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Reactions and Computational Studies of Andrographolide Analogues with Glutathione and Biological Nucleophiles

穿心蓮內酯同系物與谷胱甘肽等生物親核劑的反應和計算研究

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ABSTRACT

The aims of this work were to investigate the reactivity of the alpha methylene lactone moiety of andrographolide and its analogues to nucleophiles of biomolecules, and to explore the structural activity relationship of these compounds. The efforts were focused on search for molecular targets that react well with andrographolide.

Andrographolide is a diterpenoid component isolated from *Andrographis paniculata* which is a traditional herbal medicine claimed to be effective against an array of diseases. Here, only the anticancer activity of andrographolide was pursued in this study. It is well documented that the anticancer activity of andrographolide is due to the alpha methylene lactone. Alkylation of biological nucleophiles, especially sulphydryl groups, by the α,β-unsaturated carbonyl structure in a Michael addition, has been regarded as the major reaction which lead to the cytotoxic effect of the alpha methylene lactone structure of andrographolide.

Reactions between andrographolide and L-cysteine were studied at 37°C in different pH values by indirectly monitoring the free sulphydryl group of cysteine. Andrographolide was able to scavenge the thiol group and the reaction rates were enhanced with the pH value at the range from 6.0 to 7.0. This result indicates that andrographolide can interact with the thiol group in biomolecules. In order to reveal the interaction between andrographolide and biomolecules, the bimolecular reaction between andrographolide and glutathione was investigated under a condition mimicking *in vivo* environment. Stoichiometric analysis indicates that the reaction between these two reactants is 1 to 1 at pH 7.0. The reaction rate followed a second-order kinetic. Using a micro-liquid-liquid extraction method followed by HPLC separation, two major products

were isolated and identified, their chemical structures were determined as 14-deoxy-12-(glutathione-amino)-andrographolide and 14-deoxy-12-(glutathione -S-yl)-andrographolide.

When computational chemistry was applied to explore the structural reactivity of andrographolide and its analogues to L-cysteine in both gaseous and aqueous phases, it was found that the 16-carbonyl, 12,13-olefin bond and 14-hydroxyl on the alpha methylene lactone of the andrographolide are the key structural moieties which are responsible for the activity of andrographolide. The trend of the computational reactivity of these pharmacophores was in good agreement with the cytotoxicity of their parent compounds reported in experimental literatures. When the reactivity of some natural compounds, such as several sesquiterpenes and diterpenes, was modeled using similar *ab initio* method, it was found that the calculated results were also in good agreement with the bioactivity of these natural compounds reported in literatures. Based on the above studies, potential macromolecules were envisaged to be proteins or peptides which possess a cysteine residue near its active site. Therefore, the CAAX motif of proteins of CENP-E and CENP-F were investigated based on quantum chemistry calculation. Besides the thiol group, andrographolide has been reported to interact with amino group of biomolecules. However, the computational results indicate that the reactivity of andrographolide with amine was lower comparing with thiol group.

Our experimental works confirm that andrographolide did react with nucleophiles via a Michael reaction at the unsaturated lactone moiety of andrographolide. By using HPLC, the reactants were isolated and identified. Thus, the computational studies described in this thesis provide good evidence of structural activity relationship for andrographolide and its analogues to protein molecule.

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