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Quantitative Structure-Cytotoxicity Relationship Analysis of Betulinic Acid and its Derivatives by Semi-empirical Molecular-orbital Method

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Abstract. *A semi-empirical molecular-orbital method (CAChe) demonstrates that the cytotoxicity of betulinic acid derivatives can be predicted by several physical parameters (such as heat of formation, hydrophobicity (log P), water-solubility, ionization potential, electron affinity, dipole moment), but not by molecular size (maximum length and width). The present study demonstrates how this method can be applied to estimate the cytotoxic activity of structurally-related compounds.*

Betulinic acid [3β-hydroxy-lup-20(19)lupaen-28-carbonic acid] (**[3]** in Figure 1) is a lupane-type triterpene which was first isolated from the stem bark of an East African evergreen tree, *Ziziphus mauritiana* Lam. (Rhamnaceae), but is also found abundantly in a variety of plants (1). It induced apoptosis in melanoma (2) and neuroectodermal tumors (3), independently of the ligand/receptor system, through a reactive oxygen species-dependent mitochondrial-cytochrome c-caspase pathway (references cited in ref. 4). We have recently reported that 5 betulinic acid derivatives **[1-5]** (Figure 1) induced the cytotoxicity of murine B16 melanoma cells, accompanied by apoptosis characterized by production of apoptotic bodies (increase of sub G1 cell population), DNA fragmentation and dissipation of mitochondrial membrane potential (4). Among these compounds, **[1]** (MW=426, 50% cytotoxic concentration (CC_{50}>234.7 μM), **[2]** (MW=442, CC_{50}=226.2 μM) and **[3]** (MW=456, CC_{50}=166.6 μM) showed little or no cytotoxicity, whereas **[4]** (MW=472, CC_{50}=67.8 μM) and **[5]** (MW=470, CC_{50}=47.9 μM) were more cytotoxic (4). In this paper, a direct molecular structure-cytotoxicity (CC_{50}) relationship using a semi-empirical molecular-orbital method is demonstrated.

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Materials and Methods

Calculation. The most stable structure of derivatives **[1-5]** was calculated by CONFLEX (Confluex Co. Ltd., Tokyo, Japan). The optimization of the structure was done by the semi-empirical molecular-orbital method (MOPAC, PM3, non-COSMO), using the CAChe Worksystem 4.9 (Fujitsu Co. Ltd., Tokyo, Japan). Molecular characteristics (heat of formation, ionization potential, electron affinity, dipole moment and maximum length and width of the molecule) delineated by these calculations were used to establish the relationship with the CC_{50} value (5). The octanol-water distribution coefficient (log P) and solubility were calculated by ACD-Log P (Fujitsu) and ACD-Solubility (Fujitsu), respectively. The relationship between the physical properties and molecular size, determined from molecular structures, and the CC_{50} were investigated, using the CAChe Worksystem 4.9 project reader.

Results and Discussion

Figure 1 shows the most stable structures of 5 betulinic acid derivatives **[1-5]**, from which the heat of formation and 7 other parameters were calculated. Figure 2 summarizes the relationship between each parameter and the CC_{50}. Figure 2A shows the relationship between the heat of formation and CC_{50}. These 2 parameters produced a well-fitted regression line. A relatively high correlation coefficient (r^2=0.786) suggests that the heat of formation is a good

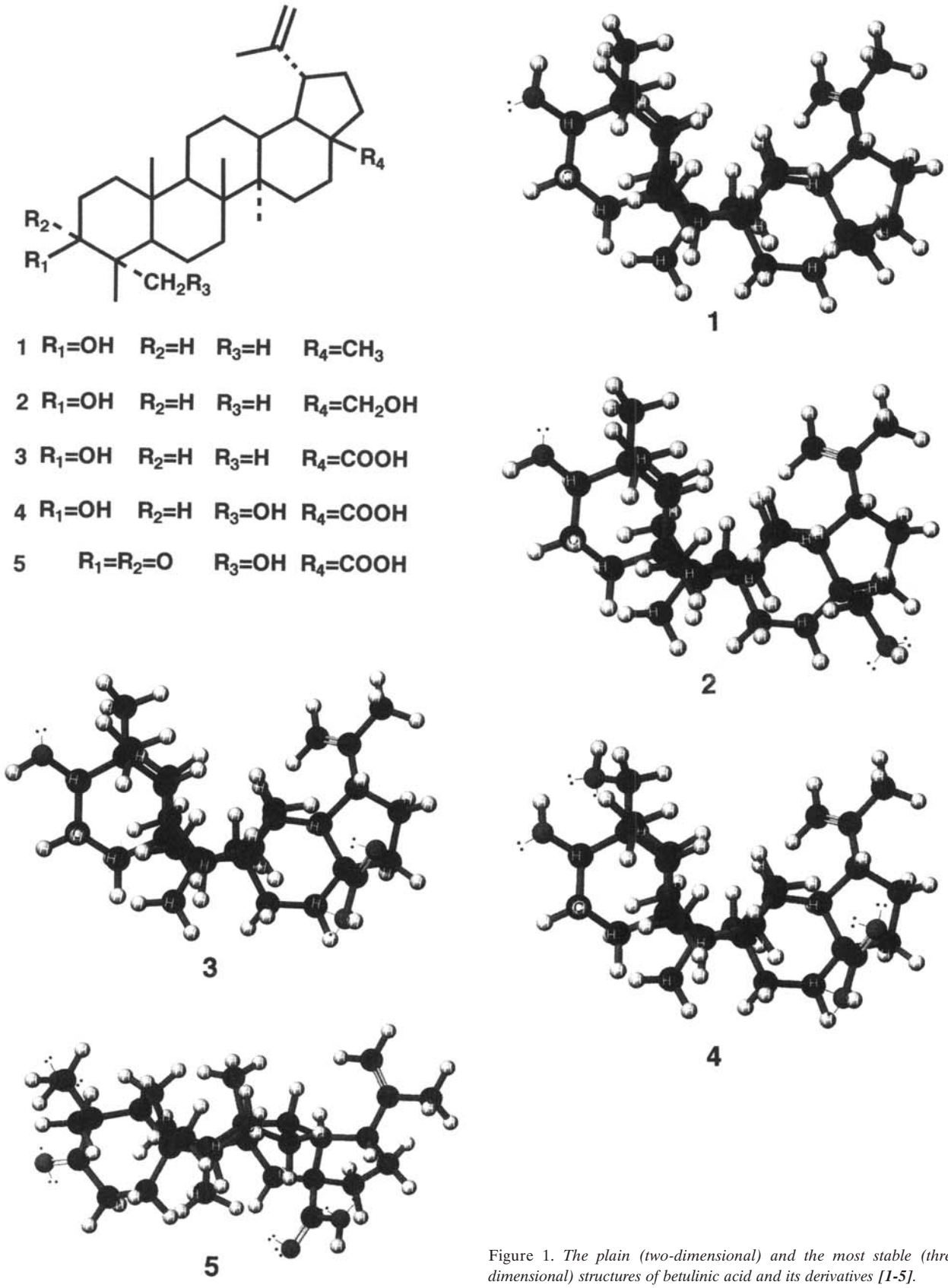

Figure 1. *The plain (two-dimensional) and the most stable (three-dimensional) structures of betulinic acid and its derivatives* ***[1-5]****.*

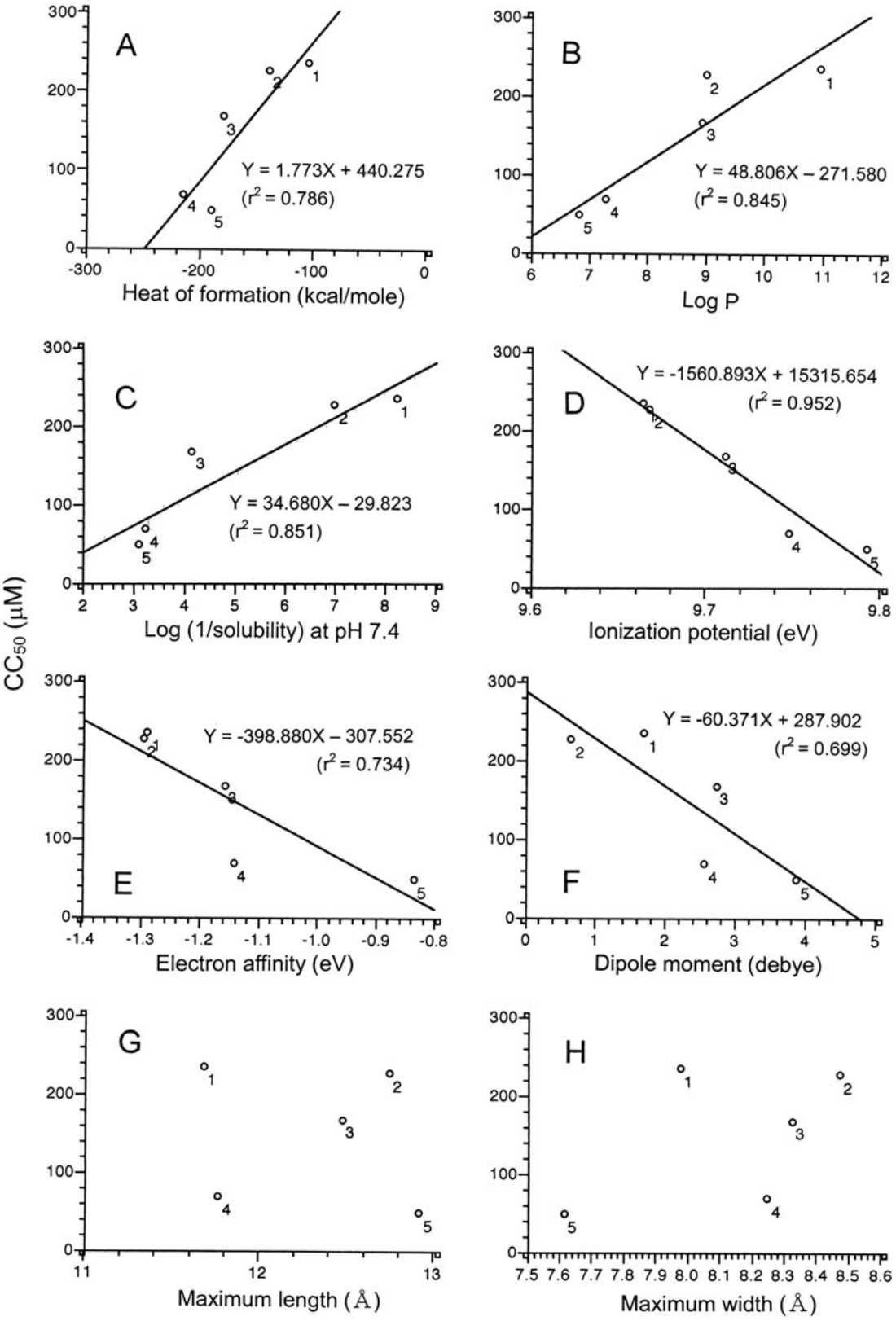

Figure 2. *Relationship between heat of formation (A), log P (B), water-solubility (C), ionization potential (D), electron affinity (E), dipole moment (F), maximum length (G) or maximum width (H) and cytotoxicity of betulinic acid and its derivatives* ***[1-5]****.*

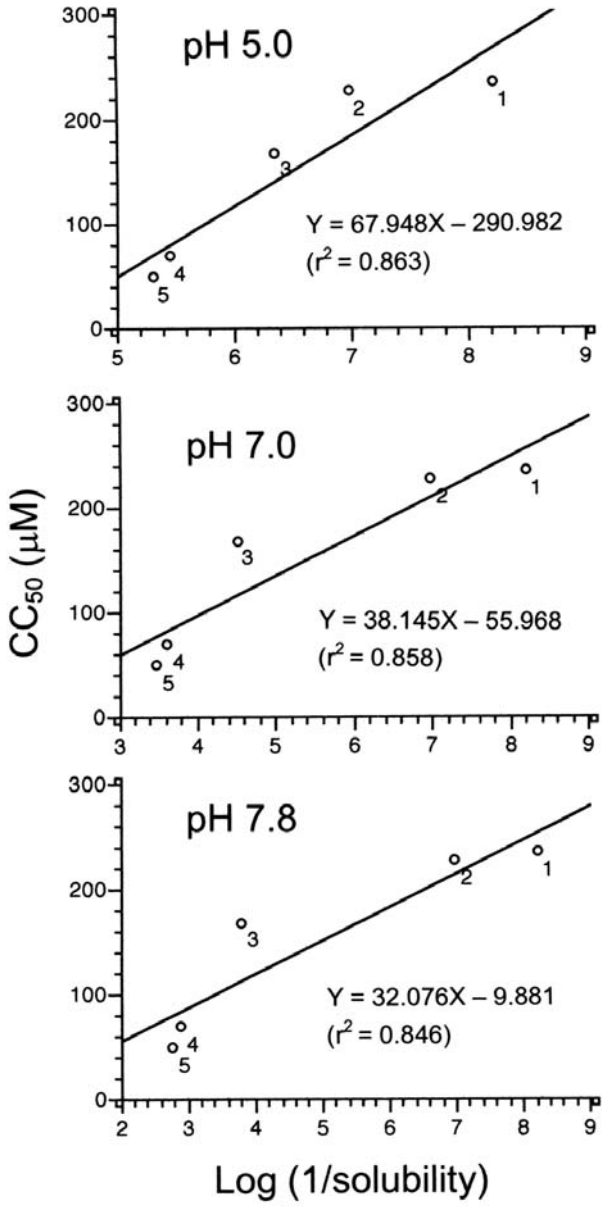

Figure 3. *Effect of pH on the solubility and cytotoxicity of betulinic acid and its derivatives* ***[1-5]****.*

factor to predict the cytotoxic activity of betulinic acid derivatives.

Figure 2B shows the relationship between the hydrophobicity (measured by log P) and the CC_{50}. Log P is an important parameter that defines the hydrophilicity or hydrophobicity of a molecule. In general, the cytotoxicity of many drugs, including gallic acid (6), vitamin K (7) and flavonoids (8, 9) and *tert*-butyl-substituted phenols (10), becomes maximum when log P reaches around 2 to 3. With an increase or decrease of the log P from this optimum value, the cytotoxicity declines. All compounds **[1-5]** were highly hydrophobic (log p>6.8) and were, therefore, unable to stably interact with water molecules, regardless of a relatively good correlation coefficient (r^2=0.845) (Figure 2B).

Figure 2C shows the good correlation between the water-solubility measured at pH 7.4 and the CC_{50} (r^2=0.851). Since the intracellular milieu of apoptotic cells is acidic (11), we investigated the effect of pH on the solubility of the betulinic acid derivatives (Figure 3). The solubility declined when the pH of the solvent was reduced from pH 7.4 to pH 5.0. The water-solubility, however, might be a useful parameter because of a good correlation coefficient between it and the CC_{50} values of the 5 betulinic acid derivatives (r^2=0.846-0.863), regardless of pH (Figure 3).

Similarly, the ionization potential (r^2=0.952) (Figure 2D), electron affinity (r^2=0.734) (Figure 2E) or dipole moment (r^2=0.699) (Figure 2F) produced well-fitted correlation with the CC_{50}. These 3 factors can also be used for predicting the biological activity of betulinic acid derivatives.

However, in terms of the correlation between the molecular size and the CC_{50}, no clear-cut correlation was found between the maximum length (Figure 2G) or width (Figure 2H) and the CC_{50}, implicating that these factors may not be appropriate for the study of structure-activity relationships.

We demonstrated for the first time, that the cytotoxic activity of 5 betulinic acid derivatives **[1-5]** can be predicted by several physical parameters (such as heat of formation, log P, water-solubility, ionization potential, electron affinity, dipole moment), but not by the molecular size (maximum length and width). This result is consistent with the fact that membrane permeability depends on the lipophilicity (log P) (12). The lack of a clear-cut correlation between the molecular size and the CC_{50} may be due to the relatively narrow range of molecular sizes of the 5 compounds (Figure 1). Thus, the present study demonstrates how this semi-empirical molecular-orbital method can be applied to estimate the cytotoxic activity of structurally-related butelinic compounds.

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