壹、甜菊糖苷;類似物之合成與生物活性探討； 貳、斑蝥胺素衍生物之細胞毒性探討

I. Study on Synthesis and Biological Activities of Stevioside Analogues;II. Study on Cytotoxicity of Cantharidinimide Derivatives

中文摘要

壹、修飾天然化合物爲藥物之設計，均導因於其增加活性之特性。結合活性天然藥物之化學與藥理活性的研究，爲提供現代化生物有機化學及藥物化學研究重要的指引。重新發現甜菊糖苷(stevioside)之藥用研究起因於其爲天然來源及其不斷增加之應用。

甜菊糖爲具有 ent-kaurane 結構之糖苷，源自菊科植物 Stevia rebaudiana Bertoni，其用途不僅爲食品添加之甜味劑，並具有降血壓、降血糖、抗發炎及抑制腫瘤增生之作用。因此菊糖類似物之結構活性相關性之建立將是開發其活性重要之課題。

經由 PyBOP 偶合活化酯法，與烷基胺類及烷基雙胺類化合物反應，合成 22 個新穎之甜菊二糖苷類似物及其結構相關之甜菊醇及異甜菊酮等之雙體化合物。所有合成之化合物，均經過 IR、1H NMR、13C NMR、FABMS 及 LC/MS/MS(ESI)等光譜解析，其結果顯示胺類之親核性、鹼性、立體之遮蔽效應及反應之溫度會影響甜菊二糖苷醯胺化合物之形成。糖苷並不會阻礙醯胺之偶合反應。

所有合成之化合物均進行人類癌細胞及人類正常肺纖維母細胞之細胞毒性檢測。其結果顯示於癌症細胞株之細胞毒性，與這些化合物之不同部位之結構有關。合成之化合物並以「改良式微孔盤抗生素敏感性檢測法(MMAST)」進行革蘭氏陽菌性及革蘭氏陰性菌之抗菌性試驗。其中化合物 2t 較青黴素 G 顯現對枯草桿菌(BCRC 10029)更強之抗菌活性。由本研究對於雙體及糖苷結構之結構活性相關性，指出可能與分子辨識與專一性接受體反應有關。我們推測不同之鏈結長度，可能影響與接受體之配位結合，而促進其生理活性。

貳、斑蝥素存在於 Mylabris caraganae 及其它昆蟲體內，並具有極強之抗癌活性、造成白血球增生及抑制蛋白質磷酸水解酶之活性。而其刺激性及發疱性之副作用及毒性，限制了斑蝥素於治療上之用途。於先前之研究中顯示，以斑蝥素與不同之胺類化合物，於三乙基胺鹼性下加熱 200oC 催化反應，而合成一系列之胺基噻唑或噻二唑斑蝥胺素化合物；而此類似物中，具有較高溶解度之化合物，亦對人類肝癌細胞株顯現較強之細胞毒性，此結果促使本實驗室進一步備製其它之斑蝥胺素衍生物。於本實驗中自行合成脂肪類之斑蝥素胺素 3a—3c，並與芳香類、吡啶類斑蝥素胺素，共 21 個衍生物與斑蝥素，測試此類化合物對肝癌及血癌細胞株(Hep G2 及 HL-60)之細胞毒性；細胞毒性測試顯示斑蝥素 1 較強之毒性與細胞毒性，斑蝥胺素衍生物顯現之毒性較低，但仍具有相當之抑制活性。可預測此

類化合物中降低胺取代基之誘導電負性，將降低產率及增加細胞毒性之表現。其結果並顯示具有硝基吡啶及硝基苯結構之班蝥胺素，具有與班蝥素相當之抑制活性。

英文摘要

I.The medicinal preparations based on the modification natural compounds frequently exceeded the native substances in the activity. In this connection investigation into the chemistry and pharmacological properties of bioactive natural substances has become an important direction in the modern bioorganic and medicinal chemistry. The renewed interest in Stevioside reflects the general trends observed in medicine were remedies of the natural origin are finding an increasing application.

Stevioside 3, an ent-kaurene glycoside extracted from Stevia rebaudiana Bertoni (Compositae), is used not only as a food additive for sweetening purposes but for anti-hypertensive, anti-hyperglycemia, anti-inflammatory and suppressed the tumor-promoting effects. Thus, stevioside analogues would be of interest to exploit its potential activities and to establish structure activity relationships.

Twenty-two novel steviolbioside analogues and their related steviol and isosteviol dimers are prepared using aliphatic alkylamines and alkyldiamines by the activated esters method (PyBOP coupling). The structures of these compounds were established using spectroscopic analysis including IR, 1H NMR, 13C NMR, FABMS, and LC/MS/MS(ESI). The results obtained, however, strongly confirm the influence of amine nucleophilicity, basicity, the steric hiderance and reaction termerature somewhat influenced the formation of steviolbioside carboxamides. Glycoside sugars did not seem to perturb the coupling reactions of carboxamides.

The cytotoxicities of all of the synthesized compounds to cancer cell lines and human embryo lung cells are examined. These results suggest that the cytotoxic effect on cancer cells involves many different structural domains of the compounds. The antibacterial activities of these synthesized compounds were also detected using modified microplate antibiotic susceptibility testing method (MMAST) with both gram-positive and gram-negative bacteria. The results indicated that compound 2t is more-potent antibacterial effect than penicillin G on Bacillus subtilis (BCRC 10029). Prior to this study, the available information on dimerization and glycodic moiety of SAR indicated that molecular recognition involved specific receptor interactions. We propose that the various chain lengths can change the binding of ligands to their respective receptors and thereby improve biological activities.

II.Cantharidin is found in Mylabris caraganae and various other insects and has an extremely high potency with antitumor properties, causes leukocytosis and inhibition of protein phosphatase activity. However, it is rarely employed to the therapeutic

treatment owing to the irritation and vesication side effects and as toxic properties. In our previous studies, a series of N-thiazolyl and thiadiazolyl cantharidinimides were prepared starting from cantharidin with various amines in triethylamine by heating to ca. 200 oC, and the analogues with better solubility showed cytotoxicity against human hepatocellular carcinoma cell lines, which encouraged us to prepare cantharidinimide derivatives. This present study, the aliphartic cantharidinimide 3a—3c is synthesized and the cytotoxicity of aliphartic, aryl, and pyridyl of twenty-one derivatives and cantharidin is investigated against Hep G2 and HL-60 cell lines. Cytotoxicity tests shows that Cantharidin 1 is more toxic and exhibits greater cytotoxicity, while synthesized cantharidinimides is less toxic but also exhibits inhibitory effects. It can be suspected that the decrease with the inductive electron negative effect of the N-substituted group will decrease the yield and increase the cytotoxicity. The results suggest that the presence of a nitropyridyl or nitrophenyl moiety display strong inhibitory effects on both cell lines as did cantharidin.