

Structure Identification of Stilbenoid Compound Isolated from the Stem of *Gnetum gnemon* L. Using Spectroscopic Methods

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ABSTRACT

In this research, the stem of *Gnetum gnemon* L. (Local name-Hyin-byin) was used for the isolation and structure assignment of stilbenoid compound. From the phytochemical screening of the extracts of the sample, many phytochemical constituents such as alkaloids, flavonoids, terpenoids, steroids, glycosides, reducing sugars, lipophilics, polyphenols, tannins and phenolic compounds were present. The compound **I** was isolated as brown amorphous powder by thin layer and column chromatographic separation techniques. In addition, the complete structure of stilbenoid compound **I** was identified by applying 1D and 2D NMR spectroscopy as well as Mass spectrometry. The isolated stilbenoid compound was assigned as Gnetin C (dimer of resveratrol) from the comparison of ¹³C NMR spectral data with literature.

Keywords : *Gnetum gnemon* L., NMR spectroscopy, MS spectrometry, stilbenoid, resveratrol dimer

1 INTRODUCTION

Plants have been used as one of the major sources of traditional medicines since prehistorical time and thus numerous drugs are derived from various plants. [1] Medicinal plants provide the primary health care service to the rural people because they are the potential source of secondary metabolites. [2] According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. [3] The uses of medicinal plants are effective, safe due to low side effects, low costs, and ease of availability. [1, 4, 5] From the survey (1993) of World Health Organization (WHO), the medicinal practitioners treat about 85% of patients in Myanmar, 90% in Bangladesh and 80% in India. [4]

Gnetum gnemon L. (Gnetaceae) is an indigeneous plant that is distributed in the tropical lowlands of the world from Northeastern South America, tropical West Africa, and South China to Southeast Asia and has been traditionally used as only as an ingredient for sour vegetables or as a snack. [5- 9] The seeds of *Gnetum gnemon* are especially nutritious, and are a major staple food in some areas. [8] Various species has been used for the treatment of rheumatitis, arthritis, bronchitis and asthma in folk medicines. [9] This plant has been used to reduce constipation and inflammation and its leaf sap is used to treat eye complications. [10] The scientific literature review revealed the uses of the ripe fruits for stomach cleansing and prevention of constipation and antimicrobial activity of seed and peel, and antioxidant property seed and plant. The edible parts of *G. gnemon* were reported for their antioxidant and DNA damage prevention activities. [10, 11] The seed extracts of this plant has been reported to have a broad spectrum of pharmacological effects. [6-8] The seed extract produced an antimetabolic syndrome effect by suppressing a gain in body weight and improving insulin resistance. [6] The antidiabetic properties of the fruit extracts were also reported. [10]

The Gnetaceae family is known to contain stilbenoid group including resveratrol, gnemonols A-L, gnemonosides K, and gnetin E. These compounds were also successfully isolated the stem and root of *Gnetum gnemon* L. [12] The seeds are very rich in resveratrol dimers such as gnetin C, Gnetin L, gnemonosides A, C, D, and resveratrol and its glucosides, gnemonoside A and gnemonoside D, and also contain trans-resveratrol and its glucoside, trans-piceid. [7, 8, 12] In addition, gnemonols K and L (resveratrol trimers), M (isorhapontigenin dimer), and gnemonoside K (glucoside of resveratrol trimer) were isolated from the seeds of this plant. [13] In this research work, one Myanmar traditional indigenous medicinal plant, Hyin-byin, (*Gnetum gnemon*) grown in Myataung village, Moekaung Township, Kachin State was selected for the isolation and structure identification of organic compound.

2 MATERIALS AND METHODS

2.1 General Experimental Procedures

All solvents were distilled prior to be used. Precoated silica gel (Merk. Co. Inc, Kiesel gel 60 F₂₅₆) plate was applied for thin layer chromatography. Iodine vapor and UV- lamp (Lambda – 40, Perkin – Elmer Co, England) were used as visualizing agents in column chromatography. FT-IR spectrometer (Shimadzu, Japan) was used for the identification of the functional groups of the isolated compound. ¹H- and ¹³C NMR spectroscopy was carried out on Bruker AVANCE 600, Germany at 600 MHz and 150 MHz respectively. Chemical shifts values are given in δ -value (ppm) with tetramethylsilane (TMS) as internal standard. Mass Spectrometer (Thermo Quest Finnigan TSQ 7000) was employed to assign the molecular formula of the compound.

2.2 Plant Materials

The stems of *Gnetum gnemon* L. were collected from Myataung village, Moekaung Township, Kachin State, Myanmar. The plant was screened and identified by authorized botanist from Botany Department, Mandalay University, Myanmar. The collected sample was washed properly with water to remove any contaminants.

2.3 Preliminary Phytochemical Screening of the Extracts from the Stem of *Gnetum gnemon* L.

The phytochemical tests of the leaves of *Ocimum sanctum* were carried out at Department of Chemistry, University of Mandalay, Myanmar to detect the different kinds of chemical constituents in the sample. [14- 17]

2.4 Extraction and Isolation of Pure Organic Compound

The air dried stems samples of *Gnetum gnemon* L. (500 g) was percolated with ethanol (2 L) for two months and dried extract (2.4 g) was obtained. The solution was filtered and evaporated to concentrate at room temperature. The filtrate was extracted with ethyl acetate and then concentrated to dryness in vacuo to give dried extract (2.61 g). The ethyl acetate extract was chromatographed over a silica gel column, eluted with a gradient solvent system of increasing polarity (n-hexane only, n-hexane: ethyl acetate, then ethyl acetate only) gives 313 fractions. The progress of separation was monitored by thin layer chromatography using n- hexane: ethyl acetate mixtures. Then, the fractions of the same R_f value were combined and (11) combined fractions were obtained. The combined fraction V (36.6 mg) was subjected to repeated column chromatography over silica gel eluted with the same solvent system to afford a brown amorphous powder compound (13 mg). [18] The structure of compound **I** was identified by ¹H-NMR, and ¹³C-NMR, DEPT, COSY, HSQC, HMBC spectroscopy and Mass spectrometry.

3 RESULTS AND DISCUSSION

3.1 Preliminary Phytochemical Tests of the Stem of *Gnetum gnemon* L.

According to the phytochemical tests of the crude extracts from the stem of *Gnetum gnemon*, the sample contains many chemical constituents alkaloids, flavonoids, steroids, glycosides, phenols, polyphenols, tannins, reducing sugars, saponins and terpenes respectively.

3.2 Structure Identification of Isolated Compound **I**

The EtOAc extract of the stem of *Gnetum gnemon* Linn was chromatographed on silica gel by using n-hexane and ethyl acetate as eluents from non-polar to polar. Compound **I** was obtained as brown amorphous powder. The molecular mass of the isolated compound is 454 Da according to MS spectrum, indicating the molecular formula (C₂₈H₂₂O₆). [19] The FT-IR spectrum indicated the characteristic signal bands of stilbenoid compound [(3267.5 cm⁻¹, O-H stretching), (3037.99 cm⁻¹, sp² hydrocarbons), (1608.69 and 1512.24 cm⁻¹, C=C stretch of aromatic hydrocarbons), (1163.11 cm⁻¹, C-O-C stretching of ether)]. [20]

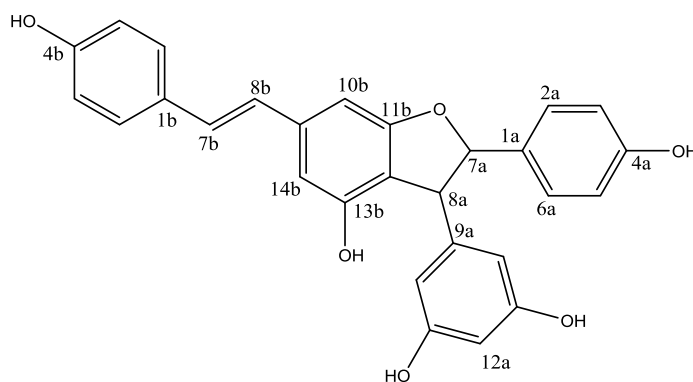
The ¹³C-NMR spectrum revealed totally 22 carbon signals indicating 28 carbons in the compound. Five carbon signals between δ 161.44, 157.23, 157.19, 154.52 and 158.39 ppm represented aromatic carbon atoms directly connected with oxygen atoms where that at δ 158.39 represented two carbon atoms. In addition, the sp³ carbinol carbon signal was found at δ _C 92.02 ppm reflecting its proton at δ _H 5.3 ppm. The ¹H- and ¹³C-NMR spectrum data (Table 1) exhibited three sets of AX-type hydrogen [δ 7.40 (2H, d, *J* = 8.6 Hz), δ 6.76 (2H, d, *J* = 8.6 Hz); δ 7.10 (2H, d, *J* = 8.4 Hz), δ 6.75 (2H, d, *J* = 8.4 Hz); δ 7.0 (1H, d, *J* = 16.5 Hz), δ 6.9 (1H, d, *J* = 16.5 Hz) (trans- alkene group)]; one set of AX₂-type *meta*-coupled aromatic proton signals [δ 6.06, 5.99 (1H, d, *J* = 2.0 Hz) and 6.05 (1H, t, *J* = 2.0 Hz); and two singlet aromatic hydrogens [δ 6.67 (1H, s), δ 6.48 (1H, s)]. Compound **I** showed two aliphatic signals at δ 5.3 (1H, d, *J* = 4.5 Hz), δ 4.2 (1H, d, *J* = 4.5 Hz).

Complete assignment of all protons and carbons was confirmed by ¹H-¹H COSY. All one- and two-dimensional NMR spectroscopic spectral data indicated that Compound **I** is the stilbenoid compound. [21, 22] From the comparison of the ¹³C NMR spectral data of the compound **I** (experimental data) with literature, the compound **I** could be assigned as Gnetin C (dimer of resveratrol).

Table (1) ^1H and ^{13}C NMR Spectral Data of the Compound **I** and that of Gnetin C from Literature (at 600 and 150 MHz, resp. in DMSO; 296 K; δ in ppm, J in Hz)

Position C/H	DEPT	δ_{H} (DMSO, d_6)	δ_{C} (DMSO, d_6)	^1H - ^1H COSY correlation	J correlation (HMBC)	δ_{C} (Acetone, d_6) [23]
1a	C		132.12		H-3a, 5a, 7a, 8a	129.2
2a	CH	7.10 (d, $J = 8.4$ Hz)	126.78	H-3a		127.0
3a	CH	6.75 (d, $J = 8.4$ Hz)	115.27	H-2a		115.57
4a	C		157.23		H-2a, 6a, 3a, 5a	156.9
5a	CH	6.75 (d, $J = 8.4$ Hz)	115.27	H-6a		115.7
6a	CH	7.10 (d, $J = 8.4$ Hz)	126.78	H-5a		127.0
7a	CH	5.3 (d, $J = 4.5$ Hz)	92.02	H-8a	H-2a	92.8
8a	CH	4.2 (d, $J = 4.5$ Hz)	54.29	H-7a		55.0
9a	C		144.99		H-8a	145.3
10a	CH	5.99(d, $J = 2.0$ Hz)	105.24		H-8a, 14a	106.2
11a	C		158.39		H-10a	158.4
12a	CH	6.05 (t, $J = 2.0$ Hz)	100.87		H-10a, 14a	101.4
13a	C		158.39		H-14a	158.4
14a	CH	6.06 (d, $J = 2.0$ Hz)	105.24		H-8a, 10a	106.2
1b	C		131.71		H- 3b, 5b	133.0
2b	CH	7.40 (d, $J = 8.6$ Hz)	127.85	H-3b		127.9
3b	CH	6.76 (d, $J = 8.6$ Hz)	115.51	H-2b		115.5
4b	C		157.19		H-3b, 5b, 2b,6b	156.9
5b	CH	6.76 (d, $J = 8.6$ Hz)	115.51	H-6b		115.5
6b	CH	7.40 (d, $J = 8.6$ Hz)	127.85	H-5b		127.85
7b	CH	7.0 (d, $J = 16.5$ Hz)	128.08	H-8b	H-2b, 6b	127.9
8b	CH	6.9 (d, $J = 16.5$ Hz)	125.48	H-7b		128.4
9b	C		139.59		H-7b, 8b	140.4
10b	CH	6.67 (s)	97.65		H-8b, 14b	98.7
11b	C		161.44		H-10b, 7a, 8a	162.1
12b	C		114.01		H-10b, 14b, 7a, 8a	114.3
13b	C		154.52		H-14b	154.3
14b	CH	6.48 (s)	107.13		H-8b, 10b	107.3

(s = singlet, d = doublet)

Figure 1. Structure of stilbenoid type compound **I**

4 CONCLUSION

The stem of *Gnetum gnemon* Linn was used for the determination of phytochemical constituents and the structure assignment of the isolated organic compound. The stem of this plant consists of many phytochemical compounds such as alkaloids, flavonoids, terpenoids, steroids, glycosides, reducing sugars, lipophilics, polyphenols, tannins and phenolic compounds. The compound **I** could be isolated as brown amorphous powder from the sample using chromatographic separation methods such as thin layer and column chromatography.

The FT-IR spectrum showed the characteristic signal bands of stilbenoid compound [(3267.5 cm^{-1} , O-H stretching), (3037.99 cm^{-1} , sp^2 hydrocarbons), (1608.69 and 1512.24 cm^{-1} , C=C stretch of aromatic hydrocarbons), (1163.11 cm^{-1} , C-O-C stretching of ether)]. The MS displayed (M⁺) at m/z 454 (corresponding to $\text{C}_{28}\text{H}_{22}\text{O}_6$). There is only two aliphatic signals at δ 5.3 (1H, d, J = 4.5 Hz) and δ 4.2 (1H, d, J = 4.5 Hz) relating the carbons at δ 92.02 and 54.28 in compound **I**. Compound **I** showed the presence of trans alkene group from the ^1H NMR spectral data [δ 7.0 (1H, d, J = 16.5 Hz), δ 6.9 (1H, d, J = 16.5 Hz)].

Careful interpretation of all the cross signals in the COSY, HSQC and HMBC spectra combined with the coupling constants of the signals in the ^1H NMR spectrum giving the complete structure assignment of the compound **I**. All NMR spectroscopic analytical data indicated that Compound **I** is the stilbenoid compound. The isolated stilbenoid compound **I** could be assigned as dimer of resveratrol compound (Gnetin C) by comparing ^{13}C NMR spectroscopic data with literature.

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