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IPOSTERIDE, NEW ANTIMICROBIAL STEROIDAL GLUCOSIDE FROM *IPOMOEA TURPETHUM* L.

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ABSTRACT

Iposteride (**1**), the new steroidal glucoside is isolated from the ethyl acetate derivation solvent sub-portion of the methanolic concentrate of *Ipomoea turpethum* Linn. Some known compounds stigmasterol (**2**), β-sitostenone (**3**), rosmarinic acid (**4**) and methyl rosmarinate (**5**), were accounted first time from this specie. Structures of the compounds were illustrated with assistance of spectroscopic systems particularly 2D-NMR. Compound **1** indicated noteworthy antibacterial action against one Gram negative and four Gram positive bacterial strains. It also showed significant anti fungal activity.

Keywords: *Ipomoea turpethum*, Convolvulaceae, steroidal glucoside, iposteride, antimicrobial activity.

INTRODUCTION

The family Convolvulaceae comprises 40 genera and 1200 species. It is represented by 13 native and cultivated genera in Pakistan. It is dominated by herbaceous or twining or climbing woody plants that frequently have funnel-shaped blooms and heart-shaped leaves (Austin and Huáman, 1961). One of the genus of family Convolvulaceae is *Ipomoea* which is represented by 500-600 species mostly occurring in the tropical zones although some species also grow in temperate zones (Cao *et al*., 2005). These are mostly dispersed all through the South and Central American countries just as Tropical Africa (Austin and Huáman, 1961). The genus *Ipomoea* is represented by 20 species in Pakistan (Cao *et al*., 2005). Some of these have been reported to possess analgesic, antimicrobial, spasmolitic, hypotensive, spasmogenic, anticancer and psychotomimetic activities (Meira *et al*., 2012). One of the species of the genus *Ipomoea* is *I. turpethum* L.which is a medicinal plant, used in both Unani and Ayurvedic systems of medicine. It is native to Asia; India, Nepal, Bangladesh, Pakistan, Sri Lanka, China, Taiwan and Myanmar (Ahmad *et al*., 2017). The literature survey revealed that only an acrylamide derivative has so far been accounted from this specie (Harun-Or-Rashid *et al*., 2002). The chemotaxonomic and ethno pharmacological significance of genus *Ipomoea* incited us to do systematic pharmacochemical studies on this specie. Herein the separation and structure explaination of iposteride (**1**) is reported, along with stigmasterol (**2**) (Arora and Kalia, 2013), β-sitostenone (**3**) (Chen and Wang, 2010), rosmarinic acid (**4**) and methyl rosmarinate (**5**) (Eun-Rhan and Mei, 2004) revealed out first time from this specie. The compound **1** demonstrated critical antibacterial action against one Gram negative and four Gram positive bacterial strains. Compound **1** additionally demonstrated good antifungal action against different strains.

MATERIAL AND METHODS

General Experimental Procedures

Determination of melting point was carried out on a Buchi equipment and these are uncorrected. UV and IR analyses were done on Hitachi-3200 spectrophotometer and JASCO 302-A spectrophotometer respectively. JASCO DIP-360 polarimeter was used to estimate Optical rotations. NMR analysis was carried out through Bruker AV-500MHz spectrometer using $CDCl_3$ with TMS (internal standard). Coupling constants *J* are described in Hz with chemical shifts δ in ppm relative to TMS. JEOL JMS-HX-110 mass spectrometer was utilized for HREIMS. Coating material of TLC plates was Si 60 F_{254} (E. Merck). The particle size of silica gel for column chromatography was 230–400 mesh (E. Merck).

Plant material

The *Ipomoea turpethum* Linn. plant (30 kg) was received from South Africa in 2014 and identified by Dr. Zehra Yaqeen, Pharmaceutical Research Centre, PCSIR Laboratories Complex Karachi; a voucher specimen was delivered to its Herbarium (No. MP 1-17).

Extraction and isolation

Whole plants (30 kg) were dried under shade, ground in powder form. Extraction of compounds was carried out with MeOH (3×30 L) at ambient temperature. A residue (500 g) was obtained after evaporation of combined MeOH extract, which was segregated into sub-fractions soluble in *n*-hexane (90 gm), chloroform (150 gm), ethyl acetate (200 g), *n*-butanol (220 g) and H_2O (50 g). Column chromatography technique was applied to further treat EtOAc fraction using silica gel with eluting solvents comprised of *n*-hexane and ethyl acetate with increasing order of polarity. Six major F_1-F_6 fractions were produced. The eluting solvent for fraction F_1 was *n*-hexane:EtOAc of 8:2 ratio. It was again chromatographed on silica gel containing column using eluent *n*-hexane and EtOAc (7:3) to separate compound **3** (20 mg). Fraction F_3 which was received through *n*-hexane:EtOAc (6:4) was further passed through another column of silica gel using eluent comprised of binary solution of *n*-hexane and EtOAc in increasing order of polarity. Further chromatography of sub-fraction with eluting agent *n*-hexane:EtOAc (5:5) gave compound **2** (15 mg). The eluting solvent for fraction F_5 was *n*-hexane:EtOAc (4:6), It was then passed through silica gel column and provided compounds **5** (35 mg) from top fraction and **4** (28 mg) tail fraction through eluent *n*-hexane and EtOAc (2.5:7.5). F_6 fraction (eluent *n*-hexane:EtOAc (3:7)) was reprocessed through column using *n*-hexane:EtOAc (2.5:7.5) to produce iposteride (**1**) (5 mg).

Iposteride (1)

Colorless amorphous solid, mp 167-169°C and $[\alpha]_D$ +135° (*c* 0.02, MeOH). IR (KBr): υ_{max} = 3050, 1720, 1640-1610, 1455 cm^{-1}. The UV (MeOH): λ_{max} (log ε): 270 (3.14), 282 (3.58), 290 (3.22) nm. EI-MS (*m/z*, $I_{rel.}$, %): 396 [M–galloylglucose–H^+, 8], 363 (100), 271 (51), 253 (35), 211 (45), 170 (22). NMR, see Table 1. HRFABMS (positive mode): $[M + H]^+$ at *m/z* = 711.4108 (calculated for $C_{41}H_{59}O_{10}$, 711.4104 $[M + H]^+$).

Hydrolysis of compound 1

A solution of 4 mg of compound **1** was prepared in 5 mL MeOH and the solution was refluxed with 2mL of 1N HCl for 4h. The refluxed matter was concentrated under decreased pressure and diluted and extracted with H_2O and EtOAc, respectively. The EtOAc extract contained two aglycones: ergosterol (m.p. 167–169°C; $[\alpha]_D$ – 135°) (Headley *et al*., 2002) and gallic acid (mp 235-239°C) (Abri and Maleki, 2016). Both of these were analyzed and identified by co-TLC, physical and spectral data. The aqueous phase containing glycone which was identifiable as D-glucose through co-TLC with a reliable specimen and the sign of its optical rotation value $[\alpha]_D$ + 52.5°.

Antimicrobial bioassay

Agar well diffusion method was applied in determination of antibacterial activity (Atta-ur-Rahman *et al*., 1999). The inhibition zones of the isolated compound were measured against 9 bacterial strains, six Gram-positive (*Bacillus subtillis* ATCC 11774; *Streptococcus fecalis* ATCC 29212; *Staphylococcus aureus* ATCC 25923; *Cornybacterium diphtheria* ATCC 27010; *Streptococcus saprophyticus* ATCC 15305; *Streptococcus pyogenes* ATCC 12344) and three Gram-negative (*Shigella flexneri* ATCC 12022; *Salmonella typhi* ATCC 10749; *Escherichia coli* ATCC 25922). The volume of sample was one hundred µL containing 10^7 CFU/mL. It was applied on Muller Hinton medium (MHI) for 24 h time and 37°C temperature. A sterile disc (6 mm) containing different concentration of test compound (50µg, 100 µg and 150 µg) was prepared from stock solution containing solvent DMSO (10 µl). The sensitivity of each microbial specie under test estimated using Amoxicillin (10 µg per disc) as a positive control. The inhibitory zones against all test organisms were measured in mm to assess the antimicrobial activity. DMSO was used as negative control. All data of antibacterial activity was average of triplicate readings.

Agar tube dilution method was used to analyze antifungal activity (Choudhary *et al*., 1995). In this test, standard sample of drugs along with compound **1** (each 200 lg/ml of Sabourd Dextose Agar) tested against *Aspergillus flavus* ATCC 32611, *Pseudallescheria boydri* ATCC 44330, *Trichophyton schoen leinii* ATCC 22775, *Candida albicans* ATCC 10231, *Microsporum canis* ATCC 36299 and *Rhizoctonia solani* ATCC 76131.

RESULTS AND DISCUSSION

Iposteride (**1**) was acquired as colorless amorphous solid, which showed positive results for a steroid in Lieberman–Burchard and Salkowski tests (Gołembiewska *et al*., 2013). The molecular formula $C_{41}H_{58}O_{10}$ was established through positive mode HRFABMS presenting a quasimolecular-ion $[M + H]^+$ peak (*m/z* 711.4108). IR

analysis exposed existence of hydroxyl (3450 cm-1), olefinic (1640, 1610 cm^{-1}), aromatic (1455 cm-1) and ester carbonyl (1720 cm^{-1}) functional groups. In UV spectrum, maxima at 270, 282, and 290 nm illustrate 5, 7 conjugated diene containing steroidal skeleton (Shapiro and Gealt, 1982). The mass fragmentation pattern of compound **1** was indicative of sterols (Headley *et al*., 2002) exhibiting an intense peak at *m/z* 396, due to loss of 6-*O*-galloylglucose residue. It further showed diagnostic fragments at *m/z* 271 $[396–\text{side chain}]^+$, 253 $[396–\text{side chain}, –H_2O]^+$ and 211 $[396–\text{side chain}, –C_3H_6, –H_2O]^+$ (Shapiro and Gealt, 1982). The ^{13}C NMR (DEPT and BB) spectra presented 41 signals containing 8 methylene, 6 methyl, 9 quaternary and 18 methine carbon (table 1). Chemical shifts at δ_C 138.4, 138.0, 130.2, 141.2, 121.4 and 117.8 indicated the presence of olefinic carbons and a downfield signal at δ_C 167.2 was attributed to ester carbonyl. Signals of oxymethylene and oxymethine carbons of the sugar residue appeared in the range δ_C 78.5-62.4 while the anomeric carbon resonated at δ_C 102.4. The aromatic signals between δ_C 145.8-107.5 were referred to quaternary carbons and methines of galloyl moiety. The characteristic shift at δ_C 17.7 assigned to C-28, established the configuration at C-24 as *R* (Wrigh, 1979). Singlets of two tertiary methyls at δ_H 0.69 and 1.20 and four secondary methyls at 1.15 (d, J = 6.5 Hz), δ_H 1.05 (d, J = 6.5 Hz), 0.90 (d, J = 6.2 Hz) and 0.93 (d, J = 6.2 Hz) were noticed in proton NMR. Doublets of conjugated olefinic protons resonated at δ_H 5.30 (J = 8.0 Hz) and 5.48 (J = 8.0 Hz), while signals of the *trans* disubstituted double bond were detected at δ_H 5.24 (J = 8.0, 15.0 Hz) and 5.30 (J = 7.5, 15.0 Hz). The signals for doublet of anomeric proton at δ_H 5.02 (J = 7.2 Hz) while those of oxymethylene and oxymethine of sugar portion were seen in the range δ_H 4.69-3.60. The assignment of *β*-linkage to the hexose moiety was carried out after considering the higher coupling constant of anomeric proton. Galloyl moiety was confirmed from magnetic equivalent protons at H-2″ and H-6″ showing resonance together as singlet at δ_H 7.09. Acidic hydrolysis of **1** yielded aglycones which could provide identification as gallic acid and ergosterol (ergosta-5, 7, 22-trien-3*β*-ol). Its spectral and physical data demonstrated complete concurrence to those presented in literature. Glycone was analyzed as D-glucose through co-TLC with pure sample and the sign of its value of optical rotation. The presence of 6-*O*-galloyl glucose moiety at C-3 position was authenticated by ^3J correlation of H-3 at δ 3.82 with anomeric carbon (δ 102.4) in HMBC experiments (Table 1). ^3J correlations of oxymethylene protons with carbonyl carbon of galloyl moiety (δ 167.2) proved the existence of galloyl moiety. Methyl protons at δ 1.15 showed ^3J correlations with C-17 (δ 56.7) as well as C-22 (δ 138.0) and could therefore assigned to C-21. Their connectivity with olefinic carbon allowed us to assign one of disubstituted double bond to C-22. The methyl protons at δ 0.99 presented ^2J correlation with C-27(δ 17.7) while ^3J correlations with C-23 (δ 130.2), C-25 (δ 33.1). The configuration at C-24 was referred *R* by correlating ^{13}C NMR data of C-20 to C-25 with those of ergosterol (Wrigh, 1979). $^1H–^1H$ COSY spectrum explained the vicinal coupling between H-6 and H-7. The structure of iposteride (**1**) could be designated as (24 *R*)-methylergoesta-5, 7, 22*E*-triene-3*β*-(6-*O*-galloylglucose) on the basis of these collected evidence (Fig. 1).

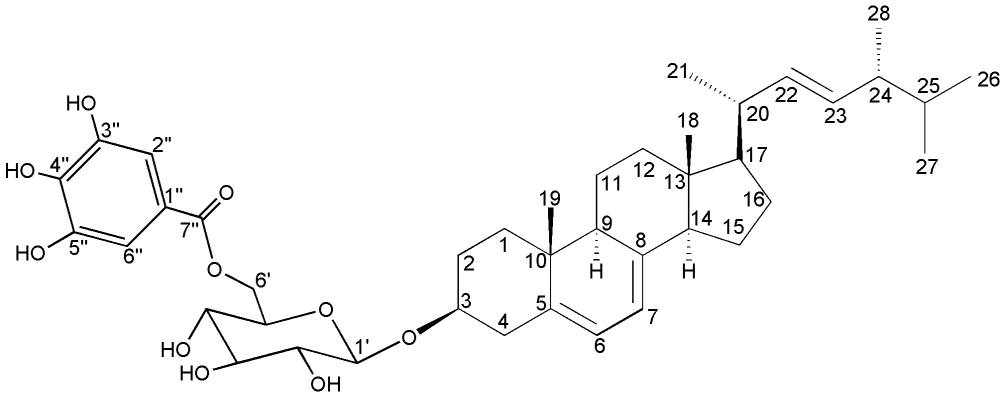

Fig. 1: Structure of compound **1**

The Agar well diffusion technique was applied for the determination of antibacterial activity of the isolated compound. Significant activity of Compound **1** was observed against four Gram-positive (*Cornybacterium diphtheria, Bacillus subtillis, Streptococcus fecalis* and *Streptococcus pyogenes*) and one Gram-negative (*Escherichia coli*) bacterial strains (Table 2).

Antifungal activity of compound **1** was examined by Agar tube dilution method. The compound **1** exhibited significant activity against *Trichophyton schoen leinii, Pseudallescheria boydri* and *Rhizoctonia solani*; moderate activity against *Aspergillus flavus* and *Microsporum canis* and weak activity against *Candida albican* (Table 3)

Table 1. ^{13}C NMR (125 MHz), ^{1}H (500 MHz) data of **1** ($CDCl_3$, δ, ppm, J /Hz).

No.	δ_H [mult., *J* (Hz)]	δ_C (mult.)	HMBC
1α/β	1.62 m; 1.02 m	40.1 (CH_2)	2, 3, 5, 10
2α/β	1.74 m; 1.45 m	32.7 (CH_2)	1, 3, 4, 10
3	3.82 m	71.4 (CH)	1, 2, 4, 5, 1′
4α/β	2.32 m; 2.40 m	41.5 (CH_2)	2, 3, 5, 6, 10
5	-	138.4 (C)	-
6	5.48 m	121.4 (CH)	4, 5, 7, 8, 10
7	5.30 m	117.8 (CH)	5, 6, 8, 9, 14
8	-	141.2 (C)	-
9	1.18 m	46.1 (CH)	5, 8, 10, 11, 19
10	-	38.6 (C)	-
11	1.50 m; 1.98 m	25.8 (CH_2)	8, 9, 10, 12, 13
12	1.37 m; 1.95 m	38.4 (CH_2)	9, 11, 13, 14, 18
13	-	44.3 (C)	-
14	1.63 m	51.5 (CH)	8, 9, 12, 13, 15, 16
15α/β	1.73 m; 1.22 m	25.7 (CH_2)	8, 13, 14, 16, 17
16α/β	1.79 m; 1.34 m	26.4 (CH_2)	14, 15, 17, 20
17	1.48 m	56.7(CH)	14, 16, 20, 21, 22
18	0.69 s	12.0 (CH_3)	12, 13, 14, 17
19	1.20 s	18.5 (CH_3)	1, 5, 9, 10
20	2.09 m	37.9 (CH)	16, 17, 21, 22, 23
21	1.15 d (6.5)	20.2 (CH_3)	17, 20, 22
22	5.24 dd (8.0, 15.0)	138.0(CH)	17, 20, 21, 23, 24
23	5.30 dd (7.5, 15.0)	130.2 (CH)	20, 22, 24, 25, 28
24	1.75 m	41.7 (CH)	22, 23, 25, 26, 28
25	1.69 m	33.1 (CH)	23, 24, 26, 27, 28
26	0.90 d (6.5)	18.7(CH_3)	24, 25, 27
27	0.93 d (6.2)	20.7 (CH_3)	24, 25, 26
28	0.99 d (6.5)	17.7 (CH_3)	23, 24, 25
1′	5.02 d (7.2)	102.4 (CH)	3, 2′, 3′
2′	3.60 m	78.2 (CH)	1′, 3′, 4′
3′	3.64 m	77.5 (CH)	1′, 2′, 4′, 5′
4′	4.10 m	74.7 (CH)	2′ 3′, 5′, 6′
5′	4.23 m	78.5 (CH)	3′, 4′, 6′
6′	4.55 dd (2.0, 11.5) 4.69 dd (6.2, 11.5)	62.4 (CH_2)	4′, 5′, 7″
1″	-	118.2 (C)	-
2″/6″	7.09 s	107.5 (CH)	1″, 3″, 4″, 7″
3″/5″	-	145.8 (C)	-
4″	-	136.8 (C)	-
7″	-	167.2 (C)	-

Plants have been used customarily for the cure of systemic and topical diseases. Research provides evidence regarding inhibitory effect of these plants on growth of many microorganisms. Such plants may contain antifungal, antiparasitic, antihistamine and antibacterial compounds (Ficker *et al*., 2003; Jones *et al*. 2000; Omar *et al*., 2000; Islam *et al*., 2001). A portion of these customarily utilized plants may prompt advancement of recent antifungal operators which are in expanding request because of resistance from conventional medications (White *et al*., 1998). It is additionally vital to record the pharmacological and phytochemical bases of customary medications to assess their adequacy and safety and to archive biological activity of species.

Nine types of bacteria used in this study, the Iposteride compound showed antibacterial activity against *B. subtillis, S. Faecalis, C. diphtheria, S. pyogenes, E.coli* while no activity was observed against *S. aureus, Shigella*

flexneri and *S. typhi*. Maximum activity was observed at concentration of 100 µg of sample and no further change was observed by increasing concentration of sample 1.

Table 2. Analysis of Antibacterial Activity of compound **1.**

Name of Bacterial strains	Zone of Inhibition (mm)			
Bacterial strains	**Compound 1 Conc. (µg)**			
	50	100	150	Amoxicillin
Bacillius subtillis	+	14 ± 0.3	+	17 ± 0.45
Streptococcus faecalis	+	16 ± 0.0	+	20 ± 0.0
Staphylococcus aureus	-	-	-	02 ± 0.12
Cornybacterium diphtheria	+	12 ± 0.17	+	16 ± 0.25
Streptococcus saprophyticus	-	-	-	19 ± 0.19
Streptococcus pyogenes	+	9 ± 0.21	+	11 ± 0.20
Escherichia coli	+	15 ± 0.0	+	18 ± 0.50
Shigella flexneri	-	-	-	14 ± 0.27
Salmonella typhi	-	-	-	11 ± 0.0

Conc. of standard drug = 10µg per disc; Zone of inhibition measured in mm
Data are mean of three replicate (n= 3) ± standard error

Table 3. *In vitro* fungicidal bioassay of compound **1.**

Organism	Inhibition (%)	Standard drugs	Inhibition by standard drugs (%)
Pseudallescheria boydri	75 ± 0.61	Benlate	100 ± 0.0
Trichophyton schoen leinii	82 ± 0.5	Miconazole	90 ± 0.10
Microsporum canis	58 ± 0.0	Miconazole	100 ± 0.0
Candida albicans	18 ± 0.2	Benlate	100 ± 0.0
Rhizoctonia solani	78 ± 0.15	Nystatin	90 ± 0.2
Aspergillus flavus	62 ± 0.33	Amphotericin-B	100 ± 0.12

Data are mean of three replicate (n= 3) ± standard error

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