

Mass Fragmentation Pattern of New Zygophyllosides from *Zygophyllum propinquum* Decne

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Abstract: Four new saponins were isolated from the whole plant of *Zygophyllum propinquum* Decne and purified by column chromatography and reverse phase high performance liquid chromatography (HPLC) and analyzed by fast atom bombardment mass spectrometry (FAB-MS). The molecular weight information about the structure architecture of saponins were obtained from characteristic peaks $[M-H]^+$ and $[M-H-SO_3Na]^-$. The new pentacyclic triterpenoidal saponins of ursane series were named Zygophylloside T (1), Zygophylloside U (2), Zygophylloside V (3) and Zygophylloside W (4). The fragmentation pattern could provide information on the linkage position of sugar in aglycone and number of sulphate groups.

Key words: *Zygophyllum propinquum* Decne • Saponins • Zygophyllosides • Mass Fragmentation

INTRODUCTION

Saponins form stable foam in water and occur in wide variety of plants and to a lesser extent in marine animals. There are 90 families and 472 plant species containing saponins [1, 2]. Some saponins have properties of hemolysis, bitterness, complex formation with cholesterol and fish poisoning [3-6]. Saponins consist of sugar moieties linked to aglycone, which are classified into triterpenoidal, steroidal and steroidal alkaloid types.

Saponins are high molecular weight glycosides consisting of a sugar moiety linked to a triterpenoid or steroid aglycone. The aglycone or non-saccharide portion of saponin is called the genin or sapogenin. All the saponins have in common attachment of one or more sugar chain to the aglycone. Mono-desmosidic saponins have sugar chain normally attached at C-3 position while bidesmosidic saponins have two sugar chains, one attached through an ether linkage at C-3 and one attached through an ester linkage at C-28 (triterpenoidal saponin) or an ether linkage at C-26 (furostanyl saponins). Tridesmosidic saponins have three sugar chains and are seldom formed.

Saponins are extremely widely distributed in plant kingdom. The saponin constitutes an important group of natural products of wide spectra of biological activity.

Zygophyllum propinquum Decne (Syn. *Zygophyllum coccinium* L.) belong to family Zygophyllaceae [7]. It is found in Sindh and Baluchistan provinces of Pakistan. It is known to cause lowering of blood sugar, used as diuretic, antipyretic and local anesthetic [8].

The structures of the saponins isolated from plants and marine animals have been investigated [9] and researchers have applied several analytical methods. Although NMR methods have successfully been used, the utility of NMR is limited due to the amount of material required and solubility of the sample. For the determination of molecular weight several other mass spectrometric methods have been used. Mass Spectrometry (MS) has been a major analytical tool due to its convenience and high sensitivity in the structure analysis. FAB (Fast Atom Bombardment) positive and negative is used in the structural determination of triterpenoid saponin from *Zygophyllum propinquum*. In FAB studies, an accelerated beam of atoms (or ions) is fired from a gun towards a target which has been

preloaded with viscous liquid (matrix – usually glycerol or thio-glycerol) containing the sample to be analyzed. When the atom beam collides with the matrix, kinetic energy is transferred to surface molecules, a large number of which are sputtered out of the liquid into a high vacuum of ion source. Ionization of these molecules can be recorded by an appropriate choice of instrumental parameters but negative ions have been proved to be more useful on the whole for saponin work [10].

MATERIALS AND METHODS

Plant Material: *Zygophyllum propinquum* Decne was collected from Baluchistan province of Pakistan. The plant was identified by plant taxonomist and a voucher specimen was deposited in the herbarium of Department of Botany, University of Karachi Pakistan. The whole plant material was kept for drying in *shade* for two weeks at ambient temperature. The dried plant material was pulverized to fine powder.

Extraction and Isolation: The powder plant material (20 kg) was extracted with 100 liters of commercial grade methanol. The methanolic extract was concentrated under vacuum afforded a gummy residue (800 g). The residue was partitioned between ethyl acetate and water. The aqueous extract was further extracted with butanol. The butanolic extract was subjected to column chromatography using silica gel as adsorbent and mobile phase of chloroform: methanol (4:1). The fraction obtained were further subjected to column chromatography on Rp-18 column and purified on PTLC using mobile phase of butanol acetic acid and water (12:3:7) using iodine as the locating reagent. The partially purified saponins were purified on HPLC using RP-18 column and mobile phase of water: methanol (30:70).

Instrumentation: The FAB negative spectra were recorded with a HX 110 Mass Spectrometer (JEOL Tokyo Japan). The ion source was operated at 10KV accelerating voltage with a mass resolution of 1000 (10 %) valley. Xenon was used as carrier gas and glycerol as matrix. Sample were dissolved in methanol and mixed with 1 il of glycerol.

RESULTS AND DISCUSSION

FAB mass spectrometry is a more reliable technique used for the structure elucidation of saponins. It provides information about the molecular weight and number of

Table 1: The FAB negative ion fragments of new saponins isolated from *Zygophyllum propinquum* Decne

Compound	Fragments	m/z
Zygophylloside T	[M-H] ⁻	1035
	[M-H-Glc] ⁻	873
	[M-H-Glc-Glc] ⁻	711
	[M-H-Glc-Glc-CO ₂] ⁻	665
	[M-H-Glc-Glc-Glc.A-CO ₂] ⁻	456
Zygophylloside U	[M-H] ⁻	909
	[M-H-GlcA] ⁻	732
	[M-H-GlcA-GlcA-SO ₃ -Na] ⁻	455
Zygophylloside V	[M-H] ⁻	961
	[M-H-SO ₃ -Na] ⁻	858
	[M-H-SO ₃ -Na-CO-H ₂ O] ⁻	650
	[M-H-SO ₃ -Na-Glc-Glc-SO ₃] ⁻	456
Zygophylloside W	[M-H] ⁻	975
	[M-H-SO ₃ -Na] ⁻	873
	[M-H-SO ₃ -Na-Glc] ⁻	711
	[M-H-SO ₃ -Na-Glc-SO ₃] ⁻	665

sugar in aglycone. The saponins are characterizes by the formation of peak [M-H]⁻-preponderantly but can also be formed by the addition of an anion that is [M+ anion]. In case of positive FAB spectrometry are characterized by [M+H]⁺ and sodium adduct molecule [M+ Na]⁺. Complementary nature of information can be obtained from positive and negative mode of FAB, however negative ion FAB have been proved to be more useful for saponins work. Typical FAB negative spectra and its fragmentation pattern of the four new sulphated saponins are given in Figs. (1-4).

Fragmentation pattern of Zygophylloside T (1): Zygophylloside T gave a molecular ion peak at m/z 1035 [M-H]⁻ and other diagnostic peaks at m/z 873, 711 and 456 in the FAB negative mode. Prominent peak at m/z 873 [M-Glc]⁻ and m/z 711 [M-Glc-Glc]⁻ have been observed due to loss of hexose in the negative FAB-MS (Table 1). The peak at m/z 711 is a characteristic diagnostic peak revealed the presence of hexose-SO₃H intact with the saponin. It has also indicated that the saponin is attached with three sugars containing only one SO₃ group [11]. The peak at m/z 456 showed the aglycone part of the saponin.

Fragmentation pattern of Zygophylloside U (2): Zygophylloside U gave molecular ion peak at m/z 909 [M-H]⁻, 733 [M-Glc A]⁻ and 456 [M-H-Glc A-Glc A-SO₃H]⁻ are the main diagnostic peaks in the negative FABMS Spectrum. The presence of peak at m/z 733 [M-Glc A]⁻

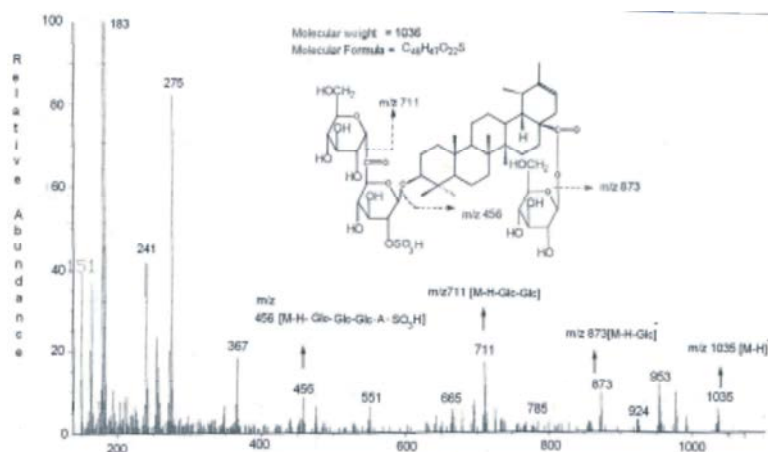


Fig. 1: Negative ion FAB mass spectra of isolated Zygophylloside T (1) and its Fragmentation Pattern

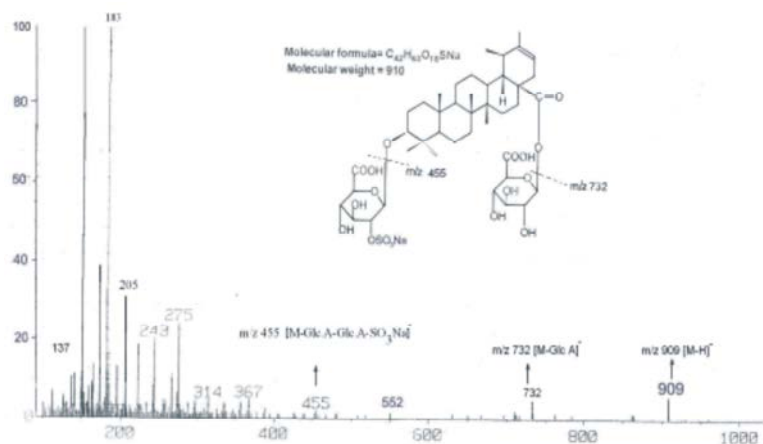


Fig. 2: Negative ion FAB mass spectra of isolated Zygophylloside U (2) and its Fragmentation Pattern

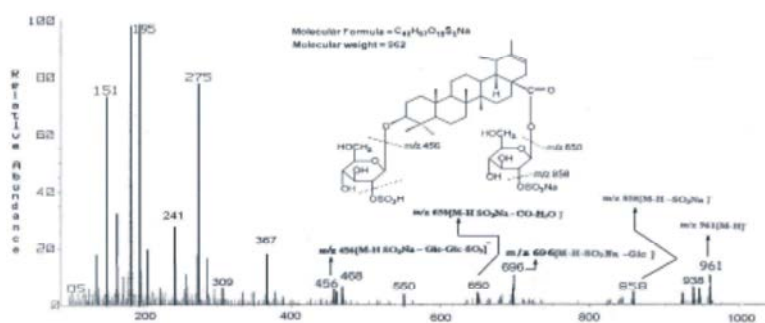


Fig. 3: Negative ion FAB mass spectra of isolated Zygophylloside V (3) and its Fragmentation Pattern

revealed the presence of one hexose and SO_3Na in the intact saponins thereby confirming that the saponins contains two sugars and one SO_3Na . The peak at m/z 456 is the triterpene portion formed by the loss of hexose and SO_3Na . The exact position and location of SO_3Na was determined by single and 2D NMR spectroscopy [12].

Zygophylloside V (3): Zygophylloside V gave a molecular ion peak at m/z 961 [M-H]⁻, 858 [M-H- SO_3Na]⁻, 696 [M-H- SO_3Na Glc]⁻, 650 [M-H- SO_3Na Glc-CO-H₂O]⁻ and 456 [M-H- SO_3Na Glc-Glc- SO_3]⁻. The fragmentation pattern provide conclusive information about the number of sulphate and sugars attached in the saponin. The peak at m/z 858 [M-103]⁻ clearly demonstrate the loss of [Na+HSO₃]⁻ ion from

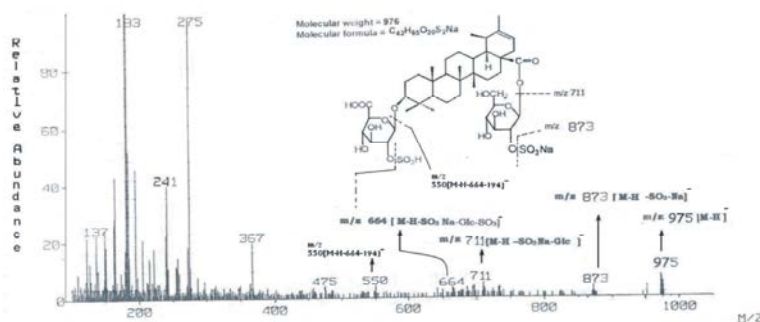


Fig. 4: Negative ion FAB mass spectra of isolated Zygophylloside W(4) and its Fragmentation Pattern

the saponin. Elimination of [M-H-SO₃Na Glc]-at m/z 696 provide information that the saponin contain another sulphate and glucose unit with the intact saponin. Decarboxylation and dehydration [CO-H₂O]-further produce peak at m/z 650 [M-H-SO₃Na Glc-48]⁻.

Zygophylloside W (4): Zygophylloside W showed a molecular ion peak at m/z 975 [M-H]⁻, 873 [M-H-SO₃Na]⁻, 711 [M-H-SO₃Na-Glc]⁻, 664 [M-H-SO₃Na-Glc-SO₃]⁻ and 550 [M-H-SO₃Na-Glc-SO₃-GlcA]⁻. The fragmentation pattern of Zygophylloside V and Zygophylloside W have similar fragmentation pattern. Peak at m/z 873 was observed due to loss of [M-H-SO₃Na]-in case of Zygophylloside W while loss of same fragment was observed at 858 in case of Zygophylloside V. Peak at m/z 711 [M-H-SO₃Na-Glc]-is characteristic diagnostic peak of sulphated saponin. This peak was not observed in Zygophylloside V and it rather appeared at m/z 696 [M-H-SO₃Na Glc]⁻. Typical peak at m/z 550 showed the presence of glucuronic acid attached with the saponin [13].

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