

Neuritogenesis of Herbal Geniposide-Related Compounds in PC12h Cells

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Previously, we have reported that geniposide, a compound isolated from an extract of *Gardenia fructus*, has neuritogenic activity in PC12h cells, a subclone of the rat pheochromocytoma cell. In this study, we have examined the effects of seven geniposide-related compounds (S-1, 6 α -hydroxygeniposide; S-2, 6 β -hydroxygeniposide; S-3, 6 α -methoxygeniposide; S-4, 6 β -methoxygeniposide; S-5, loganin; S-6, 7-ketologanin; and S-7, syringopicroside) isolated from various medicinal herbs. The geniposide-type iridoids S-1, S-2, S-3, and S-4, and S-7 induced neurite outgrowth that was similar or more potent to that of geniposide. S-2 and S-4, which are optical isomers of S-1 and S-3, respectively, were particularly potent. The 2 loganin-type iridoids, S-5 and S-6, showed less activity than geniposide. The neuritogenic activity of geniposide-type iridoids appears to be not necessarily correlated directly to their hydrophobicity. These results suggest that geniposide-type iridoids have potent neuritogenic activity and that specific configurations for the interactions between iridoid compounds and the target molecule are necessary for neuritogenic function.

Key words — iridoid compounds, optical isomer, neuritogenic activity, PC12h cells

INTRODUCTION

Iridoids have been reported to possess various biological activities, such as antimicrobial,¹⁾ anticancer,²⁾ hemodynamic,³⁾ choleric,⁴⁾ and hepatoprotective effects.⁵⁾ Furthermore, we have previously reported that several iridoid compounds, specifically geniposide, gardenoside, catalpol and aucbin, and their hydrolysates, have neuritogenic activity in PC12h cells,⁶⁾ a subclone of the rat pheochromocytoma cell.⁷⁾ In particular, genipin, the aglycon of geniposide, potently induces neurite outgrowth. Recently, we have reported that genipin also increases survival in Neuro2a cells, a neuronal cell line, exposed to serum deprivation-induced cytotoxicity.⁸⁾ Thus, genipin is believed to have neurotrophic factor-like activity, like that of nerve growth factor (NGF) or other neurotrophin, and therefore may act as a regenerative drug in various neurodegenerative diseases.

However, genepin is not without defects. The dihydropyran ring in genipin is easily broken, and the dialdehyde produced reacts strongly with amino and/or thiol residues in coexisting components such as proteins, amines, and several SH-compounds.⁹⁾ As a result, adducts of genipin lose their neuritogenic activity.

Therefore, in the present study, we have evaluated the neuritogenic activity of several geniposide-type and loganin-type iridoid compounds in PC12h cells in order to obtain stable and more active compounds that may potentially become regenerative drugs.

MATERIALS AND METHODS

Materials — The iridoid compounds used in this study (Fig. 1) were prepared as previously reported. 6 α -Hydroxygeniposide (S-1), 6 β -hydroxygeniposide (S-2), 6 α -methoxygeniposide (S-3), and 6 β -methoxygeniposide (S-4) were obtained from the leaves of *Gardenia jasminoides* cv. *fortuneana* HARA.¹⁰⁾ Loganin (S-5) and 7-ketologanin (S-6) were obtained from the leaves of *Lonicera caerulea* var.

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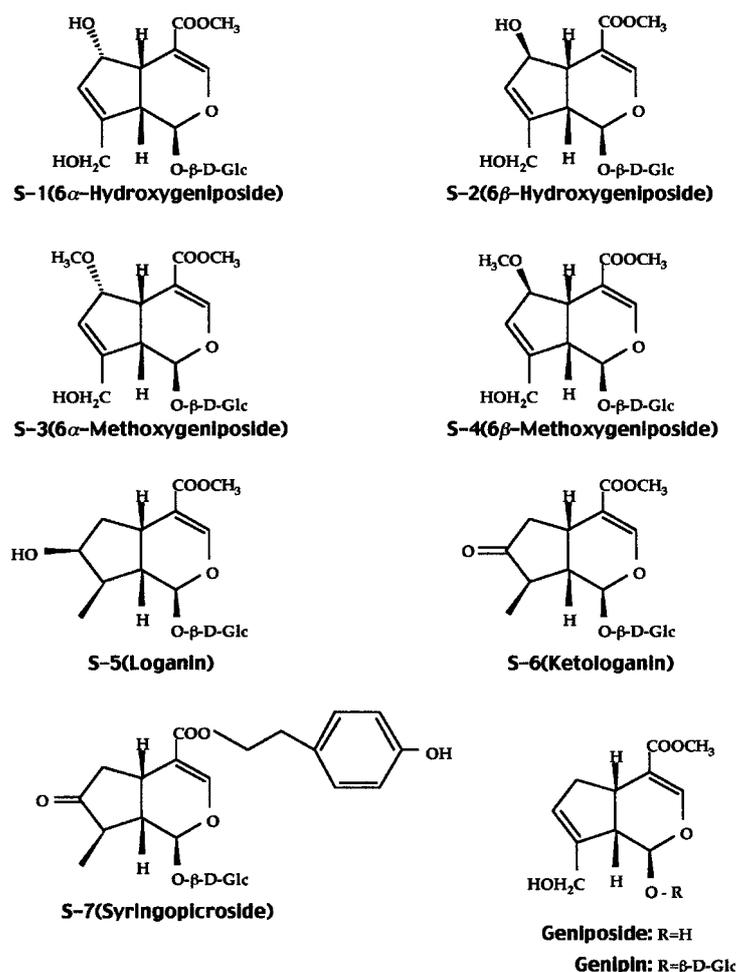


Fig. 1. Chemical Structures of Geniposide-Type and Loganin-Type Iridoid Compounds

emphyllocalyx Nakai.^{11,12} Syringopicroside (S-7) was obtained from the leaves of *Syringa reticulata* (Blume) HARA.¹³ All other reagents were the highest quality available.

Evaluation of Neurite Outgrowth — PC12h cells were generously donated by the late Dr. H. Hatanaka of Osaka University, Japan, and were cultured in Dulbecco's modified Eagle medium supplemented with precolostrum calf serum and heat-inactivated horse serum for growth, as described previously.¹⁴ After 24 hr of culture, the culture medium was replaced by serum-free medium with test compounds to treat the cells as specified in the results. Iridoids were dissolved in dimethylsulfoxide (DMSO) before use and the final concentration of vehicle (DMSO) was 0.1% or less. The neurite outgrowth of these cells was evaluated by measuring the length of the longest neurite of individual cells following 48 hr of treatment with the test compounds, as described previously.¹³ One hundred cells

in ten random fields in two culture dishes were averaged for each treatment.

Characterization of Hydrophobicity — Reverse-phase high performance liquid chromatography (HPLC) was performed on an ODS column (Puresil 5 μ C18, 120 \AA , 0.46 \times 15 cm) using a Waters 610 Fluid Unit (Millipore Waters) with a 468 Tunable Absorbance Detector (eluent, various % acetonitrile containing 0.1 % trifluoroacetic acid (TFA); flow rate, 1 ml/min; detection, UV at 240 nm).

RESULTS AND DISCUSSION

All four geniposide-type iridoids (S-1, S-2, S-3 and S-4) significantly induced neurite outgrowth in PC12h cells (Fig. 2). The extent of the activity was similar or more potent than that observed with geniposide. Two loganin-type iridoids, S-5 and S-6,

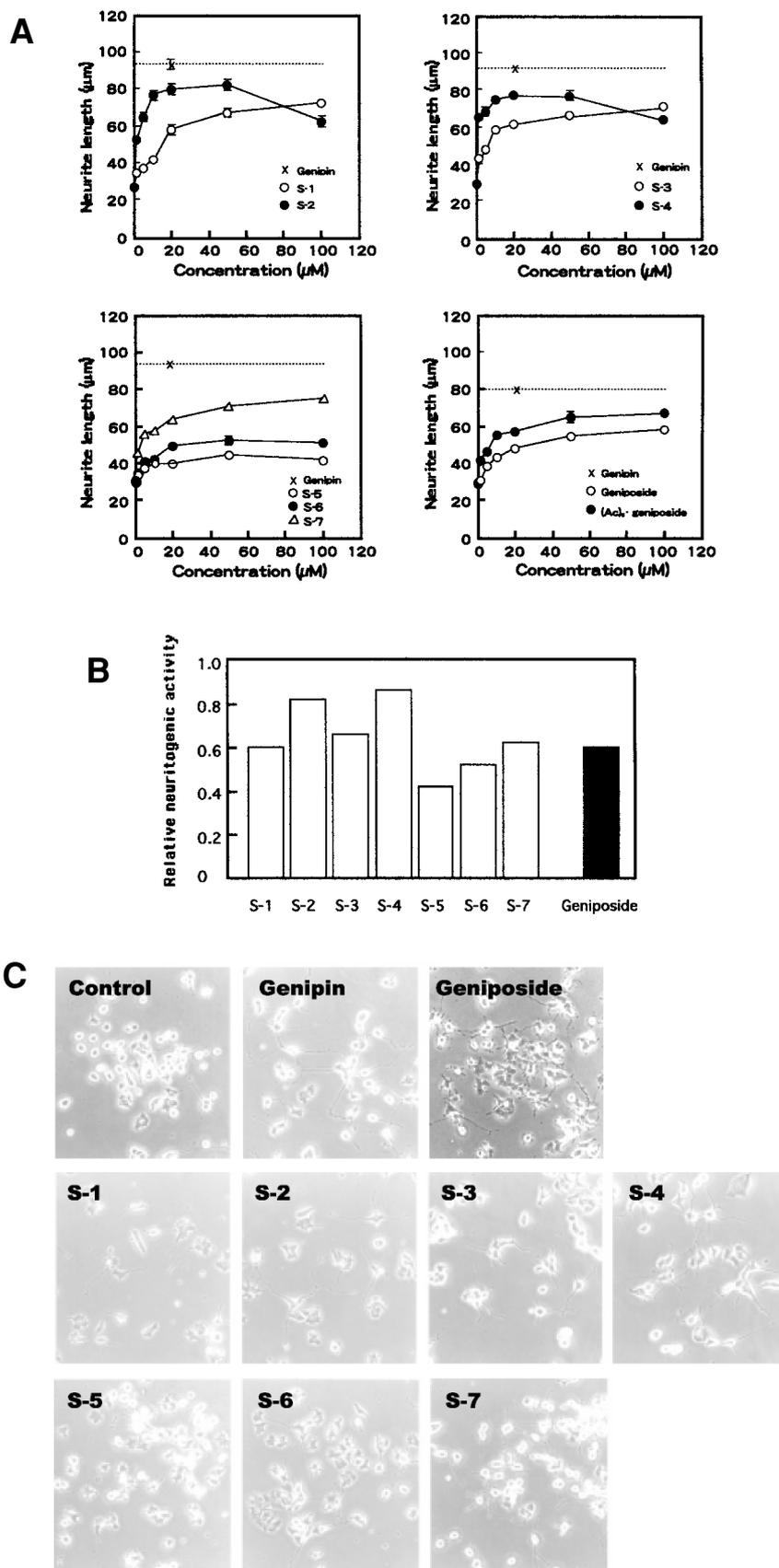


Fig. 2. Neuritogenic Activities of Geniposide-Type Iridoids and Loganin-Type Iridoids in PC12h

Cells were cultured with or without (control) concentrations of test compounds as indicated for 2 days. (A) Morphological images after treatment for 2 days. (B) Neuritogenic activity. Neurite outgrowth was measured as described in Materials and Methods for 100 cells in each group. The values shown are the mean \pm SEM. (C) Comparison of the relative activity at 20 μ M for each compound. The values shown are relative to the value of genipin in (B).

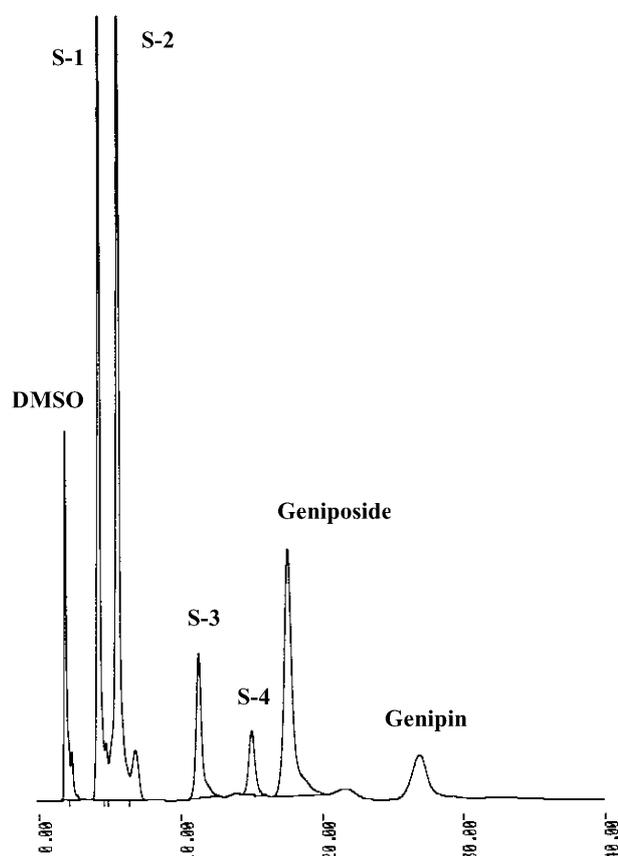


Fig. 3. Hydrophobicity of Geniposide-Type Compounds by Reverse Phase HPLC

Sample mixtures containing appropriate amounts of S-1, S-2, S-3, S-4, geniposide, and genipin were applied to an ODS column (0.46 × 25 cm) and eluted isocratically with 10% acetonitrile/0.1% TFA at a flow rate of 1 ml/min, with the absorption monitored at 240 nm.

showed less activity than geniposide. S-7, which has a similar configuration to S-6, had more potent neuritogenic activity than the loganins. As has been previously observed, genipin, an aglucon of geniposide, was more potent.⁶⁾ It seems that the difference in the activity is due to the higher hydrophobicity of genipin compared to geniposide, and that there is a correlation between the hydrophobicity and the neuritogenic activity of iridoids. Therefore, we analyzed the hydrophobicity of the iridoid compounds used in this study by HPLC with an ODS column. The hydrophobicity was, in decreasing order, S-1, S-2, S-3 and S-4, but the polarity of all of these compounds was higher than geniposide (Fig. 3). However, as shown in Fig. 2, these compounds had neuritogenic activity that was similar or more potent than that of geniposide. On the other hand, the activities of S-5, S-6 and S-7 were similar or less than that of geniposide, even though the hy-

drophobicity of these three compounds is similar or greater than that of geniposide (data not shown). Thus, the neuritogenic activity of geniposide-type iridoids appears to not be necessarily correlated directly to their hydrophobicity. The neuritogenic activities of S2 and S4 in particular were not well correlated to the hydrophobicity.

We believe that the reason for this was the relation to the configuration rather than the polarity of S-1, S-2, S-3, and S-4. Interestingly, S-2 and S-4 are optical isomers of S-1 and S-3, respectively, at the C₆ carbon position (Fig. 1). In other words, the active forms, S-2 and S-4, are both β -type, while the less active forms, S-1 and S-3, are both α -type. Thus, this can be interpreted to indicate that the configuration of a geniposide-related compound, especially at the C₆ position, is an important factor with respect to the interaction and activation of the target molecules for neuritogenesis. We have previously suggested that the target molecule of genipin/geniposide for the induction of neuritogenic activity is nNOS.¹⁵⁾ Therefore, the results of the present study strongly suggest that geniposide-type iridoids have potent neuritogenic activity and that specific configurations for the interactions between iridoid compounds and the target molecule are necessary for the manifestation of neuritogenic function.

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