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Structural analysis of isosteviol and related compounds as DNA polymerase and DNA topoisomerase inhibitors

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Abstract

Isosteviol (*ent*-16-ketobeyeran-19-oic acid) is a hydrolysis product of stevioside, which is a natural sweetener produced in the leaves of *Stevia rebaudiana* (Bertoni) Bertoni. In this report, we prepared isosteviol and related compounds from stevioside by microbial transformation and chemical conversion and assayed the inhibitory activities toward DNA metabolic enzymes and human cancer cell growth. Among twelve compounds obtained, only isosteviol (compound **3**) potently inhibited both mammalian DNA polymerases (pols) and human DNA topoisomerase II (topo II), and IC_{50} value for pol α was 64.0 μM. This compound had no inhibitory effect on higher plant (cauliflower) pols, prokaryotic pols, human topo I, and DNA metabolic

Abbreviations: topo, DNA topoisomerase; pol, DNA polymerase; dTTP, 2′-deoxythymidine 5′-triphosphate; dNTP, deoxyribonucleoside triphosphates; DMSO, dimethylsulfoxide; dsDNA, double stranded DNA; EtBr, ethidium bromide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

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enzymes such as human telomerase, T7 RNA polymerase, and bovine deoxyribonuclease I. With pol α, isosteviol acted non-competitively with the DNA template-primer and nucleotide substrate. Isosteviol prevented the growth of human cancer cells, with LD_{50} values of 84–167 μM, and 500 μg of the compound caused a marked reduction in TPA (12-*O*-tetradecanoylphorbol-13-acetate)-induced inflammation (inhibitory effect, 53.0%). The relationship between the structure of stevioside-based compounds and these activities were discussed.

Keywords: Isosteviol; DNA polymerase; DNA topoisomerase; Enzyme inhibitor; Cytotoxicity; Anti-inflammation

Introduction

Stevioside (compound **1**) is a glycoside formed by three glucose molecules and steviol (compound **2**), a diterpenic carboxylic alcohol (Fig. 1). Stevioside is a high-intensity sweetener that tastes about 300 times sweeter than sucrose (0.4% solution). In many countries, it is used as a low-calorie sweetener in a wide range of food products and beverages. Stevioside has been used for several years as a sweetener in South America, Asia, Japan, China, and different countries of the European Union, while in the United States, it has been used as a dietary supplement since 1995. *Stevia rebaudiana* (Bertoni) Bertoni products are approved for sweetening purposes in Brazil, Korea, and Japan (Kinghorn, 2002). The advantages of stevioside as a dietary supplement for human subjects are manifold: it is stable, it is non-calorific, it maintains dental health by reducing the intake of sugar, and it has possibilities for use by diabetic and phenylketonuria patients and obese persons.

We have reported that novel anti-TPA-induced inflammatory compounds, a novel terpeno benzoic acid (i.e., myrsinoic acid A (MAA)), novel triterpenoids (i.e., tormentic acid (TA) and euscaphic acid (EA)) and some phenolic compounds such as curcumin were inhibitors of mammalian DNA polymerase α, β and λ (pol α, β and λ) (Mizushina et al., 2000, 2003; Murakami et al., 2002). Although tumor promoters are compounds that promote tumor formation (Hecker, 1978), these compounds also cause inflammation and are generally used as artificial inflammation inducers for the screening of anti-inflammatory agents (Fujiki and Sugimura, 1987). Tumor promoter-induced inflammation can be distinguished from acute inflammation, which is exudative and accompanied by fibroblast proliferation and granulation. TPA not only causes inflammation but influences mammalian cell growth (Nakamura et al., 1995), suggesting that the molecular basis of the inflammation is the pol reaction related to cell proliferation. Here, we investigated the mode of action of stevioside, isosteviol and related compounds against pols and DNA metabolic enzymes, which are responsible for DNA replication leading to cell proliferation and DNA repair/recombination and, then, the relation between the degree of the cell growth inhibitory effect and the anti-inflammatory activity.

We report here that one of the compounds, isosteviol (compound **3**), is a potent inhibitor of mammalian pols and human DNA topoisomerase II (topo II). Pols and topos were suggested to be important cellular targets for chemical intervention in the development of anti-cancer agents. Isosteviol inhibited both the growth of human cancer cells and the TPA-induced inflammation. Therefore,