International Journal of Multidisciplinary Research and Development

Volume: 2, Issue: 8, 143-145
Aug 2015
www.allsubjectjournal.com
e-ISSN: 2349-4182
p-ISSN: 2349-5979
Impact Factor: 3.762

**NM Mofiz Uddin Khan**
Dhaka University of Engineering and Technology, Gazipur-1700, Bangladesh.

**Nazneen Akter**
Bangladesh Standards and Testing Institute (BSTI), Dhaka, Bangladesh.

**Md Sagar Hossain**
Department of Applied Chemistry and Chemical Engineering, University of Dhaka, Dhaka-1000, Bangladesh.

**Md Habibur Rahman**
Department of Chemistry, University of Dhaka, Dhaka-1000, Bangladesh.

**Correspondence**
**NM Mofiz Uddin Khan**
Dhaka University of Engineering and Technology, Gazipur-1700, Bangladesh.

# New stigmasterol and long chain alcohol from *Tinospora cordifolia*

**NM Mofiz Uddin Khan, Nazneen Akter, Md Sagar Hossain, Md Habibur Rahman**

**Abstract**
Two compounds were isolated and characterized from dichloromethane extract of *Tinospora cordifolia*. The compounds are known as 1-octacosnol (1) and stigmasta-5, 22, 25-triene-2, 3-diol (2). The structures were elucidated by spectroscopic analysis with $^{1}H$ NMR, $^{13}C$ NMR and IR. The 1-octacosanol has potential benefit against Parkinson disease. It also inhibit the production of cholesterol. The compound (2) stigmasta-5, 22, 25-triene-2, 3-diol appears to be the first report of its occurrence.

**Keywords:** Octacosanol, Parkinson, Cholesterol, NMR and IR.

## 1. Introduction
*Tinospora cordifolia* (local name: Gulancha, family: Menispermaceae) is a tropical herb with glabrous fleshy stem. From Wikipedia it was found that the family comprises 72 genera and 450 species which are found in the low-lying tropical regions. Including Bangladesh its widely spread species are also found in India, Burma, Ceylon and China [1]. The plant has different name in different region or country like Amrita, Guduchi, Giloy etc. [2]. It is a plant of significant medicinal importance which is designated as Rasayana [3]. All the parts of this plant are reported for various ethnobotanical and therapeutic uses. very much important due its medicinal value. Previous phytochemical analysis revealed that the plant contained alkaloids, flavonoids, steroids, sesquiterpenoids, aliphatic compounds, total phenols, cardiac glycosides, mixtures of fatty acids and reducing sugars. It also showed some antibacterial activity [4, 5, 6]. A wide range of compounds including aporphine, alkaloids like berberine, palmatine, tembetarine, choline, tinosporin and glycosides like tinocordiside, tinocordifolioside have been isolated from *T. cordifolia*.[7] Particle induced X-ray study of aqueous extracts showed that the trace elements K, Ca and Cl were very high, Mn was appreciable and Zn content was high in the leaves of *T. cordifolia*. Polysaccharide fraction from T. cordifolia was found to be very effective in reducing the metastatic potential of melanoma cells [7]. The notable medicinal properties reported are anti-diabetic, anti-peiodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities [8]. It is also used in treatment of rheumatism, jaundice, urinary disorders, dyspepsia, secondary syphilis, and fever [9]. The compound 1-octacosanol that isolated from the plants' stem has been subject to preliminary study for its potential for patients with Parkinson's disease [10, 11]. Studies have also found that octacosanol may inhibit the production of cholesterol [12].

## 2. Materials and Methods
### 2.1 General experimental procedure
The $^{1}H$ and $^{13}C$NMR spectra were recorded using a Bruker AMX-400 (400 MHz) instrument using $CDCl_3$ and the chemical shift was reported in ppm with respect to TMS or residual non deuterated solvent signals. IR spectrum was recorded by SHIMADZU CORPORATION CHART 200-91527.

### 2.2 Plant processing
The plant was collected from Noakhali district, Bangladesh and voucher specimen was deposited in the Department of Botany, University of Dhaka, Bangladesh. The plant was washed properly and stems of the cleaned plant was subjected to investigation. The stems were first dried at room temperature and then in oven at $40^0$ C. The stems were grinded to powder by a cyclotec grinder (200 meshes) and the powder was stored in an air tight bottle which was used throughout the investigations.

### 2.3 Extraction and Isolation

100g powder of the plant Tinospora was taken in a cleaned cloth thimbles. The thimbles containing the powder were placed in a Soxhlet apparatus. The dried powder was extracted separately and exhaustively by Soxhlet apparatus with n-hexane, dichloromethane (DCM) and methanol. All the extracts were filtered individually and concentrated in a 'Buchi Rotavapor' under reduced pressure. TLC study of DCM extract showed four spots of which two spots were turned pink indicating the presence of either steroidal or fatty material or both. Freeze dried DCM extract (1.5g) was subjected to column of VLC. The column was first eluted with 100% n-hexane followed by mixture of n-hexane and DCM of increasing polarity and finally with methanol. Fractions labeled as F-3 (Hexane: DCM- 20:80) and F-5 (Hexane: DCM- 15:85) contained single compound confirmed by TLC. Compound in F-3 is 1 and compound in F-5 is 2.

### 2.4 Spectral data and some physical properties

Compound 1: Colorless needle like crystal; soluble in chloroform and DCM; $R_f$ value-0.59; Melting point:83-85$^0$C; IR $\upsilon_{max}$ (KBr, cm$^{-1}$) : 3300, 2980, 1460, 1375, 1050 and 720; $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.88 (3H, t, J=6.4 Hz), 1.25 (-$CH_2$), 3.63 ( 2H, t,-$CH_2$OH); $^{13}$C NMR (400 MHz, $CDCl_3$): δ (ppm) 14.12 (-$CH_3$), 22.71, 25.77, 29.38, 29.46, 29.63, 29.72, 31.95, 32.86 (each, –$CH_2$) and 63.13 (-$CH_2$-OH).

Compound 2: Colorless crystal; soluble in chloroform and DCM; $R_f$ value-0.51; Melting point:86-88$^0$C; IR $\upsilon_{max}$ (KBr, cm$^{-1}$) :3450, 2980, 1640, 1450, 1370 and 1050; $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.67 (3H, s), 0.84 (3H, t), 0.99 (3H, s), 1.02 (3H, d), 1.54 (3H, s), 3.51 (1H, m), 3.67 (1H, m), 4.70 (2H, m), 5.04 (1H, dd, J=7.98Hz), 5.14 (1H, dd, J=8Hz) and 5.33 (1H, m); $^{13}$C NMR (400 MHz, $CDCl_3$): δ (ppm) 37.0 (C-1), 71.3 (C-2), 71.9 (C-3), 42.4 (C-4), 140.8 (C-5), 121.7 (C-6), 31.7 (C-7), 31.9 (C-8), 50.3 (C-9), 36.2(C-10), 21.1 (C-11), 39.8 (C-12), 42.4 (C-13), 57.0 (C-14), 24.4 (C-15),28.9 (C-16), 56.0 (C-17), 12.1 (C-18), 19.4 (C-19), 21.1 (C-20), 40.5 (C-21), 138.0 (C-22), 129.6 (C-23), 51.3 (C-24), 156.0 (C-25), 106.1 (C-26), 21.1 (C-27), 23.0 (C-28), and 12.0(C-29).

## 3. Result and Discussion

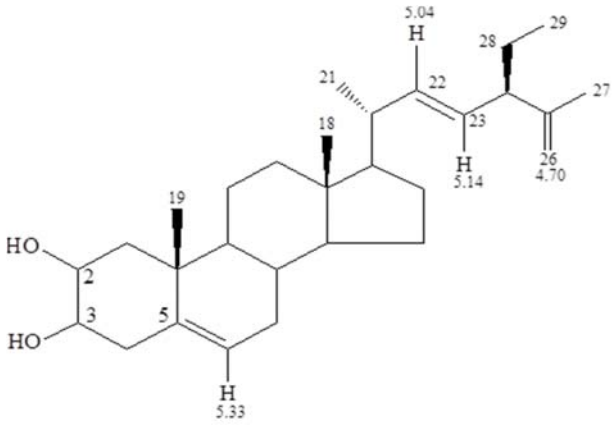
The IR spectrum of the compound **1** showed a broad -OH and C-H stretching absorption at 3300 and 2990 cm$^{-1}$ respectively. The bands at 2980, 1460 and 1375 cm$^{-1}$ were due to the presence of aliphatic C-H either stretching of –$CH_3$, -$CH_2$- or bending of –$CH_3$ in aliphatic compound. The band at 1050 cm$^{-1}$ was suggestive the presence of C-O stretching vibration. Absorption at 720 cm$^{-1}$ is suggestive for an alkyl long chain. The absence of an intense band at 1720 cm$^{-1}$ the compound 1 is neither an ester nor a carbonyl compound. It may be an alcohol.

The $^1$H NMR spectrum showed a signal at 3.63 ppm due to the oxymethylene proton -$CH_2$OH. The broad peak at 1.20-1.31 ppm was showed for the methylene protons in long chain of the molecule and the signal at 0.88 ppm indicating the presence of methyl proton. The $^{13}$C NMR showed that the total number of carbon present in the molecule was 28. The spectral analysis and reported [13] data analysis revealed that the molecule is 1-octacosanol and the structure of the molecule is -


1-OCTACOSANOL

The IR spectrum of compound 2 showed an absorption at 3450 cm$^{-1}$ indicating the presence of -OH group. The bands at 2980, 1640 cm$^{-1}$ were due to the presence of C-H stretching and >C=C< stretching. The band at 1450 cm$^{-1}$ was suggestive the presence of –$CH_2$- bending in aliphatic compound. The absorption at 1370 cm$^{-1}$ was appeared due to the -$CH_3$ group. Absorption at 1050 cm$^{-1}$ appeared for C-O stretching which was not for ester or carbonyl group as there was no sharp peak at 1720 cm$^{-1}$.

$^1$H NMR spectrum showed two 1H multiplets at 3.67 ppm and 5.33 ppm typically for H-3 and H-6 of a steroidal nucleus. The olefin protons H-22 and H-23 appeared as characteristics downfield signal at 5.04 ppm and 5.14 ppm. These two signals were observed as double doublet which indicated coupling with the neighboring methine protons. Another olefin protons appeared at 4.70 pmm for H-26.Two tertiary methyl protons' were displayed as singlet at 0.67 ppm and 0.99 ppm. Chemical shift 3.51 ppm and 3.67 ppm broad multiplets were assigned for oxymethine proton at H-2 and H-3. $^{13}$C NMR experiment revealed the presence of twenty nine (29) carbons for a steroidal skeleton. The signals for four quaternary carbons which were C-5, C-10, C-13 and C-25 appeared at 140.8 ppm, 36.2ppm, 42.4 ppm and 156.0 ppm respectively. The signal at 71.3 ppm and at 71.9 ppm were assigned for methine carbon C-2 and C-3 respectively. The spectrum contained two spectral lines at 138.0 ppm and 129.6 ppm due to double bonded carbons at C-22 and C-23 respectively. Two other double bonded carbons C-5 and C-6 were appeared at 140.8 ppm and 121.7 ppm. The signal at 105.9 ppm indicated the presence of olefinic carbon at C-26 that was reversed in the DEPT-135 indicating that the C-26 is assignable for =$CH_2$. The NMR values of the compound **2** compared with steroidal moiety of known compound 1′ (Stigmast-11,20,25-triene-3,9-diolyl)-3-(pimara-11,15-diene-3-olyl)-glycerol [14, 15]. On the basis of these evidence the structure of the compound **2** has been established as Stigmasta-5,22,25-triene-2,3-diol that is shown below:




Stigmasta-5,22,25-triene-2,3-diol

## 4. Conclusion

The plant Tinospora cordifolia is a very important medicinal plant that we found in literature. Two compounds were isolated from the stem of the plant from this study. These two compounds found from the dichloromethane extract. There are two other extracts of n-hexane and methanol extract. The compound **1** that is 1-octacosanol has some very important applications against Parkinson disease and to inhibit cholesterol production. That is why this finding could be important for pharmaceutical companies and research institutes in the development of new drugs.

## 5. References


1. Albinjose J, Jasmine E, Selvankumar T, Srinivasakumar KP. Bioactive compounds of *Tinospora cordifolia* by gas chromatography-mass spectrometry (GC-MS). Int J of Multidis Res and Development, 2015; 2(1): 88-97.
2. Tanwar S, Jain J, Verma S, Solanki D. Standardization and phytochemical evaluation of *Tinospora cordifolia*. Int J of Phar and Pharmaceu Scis, 2012; 4(1): 219-223.
3. Choudhary N, Siddiqui MB, Khatoon S. Pharmacognostic evaluation of *Tinospora cordifolia* and identification of biomarkers. Indian J of Traditional Knowl, 2014; 13(3): 543-550.
4. Savistha D, Rita M, Sonam V, Varuni G, Kirti P, Basavaraj H. Phytochemical analysis, antimicrobial and antitumour screening of endophytes of *Tinospora cordifolia*. Int J of Pharma and Bio Scis, 2012; 3(4): 533-540.
5. Nagaprashanthi Ch, Rafi KP, Gopi CK, Aleemuddin MA, Rajiya BG. *In vitro* antimicrobial activity of *Tinospora cordifolia* and its phytochemical screening. Int J of Pharm Tech Res, 2012; 4(3): 1004-1008.
6. Amit S, Neha B, Ruby G, Vikas M. Antimicrobial activity and phytochemical screening of *Tinospora cordifolia* and *Euphorbia hirta*. Int J of App Biol and Pharmaceu Tech, 2013; 4(3): 311-316.
7. Devprakash KK, Srinivasan T, Subburaju, Suhas G, Sachin S. *Tinospora cordifolia*:- A review on its ethnobotany, phytochemical & pharmacological profile. Asian J of Biochemical and Pharmceu Res, 2011; 1(4): 291-302.
8. Singh SS, Pandey SC, Srivastava S, Gupta VS, Patro B, Ghosh AC. Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi). Indian J of Pharmacology, 2003; 35: 83-91.
9. Grover D, Dutta S, Farswan AS. *Tinospora cordifolia*: Pharmacognostical and phytochemical screening. Guru Drone J of Pharm and Res, 2013; 1(1): 13-17.
10. Snider SR. Octacosanol in Parkinsonism. Annals of neurology, 1984; 16(6): 723.
11. Wang T, Liu YY, Wang X, Yang N, Zhu HB, Zuo PP. Protective effects of octacosanol on 6-hydroxydopamine-induced Parkinsonism in rats via regulation of ProNGF and NGF signaling. Acta pharmacologica sinica, 2010; 31(7): 765-774.
12. Taylor, Johanna C, Rapport, Lisa, Lockwood, Brian G. Octacosanol in human health. Nutrition, 2003; 19(2): 192-195.
13. Kim SS, Lee CK, Kang SS, Jung HA, Choi JS. Chlorogenic acid, an antioxidant principle from aerial parts of *Artemisia iwayomogi* that acts on 1,1-Diphenyl-2-picrylhydrazyl radical. Arch Pharm Res, 1997; 20(2): 148-154.
14. Ill-Min C, Ali M, Khanh TD, Choung MG, Park HJ, Ahmad A. New stigmastane steroids constituents from rice hulls of *Oryza sativa* and inhibitory activity on radish seed. Bull Korean Chem Soc, 2006; 27(1): 93-98.
15. Ali M. Techniques in terpenoid identification. Birla Publications, 2001.