives an associated energy of 1.7 kcal/mol, which more than offsets this difference. Molecular model-building shows that the $\phi(1) = 93°$ and 124° conformations can both fit into a DNA-binding model [7,12,13] – the maximal differences in the 011–N distances of 0.1 Å are not significant.

Figures 3–5 show energy maps for several hypothetical daunomycin derivatives with varying substitution patterns. Deletion of the 09 hydroxyl group (fig.3) does not result in any appreciable shift of the global energy minimum (table 1); the subsiduary minimum at $\phi(1) = 300°$ is of far too high an energy to be populated. Similarly, deletion of the $-COCH_3$ side chain at C9 produces no significant alteration in the position of the energy minimum (fig.4), although the low-energy region is now more diffuse than that for the native drug. Thus, since the relative dispositions of the nitrogen atom and the chromophore (as judged by the 011–N and 012–N distances), remain unaltered on going from daunomycin to both these putative derivatives, it may be concluded that their extent of DNA binding will be closely similar.

Deletion of the 06 hydroxyl group on the chromophore ring B results in a rather different situation (fig.5). The global minimum remains in the same area as before, with a calculated energy of –2.74 kcal/mol; however there is now a significant second minimum only 0.6 kcal/mol higher in energy, nearly 90° away

Table 1
Geometric features of the low-energy conformations of daunomycin and several hypothetical derivatives, compared with experimentally-determined values

	Torsion angles, in °		Distances in Å			Energy (kcal/mol)
	$\phi(1)$	$\phi(2)$	011–012	011–N	012–N	
Daunomycin minimum energy	93	290	2.58	8.92	10.24	–2.89
Daunomycin minus 06	121	204	2.58	7.41	7.41	–2.13
	90	288	2.58	8.91	10.21	–2.74
Daunomycin minus 09	84	287	2.58	8.90	10.19	–2.51
Daunomycin minus 06 and 09	121	203	2.58	7.40	7.48	–1.95
	84	290	2.58	8.89	10.22	–2.33
Daunomycin minus $-COCH_3$	92	286	2.58	8.91	10.17	–2.29
Daunomycin in the crystal [14]	125	292	2.58	9.02	10.29	–2.15
Carminomycin [17–19]	117	290	2.59	8.98	10.24	–2.25
N-Bromoacetyldaunomycin [16]	102	281	2.59	8.88	10.04	–2.48

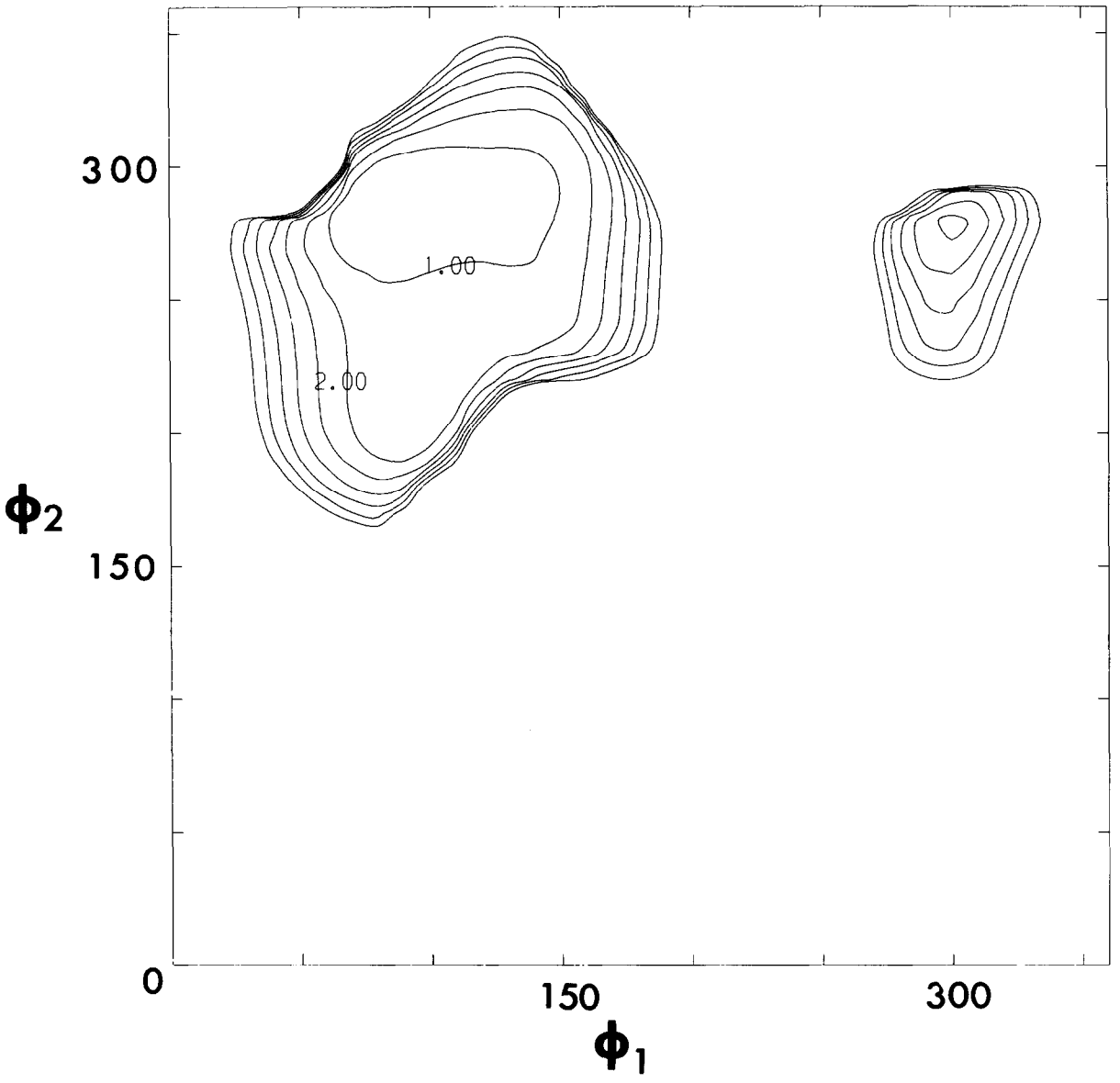

Fig.3. Energy map for daunomycin without 09.

in $\phi(2)$. The barrier to rotation between these two conformers is ~3 kcal/mol, and the subsiduary one has a relative population of 26%. Table 1 shows that the two conformers have very distinct geometric triangulation parameters. It is thus unsurprising that the $\phi(2) = 204^{\circ}$ structure cannot effectively bind to DNA with simultaneous involvement of the intercalating chromophore and the amine nitrogen atom, as judged by our model-building studies. Deletion of both 06 and 09 substituents results in an energy map displaying features of both fig.4 5 (table 1). In this case, the relative population of the minor conformer is 35%.

A study of the thermodynamics of daunomycin–DNA binding has shown [26] that the free-energy change on binding is ~–8.5 kcal/mol, with an enthalpy

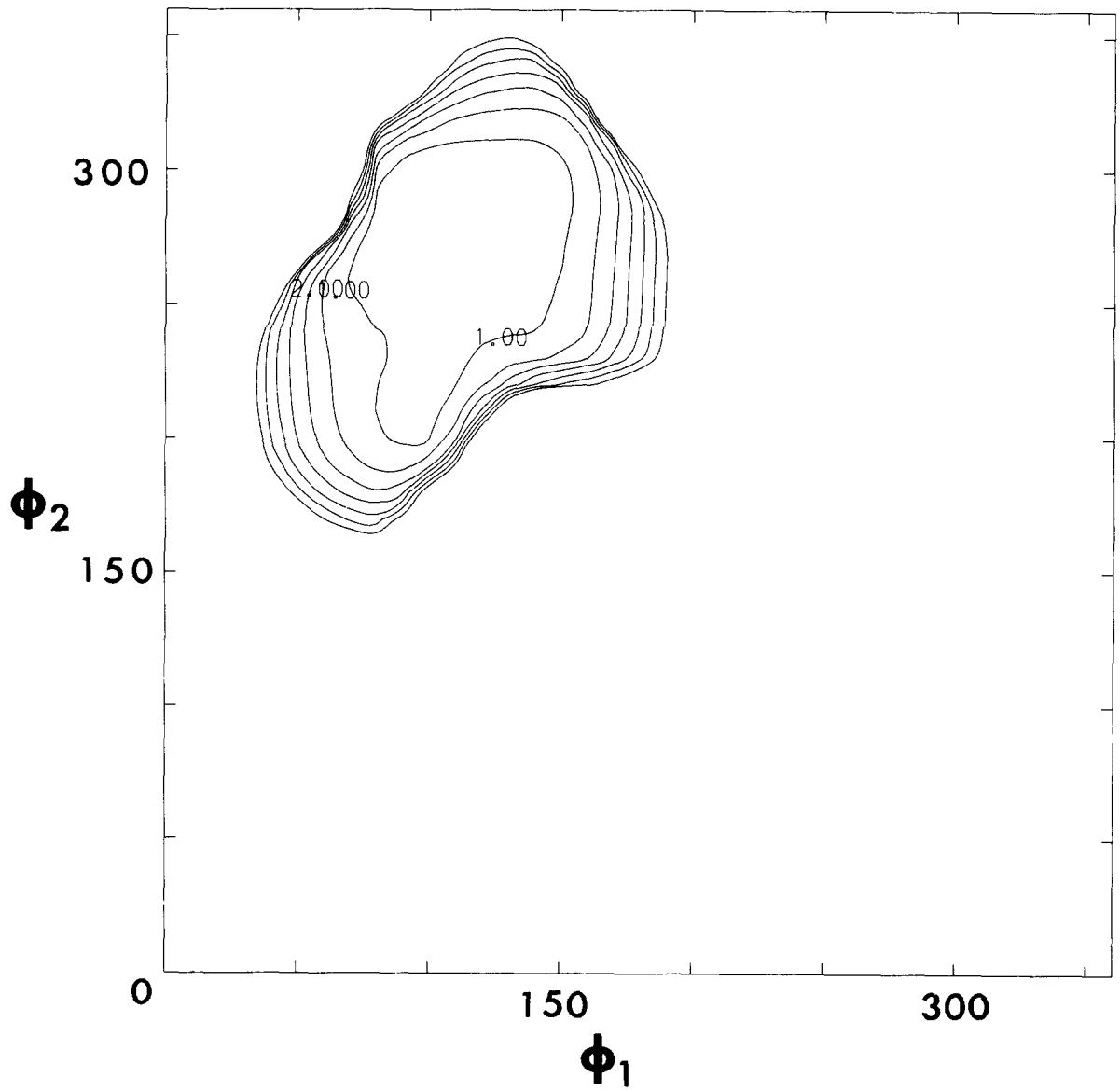

Fig.4. Energy map for daunomycin without the $-COCH_3$ side chain.

change of −5.3 kcal/mol. Thus, the barrier to rotation of the two (daunomycin minus 06) conformers is low by comparison and one would then not expect such a derivative to bind to DNA significantly less effectively than the parent drug. In view of the dihydroxyquinoid character of the daunomycinone chromophore being implicated in its cardiotoxic properties, it might be considered that amendment of this by deletion of 06 would result in a therapeutically-improved derivative. Our results suggest that such an approach to a superior drug would be fruitful if simultaneous retention of DNA affinity is sought. We thus conclude that none of the structural alterations of daunomycin investigated here will significantly affect its DNA binding capacity. However, since the biological activity of these drugs is an expression of

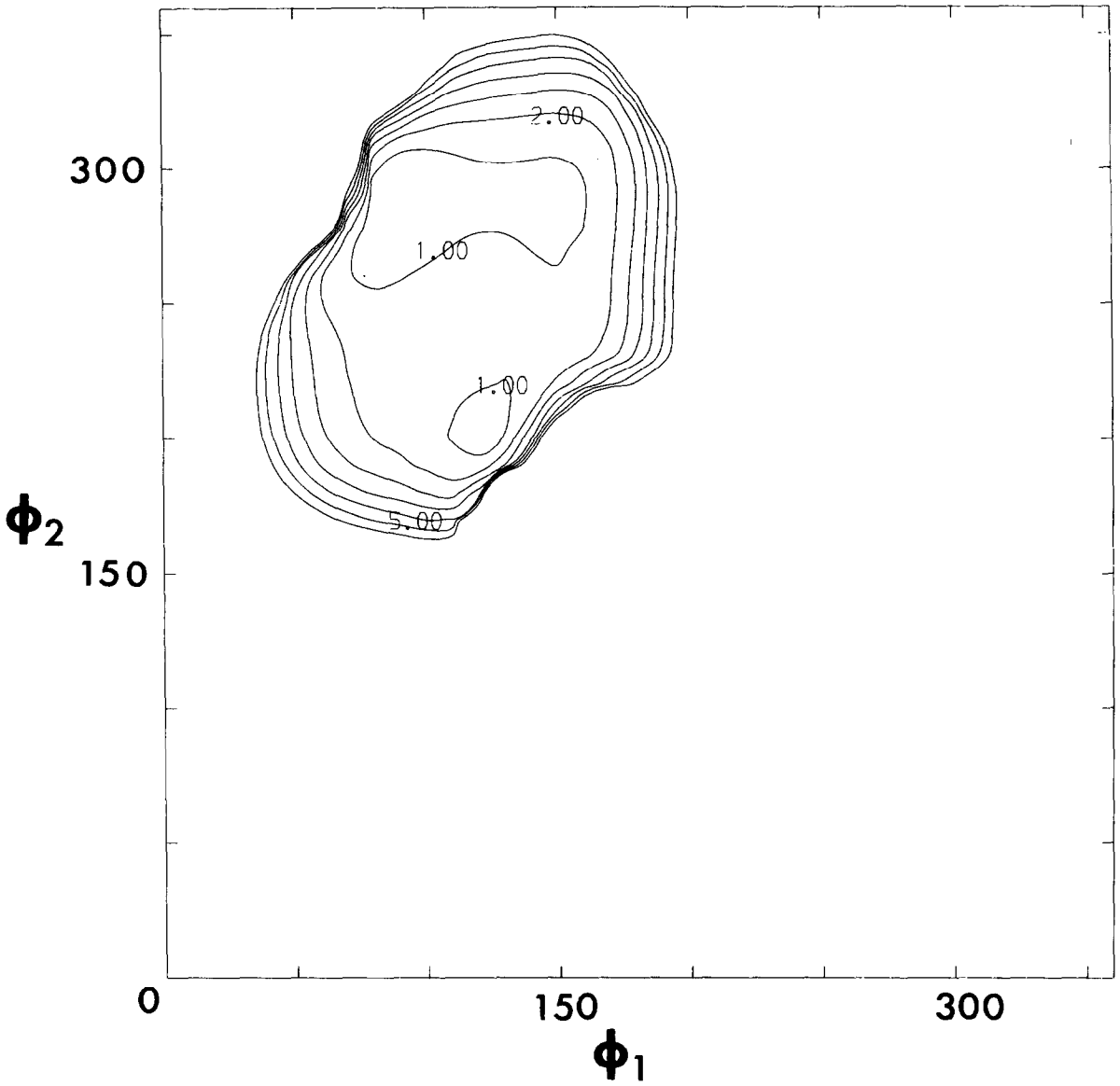

Fig.5. Energy map for daunomycin without 06.

many factors in addition to the purely conformational factors outlined above, the possession of pronounced DNA affinity does not necessarily impart useful activity.

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