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A SPINASTERYL GLYCOSIDE FROM *IPOMOEA TURPETHUM* L. HERB (STEM) GROWING IN BANGLADESH

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ABSTRACT

Chromatographic purification and spectroscopic analysis of the constituents from the stem extract of *Ipomoea turpethum* L. reported 22, 23-dihydro-α-spinasteryl-β-D glucoside (H-1) in addition to salicylic acid and *N*-p-comaryltyramine. The structures were elucidated by spectroscopic analysis including ^{1}H-NMR and ^{13}C-NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, HMQC, HMBC, UV and IR spectroscopy. 22, 23-dihydro-α-spinasteryl-β-D-glucoside is first reported from *Ipomoea turpethum*.

Key words **:** *Ipomoea turpethum*, 22, 23-dihydro-α-spinasteryl-β-D-glucoside

INTRODUCTION

Ipomoea turpethum L. (Synonym- *Operculina turpethum*); Family- Convolvulaceae, is a large, perennial and climbing herb found in Southern, South east and the Barendra region of Bangladesh, India, Srilanka and other tropical regions of the World. It produces milky juice and reported to be effective against ascites, piles, snake bites, fever, ulcer, itching, bronchitis, muscle pain, constipation, anemia and jaundice in folk medicine (Kirtikar and Basu 1994). Previous phytochemical investigations revealed the presence of some triterpenoids (Sahabuddin 1999); β sitosterol, betulin and lupeol (Nasar 1982); glycosidic resin, volatile oil (Kirtikar and Basu 1994, Wagner *et al*. 1978) and broad spectrum virus inhibitory activity (Khan 1992). Alcoholic extracts of the fresh fruits showed antibacterial activities against *Micrococcus pyogens*, var. aurus and *E. coli* (Welth of India 1966). Root and stem extracts have antioxidant and anti-cancer effect (Anbuselvum *et al.* 2007); and reported to be hepato-protective and anti-clastogenic effect (Ahmed *et al*. 2009). The present authors reported β-sitosteryl-β-D-glucoside (Daucosterol), salicylic acid and *N*-p-coumaryl tyramine from the stems of *I. turpethum* (Rashid *et al*. 2003); and evaluated the antibacterial, antifungal activity and cytotoxicity (Rashid *et al*. 2006). *N*-p-coumaryl tyramine showed significant antibacterial, antifungal activity and cytotoxicity. During the toxicological studies, it did not show any adverse

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effect on Long evans rat (Rashid *et al*. 2004). So this plant may be a rich source of important metabolite of therapeutic and pharmacological interest and have the demand of research. Authors, herein, report the existence of another glycoside 22, 23-dihydro-α-spinasteryl-β-D-glucoside (H-1) in *I. turpethum.*

MATERIALS AND METHODS

The stems of *Ipomoea turpethum* were collected from the rural areas of Rajshahi and Naogaon districts, Bangladesh during the winter season and were taxonomically identified. A voucher specimen has been maintained in the Department of Botany, Rajshahi University.

Melting point was recorded on a Gallenkamp melting point apparatus and UV spectra were recorded in MeOH on a Backman double beam spectrometer. IR spectra were obtained in KBr disc on a Perkin Elemer 1600 FTIR spectrometer. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were acquired on a JEOL JNM alpha spectrometer using TMS as an internal standard.

Air dried and pulverized stem (300 g × 3) was extracted by percolation (Rawshan 2002) in ethanol at 60°C. The ethanol extract was successively partitioned with petroleum ether, chloroform and ethyl acetate. Evaporation of solvents provided 25, 2.8 and 1.5 gm of petroleum ether, chloroform and ethyl acetate soluble materials, respectively. The chloroform solubles were fractionated by column chromatography (CC) over silica gel (Merck, mesh 60-120) using n-hexane-chloroform mixture of increasing polarities. Cent per cent chloroform afforded compound 2 (12 mg) and H-1 (10 mg) which were purified by repeated TLC and PTLC using Merck silica gel 60 (GF_{254}) on glass plates at a thickness of 0.5 mm at the multiple development of chloroform-methanol, 7 : 1 (R_f value 0.25) and 4 : 1 (R_f value 0.35), respectively. The spots on TLC and PTLC were visualized under UV light (254 and 260 nm) and spraying with 1% vanillin sulphate followed by heating at 110°C for 5 minutes. Antibacterial, antifungal and cytotoxicity studies were done according to reported method (Rashid *et al*. 2006).

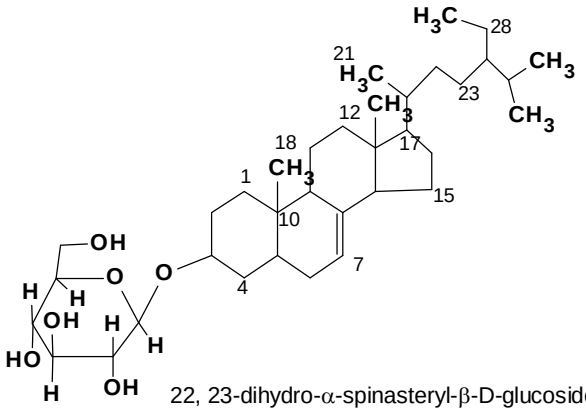
Ash white crystalline powder (MeOH), mp 284-286°C {spinasteryl-glucoside, lit. 279 - 283°C (Ariswa *et al*. 1985)}; UV (MeOH) λ_{max}: 205.2 nm; IR (KBr) ν_{max}: 1091, 1374, 1472, 2897, 3403/cm; ^{1}H-NMR (500 MHz, C_5D_5N) δ: 0.60 (3H, s, H-18), 0.74 (3H, s, H-19), 0.88 (3H, d, *J* = 6.42 Hz, H-27), 0.91 (3H, t, *J* = 6.5 Hz, H-29), 0.92 (3H, d, *J* = 6.42 Hz, H-26), 0.94 (1H, t H-9), 1.02 (3H, d, *J* = 7.0 Hz, H-21), 1.08 (1H, m, H-24), 1.09 (2H, d, *J* = 13.07 Hz, H-11), 1.10 (2H, m, H-23), 1.13 (2H, m, H-4), 1.15 (1H, t H-14), 1.16 (1H, d, *J* = 13.20 Hz, H-17), 1.27 (2H, m, H-16), 1.29 (2H, m, H-15), 1.31 (2H, m, H-28), 1.40 (2H, m, H-22), 1.72 (2H, m, H-1), 1.73 (1H, m, H-25), 1.75 (2H, m, H-6), 1.85 (2H, m, H-12), 1.94 (1H, bd, *J* = 11.3 Hz, H-5), 1.96 (1H, m, H-20), 2.50 (1H, t, *J* = 11.76 Hz, H-2), 2.75 (1H, dd, *J* = 2.0, 2.5 Hz, H-2), 3.96 (1H, m, H-3), 4.0 (1H, m,

H-5′), 4.09 (1H, t, *J* = 8.3 Hz, H-2′), 4.31 (1H, t, *J* = 8.07 Hz, H-3′), 4.32 (1H, t, *J* = 8.07 Hz, H-4′), 4.45 (1H, dd, *J* = 5.0, 11.67 Hz, H-6′), 4.59 (1H, dd, *J* = 2.50, 11.25 Hz, H-6′), 5.07 (1H, d, *J* = 8.2 Hz, H-1′), 5.36 (1H, bs, H-7); ^{13}C-NMR (125 MHz, C_5D_5N) δ: 12.0 (C-18), 12.2 (C-19), 19.0 (C-21), 19.2 (C-27), 19.4 (C-29), 20.0 (C-26), 21.3 (C-11), 23.4 (C-15), 24.5 (C-23), 26.4 (C-28), 28.6 (C-16), 29.5 (C-25), 30.3 (C-2), 30.3 (C-6), 32.1 (C-20), 34.2 (C-22), 36.4 (C-10), 37.0 (C-4), 37.5 (C-1), 39.4 (C-12), 40.0 (C-5), 42.5 (C-13), 46.1 (C-24), 50.4 (C-9), 56.3 (C-17), 56.9 (C-14), 78.1 (C-3), 121.9 (C-7), 140.9 (C-8), 102.6 (C-1′), 75.4 (C-2′), 78.5 (C-3′), 71.7 (C-4′), 78.6 (C-5′), 62.9 (C-6′).

RESULTS AND DISCUSSION

A combination of CC and repeated PTLC afforded compound H-1 and 2 from chloroform solubles and compound 3 from ethyl acetate solubles. Identification and characterization of compound 2 (Salicylic acid) and 3 (*N*-p-comaryl-tyramine) were reported in authors' previous report (Rashid *et al.* 2003). H-1 appeared as dark spot under UV light and developed bright pink to gray coloration when sprayed with 1% vanillin-sulphate followed by heating which indicates the presence of glycoside. UV spectrum λ_{max} at 205.2 nm may be due to single unsaturation. ^{1}H-NMR data exhibited two tertiary methyl proton peaks at δ 0.60 (s, H-18), 0.74 (s, H-19); three secondary methyl proton peaks at δ 1.02 (d, *J* = 7.0 Hz, H-21), 0.92 (d, *J* = 6.42 Hz, H-26), 0.88 (d, *J* = 6.42 Hz, H-27); one primary methyl proton peak at δ 0.91 (t, *J* = 6.5 Hz, H-29); one anomeric proton peak at δ 5.07 (d, *J* = 8.2 Hz, H-1′); one olefinic proton peak at δ 5.36 (bs, H-7); one oxygen substituted methine proton peak at δ 3.96 (m, H-3). These proton data suggested that compound H-1 is a steroidal glycoside (Gafur *et al*. 1997, Kojima *et al*. 1990). In ^{13}C-NMR spectrum six carbon peaks at δ 102.6 (C-1′), 75.4 (C-2′), 78.5 (C-3′), 71.7 (C-4′), 78.6 (C-5′) and 62.9 (C-6′) are similar to those of methyl β-D-glucose, which indicates the presence of a β-D-glucose unit (Zhao *et al.* 1992). In IR spectrum ν_{max} 3403/cm for O-H stretching (In β-D-glucose unit); 2897/cm for C-H stretching and 1374/cm and 1472/cm for –C = C- stretching present as the olefinic carbon. In ^{1}H-^{13}C COSY the olefinic proton 5.36 (1H, bs, H-7) is related to olefinic carbon peak 121.9 (C-7). In HMBC it showed that another olefinic tertiary carbon 140.9 (C-8) is correlated to proton peak 0.94 (1H, t H-9) and 1.15 (1H, t H-14). In ^{13}C-NMR spectrum 29 carbon peaks are similar to those of 22, 23-dihydro-α-spinasterol (Kojima *et al*. 1990, Ling *et al*. 2010), except C-3 (78.1 ppm instead of 71.7 ppm). The carbon peak at δ 78.1 (C-3) was shifted down field by 6.4 ppm, which indicates that the glycone part (β-D-glucose unit) is attached at C-3 which showed ν_{max} 1091/cm for C-O stretching in IR spectrum. 22, 23 dihydro derivative is confirmed by the presence of two methine proton peak at δ 1.40 (2H, m, H-22) and 1.10 (2H, m, H-23) instead of two olefinic proton peak at 5.07, 5.21 (each 1H, dd, *J* = 15.1, 9.0 Hz, H-22, 23) in alpha-spinasteryl-β-D-glucoside (Kojima *et*

al. 1990, Gafur *et al.* 1997). Hence H-1 is characterized and identified as the 22, 23-dihydro-α-spinasteryl-β-D-glucoside.



22, 23-dihydro-α-spinasteryl-β-D-glucoside

α-spinasterol, 22, 23-dihydro-α-spinasterol (Janson *et al*. 2009, Elvia *et al*. 2010, Jeong *et al*. 2004, Ling *et al.* 2010), and α-spinasteryl-β-D-glucoside (Kojima *et al*. 1990, Gafur *et al.* 1997) were reported from various plant sources. But 22, 23-dihydro-α-spinasteryl-β-D-glucoside was not reported earlier. Structurally related compound β-sitosterol have been reported from *I. turpethum* (Nasar 1982). Therefore, isolation of 22, 23-dihydro-α-spinasteryl-β-D-glucoside supports its placement in *I. turpethum*.

The antibacterial, antifungal and cytotoxicity studies of H-1 did not show any significant activity (data not shown). But α-spinasterol was reported to be a potent inhibitor of glomerular mesengial cell proliferation caused by high ambient glucose and control the streptozocin induced diabetic nephropathy in mice (Jeong *et al*. 2004) and have the antioxidant property (Elvia *et al*. 2010). But it is yet to be known that its dihydro-glycoside possesses such kinds of activity or not, may be interesting for further research.

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