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GLUCOSYLATED ISOFLAVONES AS DNA TOPOISOMERASE II POISONS

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Since topoisomerase poisons allow the enzyme to cut and covalently bind to DNA but abort the subsequent rejoining of the molecule after relieving the torsional stress. To study their action we have made use of a supercoiled form of the pRYG plasmid that bears a specific topoisomerase recognition and binding region. The conversion of the supercoiled circular double-stranded DNA to the linear and open circle forms in the presence of a topoisomerase II poison and a denaturation step by proteinase K-SDS is indicative of the efficiency of our test agents to stabilize the cleavable complex. Using this system, three glucosylated isoflavones (6′-methoxypseudobaptigenin-7-*O*-β-glucoside, genistin, and daidzin) isolated from cytotoxic chloroform and ethyl acetate extracts of *Retama sphaerocarpa* Boissier, were found to have the ability to stabilize the cleavage complex human DNA topoisomerase II.

Keywords: Retama sphaerocarpa; Topoisomerase II; Isoflavones; Genistin; Daidzin, 6′-methoxypseudobaptigenin-7-*O*-β-glucoside

INTRODUCTION

DNA topoisomerases are essential enzymes that govern DNA topology during fundamental nuclear metabolic processes. Chemicals agents able to interfere with DNA topoisomerases are widespread in nature, and some of them have outstanding therapeutic efficacy in human cancer and infectious diseases.

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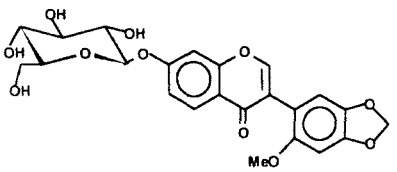
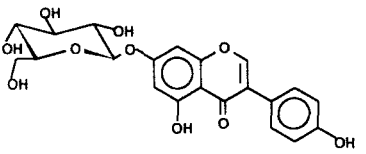
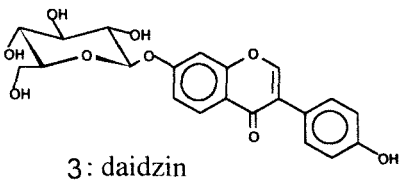
Several plant-derived flavonoids have been previously reported to inhibit certain regulatory enzymes including protein kinase C,[1] reverse transcriptase,[2] and DNA topoisomerase (topo) I and II.[3,4] The isoflavone genistein has been found to be a potent and specific *in vitro* inhibitor of the activity of the epidermal growth factor receptor tyrosine kinase[5] and it has been reported to stabilize the covalent topo II-DNA cleavage complex and thus function as a topo II poison.[6,7] Stabilization of the cleavage complex on DNA may not be directly cytotoxic. It appears that there must be some secondary event to generate the toxic DNA lesion. One attractive model that has experimental support considers that collision of DNA replication forks with cleavage complexes causes the complex to fall apart without rejoining DNA, thereby generating lethal double strands breaks.[8]



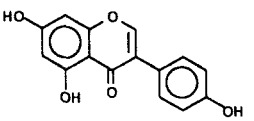
1: 6′-methoxypseudobaptigenin-7-*O*-β-glucoside



2: genistin



3: daidzin



4: genistein

In order to identify new topoisomerase poisons from plant metabolites and as part of our continuing search for flavonoids,[9–11] we have found that three glucosylated isoflavones (6′-methoxypseudobaptigenin-7-*O*-β-glucoside, genistin, and daidzin) isolated from a cytotoxic chloroform and ethyl acetate extracts of *Retama sphaerocarpa* Boissier have the ability to induce a cleavage complex with topoisomerase II.

MATERIALS AND METHODS

Enzymes, Nucleic Acids and Chemicals

Purified enzymes and supercoiled pUC19 were purchased from TopoGen, Inc. (Columbus, OH, USA). Proteinase K and genistein were from Sigma

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Chemical Co. Stock solutions of these drugs were dissolved in dimethylsulfoxide at 40 mM and were diluted in water containing 2.5% dimethylsulfoxide before use. The glycosylated isoflavones used in the present study, 6′-methoxypseudobaptigenin-7-*O*-*β*-glucoside, genistin and daidzin were isolated from cytotoxic chloroform and ethyl acetate extracts from *Retama sphaerocarpa* according to the method of López-Lázaro *et al.*[9]

DNA Cleavage Reactions with Topoisomerase II

Cleavage buffer contained 30 mM Tris–HCl, pH 7.6, 60 mM NaCl, 15 mM mercaptoethanol, 8 mM $MgCl_2$, 3 mM ATP. In the cleavage reaction (20 μL) containing water, cleavage buffer, glucosilated isoflavones dissolved in 2 μl dimethylsulfoxide/H_2O (2.5%), pUC19 DNA (0.25 μg in 1 μL of buffer), and 2 μl (4 units) of topoisomerase II storage buffer, the components were mixed in this order in ice/water. Reactions were carried out by incubation at 37°C for 30 min, terminated by the addition of 2 μl SDS 10% and 1 μl proteinase K 20 μg/ml and followed by an additional 15 min incubation at 37°C. Subsequently, the samples were extracted with chloroform : isoamyl alcohol, and 2 μl bromophenol blue. Samples were loaded on 1% agarose gels and electrophoresced at 6 V/cm for 2.5 h in Tris–acetate–EDTA buffer. Gels were stained with ethidium bromide and washed in a large amount of water. The DNA band was visualized under UV light and photographed with Polaroid type 667 positive/negative film. For the quantitative determination of topo II activity, videoimpression was measured densitometrically using PCBAS software. After integration of the three bands, linear and nicked open circle (OC) forms were expressed as a percentage of total DNA.

RESULTS

The gel presented in Figure 1 shows the stabilization effects of the three isoflavones on cleavage complex formation at concentrations of 250 and 500 μM. It is observed that the three glucosylated isoflavones in the presence of topoisomerase II induces formation of OC and linear plasmid DNA in a fashion similar to genistein tested at 100 μM. The isoflavones were shown to be inactive at this concentration of 100 μM.

To obtain quantitative data, the amount of linear and OC DNA was measured densitometrically by videoimpression with PCBAS software.

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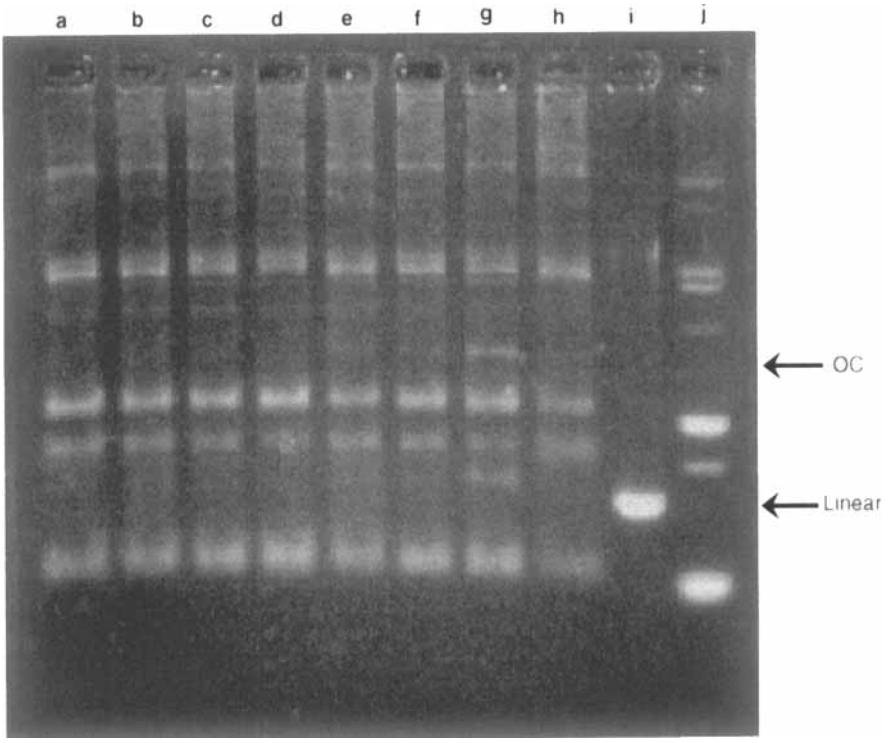

FIGURE 1 DNA-Topoisomerase II mediated DNA cleavage by 6′-methoxypseudobaptigenin-7-*O*-*β*-glucoside (a: 500 μM, b: 250 μM), daidzin (c: 500 μM, d: 250 μM), genistin (e: 500 μM, f: 250 μM) and genistein (g: 100 μM); TopoII plus DNA pRYG (h), DNA linear (i), DNA pRYG (j). Cleavage of DNA was analyzed by agarose gel assay described in "Materials and Methods".

DISCUSSION

As shown in Figure 2, genistin and daidzin showed comparable cleavage activities which were lower than that observed for genistein. In contrast, 6′-methoxypseudobaptigenin-7-*O*-*β*-glucoside had no effect on topo II activity at a concentration of 250 μM. These results suggest that the introduction of a voluminous hydrophilic substituent (glucose) in the 7-position of the isoflavone structure reduces the stabilization effect on cleavage complex formation, most likely due to a decrease in the interaction with topoisomerase II-DNA. Therefore, topoisomerase-mediated DNA damage seems to be a possible candidate mechanism, by which 6′-methoxypseudobaptigenin-7-*O*-*β*-glucoside, genistin, and daidzin may exert their cytotoxic activity.

Recent research has confirmed that many common foods contain non-nutritive components, such as flavonoids, that may provide protection

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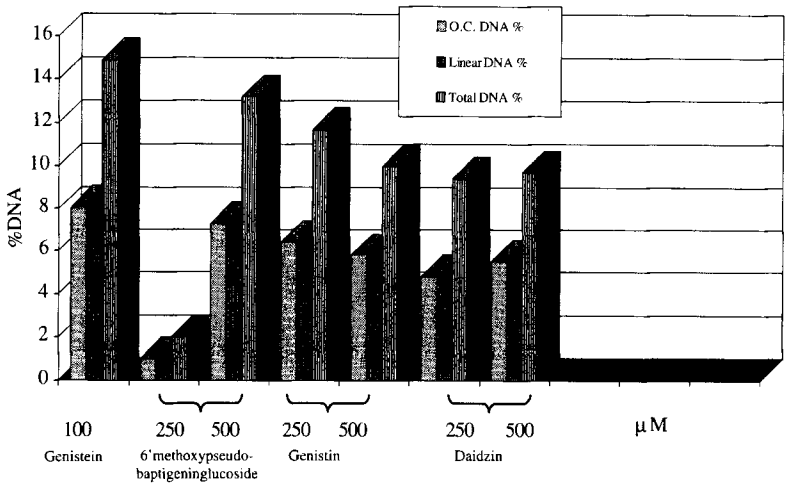

FIGURE 2 Quantitative comparison of topoisomerase II-mediated DNA cleavage induced by genistein, 6′-methoxy-pseudobaptigenin-7-*O*-β-glucoside, genistin and daidzin. The percentage of DNA cleavage by topoisomerase II in the presence of drugs was determined by scanning the gels with densitometer (OC: open circular).

against chronic diseases including some forms of cancer. Epidemiological investigations support this hypothesis, because the high level of these compounds are found in countries or regions with low cancer incidence. These epidemiologic studies consistently show the cancer protective effect of fruit and vegetable consumption, but there is little understanding of which phytochemicals account for this observation.[12]

Although these three isoflavones are not potent topoisomerase II poisons, our results contribute to the view concerning the protective anticancer properties of this kind of chemopreventer, and show that a new phytochemical (6′-methoxypseudobaptigenin-7-*O*-β-glucoside) has a possible anticancer action.

Acknowledgements

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