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A New Ceramide Along With Eight Known Compounds from the Roots of *Artemisia incisa* Pamp

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Abstract: A new compound **(1)** (named as artemceramide-B) together with eight known compounds (taraxerol **(2)**, taraxerol acetate **(3)**, β-sitosterol **(4)**, stigmasterol **(5)**, *trans*-ethyl caffeate, dracunculin **(7)**, scoparone **(8)** and isoscopoletin **(9)** were isolated from an ethanolic extract of the roots of *Artemisia incisa* Pamp (Asteracae). The structures of the compounds were determined through IR, 1D NMR (^{1}H NMR, ^{13}C NMR) and 2D NMR (COSY, NOESY, HSQC and HMBC) analyses. Accurate mass analyses were done with EI-MS, ESI-MS and acid methanolysis of compound **1** followed by GS-MS studies. The relative stereochemistry of artemceramide-B was determined by comparing its specific rotation and spectroscopic data with the literature. Compounds **1-9** were tested for their anti-bacterial potential against five bacteria strains; *Staphylococcus epidermidis, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis* and *Escherichia coli*. Compound **1** (new) (MIC: 0.0157, 0.0313 mg/mL) and **7 (**MIC: 0.0815, 1.000 mg/mL) showed excellent activities against *S. epidermidis* and *S. aureus* while compound **9** showed excellent activities (MIC: 0.0700, 1.234, 1.890 and 2.286 mg/mL) against *S. epidermidis, S. aureus, K. pneumoniae* and *E. coli*, respectively. Compound **6** (MIC: 2.000 mg/mL) was found to be active against *E. coli* while neither of the compounds showed potential activity against *B. subtilis.*

Keywords: *A. incisa*; Asteraceae; artemceramide-B; methanolysis.

1. Introduction

Artemisia belongs to the most assorted genus of the family Asteraceae, consisting of over 500 species, with 38 species reported in Pakistan [1]. Various classes of secondary metabolites including oxygenated aliphatic hydrocarbons, phenolic hydrocarbons, polyacetylenes, furans, terpenes, flavonoids, coumarins and alkamides are reported from *Artemisia* [2]. Most of the *Artemisia* compounds are pharmacologically reported as antimalarial, antihyperglycemic, antiulcerogenic,

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antihistamine, antispasmodic, antifungal, antibacterial, antioxidant, antiallergic, insecticidal, anticancer and anthelmintic [3]. More than 1000 biodynamic compounds have been reported from the genus so for [4]; we are reporting the ceramides class for the first time from *A. incisa*. The ceramides, cerebrosides, sphingosine, glycospingolipids and sphingomyelin derivatives (sphingolipids) are essential part of the eukaryotic cell membrane and play a key role in the antigen antibody reactions and transmission of biological information [5]. Recently, some sphingolipids have shown anti-tumor, cytotoxic, immunostimulatory, anti-hepatotoxic, antiviral, antifungal, anti-ulcergenic and Ca^{2+}-ATPase activities [6-7].

We are reporting the isolation and structural elucidation of a new compound; named as artemceramide-B **(1)** together with known compounds **2-9** from *A. incisa* and the antibacterial screening (sensitivity and MIC mg/mL) of the compounds **1-9** against *Staphylococcus epidermidis, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis* and *Escherichia coli*.

2. Materials and Methods

2.1. Plant collection and Identification

The roots of *Artemisia incisa* were collected in October, 2010 from Dir (lower), Pakistan. The plant was identified by Prof. Dr. Tanveer Akhtar, Department of Botany, University of Kashmir, Muzaffarabad, Pakistan. The plant specimen (voucher # Bot. 2020 (PUP) was deposited in herbarium of the Department of Botany, University of Peshawar, Khyber Pakhtunkhwa, Pakistan.

2.2. Extraction, fractionation and isolation from the roots of Artemisia incisa

Air dried roots (1.5 kg) of *Artemisia incisa* were macerated in 5 L of methanol for seven days. The extract was concentrated under *vacuo*, yielding a gummy residue (72 g) which was subjected to silica gel vacuum liquid chromatography (VLC), eluted with dichloromethane followed by methanol yielding fractions of dichloromethane (24 g) and methanol (14 g) respectively.

The dichloromethane fraction (24 g) was subjected to silica gel column chromatography (CC) eluted with *n*-hexane:ethyl acetate (10:0, 9:1.5, 8:3 and 6:4), yielding five sub-fractions **A-E**. The sub-fraction **A** (0.8 g) was a mixture of oxygenated hydrocarbons and was not pursued further. The sub-fractions **B** (4.5 g) and **C** (5.7 g) were combined and subjected to CC, eluted with *n*-hexane:ethyl acetate (8:1.5, 8:2, 7:2.5, 7:3), yielding compounds **2** (55 mg), **3** (80 mg), **4** (38 mg) and **5** (105 mg) respectively. The sub-fraction **D** (2.3) was passed through sephadex LH-20 column eluted with dichloromethane:methanol (5:5), yielding compounds **6** (12 mg) and **7** (4 mg). The sub-fraction **E** (8.2 g) was subjected to CC eluted with *n*-hexane:ethyl acetate (6:3.8), yielding compound **8 (**14 mg). The methanolic fraction (14 g) on CC eluted with *n*-hexane:ethyl acetate (5:4), yielding compound **9** (15 mg) while further elution with dichloromethane:methanol (9:1), yielding a new compound **1** (20 mg**)**.

2.3. *Antibacterial sensitivity testing of the compounds (**1-9**)*

The antibacterial activity against the five selected bacterial strains was performed using the agar well diffusion method of Akinpelu [16]. Using 1 mL pipette, about 0.2 mL of the broth culture of the test organisms on to the sterile molten test agar at 45˚C (18 mL)(diagnostic sensitivity test agar, Biotech Ltd.). The agar prepared was decanted into sterilized labeled (with type of the pathogen) Petri dishes. On solidification a required number of holes of about 5 mm were made. The solution of the compounds were added to the wells using Pasteur pipettes. Streptomycin phosphate (1.0 mg/mL) was used as a standard. The plates after 2 hours were incubated for 24 hours at 37˚C. The plates were then streaked with dried bacterial isolates and incubated at 37 ˚C. After 72 hours, the clear zone of inhibition was measured in mm and same procedure was employed for all the compounds **(1-9).**

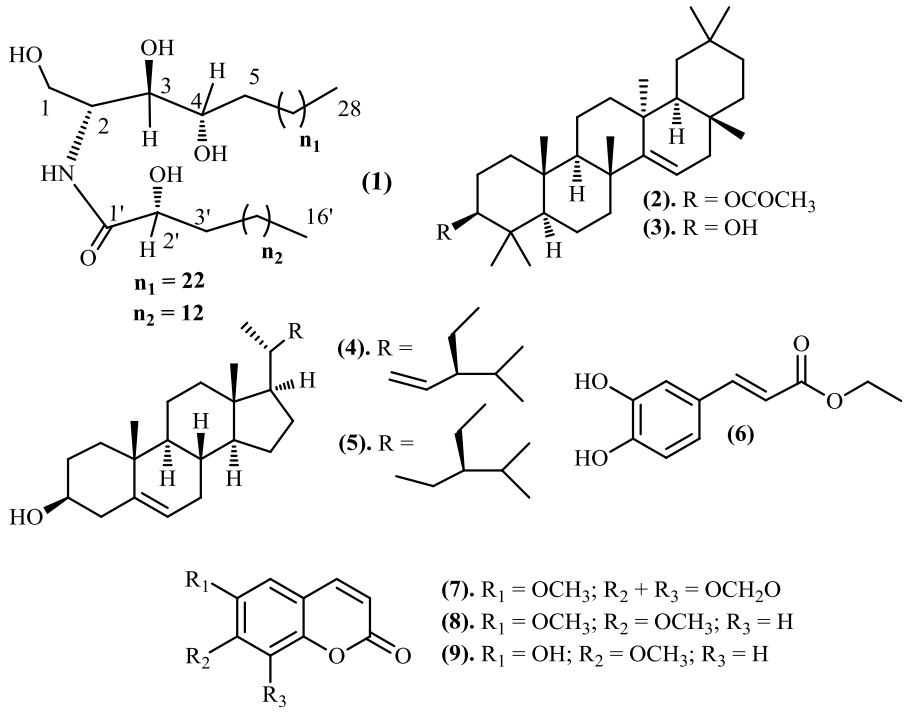

Figure 1. Structures of the compounds **1-9**

*2.4. Determination of minimum inhibitory concentration (MIC mg/mL) of the selected compounds **(1, 6, 7** and **9)***

The minimum inhibitory concentration (MIC) for some of the more active compounds (**1, 6, 7** and **9**) was determined using the method of Russell and Furr [17]. various concentrations (0.0078 - 1.000 mg/mL) of the compounds (**1, 6, 7** and **9**) in DMSO (solvent) were prepared using a two-fold dilution method. Then the procedure given above was followed. The lowest concentration preventing the growth was taken as the MIC for the compound.

2.5. General experimental procedures

Column chromatography (CC) was performed on silica gel (60-200 mesh, Merk, Germany) and Sephadex LH-20 (Amersham Biosciences, Sweden). Thin layer chromatography (TLC) was done on precoated sheets of silica gel (0.20 mm thick, 20×20 cm, Merck, Germany) on aluminum backing. Visualization of spot on TLC was done using vanillin/H_2SO_4 or ceric sulphate reagents followed by heating. The ID and 2D NMR data were obtained with a Bruker instrument (Avance DRX-500, Germany) operating at a frequency of 500.13 (^{1}H) and 125.62 MHz (^{13}C) at a temperature of 296.0 K. Optical rotations were measured with a Perkin-Elmer 243B polarimeter (USA) in pyridine and methanol at 25 °C. IR spectra were recorded with a Nicolet Avatar FT-IR spectrophotometer (Thermo scientific, USA). The positive ESI-MS data were obtained with a LCQ fleet LC-MS system by direct inlet using methanol as a solvent (Thermo scientific, USA). The EI-MS and GC analyses were done with a Shimadzu QP-5000 instrument [GC conditions: DB-1 capillary column (30 m × 0.25 mm); injector temperature, 270 °C; column temperature, 60-260 °C; temperature increase rate, 10 °C min^{-1}; He at 15 mL min].

*2.6. The methanolysis of compound **1***

Compound **1** (14 mg) was refluxed with 80 % aqueous methanol (16 m1) and 1.0 N HCl (3mL) at 85 °C for 18 h. the components of the mixture were compared on TLC using MeOH:CH_2Cl_2 (1:9). The R_f values of artemceramide-B (**1**), long chain base (LCB) and fatty acid methyl ester (FAME) were 0.55, 0.35 and 0.91 respectively. The solution obtained was extracted three times with

n-pentane. The *n*-pentane solution was dried over anhydrous Na_2SO_4 and then concentrated under *vacuo*, yielding a FAME (6.5 mg). The FAME was subjected to GC-MS analysis, Peak l (t_R 14.26 min, 1.2 %, 2-hydroxypalmitic acid methyl ester), El-MS *m/z*: 286 $[M]^+$(2), 268 $[M-H_2O]^+$ (0.5), 227 $[M-CH_3OCO]^+$ (8), 208 (1), 182 $[M-CH_3OCO-CH_2OHCH_2]^+$ (0.5), 159 (1), 145(2), 127 $[C_9H_{19}]^+$ (3), 125 (4), 111 (6), 97 (20), 90 $[CHOC(OH)=CHOH]^+$ (21), 83 (27), 69 (30) and 57 (81). The FAME was obtained as a white amorphous powder, m.p. 76 °C. The specific rotation of the FAME; $^{25}[\alpha]^D$ -5.0 (c 0.1, chloroform) was compared with the reported analogues of α-hydroxy fatty acid methyl ester having levo (-) specific rotation and an absolute *Rectus* configuration (*R*) [15], therefore, the structure of FAME was elucidated as (*R*)-2-hydroxypalmitic acid methyl ester [14].

3. Results and Discussion

3.1. Structure elucidation

Phytochemical investigation of dichloromethane and methanol fraction of *A. incisa* resulted in the isolation of a new compound; artemceramide-B **(1)** (Figure 2**)** and eight known compounds; taraxerol acetate **(2)** [8], taraxerol **(3)** [9], stigmasterol **(4)** [10], β-sitosterol **(5)** [11], *trans*-ethyl caffeate **(6),** dracunculin **(7)** [12], scoparone **(8)** [13], and isoscopoletin **(9)** [13] **(**Figure 1**)**. The plant of *A. incisa* was phytochemically unexplored, however, among the biological activities, *in vitro* assessment of the protection from oxidative stress by various fractions of the extract of *Artemisia incisa* have been reported [18].

Compound **1** was obtained as a white amorphous powder, m.p. 141–142 °C and $^{20}[\alpha]_D$ +7.98 (*c* 0.07, pyridine). The molecular formula of $C_{44}H_{89}NO_5$ was deduced from HR-ESIMS (positive ion mode) which showed the pseudo molecular ion peak at *m/z* 712.6093 $[M+H]^+$. The IR showed absorptions for NH st. at 3401, OH st. at 3365.4, 3328.5, 3246.6 and 3218.6, CONH st. at 1621.8 and $(CH_2)_n$ st. at 963.3, 720.3 $cm.^{-1}$ The ^1H and ^{13}C NMR (Table 1) spectra of compound **1** gave signals characteristics of two long chain aliphatic moieties which consist of methylenes (δ_H 1.26, 56H, m, 1.78, 4H, m and 1.70, 4H, m; δ_C 29.16–29.99, 31.92 and 22.58) and two terminal methyls (δ_H 0.84, 6H, t, J = 7.0 Hz; δ_C 13.77) linked through a secondary amide linkage (δ_H 8.59, 1H, d, J = 9.0 Hz; δ_C 174.95), proposing the structure nature of ceramide. This was further supported by methanolysis of compound **1**, yielding a long chain base (LCB) and α-hydroxy fatty methyl ester (FAME). On GC-MS analysis, the FAME was determined to be methyl 2-hydroxy palmitate [14], the absolute configuration at C-2 was determined to be *R*, from the specific rotation $^{20}[\alpha]_D$ -1.40 (*c* 0.10, chloroform) [15]. The characteristic ^1H and ^{13}C NMR signals for diastereotopic oxymethylene (δ_H 4.51, 1Ha, dd, J = 10.5, 5.0 Hz and 4.43, 1Ha, dd, J = 10.5, 5.0 Hz; δ_C 61.74), an amidomethine (δ_H 5.11, 1H, m; δ_C 52.69), oxymethines (δ_H 4.36, 1H, dd, J = 11.0, 4.5 Hz and 4.30, 1H, m; δ_C 76.54 and 72.72), diastereotopic methylene (δ_H 1.98, 1Ha, m and 2.19, 1Hb, m; δ_C 33.84), methylene (δ_H 2.2.1, 2H, m; δ_C 26.36) and hydroxyl protons (6.25, 1H, br. s; 6.71, 1H, br. s and 6.72, 1H, br. s), proved the presence of phytosphingosine moiety. Similarly, the characteristic ^1H and ^{13}C NMR signals for oxymethine (δ_H 4.62, 1H, dd, J = 7.6, 3.5 Hz; δ_C 72.16), diastereotopic methylene (δ_H 2.20, 1Ha, m and 2.23, 1Hb, m; δ_C 35.37), methylene (δ_H 1.98, 1H, m; δ_C 25.48) and hydroxyl proton (δ_H 7.65, 1H, br. s) showed the presence of α-hydroxy fatty acid moiety.

In the COSY (^1H-^1H) spectrum, the amidomethine proton at δ_H 5.11 (H-2) showed cross peaks with the diastereotopic oxymethylene protons at δ_H 4.51; 4.43 (2H-1), amidoproton at δ_H 8.59 (NHCO) and oxymethine proton at δ_H 4.36 (H-3). The oxymethine proton at δ_H 4.36 (H-3) have correlation with the oxymethine proton at δ_H 4.30 (H-4). In the HMBC spectrum, the hydroxyl protons at δ_H 6.25, and 6.71, 6.72 showed ^2J correlations with the carbons at δ_C 61.74 (C-4) and 76.54 (C-2), 72.72 (C-3) respectively which showed their corresponding position of attachment at sphingosine moiety. The amidomethine proton at δ_H 5.11 (H-2) showed ^2J HMBC correlation with the carbons at δ_C 61.74 (C-1) and 76.54 (C-3) while ^3J HMBC correlation with the carbon at δ_C 72.72 (C-4). Similarly, the oxymethine proton at δ_H 4.36 (H-3) showed ^2J HMBC correlation with the carbons at δ_C 72.72 (C-4) and 52.69 (C-2) while ^3J HMBC correlation with the carbons at δ_C 61.74 (C-1) and 33.84 (C-5). Thus both the COSY and HMBC correlations established the partial structure of sphingosine as 2-amido-1,3,4-triol sphingosine. The hydroxyl protons at δ_H 7.65 showed correlation with the carbon at δ_C 72.16

which showed their position of attachment at the α-carbon (C-2′) of fatty acid moiety. The oxymethine proton at δ_H 4.62 (H-2′) have ^2J HMBC correlation with the carbons at δ_C 174.95 (C-1′) and 35.37 (C-3′) while ^3J HMBC correlation with the carbons at δ_C 25.48 (C-4′). These correlations as well as the ^3J HMBC correlation of the protons at δ_H 2.20; 2.23 (2H-3′) with the carbonyl at δ_C 174.95 (C-1′) showed the presence of α-hydroxy fatty acid moiety. In the COSY spectrum, the oxymethine proton at δ_H 4.62 (H-2′) showed cross peaks with the diastereotopic methylene protons at δ_H 2.20; 2.23 (2H-3′) which proved the presence of α-hydroxy fatty acid moiety. The amide linkage between the sphingosine and fatty acid moieties was deduced from the HMBC correlation of the NH proton at δ_H 8.59. The NH proton showed ^2J correlation with the carbons at δ_C 52.69 (C-2) and ^3J correlation with the carbons at δ_C 61.74 (C-1) and 76.54 (C-3) of sphingosine moiety. Similarly the NH proton showed ^2J correlation with the carbonyl at δ_C 174.95 (C-1′) of fatty acid moiety.

The EI-MS of the FAME obtained from the methanolysis of compound **1** gave $[M]^+$ at *m/z* 286 corresponding to (*R*)-2-hydroxypalmitic acid methyl ester [14]. Therefore, the phytosphingosine moiety was declared to be a C_{28} aliphatic-1,2,3-triol amine (2-aminooctacosane-1,3,4-triol).

The NOESY correlation of compound **1** showed two groups of protons; (H-2, H-2′, H-4, OH-3′) and (H-3, OH-4, OH-2′, NH-1′) while the optical rotation of compound **1** was recorded as $^{20}[\alpha]_D$ +7.98 (*c* 0.07, C_5H_5N).

So, by comparing the data obtained from optical rotation and NOESY correlations (Figure 3) as well as the characteristic ^{13}C NMR chemical shifts of compound **1** (at carbons; C-1′ (174.95), C-2′ (72.16), C-1 (61.74), C-2 (52.69), C-3 (76.54) and C-4 (72.72) **(**Table 1**)** with the ceramide analogue; (*R*)-2-hydroxy-N-((*2R,3R,4S*)-1,3,4-trihydroxynonacosan-2-yl-pentacosanamide, $^{20}[\alpha]_D$ +10.2 (*c* 0.09, C_5H_5N) [15] it was deduced that the relative configurations at the carbons; C-2, C-3, and C-4 of sphingosine moiety of compound **1** were *2R, 3R,* and *4S* respectively.

The overall information obtained from the physicochemical constants and spectral data of IR, ESI-MS, EI-MS, 1D- and 2D-NMR, acid-methanolysis followed by GC-MS analyses, the structure of compound **1** was established as (*2′R*)-2′-hydroxy-2N-((*2R,3R,4S*)-1,3,4-trihydroxyoctacosan-2-yl)hexadecanamide. The compound **1** was found to be new after searching the literature and named as artemceramide-B. The structure of artemceramide-B **(1)** along with important COSY, HMBC correlations are given in Figure 2 while NOESY correlations in Figure 3.

*3.2. Physical data of known compounds (**2-9**)*

Compound **2 (**taraxerol acetate**)** [8] was isolated as white crystals, m.p. 282–283 °C (lit. m.p. 282–283 °C). The molecular formula of $C_{32}H_{52}O_2$ was established from EI-MS with *m/z* 468.39 $[M]^+$. Compound **3** (taraxerol) [9] was obtained as white crystals, m.p. 302-304 °C (lit. m.p. 304–305 °C). The molecular formula of $C_{30}H_{50}O$ was established from EI-MS with *m/z* 426.38 $[M]^+$. Compound **4** (stigmasterol) [10] was isolated as white crystals, m.p. 165–166 °C (lit. m.p. 165–167 °C). The molecular formula of $C_{29}H_{48}O$ was established from EI-MS with *m/z* 412.37 $[M]^+$. Compound **5** (β-sitosterol) [11] was isolated as white crystals, m.p. 135–136 °C (lit. m.p. 136 °C). The molecular formula of $C_{29}H_{50}O$ was established from EI-MS with *m/z* 414.39 $[M]^+$. Compound **6** (*trans*-ethyl caffeate) [12] was obtained as white needles like crystals, m.p.148–150 °C (lit. m.p. 149–150 °C). The molecular formula of $C_{11}H_{12}O_4$ was deduced from EI-MS with *m/z* 208.073 $[M]^+$. Compound **7** (dracunculin) [12] was obtained as colorless needles, m.p. 220–222 °C (lit. m.p. 219–221 °C). The molecular formula of $C_{11}H_8O_5$ was obtained from the EI-MS with *m/z* 220.037 $[M]^+$. Compound **8** (scoparone) [13] was isolated as greenish white needles, m.p. 143–144 °C (lit. m.p. 144 °C). The molecular formula of $C_{11}H_{10}O_4$ was deduced from the EI-MS with *m/z* 206.058 $[M]^+$. Compound **9** (isoscopoletin) [13] was obtained as white yellow crystals, m.p. 184–185 °C (lit. m.p. 185 °C). The molecular formula of $C_{10}H_8O_4$ was obtained from the EI-MS with *m/z* 192.042 $[M]^+$. The complete structure assignments of the known compounds (**2-9**) were achieved by comparing their spectral data (1D and 2D NMR, IR and MS) with the literature.

Table 1. ^{1}H-NMR (500.13 MHz), ^{13}C-NMR (152.62 MHz) data of compound **1** in pyridine-*d5*, δ in ppm and *J* in Hz.

Position	δ_H	δ_C
NH	8.59 (1H, d, *J* = 9.0)	---
1	4.51 (1Ha, dd, *J* = 10.5, 5.0); 4.43 (1Hb, dd, *J* = 10.5, 5.0)	61.74
2	5.11 (1H, m)	52.69
3	4.36 (1H, dd, *J* = 11.0, 4.5)	76.54
4	4.30 (1H, m)	72.72
5	1.98 (1Ha, m); 2.19 (1Hb, m)	33.84
6	2.2.1 (2H, m)	26.36
1-OH	6.71 (1H, br. s)	---
3-OH	6.72 (1H, br. s)	---
4-OH	6.25 (1H, br. s)	---
1′	---	174.95
2′	4.62 (1H, dd, *J* = 7.6, 3.5)	72.16
3′	2.20 (1Ha, m); 2.23 (1Hb, m)	35.37
4′	1.98 (1Hb, m)	25.48
7–25 and 5′–13′	1.26 (56H, m)	29.16–29.99
26/14′	1.78 (4H, m)	31.92
27/15′	1.70 (4H, m)	22.58
28/16′	0.84 (6H, t, *J* = 7.0)	13.77
2′-OH	7.63 (1H, br. s)	---

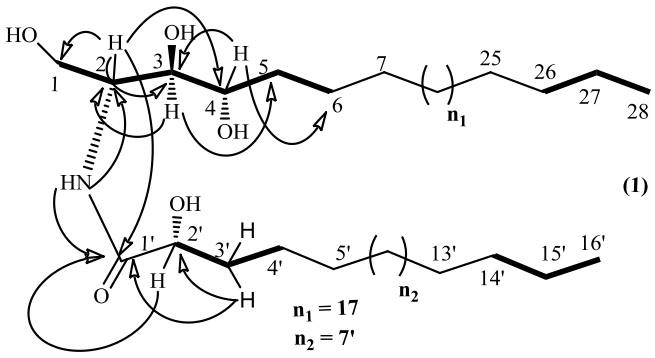

Figure 2. Important COSY (^{1}H ——— ^{1}H) and HMBC (^{1}H ⟶ ^{13}C) correlations of artemceramide-B **(1)**

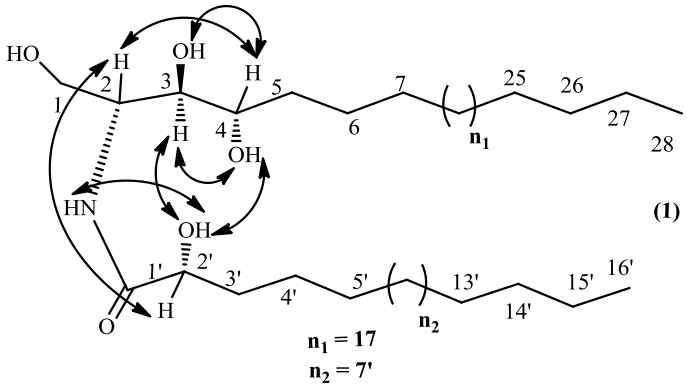

Figure 3. Important NOESY (^{1}H ⟷ ^{1}H) correlations of artemceramide-B **(1)**

3.3. Result of the antibacterial screening (sensitivity testing) and minimum inhibitory concentration for some selected compounds

The analyses of data from the Table 2 and 3 showed that the new compounds **1** (MIC: 0.0157, 0.0313 mg/mL) have enhanced potential against *S. epidermidis* and *S. aureus* as compared to streptomycin and tetracycline. The high potential of compound **1** might be due to the fact that it is a polar lipid bearing four hydroxyl groups and an amide linkage between two long aliphatic chains. The compound **9** showed high activity (MIC: 0.0700, 1.234, 1.890 and 2.286 mg/mL) against *S. epidermidis, S. aureus, K. pneumoniae and E. coli* respectively, which showed that coumarins bearing active phenolic groups might be the good choice against these bacterial strains. Compound **6** (MIC: 2.000 mg/mL) was found to be active against *E. coli* while neither of the compounds showed good activity against *B. subtilis.* The sterols **4, 5** and triterpenes **2, 3** were found to be inactive**.** Due to very high potential of the compound **1** (MIC: 0.0157, 0.0313 mg/mL) and **9** (MIC: 0.0700, 1.234), it is recommended for in *in-vivo* testing against the bacterial strains of *S. epidermidis* and *S. aureus.* The results are mean values of five replicates ± S.D.

Table 2. Result of the antimicrobial screening (sensitivity testing) of the compounds **1-9** against the five bacterial strains.

Compound name	***S. epidermidis***	***S. aureus***	***K. pneumoniae***	***B. subtilis***	***E. coli***
	Zone of inhibition in mm at 2 mg/mL				
Artemceramide-B **(1)**	29	26	10	NA	16
Taraxerol acetate **(2)**	NA	NA	NA	NA	NA
Taraxerol **(3)**	NA	NA	NA	NA	NA
Stigmasterol **(4)**	NA	NA	NA	NA	NA
β-Sitosterol **(5)**	NA	NA	NA	NA	NA
trans-Ethyl caffeate **(6)**	09	NA	16	18	26
Dracunculin **(7)**	17	20	10	15	18
Scoparone **(8)**	14	12	NA	12	09
Isoscopoletin **(9)**	28	20	16	14	18
Streptomycin (1 mg/mL)	18	21	NA	20	NA
Tetracycline (1 mg/mL)	12	NA	12	22	18

NA = Not active

Table 3. Minimum inhibitory concentrations (MICs) for some selected compounds **1**, **6, 7** and **9** against the five bacterial strains.

Compound name	***S. epidermidis***	***S. aureus***	***K. pneumoniae***	***B. subtilis***	***E. coli***
			MIC (mg/mL)		
Artemceramide-B **(1)**	0.0157	0.0313	NA	NA	NA
trans-Ethyl caffeate **(6)**	NA	NA	NA	NA	2.000
Dracunculin **(7)**	0.0815	1.000	NA	NA	NA
Isoscopoletin **(9)**	0.0700	1.234	1.890	NA	2.286
Streptomycin (mg/mL)	0.0625	1.000	NA	0.2500	NA

NA = *Not active*

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References

[1] M.A. Muhammad, Q. Hayat and A.S. Mumtaz (2010). A study on elemental contents of medicinally important species of *Artemisia* L. (Asteraceae) found in Pakistan, *J. Med. Plants Res.* **4**, 2256.

[2] B. Saadalia, D. Borikya, M. Blaghenb, M. Vanhaelenc and M. Talbi (2001). Alkamides from *Artemisia dracunculus*, *Phytochemistry* **58**, 1083.
[3] K.S. Bora and A. Sharma (2011). The Genus *Artemisia*: A comprehensive review, *Pharm. Biol.* **49**, 101.
[4] R.X. Tan, W.F Zheng and H.Q. Tang (1998). Biologically active substances from the genus *Artemisia*, *Planta Med.* **64**, 295.
[5] P. Muralidhar, P. Radhika, N. Krishna, D. Venkata Rao and C.B. Rao (2003). A Review, sphingolipids from marine organisms, *Nat. Prod. Sci.* **9**, 117.
[6] H.E. Carter, F. Glick, W. Norris and G.E. Phillips (1942). Structure of sphingosine, *J. Biol. Chem.* **142**, 449.
[7] S. Kawatake, M. Inagaki and R. Isobe (1997). Biologically active glycosides from Asteroidea. Glycosphingolipids from the starfish *Luidia maculata*, 1-structure of a new sulfatide molecular species, *Liebigs Ann. Recueil*. **37**, 1797.
[8] S.B. Mahato and A.P. Kundu (1994). ^{13}C NMR spectra of pentacyclic triterpenoids; a compilation and some salient features, *Phytochemistry* **37**, 1517.
[9] B.D. Paul, G.S. Rao and G.J. Kapadia (1974). Isolation of myricadiol, myricitrin, taraxerol, and taraxerone from *Myrica cerifera* L. root bark, *J. pharm. Sci.* **63**, 958.
[10] H.Q. Tang, J. Hu, L. Yang and R.X. Tan (2000). Terpenoids and flavonoids from *Artemisia* species, *Planta Med.* **66**, 391.
[11] G.D. Brown (2010). The biosynthesis of Artemisinin (Qinghaosu) and the phytochemistry of *Artemisia annua* L. (Qinghao), *Molecules* **15**, 7603.
[12] O. Hofer, G. Szabo, H. Greger and A. Nikiforov (1986). Leaf coumarins from the *Artemisia laciniata* group, *Liebigs. Annalen der Chemie*. **12**, 2142.
[13] R.D.H. Murray (1997). Naturally occurring plant coumarins, *Prog. Chem. Org. Nat. Prod.* **72**, 1.
[14] L.T. Jun, W.U. Ping, L. Mei-fang and W.E.I. Xiao (2005). Ceramides from the roots of *Serratula chinensis*, *J. Trop. Subtrop. Bot.* **13**, 403.
[15] D.Z. Chen, H.B. Xiong, K. Tian, J.M. Guo, X.Z. Huang and Z.Y. Jiang (2013). Two new sphingolipids from the Leaves of *Piper betle* L., *Molecules* **18**, 11241.
[16] D.A. Akinpelu (1999). Antimicrobial activity of the crude extract of *Vernonia amygdalina* leaves, *Fitoterapia* **71**, 75.
[17] A.D. Russell and J.R. Furr (1977). The antibacterial activity of a new chloroxylenol preparation containing ethylenediamine tetraacetic acid, *J. Appl. Bacteriol.* **43**, 253.
[18] A.U. Rahman, G. Sehrish, A.A. Muhammad, T. Shahzadi, T. Riaz, S.Z. Siddiqui and A. Muhammad (2011). In vitro assessment of the protection from oxidative stress by various fractions of *Artemisia incisa* Pamp, *J. Serbian Chem. Soc.* ***76***, 1379.