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Secondary Metabolites from *Glycine soja* and Their Growth Inhibitory Effect against *Spodoptera litura*

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Supporting Information

ABSTRACT: The wild soybean (*Glycine soja* Sieb. et Zucc) has been reported to be relatively resistant to insect and pathogenic pests. However, the responsible secondary metabolites in the aerial part of this important plant are largely unknown. From the aerial part of *G. soja*, 13 compounds were isolated and identified, including seven isoflavonoids (**1**−**7**), a cyclitol (**8**), two sterol derivatives (**9** and **10**), and three triterpenoids (**11**−**13**). Compound **7** is a new isoflavonoid, and compounds **9** and **10** are reported as natural products for the first time. The growth inhibitory activity of **1**, **3**, **4**, and **8** against the larvae of *Spodoptera litura* was investigated. The most abundant isoflavonoid in the aerial part of *G. soja*, daidzein (**1**), which could not be metabolized by *S. litura*, was found to inhibit the insect larvae growth significantly in 3 days after feeding diets containing the compound. Compounds **3**, **4**, and **8**, which could be partially or completely metabolized, were inactive. Our results suggested that the isoflavonoid daidzein (**1**) might function as a constitutive defense component in *G. soja* against insect pests.

KEYWORDS: *Glycine soja*, secondary metabolites, isoflavonoids, growth inhibition, *Spodoptera litura*

INTRODUCTION

The wild soybean *Glycine soja* Sieb. et Zucc, distributed mainly in most parts of China, is considered to be the ancestor and the most important gene source of cultivated soybean (*Glycine max*).[1] In natural habitats, *G. soja* usually grows in harsh conditions such as on the edges of crop fields, roadsides, and riverbanks and faces many biotic and abiotic stress factors like herbivory, pathogen infection, soil salinization, and water deficiency.[2]

A large number of reports have shown that *G. soja* is relatively resistant to insect and pathogenic pests,[3,4] which is presumably related to the defensive function of the secondary metabolites accumulated in its aerial part. A literature survey indicated that numerous studies about *G. soja* focused on its agronomic, biochemical, and ecological characters, pest and disease resistance properties, and molecular biology, while only a few investigations on the chemistry of this important plant and even less investigation on its ecological correlations with the plant were found. The seeds of *G. soja* have been well-known to contain isoflavonoids such as daidzein glycosides, genistein glycosides, genistein, daidzein, 6-OH daidzein, and glycithin glycosides.[5] The seed coat of *G. soja*, however, was found to be rich in three flavonoids, epicatechin, cyanidin 3-*O*-glucoside, and delphinidin 3-*O*-glucoside,[6] and epicatechin was suggested to be functionally related to coat-imposed hardseededness. In addition, Zhou et al.[7] reported that isoflavonoids played an important role in the adaption of *G. soja* to saline environment. Shi et al. found that the wax content of *G. soja* was correlated with its resistance of soybean mosaic virus (SMV) disease.[8] However, to our surprise, so far, the secondary metabolites accumulating in the aerial part of *G. soja* still remain uninvestigated, and their possible ecological functions are also largely unknown. Therefore, we carried out a phytochemical investigation on the aerial part of this plant and tested the growth inhibitory activity of the major components against larvae of *Spodoptera litura*, one of the insect pests of *G. soja*, and analyzed their metabolites in the insect excrements. Here, we report the isolation and structure elucidation of the secondary metabolites of *G. soja* and their biological activity.

MATERIALS AND METHODS

General Experimental Procedures. Column chromatography was performed on 200−300 mesh silica gel (Qingdao Marine Chemical Factory, Qingdao, People's Republic of China). Sephadex LH-20 (Amersham Phamacia Biotech, Sweden) and MCI gel CHP-20P (75−150 μm, Mitsubishi Chemical Corp., Tokyo, Japan). Melting points were measured on a XRC-1 micromelting point apparatus (Beijing, China) and were uncorrected. Optical rotations were obtained on a Jasco P-1020 spectropolarimeter (Jasco, Tokyo, Japan). UV spectroscopic data were measured on a Shimadzu-210A double-beam spectrophotometer (Shimadzu, Tokyo, Japan). IR spectra of samples in KBr discs were recorded on a Bruker-Tensor-27 spectrometer (Karlsruhe, Germany) with KBr pellets. NMR spectra were carried out on either a Bruker AM-400 or a DRX-500 or an Avance III-600 spectrometer (Karlsruhe, Germany) with TMS as an internal standard.

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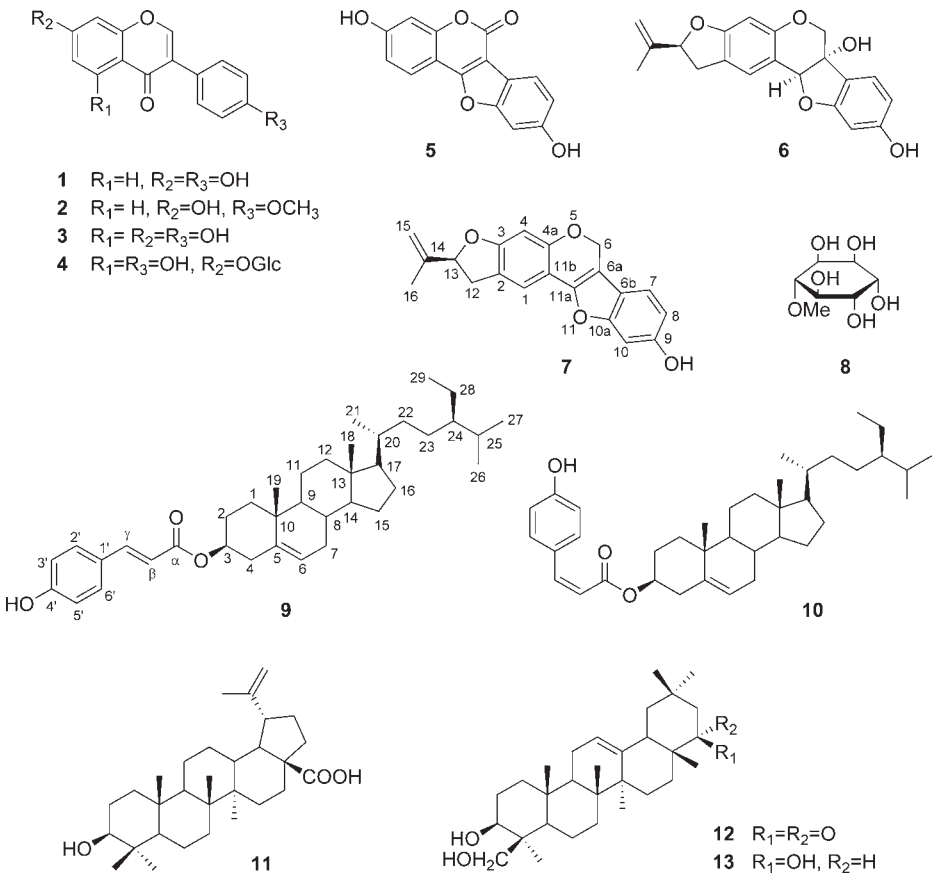

Figure 1. Chemical structures of compounds **1**−**13** isolated from *G. soja*.

Mass spectra were obtained on a Waters AutoSpec Premier P776 spectrometer (Waters Corp., MA).

Plant Material. Two samples of the aerial part of *G. soja* were collected, one from Heilongjiang province in April 2009 and the other from Tianjin Dahuangbao Wetland Natural Reserve in September 2010. Samples were identified by one of the authors (Ting-Shuang Yi), and authentic samples were kept in Plant Germplasm and Genomics Center, Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences.

Insects. Fourth-instar larvae of *S. litura* were purchased from Plot-scale Base of Biopesticides, Institute of Zoology, Chinese Academy of Sciences.

Extraction and Isolation. After it was air dried, the sample collected from Heilongjiang (500 g) was powdered and extracted three times with MeOH (5 L) at ambient temperature. The solvent was evaporated in vacuo, and then, the residue (43 g) was partitioned between water and EtOAc. The EtOAc fraction (28 g) was further divided into six fractions using silica gel chromatography (200−300 mesh, 100 g), successively eluted with $CHCl_3$/$(CH_3)_2CO$ (9:1, 8:2, 7:3, 1:1, and 0:1) and MeOH to give fractions A, B, C, D, E, and F. Compounds **1** (315 mg), **4** (189 mg), and **8** (980 mg) (Figure 1) were obtained, respectively, from fractions B, D, and E through recrystallization. Fraction A was subjected to MCI gel column chromatography eluting with methanol−water (80−100%) and then repeatedly chromatographed on silica gel (petroleum ether−acetone, 4:1) and Sephadex LH-20 (acetone) columns to give compounds **5** (10 mg) and **11** (6 mg). The other fractions (B and E) were further purified by silica gel column with chloroform−acetone (6:1) as the eluent and yielded compounds **3** (5 mg) and **12** (4 mg). The sample collected from Tianjin (3 kg) was extracted and isolated in the same way as described above. As a result, seven compounds including **5** (30 mg), **13** (40 mg), **6** (7 mg), **7** (5 mg), **2** (12 mg), **9** (10 mg), and **10** (8 mg) were obtained from fraction A with repeated MCI gel, silica gel, and Sephadex LH-20 column chromatographies. In addition, compounds **1**, **4**, and **8** were also present as major components in this sample, which were not further purified.

Daidzein (***1***). Yellowish powder. The NMR data (see the Supporting Information) were consistent with the structure of daidzein.[9]

Formononetin (***2***). Yellowish powder. The NMR data (see the Supporting Information) were consistent with the structure of formononetin.[10]

Genistein (***3***). Yellowish powder. The NMR data (see the Supporting Information) were consistent with the structure of genistein.[10]

Genistin (***4***). Yellowish powder. $[\alpha]_D^{21}$ −30.8 (*c* 0.10, pyridine) (lit.,[11] $[\alpha]_D^{23}$ −32.0 in DMSO). The NMR data (see the Supporting Information) were consistent with the structure of genistin.[10]

Coumestrol (***5***). Yellow needles; mp 353−356 °C (lit. mp 355−359).[12] The NMR data (see the Supporting Information) were consistent with the structure of coumestrol.[12]

Glyceollin III (***6***). Colorless needles; mp 147−150 °C (lit. mp 149−154 °C).[12] The ^{1}H NMR and ^{13}C NMR data (see the Supporting Information) of this compound were essentially the same with that reported for the same compound.[12,13]

Glyceollin VI (***7***). Yellow solid; $[\alpha]_D^{23}$ +42.8 (*c* 0.24, MeOH). UV (MeOH) λ_{max} (log ε): 348 (4.04), 333 (4.03), 290 (3.88), 206 (4.46) nm. IR (KBr) ν_{max}: 3425, 1624, 1484, 1128 cm^{-1}. EIMS (relative intenseity): *m*/*z* 336 ([M]$^+$, 76%), 320 (71%), 319 (100%), 305 (46%). HREIMS: *m*/*z* 320.1049 (calcd for $C_{20}H_{16}O_4$, 320.1050). ^{1}H NMR (acetone-d_6): δ 7.29 (1H, d, *J* = 8.4 Hz, H-7), 7.25 (1H, s, H-1), 6.99 (1H, d, *J* = 2.0 Hz, H-10), 6.81 (1H, dd, *J* = 2.0, 8.4 Hz, H-8), 6.36 (1H, s, H- 4), 5.52 (2H, s, H_2-6), 5.23 (1H, t, *J* = 8.7 Hz, H-13), 5.06 (1H, s, H_a-15), 4.89 (1H, s, H_b-15), 3.66 (1H, dd, *J* = 8.7, 15.6 Hz,

H_a-12), 2.98 (1H, dd, J = 8.0, 15.6 Hz, H_b-12), 1.75 (3H, s, CH_3-16). ^{13}C NMR (acetone-d_6): δ 161.7 (s, C-3), 157.1 (s, C-9), 156.2 (s, C-10a), 155.4 (s, C-4a), 147.8 (s, C-11a), 145.2 (s, C-14), 120.6 (s, C-2), 119.8 (d, C-7), 119.3 (s, C-6b), 116.9 (d, C-1), 113.1 (d, C-8), 112.0 (t, C-15), 110.2 (s, C-11b), 106.4 (s, C-6a), 99.0 (2C, d, C-4 and 10), 87.2 (d, C-13), 65.9 (t, C-6), 34.4 (t, C-12), 17.2 (q, C-16).

Pinitol (***8***). Colorless grain crystals; mp 183−187 °C (lit. mp 185−186 °C);[14] $[\alpha]_D^{21}$ +61.3 (c 0.122, H_2O) (lit.,[15] $[\alpha]_D^{23}$ +60.0 in H_2O). The NMR data (see the Supporting Information) were consistent with the structure of pinitol.[15]

Sitosterol trans-p-Coumarate (***9***). Colorless fine needles; mp 198−200 °C; $[\alpha]_D^{20}$ −7.2 (c 0.093, $CHCl_3$). UV (MeOH) λ_{max} (log ε): 313 (4.37), 227 (4.08), 202 (4.20) nm. IR (KBr) ν_{max}: 3394, 2956, 1712, 1633, 1604, 1514 cm^{-1}. EIMS (relative intenseity): m/z 560 (M^+, 5%), 396 (100%), 397 (55%), 382 (40%), 381 (24%), 255 (24%), 147 (100%). HR-EIMS: m/z 560.4232 (calcd for $C_{38}H_{56}O_3$, 560.4229). ^{1}H NMR (acetone-d_6): δ 8.95 (1H, s, 4′-OH), 7.59 (1H, d, J = 15.9 Hz, H-γ), 7.54 (2H, d, J = 8.5 Hz, H-2′ and 6′), 6.85 (2H, d, J = 8.5 Hz, H-3′ and 5′), 6.32 (1H, d, J = 15.9 Hz, H-β), 5.40 (1H, d, J = 4.0 Hz, H-6), 4.63 (1H, m, H-3), 1.07 (3H, s, CH_3-19), 0.96 (3H, d, J = 6.3 Hz, CH_3-21), 0.84(9H, overlapped, CH_3-26, 27 and 29), 0.72 (3H, s, CH_3-18). ^{1}H NMR ($CDCl_3$): δ 7.62 (1H, d, J = 16.0 Hz, H-γ), 7.42 (2H, d, J = 8.4 Hz, H-2′ and 6′), 6.84 (2H, d, J = 8.4 Hz, H- 3′ and 5′), 6.28 (1H, d, J = 16.0 Hz, H-β), 5.40 (1H, d, J = 4.0 Hz, H-6), 4.72 (1H, m, H-3), 2.38 (2H, m, H_2-4), 1.05 (3H, s, CH_3-19), 0.93 (3H, d, J = 6.4 Hz, CH_3-21), 0.84 (9H, overlapped, CH_3-26, 27 and 29), 0.68 (3H, s, CH_3-18). ^{13}C NMR (acetone-d_6): δ 166.8 (s, C-α), 161.1 (s, C-4′), 145.3 (d, C-γ), 140.7 (s, C-5), 130.8 (2C, d, C-2′ and 6′), 126.4 (s, C-1′), 123.0 (d, C-6), 116.6 (2C, d, C-3′ and 5′), 115.5 (d, C-β), 74.0 (d, C-3), 57.4 (d, C-14), 56.7 (d, C-17), 50.9 (d, C-9), 46.5 (d, C-24), 42.9 (s, C-13), 40.4 (t, C-4), 39.9 (t, C-12), 37.7 (t, C-1), 37.2 (t, C-22), 36.8 (s, C-10), 34.5 (d, C-20), 32.6 (t, C-2), 32.5 (t, C-16), 29.7 (d, C-8), 28.8 (d, C-25), 28.5 (t, C-7), 26.6 (t, C-23), 24.8 (t, C-15), 23.6 (t, C-28), 21.6 (t, C-11), 20.0 (q, C-26), 19.6 (q, C-19), 19.2 (q, C-27), 19.1 (q, C-21), 12.2 (q, C-29), 12.1 (q, C-18). ^{13}C NMR ($CDCl_3$): δ 167.3 (s, C-α), 158.3 (s, C-4′), 144.6 (d, C-γ), 139.9 (s, C-5), 130.1 (2C, d, C-2′ and 6′), 127.1 (s, C-1′), 122.9 (d, C-6), 116.1 (2C, d, C-3′ and 5′), 115.9 (d, C-β), 74.2 (d, C-3), 56.9 (d, C-14), 56.2 (d, C-17), 50.2 (d, C-9), 46.0 (d, C-24), 42.5 (s, C-13), 40.8 (t, C-4), 39.9 (t, C-12), 38.4 (t, C-1), 37.2 (t, C-22), 36.8 (s, C-10), 36.4 (d, C-20), 34.1 (t, C-2), 32.1 (t, C-16), 32.0 (d, C-8), 28.9 (d, C-25), 28.5 (t, C-7), 26.7 (t, C-23), 24.9 (t, C-15), 23.7 (t, C-28), 21.7 (t, C-11), 20.1 (q, C-26), 19.6 (q, C-19), 19.3 (q, C-27), 19.2 (q, C-21), 12.2 (q, C-29), 12.1 (q, C-18).

Sitosterol cis-p-Coumarate (***10***). Ccolorless fine needles; mp 139−143 °C; $[\alpha]_D^{20}$ −50.1 (c 0.286, $CHCl_3$). UV (MeOH) λ_{max} (log ε): 308 (3.94), 203 (3.98) nm. IR (KBr) ν_{max}: 3527, 3311, 2937, 1713, 1665, 1606, 1588, 1512, 1450 cm^{-1}. EIMS (relative intenseity): m/z 560 (M^+, 2%), 396 (41%), 397 (30%), 255 (53%), 254 (44%), 165 (55%), 147 (76%), 107 (100%). HR-EIMS: m/z 560.4232 (calcd for $C_{38}H_{56}O_3$, 560.4229). ^{1}H NMR (acetone-d_6): δ 8.82 (1H, s, 4′-OH), 7.78 (2H, d, J = 8.5 Hz, H-2′ and 6′), 6.87 (1H, d, J = 13.0 Hz, H-γ), 6.85 (2H, d, J = 8.5 Hz, H-3′ and 5′), 5.78 (1H, d, J = 13.0 Hz, H-β), 5.43 (1H, d, J = 5.1 Hz, H-6), 4.61 (1H, m, H-3), 1.07 (3H, s, CH_3-19), 0.99 (3H, d, J = 6.4 Hz, CH_3-21), 0.86 (9H, overlapped, CH_3-26, 27 and 29), 0.75 (3H, s, CH_3-18). ^{13}C NMR (acetone-d_6): δ 166.2 (s, C-α), 159.5 (s, C-4′), 143.8 (d, C-γ), 140.7 (s, C-5), 133.7 (2C, d, C-2′ and 6′), 127.4 (s, C-1′), 123.1 (d, C-6), 117.2 (2C, d, C-3′ and 5′), 115.6 (d, C-β), 74.2 (d, C-3), 57.5 (d, C-14), 56.9 (d, C-17), 51.0 (d, C-9), 46.6 (d, C-24), 43.0 (s, C-13), 40.5 (t, C-4), 38.9 (t, C-12), 37.8 (t, C-1), 37.3 (t, C-22), 36.9 (s, C-10), 34.6 (d, C-20), 32.6 (t, C-2), 32.5 (t, C-16), 29.7 (d, C-8), 28.9 (d, C-25), 28.5 (t, C-7), 26.7 (t, C-23), 24.9 (t, C-15), 23.7 (t, C-28), 21.7 (t, C-11), 20.1 (q, C-26), 19.6 (q, C-19), 19.3 (q, C-27), 19.2 (q, C-21), 12.3 (q, C-29), 12.2 (q, C-18).

Betulinic Acid (***11***). White powder; $[\alpha]_D^{21}$ +7.5 (c 0.10, pyridine) (lit.,[16] $[\alpha]_D^{23}$ +7.9 in pyridine). The NMR data (see the Supporting Information) were consistent with the structure of betulinic acid.[16]

Soyasapogenol E (***12***). White powder. The NMR data (see the Supporting Information) were consistent with the structure of soyasapogenol E.[17,18]

Soyasapogenol B (***13***). White powder; $[\alpha]_D^{21}$ +92.0 (c 0.13, $CHCl_3$) (lit.,[19] $[\alpha]_D^{23}$ +90.7 in $CHCl_3$). The NMR data (see the Supporting Information) were consistent with the structure of soyasapogenol B.[17,18]

Larval Growth Inhibition Assay. The effect of the major constituents of *G. soja* on larval growth of *S. litura* was evaluated as described by Maistrello et al.,[20] with slight modifications. Leaf discs were punched out from glandless fresh cabbage leaves, randomized, and arranged (2 discs/dish) on moistened filter paper inside glass Petri dishes. Alternate discs were treated on their rear surface with either 20 μL of MeOH or 200 μg of sample dissolved in 20 μL of MeOH applied with a microliter syringe. Larvae that had been deprived of food for 4 h were then placed in the dishes after being weighed. After 4 h, the larvae were transferred to either fresh untreated leaves or an artificial diet. This procedure was repeated every 24 h, and for each individual, the weight was recorded. Seven replicates were set for each treatment, and the entire experiment was repeated three times. Excrements of the treated larvae and the control were separately collected for metabolite analysis.

Metabolite Analysis by HPLC. The collected excrement was extracted with methanol under ultrasonic bath for 3 × 20 min and then centrifuged. The supernatant was analyzed by high-performance liquid chromatography (Aglient 1200, Waldbronn, Germany), equipped with a quaternary pump, a vacuum degasser, an autosampler, a thermostatted column compartment, and a diode array detector. The column used was a 250 mm × 4.6 mm i.d., 5 μm, Li-Chrospher 100 RP-18, Zorbax SB-C18 (Agilent, United States) was used. The gradient elution with (A) water and (B) methanol was linear gradient 40−80% of B for compound **1**, 50−80% of B for compound **3**, and 20−60% of B for compound **4** in 0−30 min, and then isocratic 95% of B in 30.01−35 min. The flow rate was 1.0 mL/min, the injection volume was 20 μL, and the column temperature was maintained at 30 °C. The eluent was monitored at 210, 238, 254, and 280 nm. In the case of compound **8**, the metabolite was probed with TLC method ($CHCl_3$/MeOH = 4:1 as developing system) due to the lack of UV absorption.

RESULTS AND DISCUSSION

Compound **7** was obtained as a yellow solid and exhibited a purple fluorescence under UV light at 254 nm. The IR spectrum indicated the presence of a hydroxyl group (3425 cm^{-1}) and aromatic rings (1624 and 1484 cm^{-1}). The ^{1}H NMR spectrum of **7**, coupled with its HSQC spectrum, showed five aromatic protons at δ_H 7.29 (d, J = 8.4 Hz, H-7), 7.25 (s, H-1), 6.99 (d, J = 2.0 Hz, H-10), 6.81 (dd, J = 2.0, 8.4 Hz, H-8), and 6.36 (s, H-4), two olefinic methylene protons at δ_H 5.06 (s, H_a-15), 4.89 (s, H_b-15), one oxymethine proton at δ_H 5.23 (1H, t, J = 8.0 Hz, H-13), four methylene protons at δ_H 5.52 (2H, s, H_2-6), 3.66 (dd, J = 8.8, 15.2 Hz, H_a-12), and 2.98 (dd, J = 8.0, 15.6 Hz, H_b-12), and one methyl at δ_H 1.75 (3H, Me-16). In the ^{13}C NMR and distortionless enhancement by polarization (DEPT) spectra, 20 carbon signals including one methyl, three methylenes including an olefinic one, five olefinic methines, one oxymethine, and ten olefinic quaternary carbons were displayed, which were in agreement with the above observation. Careful comparison of the NMR data of **7** with those of glyceollin III (**6**),[12] a pterocarpan compound also isolated in this experiment, revealed that **7** was another pterocarpan derivative similar to **6**.

The major difference between **7** and **6** was at C-6a and C-11a. Unlike the case for **6**, a double bond was formed between C-6a and C-11a in **7**, as revealed by the absence of oxy-quaternary carbon at δ 76.7 and oxymethine carbon at δ 86.3, and the presence of two more olefinic quaternary carbons at δ 147.8 and 106.4, ascribable to C-11a to C-6a, respectively, according to their HMBC correlations (Figure 2) in **7**, as well as the obviously upfield-shifted C-6 (from 70.7 in **6** to 65.9 in **7**). Considering that compound **7** was likely biosynthesized from **6** through dehydration, the two compounds should have the same stereochemistry at C-13. Unambiguous assignment of **7** was achieved through 2D NMR [^{1}H−^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC)] experiments. Compound **7** was therefore identified as 6a,11a-dehydrate of glyceollin III and named glyceollin VI (Figure 1).

Compounds **9** and **10** were obtained as white fine needles. ^{1}H and ^{13}C NMR (including DEPT) spectra of both compounds showed two obvious groups of signals, one group corresponding to sitosterol and the other group to *p*-coumaric acid. The dramatically downfield-shifted H-3 (ca. 1 ppm) and upfield-shifted carbonyl carbon (ca. 4 ppm) suggested that compounds **9** and **10** were conjugates of sitosterol and *p*-coumaric acid through esterification between 3-OH and the carboxylic group. The 2D NMR spectra of **9** and **10** including ^{1}H−^{1}H COSY, HSQC, HMBC, and ROESY, especially the ^{1}H−^{13}C long-range correlation from H-3 to the carbonyl carbon, confirmed the above deduction and established the structures of **9** and **10** as sitosterol *p*-coumarates. The difference between **9** and **10** was the configurations of their double bonds in the *p*-coumaric acid parts, with the former *trans* while the latter *cis*, as evident from the coupling constants between the two olefinic protons (16.0 Hz in **9** and 13.0 Hz in **10**). The C-3 oxy-substituents in **9** and **10** were identified as β considering that all sterol ferulates discovered so far have the same stereochemistry at C-3,[22,23] which was supported by the absence of correlation between H-3 and Me-19 in the ROESY spectra. Therefore, compounds **9** and **10** were identified as sitosterol *trans-p*-coumarate and sitosterol *cis-p*-coumarate, respectively (Figure 1).

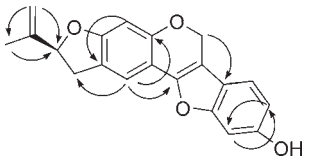

Figure 2. Significant correlations of compound **7** in HMBC spectra (H → C).

Although the synthesis of sitosterol ester of *p*-coumaric acid has been described,[21] this is the first report on the isolation of **9** and **10** as natural products. However, sterol ferulates have been isolated and identified from rice bran and wheat, rye, and corn bran oils.[22,23] It seems that conjugates of sterols and phenyl propanoids generally exist in plant kingdom, especially in crop plants.

The known compounds (Figure 1) were identified as daidzein (**1**),[9] formononetin (**2**),[10] genistein (**3**),[10] genistin (**4**),[10,11] coumestrol (**5**),[12] glyceollin III (**6**),[12,13] pinitol (**8**),[14,15] betulinic acid (**11**),[16] soyasapogenol E (**12**),[17,18] and soyasapogenol B (**13**),[17−19] respectively, by comparison of their spectroscopic and spectrometric data (^{1}H, ^{13}C NMR, and MS) with those reported in the literature. In addition, full NMR assignments of

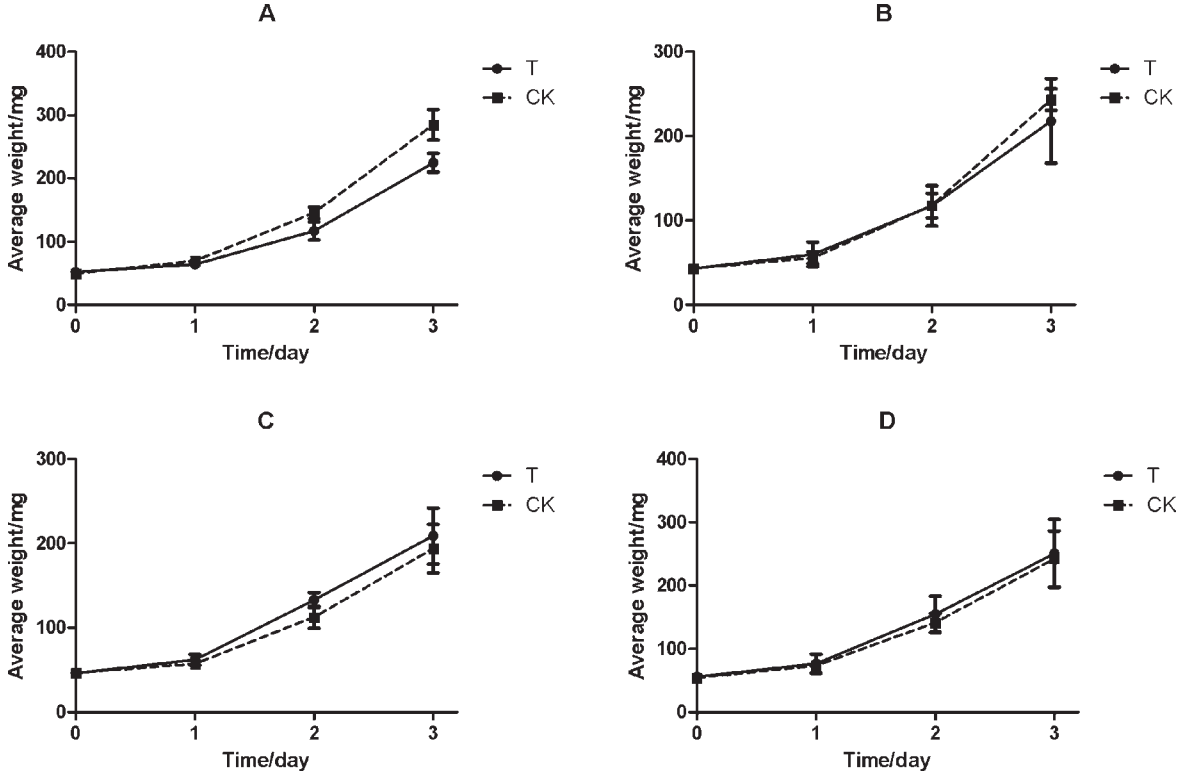

Figure 3. Growth inhibitory activity of the major compounds against *S. litura*. T, treatment; CK, control; A, daidzein (**1**); B, genistein (**3**); C, genitin (**4**); and D, pinitol (**8**).

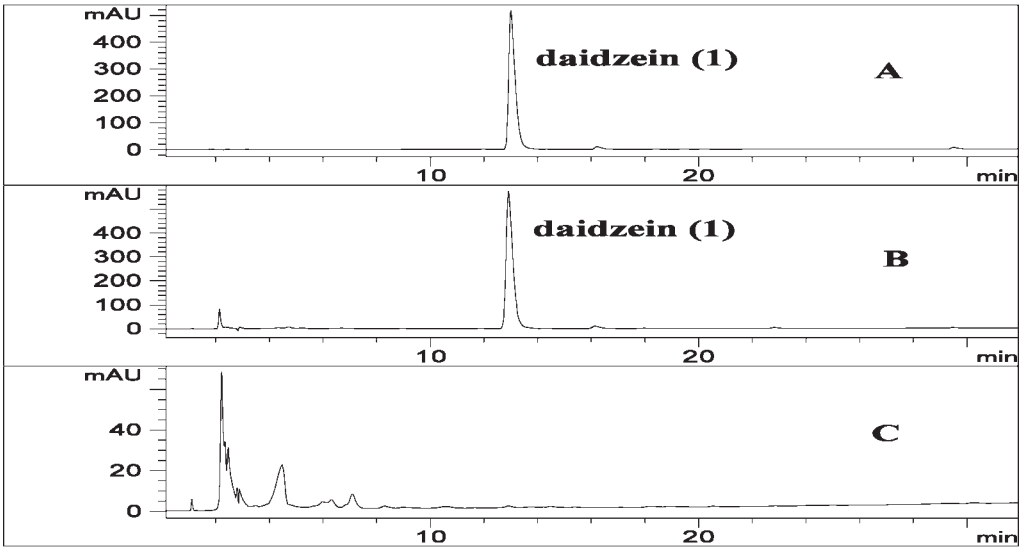

Figure 4. Chromatograms (254 nm) showing the metabolism of daidzein (**1**) in the excrements of *S. litura*. A, standard; B, treatment (excrement of *S. litura* fed with diet containing **1**); and C, control (excrement of *S. litura* fed with untreated diet).

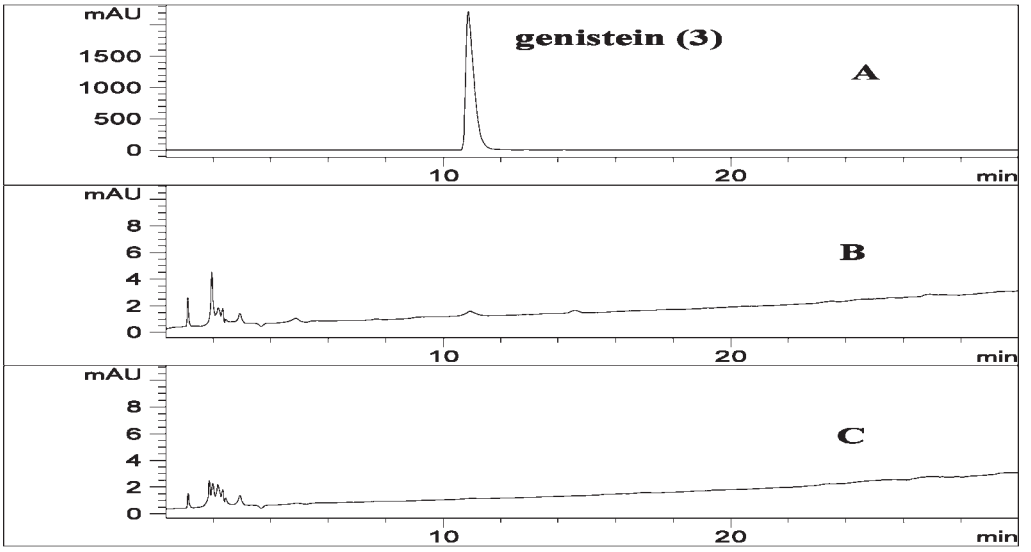

Figure 5. Chromatograms (254 nm) showing the metabolism of genistein (**3**) in the excrements of *S. litura*. A, standard; B, treatment (excrement of *S. litura* fed with diet containing **3**); and C, control (excrement of *S. litura* fed with untreated diet).

12 were achieved through 2D NMR (^{1}H−^{1}H COSY, HSQC, HMBC, and ROESY) experiments.

The major components **1**, **3**, **4**, and **8** were originally tested for their antifeedant activity against the larvae of *S. litura*, but none of them were found to be active. Then, the growth inhibitory activity of these compounds against the larvae of *S. litura* was investigated, through detection of the detrimental effect on the weight gain of *S. litura* fed with diets containing compounds. It was found that the most abundant isoflavonoid in the aerial part of *G. soja*, daidzein (**1**), inhibited the insect larvae growth significantly in 3 days (Figure 3A). Compounds **3**, **4**, and **8**, however, were inactive (Figures 3B−D).

To find out whether the fourth-instar larvae of *S. litura* could metabolize the compounds, ingest them, or just excrete them, the insect excrements were collected and analyzed for the metabolites. The results indicated that daidzein (**1**) could not be metabolized by *S. litura* at all (Figure 4), while compound **4** could be partially metabolized to a compound with retention time of 30.6 min (Figure 6), and compounds **3** (Figure 5) and **8** (figure not shown) were completely metabolized by the insects. We tried to purify and identify the metabolite of **4** with semipreparative HPLC and NMR methods but did not succeed due to paucity of sample. Because the metabolites of **3** and **8** were not detected through extensive HPLC and TLC analyses, even including HPLC-MS attempts, what these two compounds were metabolized to remains also unclear.

The metabolisms of compounds **1**, **3**, **4**, and **8** in *S. litura* were exactly in agreement with their inhibitory effects on the growth of insects. The antigrowth effect of daidzein (**1**) on insect growth suggested that this isoflavonoid might function as a constitutive defense component in *G. soja* against insect pests, which could well

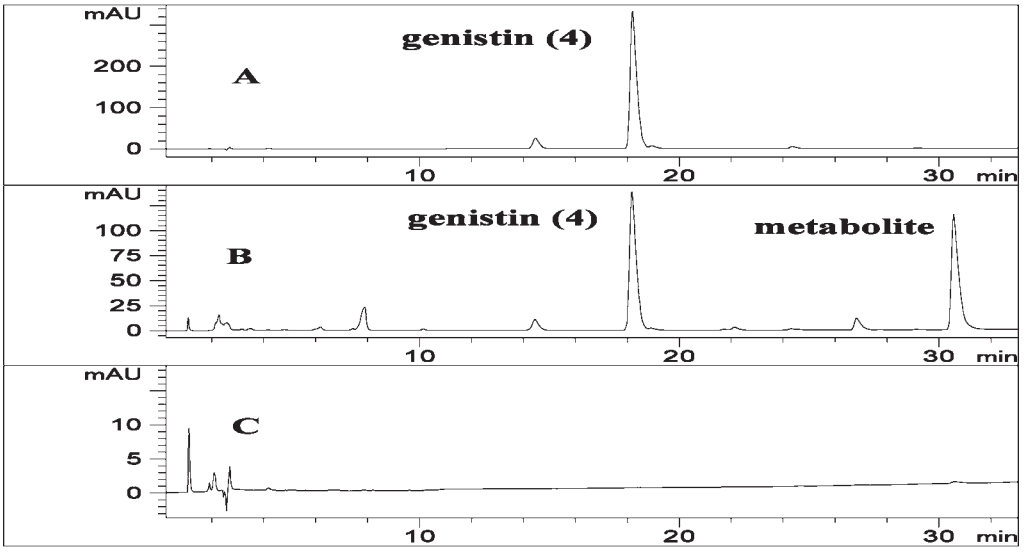

Figure 6. Chromatograms (254 nm) showing the metabolism of genistin (**4**) in the excrements of *S. litura*. A, standard; B, treatment (excrement of *S. litura* fed with diet containing **4**); and C, control (excrement of *S. litura* fed with untreated diet).

explain why the plant synthesizes and accumulates this compound as one of its most abundant secondary metabolites. For the other two isoflavonoids **3** and **4**, which are less abundant than **1** in the aerial part of *G. soja*, it is plausible that some insects such as *S. litura* have adapted to these defense compounds and evolved detoxification mechanisms in their coevolution with the plant. Although pinitol (**8**), the most abundant secondary metabolite in the aerial part of *G. soja* (ca. 0.05% dry weight), was reported to be a larvae growth inhibitor for *Heliothis zea* in leaves of cultivated soybean (*G. max*),[24] it was not active with *S. litura*, indicating that its defensive role might be insect specific. The findings in this investigation will shed more light on the relationship between the function of secondary metabolites in *G. soja* and its survival in harsh environments in its natural habitats.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H NMR and ^{13}C NMR data of known compounds and ^{1}H and ^{13}C NMR spectra of compounds **7**, **9**, and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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