III. Triterpenoids from *Grewia* sp.

3.1 Introduction

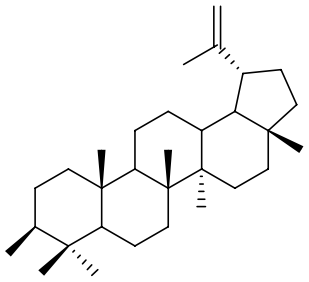
The extract of leaves and flowers of a *Grewia* sp. (MG 3692) was obtained from Madagascar rainforests as a part of the ICBG program for the isolation of active anticancer agents. After testing the extract on A2780 bioassay, it exhibited activity at 3.7 μg/mL and hence it was selected for further analysis of its chemical components. The extract yielded one new and one known triterpenoid, **1** and **2**. The isolation and structure determination of these compounds is described in this chapter.

3.1.1 Chemical Investigation of a *Grewia* sp.

Grewia sp. of the Malvaceae family (previously belonging to the Tiliaceae family) is a genus of flowering plants found in tropical and temperate regions as well. The Malvaceae family has 75 genera and about 1500 species. Plants belonging to this family are economically useful, as they provide an excellent source of naturally occurring fiber, like cotton from the genus *Gossypium*, and certain foods like okra, from *Abelmoschus esculentus*, which is used as a vegetable. The stem and roots of *Hibiscus tiawanensis*, native to Taiwan, have anti-inflammatory and antipyretic properties.[2] The dried leaf extract of *Hibiscus sabdariffa* acts as a anti-hypertensive by inhibition of angiotensin-converting enzyme (ACE).[3] Plants of the *Hibiscus* sp. have been used as an antidote for chemical and wild mushroom poisoning[4] as well as an Ayurvedic herbal shampoo in Indian medicine.

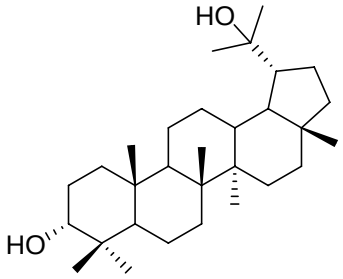
The genus *Grewia* is mainly associated with triterpenoids and alkaloids. The extracts from these plants are known to have medicinal properties.[5] The bark and roots of *Grewia*

tiliaefolia are used to treat skin diseases, hypertension, ulcers and diarrhoea.[6] Lupenol (**3.1**), isolated from this plant, is known to cause apoptosis in several cancer cells.[7]



3.1 Lupenol

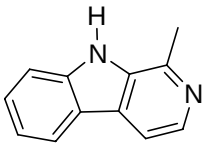
Grewin (**3.2**) isolated from the extract of *Grewia bilamellata*, exhibits antimalarial activity against *P. falciparum*.[8]



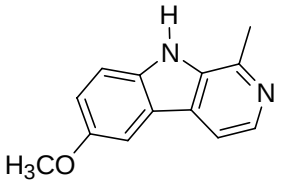
3.2 Grewin

Grewia asiatica was initially cultivated mainly for its sour fruits until its high medicinal value was discovered. The fruits of this plant are used as an astringent, an anti-inflammatory agent, for blood disorders, and a fever reducer.[9] The bark is used medicinally for the treatment of diarrhea.[9] *Grewia bicolor* is a part of Sudanese traditional medicine, and is used in the treatment of skin lesions and sometimes also as a tranquilizer.[10] The three alkaloids,

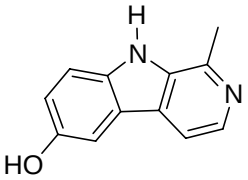
harman (**3.3**), 6-methoxyharman (**3.4**), and 6-hydroxyharman (**3.5**), isolated from the methanol extract of this plant, have antibacterial properties.[10]



3.3 Harman



3.4 6-methoxyharman

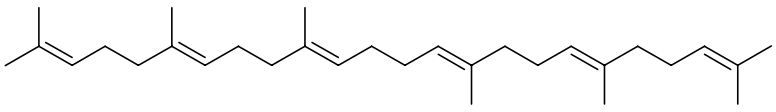


3.5 6-hydroxyharman

An extract of *Grewia villosa* extract is used in treatment of tuberculosis,[11] and this plant is also known to contain harman alkaloids. Harman alkaloids belong to the class of β-carbolines and bind strongly to receptors in the brain and affect the CNS.[12]

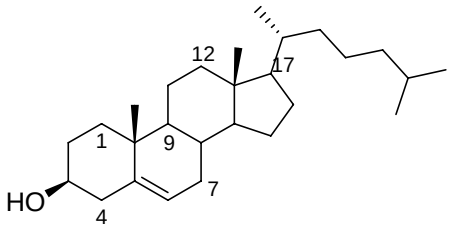
3.1.2 Structure and Chemistry of Triterpenoids

Triterpenes are a large class of compounds that include steroids and sterols. This class is present abundantly in plants and animals. They have a C30 carbon skeleton and most naturally occurring triterpenoids are biosynthesized from squalene (**3.6**).



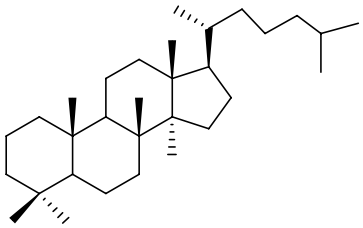
3.6 Squalene

Several steroids are formed from squalene by various cyclizations, loss of small molecules, ring expansions, or contractions. One such example of a triterpene is cholesterol (**3.7**)

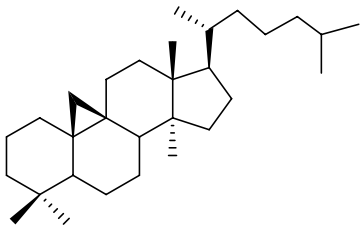


3.7 Cholesterol

Depending on the folding pattern of the squalene chain, different types of triterpenes are formed in nature. There are about 20 different groups of triterpenes known in nature. The cyclizations of chair-boat-chair-boat conformation of squalene give a protostane cation. The lanostrane skeleton (**3.8**) is derived from this cation, which forms the biological precursor for most steroids found in animals. The cycloartane skeleton (**3.9**) is also formed from the protostane cation by cyclization between C9-C19 carbons. Most plants synthesize their triterpenes from the cycloartane skeleton.[13] These triterpenes are commonly called phytosterols.[14]

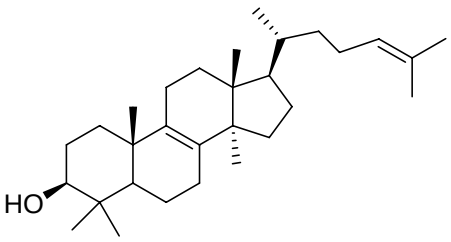


3.8 Lanostrane

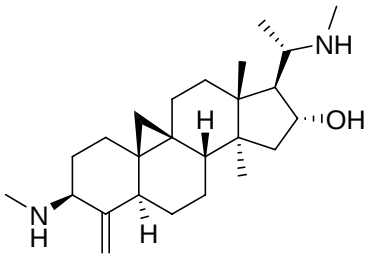


3.9 Cycloartane

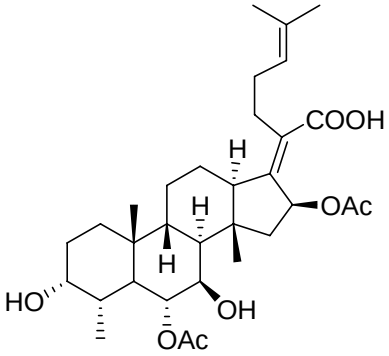
Most triterpenes have methyl groups at the C10 and C13 positions and an alkyl side chain at the C17 position. A few examples of common triterpenoids are given below.



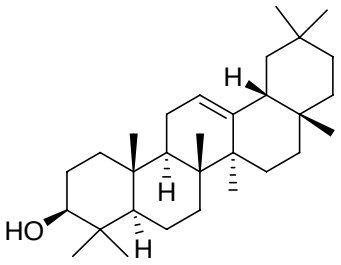
3.10 Lanosterol



3.11 Cyclobuxine



3.12 Cephalosporin



3.13 β-amyrin

About 2500 triterpenes have been studied so far, however, very few of them are investigated for there biological importance.

Recently many studies have been conducted to determine the biological activity of this class of compounds. Several triterpenoids have diverse pharmacological properties including anti-fungal,[15] anti-bacterial, and anti-mutagenic activity.[16] The triterpenes are known to inhibit the action of multi-drug resistance (MDR) protein, the activity of which leads to the failure of several potential anticancer agents.[17]

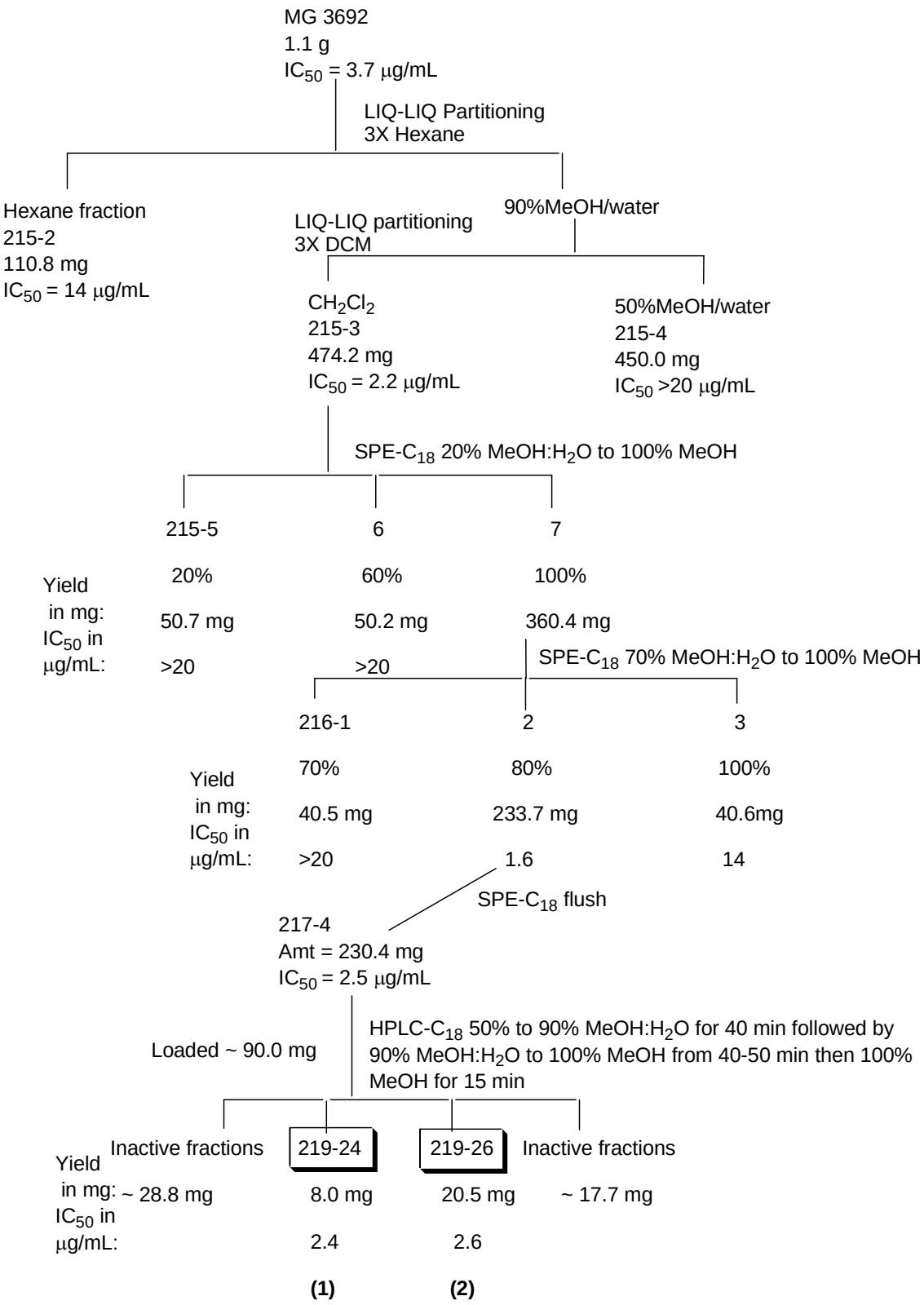
3.2 Results and Discussion

3.2.1 Isolation and structure determination of compound 1 and 7*β*-hydroxy-23-deoxojessic acid (2) from *Grewia* sp.

As a part of ICBG program in continuous search for potential anticancer agents from Madagascar rainforests, we received a crude extract of *Grewia* sp. (MG 3692) which was active in A2780 human ovarian cancer cell line (IC_{50} = 3.7 μg/mL) and hence it was selected for further investigation.

The ethanol extract of MG 3692 (1.1 g) was subjected to liquid-liquid partitioning between hexanes (3 × 100 mL) and aqueous MeOH (MeOH:H_2O, 9:1, 100 mL). The aqueous layer was then diluted to 50% MeOH (v/v) and extracted with CH_2Cl_2 (3 × 180 mL). The CH_2Cl_2 fraction displayed the highest activity (IC_{50} = 2.2 μg/mL) and hence selected to isolate its active components. This fraction was passed through a reverse phase SPE-C_{18} column and eluted with a gradient elution of 60% MeOH:H_2O to 100% MeOH. Five fractions were collected and only one was active at IC_{50} = 1.6 μg/mL. This fraction was further purified by column chromatography on a C_{18} reversed phase HPLC column using a gradient elution of 40% MeOH:H_2O to 100% MeOH. This yielded several inactive fractions as well as two pure and active fractions, 219-24, 219-26. One was a new compound, compound **1**, and the other was identified as a known compound (**2**), 7*β*-hydroxy-23-deoxojessic acid.[18]

Grewia sp.



Scheme 3.1 Fractionation tree for *Grewia* sp.

3.2.2 Structure Elucidation of Compound 1

Compound **1** was isolated as a yellowish amorphous solid. Positive ion LRFABMS gave a molecular ion peak at *m/z* 489.9 [M+H$^+$], consistent with the molecular composition of $C_{30}H_{48}O_5$. The IR spectrum of **1** showed a broad band at 3649 cm^{-1}, and a sharp band at 1542 cm^{-1} indicating the presence of hydroxyl and carbonyl groups. The ^{1}H and ^{13}C NMR spectra indicated that this compound belonged to the class of triterpenoids.

The ^{1}H spectrum of **1** in methanol-d_4 (Fig. **3.1**) indicated that the compound was pure with three oxygenated protons and six methyl groups. A highly shielded peak at δ_H 0.45 (d, J = 4.4 Hz, H-19) suggested the presence of a cyclopropyl ring.[19] The ^{13}C spectrum of compound **1** contained 30 signals: six methyls, eight methylenes, five methines, three oxygenated carbons, and eight quaternary carbon peaks.

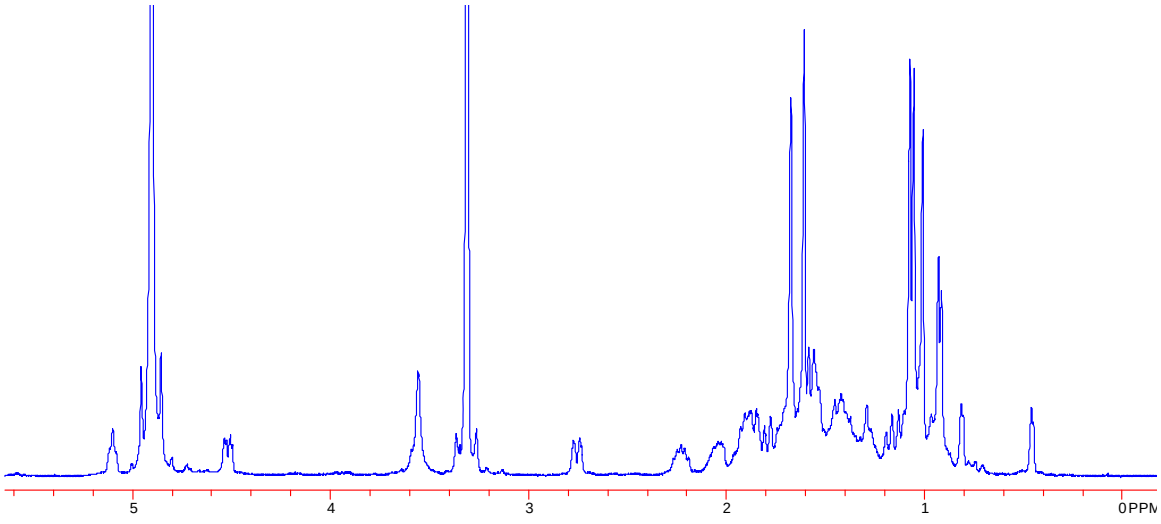


Figure 3.1 ^{1}H NMR spectra of compound **1** in methanol-d_4

The ^{1}H and ^{13}C NMR signals in methanol-d_4 showed typical signals for the cyclopropane methylene protons with peaks at δ_C/δ_H 22.0 (C-19)/0.45 (d, J = 4.4 Hz, H_β-19) and 0.81 (d, J = 4.8 Hz, H_α-19); two quaternary carbons at δ_C 29.1 (C-9) and δ_C 29.2 (C-10). Three oxygenated methine peaks were observed at δ_C/δ_H 73.5 (C-1)/3.55 (br, H_β-1); δ_C/δ_H 71.4 (C-3)/4.52 (dd, J = 10.4 Hz, 6.8 Hz, H_α-3), δ_C/δ_H 70.6 (C-7)/3.56 (br, H_β-7). From HSQC and HMBC correlations, the proton-carbon pairs were connected to each other and $^{1}J_{CH}$ correlations were determined (Table **3.1**) to obtain a cycloartane-type triterpene.

HMBC correlations for the fusion of the A/B rings was confirmed by the correlation of H-29 (δ_H 1.07, s) to C-3 (δ_C 71.4), C-5 (δ_C 37.5), C-28 (δ_C 180.6); H-5 ((δ_H 2.76, dd, J = 4.0 Hz, 14.0 Hz) to C-4 (δ_C 46.9), C-3 (δ_C 73.5), C-6 (δ_C 29.6), C-7 (δ_C 70.6), and C-19 (δ_C 22.0).

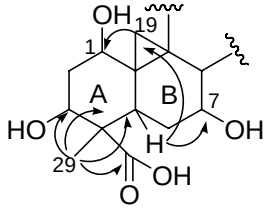


Figure 3.2 Key HMBC correlations of A/B ring fusion in fragment 1

Similarly, the B/C ring fusion was confirmed by correlation of H-19 to C-8 (δ_C 25.3), C-9 (δ_C 26.1), and C-11(δ_C 27.2), and H-8 (1.29, s) to C-7, C-11, C-14 (δ_C 30.9), and C-19 (δ_C 22.0).

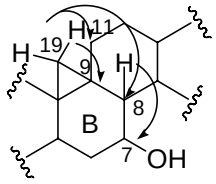


Figure 3.3 Key HMBC correlations of B/C ring fusion in fragment 2

After confirming the fusion of the C/D ring at position C-13 and C-14 (**3.5**), the attachment of the alkyl side chain was determined. A strong HMBC correlation from H-17 (δ_H 1.57, m) to C-14 (δ_C 30.9), C-21 ((δ_C 19.0), C-22 (δ_C 26.0), and C-27 (δ_C 17.8) indicated the point of attachment of the alkyl chain at C-17 thereby confirming the cycloartane skeleton (**3.20**)

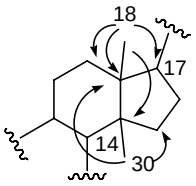


Figure 3.4 Key HMBC correlations of C/D ring fusion in fragment 3

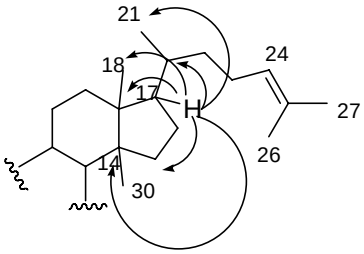
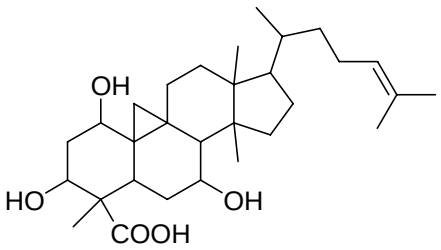


Figure 3.5 HMBC correlations for attachment of alkyl side chain at C-17

Thus from the HMBC correlations as discussed above, the flat structure of compound **1** was established as shown in figure 3.14.



3.14 Flat structure of compound **1**

The stereochemistry of compound **1** was determined from 1D and 2D ROESY spectra. The ROESY correlations of H-19 (δ_H 0.45, d, J = 4.4 Hz) to H_β-1 (δ_H 3.55, m); H-19 (δ_H 0.81, d, J = 4.5 Hz) to H_β-8 (δ_H 1.29, s) and H_β-11 (δ_H 1.39, m) as well as H-5 ((δ_H 2.76, dd, J = 4.0 Hz, 14.0 Hz) to H_α-3 (δ_H 4.52, dd, J = 10.4 Hz, 6.8 Hz) and H-7 (δ_H 3.56, m) confirmed the chair conformation of both the rings, A and B, indicating a *trans* fusion of the two rings and a *β*-orientation of the cyclopropane ring. The H-3 signal was observed as a doublet of doublets (dd) due to the axial-equatorial (J = 6.8 Hz) and diaxial (J = 10.4 Hz) interactions, suggesting an α-orientation of H-3. Correlations of H_β-8 (δ_H 1.29, m) to H_β-11 (δ_H 1.39, m), H_α-15 (δ_H 1.41, m), H_β-18 (δ_H 1.61, s) and H-19 (δ_H 0.45, d) as well as correlations of H_α-7 (δ_H 3.56, m) to H_α-30 (δ_H 1.68, s), suggested a *trans* fusion C/D rings of this triterpenoid (**3.6**).

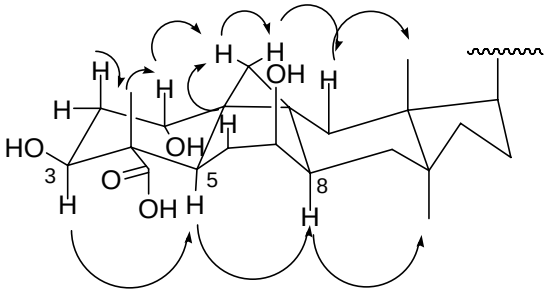


Figure 3.6 Key ROESY correlations of compound **1**

A strong correlation of H-18 to H-20 ((δ_H 1.40, m) was observed, suggesting a *β*-attachment of the alkyl side chain. After assignments of carbons and protons in compound **1** from 1D

and 2D spectra, the final structure of compound **1** was assigned as shown in Figure 3.7. This compound has been identified in nature for the first time.

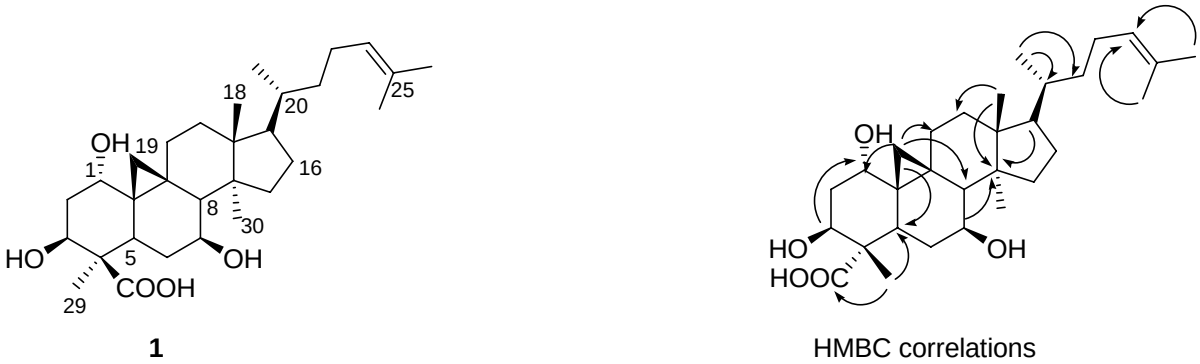


Figure 3.7 Final structure and key HMBC correlations of compound **1**

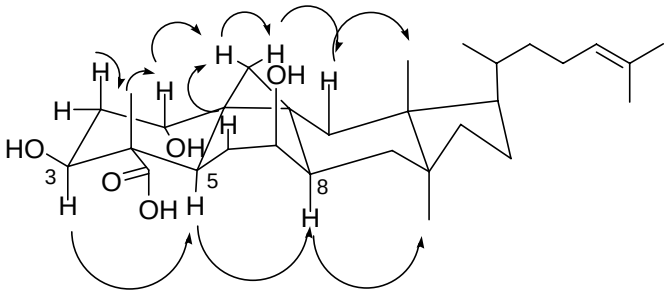


Figure 3.6 Key ROESY correlations of compound **1**

Table 3.1 $^{1}H^{a}$ and $^{13}C^{b}$ NMR data for compound **1**

	Compound 1	
Position	^{1}H NMR[c]	^{13}C NMR[c]
1	3.55[d]	73.5
2	1.05 m, 1.84[d]	37.7
3	4.52 dd (6.8, 10.4)	71.4
4		46.9
5	2.76 dd (4.0, 14.0)	37.5
6	1.91[d], 1.29[d]	29.7
7	3.56[d]	70.6
8	1.29[d]	25.3
9		26.1
10		29.2
11	1.39[d], 2.21[d]	27.2
12	1.15[d], 1.45[d]	33.9
13		56.0
14		30.9
15	1.41[d], 1.84[d]	37.9
16	1.54[d]	38.4
17	1.57[d]	53.0
18	1.61 s	17.8
19	0.45 d (4.4), 0.81 d (4.8)	22.0
20	1.40 m	37.2
21	0.92 d (6.4)	19.0
22	1.90[d], 2.05[d]	26.0
23	1.68[d]	34.0
24	5.10 t (12.0)	126.3
25		131.9
26	1.01 s	18.2
27	1.05 s	19.3
28		180.6
29	1.07 s	9.2
30	1.68 s	26.1

[a]δ (ppm) 400 MHz; s: singlet; br s: broad singlet; d doublet; t: triplet; m: multiplet; [b]δ (ppm) 100 MHz; [c] in methanol-d_4; [d] overlapped and unresolved signals, values obtained from HSQC and 1D TOCSY experiment.

3.2.3 Structure Elucidation of Compound 2

Compound **2** was also isolated as a yellowish amorphous solid. Positive ion HRFABMS gave a molecular ion peak at *m/z* 525.3591 [M+Na$^+$], confirming the molecular composition of $C_{31}H_{50}O_5$. The IR spectrum of **1** showed a broad band at 3655 cm^{-1} and a sharp band at 1550 cm^{-1} indicating presence of hydroxyl and carbonyl groups. The ^{1}H and ^{13}C NMR spectra indicated that this compound also belonged to the class of triterpenoids.

The ^{1}H spectrum of **2** in pyridine-d_5 indicated that the compound had three oxygenated protons and six methyl groups (Fig. **3.9**). A highly shielded doublet at δ_H 0.59 (d, J = 4.5 Hz, H-19) suggested the presence of a cyclopropyl ring.[19] The ^{13}C spectrum of compound **2** contained 31 signals: six methyls, nine methylenes, five methines, three oxygenated carbons, and eight quaternary carbon peaks.

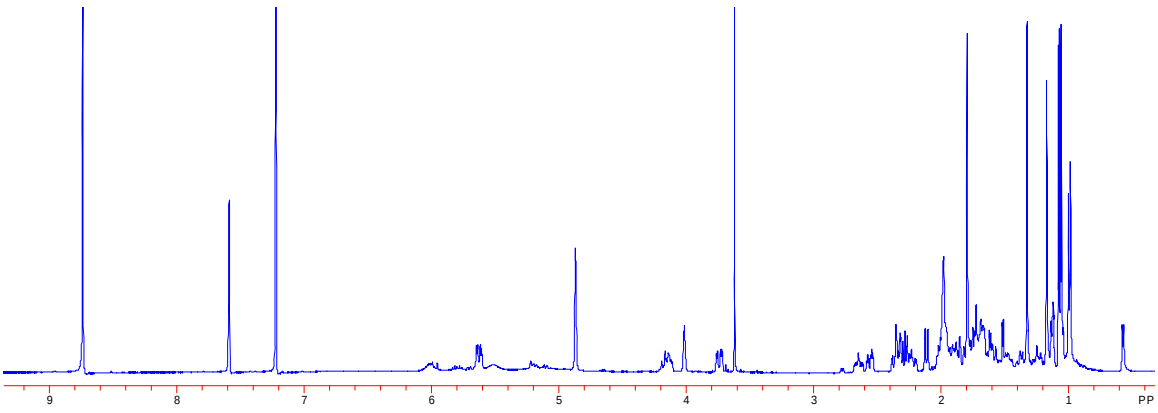


Figure 3.9 ^{1}H NMR spectra of compound **2** in pyridine-d_5

The ^{1}H and ^{13}C NMR signals in pyridine-d_5 showed typical signals for the cyclopropane methylene protons with peaks at δ_C /δ_H 28.3 (C-19)/0.59 (d, J = 4.5 Hz, H_β-19) and 1.14 (d, J = 4.8 Hz, H_α-19); two quaternary carbons at δ_C 21.4 (C-9) and δ_C 31.2 (C-10). Three oxygenated methine peaks were observed at δ_C /δ_H 73.0 (C-1)/4.02 (br, H_β-1); δ_C /δ_H 71.0 (C-3)/4.14 (br, H_α-3), δ_C /δ_H 70.0 (C-7)/5.63 (dd, J = 12.4 Hz, 4.5 Hz, H_β-7). The HSQC and HMBC correlations confirmed that compound **2** had a similar cycloartane-type skeleton as that of **1** (Fig. **3.10**).

HMBC correlations for the fusion of the A/B rings was confirmed by the correlation of H-29 (δ_H 1.80, s) to C-3 (δ_C 71.0), C-5 (δ_C 37.3), C-28 (δ_C 180.5); H-5 to C-4 (δ_C 55.8), C-3, C-6 (δ_C 34.7), C-7 (δ_C 70.0), and C-19 (δ_C 28.3).

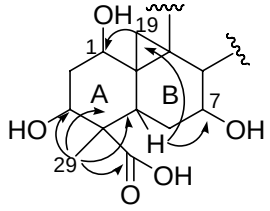
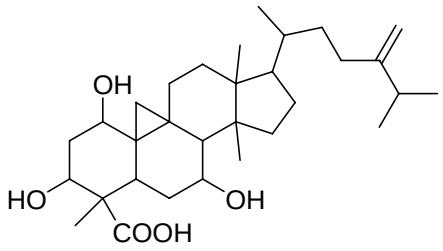


Figure 3.10 Key HMBC correlations of A/B ring fusion in fragment 1

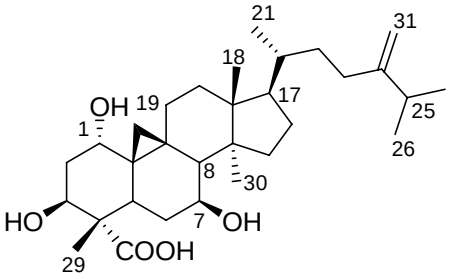
After connecting the fragments together from HMBC correlations, as discussed above, the flat structure of compound **2** was established as shown in Figure 3.15. The only difference between compound **1** and compound **2** was the position of the double bond in the alkyl side chain. The position of the double bond in **2** was confirmed from the HMBC correlations of H-25 (δ_H 2.28, m) to the two methyl doublets, C-26 (δ_C 22.6) and C-27 (δ_C 22.5).



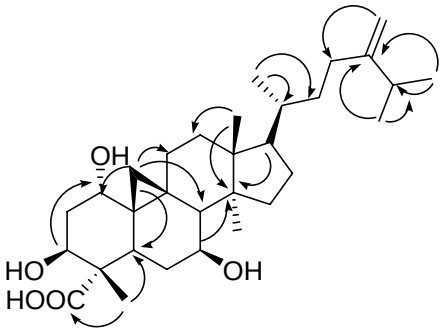
3.15 Flat structure of compound **2**

To determine the stereochemistry of compound **2**, 1D and 2D ROESY experiments were carried out and the carbon chemical shifts were compared to those in literature.[18] The *β*-orientation of H-3 (δ_H 5.63, dd) was confirmed from its coupling constants (J = 12.4 Hz, 4.5 Hz). A strong correlation of H-3 to H-5 (δ_H 3.74, dd, J = 14.0 Hz, 4.0 Hz); H-5 to H-7 (δ_H 4.14, br) and H-7 to H-8 (δ_H 2.12, br) showed *β*-orientation of these four protons. Further, the *trans* fusion of the C/D rings was established from the ROESY correlations of H-8 to H-18 (δ_H 1.18, s) and H-7 to H-30 (δ_H 1.33, s) (Fig. **3.11**)

From the ROESY data and its comparison to literature values, the structure of compound **2** was confirmed to be 7*β*-hydroxy-23-deoxojessic acid.[18] Compound **2** is a known compound; however, it has been isolated and identified from *Grewia* sp. for the first time.



2



HMBC correlations

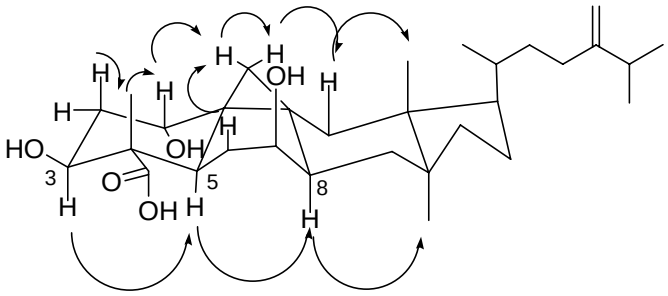


Figure 3.11 Final structure and key HMBC and ROESY correlations of compound **2**

Table 3.2 $^{1}H^{a}$ and $^{13}C^{b}$ NMR data for compound **2** and its comparison to literature values

	7β-hydroxy-23-deoxogesic acid		**Compound 2**	
Position	^{1}H NMR^{c}	^{13}C NMR^{c}	^{1}H NMR^{c}	^{13}C NMR^{c}
1	4.0 br	70.5	4.02^{d}	73.0
2	2.54 ddd (13.0, 4.5, 4.0), 2.56^{d} ddd (13.0, 8.0, 4.0)	38.8	2.35^{d}, 1.84^{d}	39.4
3	5.60 dd (12.0, 4.5)	70.5	5.63 dd (12.4, 4.5)	70.0
4		55.4		55.8
5	3.71 dd (12.5, 4.0)		3.74 dd (14.0, 4.0)	37.0
6		34.0	1.84^{d}, 2.35^{d}	34.7
7	4.12 ddd (11.0, 8.5, 4.0)	69.5	4.14 br	71.0
8	2.10 d (8.5)	54.9	2.12 d (7.6)	55.4
9		20.9		21.4
10		30.7		31.2
11		26.7	1.70^{d}, 2.65^{d}	27.2
12		33.3	1.75^{d}, 1.91^{d}	33.8
13		46.0		46.5
14		49.2		49.8
15		37.5	1.98^{d}, 1.98^{d}	38.0
16		28.8	1.38^{d} , 2.00^{d}	29.3
17		52.0	1.67^{d}	52.5
18	1.15 s	17.7	1.18 s	18.2
19	0.55 d (4.5), 1.11 d (4.5)	27.8	0.59 d (4.5), 1.14 d (4.8)	28.3
20		36.4	1.50 m	37.5
21	0.98 d (5.0)	18.7	0.92 d (6.4)	19.0
22		36.4	1.25^{d}, 1.71^{d}	35.9
23		31.6	2.24^{d}, 2.00^{d}	32.2
24		156.7		157.2
25		34.1	2.28^{d}	34.5
26	1.06 d (7.0)	22.0	1.10 d (2.4)	22.6
27	1.05 d (7.0	21.9	1.09 d (3.2)	22.5
28	1.77 s	9.7	1.80 s	10.2
29		179.9		180.5
30	1.31 s	19.0	1.33 s	19.5
31	4.85 br, 4.86 br	106.6	4.87 br, 4.87 br	107.1

^{a}δ (ppm) 400 MHz; s: singlet; br s: broad singlet; d doublet; m: multiplet; ^{b}δ (ppm) 100 MHz; ^{c} in pyridine-d_5; ^{d} overlapped and unresolved signals, values obtained from HSQC and 1D TOCSY experiment.

3.2.4 Antiproliferative activity of Compounds 1 and 2

The two compounds isolated from *Grewia* sp. were tested for their growth inhibition ability using the A2780 human ovarian cancer cell line. Compound **1** is a new compound and it exhibited strong growth inhibition. Since **1** is a new compound, no previous activity has been reported for it. Compound **2** is a known compound, but no biological activity has been previously published for it. The activity data for these two compounds is tabulated in Table **3.3**

Table 3.3. Antiproliferative activity of triterpenoids **1** and **2** against A2780 human ovarian cancer cell line

Compound	IC_{50} (μg/mL)	IC_{50} (μM)
1	2.4	4.9
2	2.6	5.2

3.3 Experimental section

3.3.1 General Experimental Procedures- The UV spectra were collected on UV-1210 and the IR spectra were measured on a MIDAC M-series FTIR spectrophotometers. 1D and 2D NMR spectra were obtained on a Varian Inova 400 spectrometer, and chemical shifts are given in ppm. Mass spectra were obtained on JEOL JMS-HX-110 instrument in the positive ion mode. HPLC was carried out using a Shimadzu LC-10AT with Analytical (5 μm, 250 × 10 mm) and preparative (8 μm, 250 × 10 mm) C_{18} Varian Dynamax columns coupled with a UV diode array detector.

3.3.2 Cytotoxicity Bioassays- The A2780 human ovarian cancer cell line antiproliferative assay was performed at Virginia Polytechnic Institute and State University as previously described.[20]

3.3.3 Plant Material- The dried leaves and flowers of *Grewia* sp. were ground and extracted with EtOH; the resulting extract was designated MG 3692 and 1.1 g was made available for this work.

3.3.4 Extraction and Isolation- 1.1 g of the dried plant material, MG 3692 was suspended in aqueous MeOH (MeOH:H_2O, 9:1, 100mL) and extracted with hexanes (3 × 100mL). The aqueous layer was then diluted to 50% MeOH (v/v) and extracted with CH_2Cl_2 (3 × 180mL). The aqueous CH_2Cl_2 fraction displayed the highest cytotoxicity (IC_{50} = 2.2 μg/mL). Hence this fraction was selected for further isolation. It was chromatographed using C_{18} SPE column eluting with 20% MeOH:H_2O to 100% MeOH which yielded five fractions. Only one fraction, IV, was active at IC_{50} = 1.6 μg/mL. Fraction IV was loaded on a C_{18} Varian Dynamax column [8 μm, 250 × 10 mm, 1.8 mL/min, gradient elution with 50% MeOH:H_2O to 100% MeOH for 65 min and thirty-four subfractions were collected. Two pure subfractions were collected and one new compound, compound (**1**) (8.0 mg, t_R 49 min) and one known compound (**2**) (20.5 mg, t_R 50 min) were isolated from this fraction.

Compound 1: yellow amorphous solid; UV (MeOH) λ_{max} (log ε) 210 nm; IR ν_{max} 3649, 2591, 1962, 1542, 785 cm^{-1}; ^1H NMR (400 MHz, methanol-d_4):H-1: 3.55, H-3: 4.52 (dd, J = 6.8, 10.4 Hz) , H-5: 2.76 (dd, J = 4.0, 14.0 Hz), H-7: 3.56, H-8: 1.29, H-17: 1.57, H_3-18: 1.61, H_2-19: 0.45 (d, J = 4.4 Hz)/0.81 (d, J = 4.8 Hz), H-20: 1.40, H_3-21: 0.92(d, J = 6.4 Hz),

H-24: 5.10 (t, J = 12.0 Hz), H_3-26: 1.01 (s), H_3-27: 1.05 (s), H_3-29: 1.07 (s), H_3-30: 1.68 (s). ^{13}C NMR (100 MHz C_5D_5N) see Table **3.1**; LRFABMS *m/z* 489.9 $[M+H]^+$ (calcd. for $C_{30}H_{49}O_5^+$, 489.69)

Compound 2: yellow amorphous solid; UV (MeOH) λ_{max} (log ε) 210 nm; IR ν_{max} 3655, 2327, 1550, 765 cm^{-1}; ^1H NMR and ^{13}C NMR (100 MHz, CD_3OD), see Table **3.2**; HRFABMS *m/z* $[M+Na]^+$ 525.3591 (calcd. for $C_{31}H_{50}O_5Na^+$, 525.3556)

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