New Sphingolipid from G*ylcine max*

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Abstract
In a study of the chemical profiling of plants used in dietary supplements to investigate the possible misidentification and authentication, the methanolic extract of *Glycine max* was evaluated chemically; one new sphingolipid was isolated by normal phase liquid chromatography. The compound was characterized by spectroscopic techniques including 2D NMR spectroscopy.
Keywords: *Glycine max*, fabaceae, sphingolipid

Introduction

The relationship between diet and human health has become a very active area of research and debate. Identification and characterization of specific diet-derived chemicals, and an increased understanding of their biological activity have given rise to increased interest in the role for natural products in disease prevention and treatment (Willer, 1994). Legumes play a pivotal role in the traditional diets of many regions throughout the world (Messina, 1999). Soybeans are unique among legumes because they are a primary source of soysaponins, flavonoids and other secondary metabolites (Kitwaga *et al.*, 1985; *Shiraiwa et al*., 1991; Fliegmann *et al.*, 2010; Akihisa *et al.*, 1994; Jay *et al.*, 1983; Berlag *et al.*, 1988). Plant-derived saponins are considered to play a significant role in plant defense systems against pathogens and herbivores. Numerous reports emphasize the fungicidal (Lee *et al*., 2001;), allelopathic (Waller *et al*., 1993), insecticidal (Nielson *et al*., 2010) and molluscicidal (Huang *et al.*, 2003) activity of various saponins. The presence of saponins in soybeans has also attracted considerable interest because of both their health benefits and adverse sensory characteristics. Soysaponins are the primary dietary sources of saponins from foods. Soysaponins have been demonstrated to possess multiple health-promoting properties, such as lowering of cholesterol by inhibiting its absorption, being anticarcinogenic, antihepatotoxic, promoting antiinfectivity of HIV, antimutagenic and immunostimulatory activities (Kinjo *et al*., 1998; Miyao *et al.*, 1998; Okubo *et al*., 1994; Baxter *et al*, 1990; Hu *et al.*, 2004; Berhow *et al*., 2000; Hostettmann and Marston, 1995). As a part of our programme to phytochemically investigate medicinal plant derived

dietary supplements, the methanolic extract of soybean led to the isolation of new sphingolipid (Fig. 1).

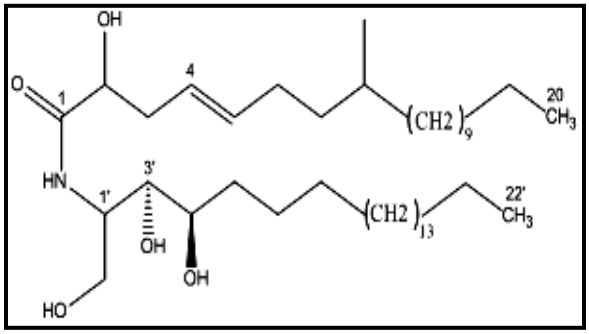

Fig. 1. Structure of compound 1

Material and Methods

General

NMR spectra were recorded on a Varian AS 500 NMR spectrometer instrument using TMS as internal standard. Chemical shifts were reported in δ units and coupling constants (J) in Hz. ESIMS was obtained on Agilent Series 1100 SL mass spectrometer. IR spectra were recorded using KBr pellet on a Bruker Tensor 27 FT-IR spectrometer. Optical rotations were measured on a Rudolph Research AutoPol IV polarimeter. Column chromatography was performed by using silica gel (40 µm mesh, JT Baker). TLC analysis was carried out on silica gel 60 F_{254} plates (Merck) and spots on TLC plates were observed under UV light (254/365 nm). Spraying reagents *p*-anisaldehyde-H_2SO_4 (Sigma-Aldrich), 10% H_2SO_4 in ethanol and water, followed by heating were used for the detection of spots.

Plant source

The soybean seeds (*Glycine max*) purchased from market and were properly identified by running the TLC/Co-TLC with the standard sample of soybean seeds.

Extraction and isolation

The finely powdered *Glycine max* seed extract (70 g) was mixed with an equal amount of silica gel and subjected to column chromatography (CC) over a silica gel (1.0 kg) column (135×6.0 cm) and eluted with $CHCl_3$-MeOH (9:1) to obtain fractions SS-A (33.2g) and SS-B (15.1g). The polarity of eluent was changed to $CHCl_3$-MeOH-H_2O (13:7:2; lower layer, labelled as solvent system A) to afford seven fractions labeled as SS-C to SS-J. Fraction SS-C (5.1 g) after recolumn-chromatography afforded **1** (58.7 mg).

Compound 1: Green amorphous powder; IR (KBr): ν_{max} = 3270 (-N-H), 2884 (-C-H) 1740 (-CO); 1281, 764 cm^{-1}; ESIMS (positive mode): *m/z* 682.1271 $[M+H]^+$ (calcd. for $C_{42}H_{83}O_5N$); ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) data, see Table 1.

Table 1. ^{1}H- (500 MHz, $CDCl_3$), ^{13}C-NMR (125 MHz, $CDCl_3$) of 1

Position 1			**Position 1**		
	δ_C	δ_H		δ_C	δ_H
1	175.0		10-19	27.1-31.9	1.25-1.31
2	78.2	4.20	20	15.1	0.90
3	39.1	2.20	1'	55.2	3.75
4	123.3	5.25	2'	62.1	3.50
5	134.4	5.41	3'	75.6	3.91
6	28.2	1.92	4'	71.1	3.29
7	36.5	1.50	5'	32.1	1.42
8	39.1	1.58	6'-21'	25.0-31.9	1.25-1.31
9	19.1	0.96	22'	15.0	0.92
N - H		8.02			

Chemical shifts are in ppm, J in parentheses are in Hertz

GC/MS

GC/MS data obtained on Varian Mass Spectrometer using VF-5 column (60 m × 0.32 mm i.d.; 0.25 µm film thickness). Column temperature programmed 5 min. at 60 ^oC, then rising at 2 and 3 ^oC upto 240 ^oC. "Injector temperature, 240 ^oC"; "ion source temperature, 250 ^oC", "interface temperature, 270 ^oC; acquisition mass range 700-40 amu; ionization energy, 70 eV. Helium was used as carrier gas with a flow rate 0.5 ml/min. The identification of peaks was accomplished by comparison of the mass spectra with those reported in the NIST library. Identification of the aliphatic chains was also done by comparison of their linear RI with those from Mass Finder library.

Results and Discussion

Compound **1** was obtained as greenish amorphous powder. The ESI/MS depicted the *m/z* 683.1271 $[M + H]^+$, corresponding to the molecular formula $C_{42}H_{83}O_5N$. The series of interconected signal in ^{1}H NMR spectrum between δ_H 1.25-1.31 revealed the presence of side chain, which was supported by the fragementation pattern of long chains at *m/z* 311.5310 $[M + H]^+$ and *m/z* 374.4508 $[M + H]^+$, which depicted the presence of icosane and docosane aliphatic moieties respectively, which were identified by GC/MS by using the corresponding standards. As the IR showed the typical N-H stretching at 3280, 1550 cm^{-1} indicating the presence of amide group interconnected two long side chains, supported by the typical signal at δ_H 8.02 in

^{1}H NMR spectrum. The resonances in ^{13}C NMR at δ_C 175.1, 123.3, 134.1 revealed the presence of carbonyl and side chain double bond. The position of the double bond was assigned to icosane side chain positioned between C_4-C_5, supported by obtaining the long chain fragement of C-16 (hexadecane) in ESI/MS/MS at *m/z* 227.4302 $[M + H]^+$. As the molecule is polyhydroxylated revealed by the ^{13}C NMR resonaces at δ_C 78.2, 75.6, 71.1 and 62.1. The position of the hydroxyls was given on the basis on fragementation pattern obtained both in ESI/MS/MS and GC/MS. Based on the above description, structure 1 (Fig. 1.) was assigned to compound **1**.

Acknowledgements

We are thankful to Council of Scientific and Industrial Research (CSIR), New Delhi, India for providing fellowship, as Pool Officer under the Grant No. IA-27401 to corresponding author.

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