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Research Article

A new steroid from chloroform extract of *Tamarix indica*

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Abstract

The chloroform soluble fraction of the *Tamarix indica* was used for the first time to purify a new steroid compound as well as four other known compounds. Modern sophisticated techniques such as ^{1}H-NMR, ^{13}C-NMR, EIMS, HREIMS, one dimensional and two-dimensional spectroscopic techniques were used to characterized for structure elucidation of the isolated molecules. The known compounds were recognized as Lupeol **(2)** Stigmasterol **(3)**, Ursolic acid **(4)**, Oleanolic acid **(5)**.

Keywords:
Tamarix indica, Steriods, Terpenoids, ^{1}D NMR and MS techniques, Computational analysis.

1. Introduction

Genus *Tamarix* is the most typical genus of family *Tamaris-caceae* , *Tamarix* is a genus bearing flowering plants of different have about 50-60 species. Mostly species of this genra are present in Africa, Asia and Europe. Height of these plants is maximum eighteen meter and have evergreen broad leaves. Plants with thin branches and grayish green leaves. The genus *Tamarix* have number of species that´s why the family was named *Tamariscaceae*. Seventy-nine type of flowering plants are present in *Tamaris-caceae* (*Tamariscinae*) family, grouped into five genera, which are generally herbs, shrubs and small trees. Phytochemical studies of this genus reveled the presence of following compounds such as sugars, proanthocyanidins alkaloids, quercetin, ellagic acid, flavonols, cyanids, terpenoids, steriods polyphenols, tannin as well as, non-steroids amines and amides, kaempferol accountable for the wide range of medicinal properties.

The speice Tamarix indica is grown in the dry areas of Asia, it is small evergreen. It has hermaphrodite flowers, oblong and rounded petals, pink and white flowers appear as compact-masses at tip of branch alternatively from March to September. It is present mainly in latitude to high altitude especially in Chita hills of Northern areas Indus River Belt, Attock, and Kala in Pakistan. Tamarix indica. has been shown a list of pharmacological activities The methanolicextracts of leafs, barks and also the other major parts of plants revealed cytotoxic, antidiarrheal and antinociceptive activities. Furthermore, the aqueous extracts of bark of this plant were used for aphrodisiacs activities [1–5].

Almost all steroid molecules share a basic structure known as the perhydro-cyclopentanophenanthrene ring system. This consists of four fused rings: three cyclohexane rings (designated as rings A, B, and C) and one cyclopentane ring (designated as ring D), which are arranged in a specific orientation.Despite their structural differences, all steroid molecules share certain properties, such as the ability to bind to specific receptors in the body and influence gene expression [6]. In addition to these established uses, research is ongoing to discover new potential applications for steroids. For example, there is interest in exploring the use of certain steroids as neuroprotective agents in conditions such as Alzheimer's disease, and as anti-aging agents due to their ability to modulate hormone levels and cellular processes. Steroids are also being investigated as potential lead compounds in drug discovery for a wide range of therapeutic areas, including cancer, inflammation, and metabolic disorders [7].

The present work describes the isolation of a new steroid compound and four known compounds from Tamarix indica. The known compound name as Lupeol (2) [8], Stigmasterol (3) [9], Ursolic acid (4) [10], Oleanolic acid (5) [11].

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2. Materials and Methods

2.1. General

Silica gel, a commonly used adsorbent material, was used in both thin layer chromatography and column chromatography. Pre-coated preparative plates with silica gel were used to separate and isolate the components of the mixture being studied in TLC. The melting points of the substances were assessed using a Buchi melting point apparatus, which utilizes glass capillaries to contain the sample. Optical rotations, which are a measure of how the sample interacts with polarized light, were measured using a digital polarimeter. Ultraviolet spectra were recorded with the help of Hitachi U-3200 spectrophotometer, while infrared spectra were obtained using an FTIR spectrophotometer. These instruments are used to determine the chemical composition of the substances by analyzing how they interact with different types of electromagnetic radiation.

The ^{1}H-NMR spectra were obtained using TMS (tetramethylsilane) as a reference in $CDCl_3$ (deuterated chloroform), while ^{13}C-NMR spectra were obtained at specific frequencies in $CDCl_3$. Nuclear Magnetic Resonance (NMR) spectroscopy is used for the identification of individual atoms in the substance being studied.

To obtain precise mass measurements, mass spectrometry was used, with glycerol and thioglycerol as matrices. Cesium iodide (CsI) was used as an internal standard. Mass spectrometry is a technique that allows for the determination of mass of individual atoms or molecules in a sample. Ceric Sulphate reagent was used to visualize the spots on TLC plates through spraying. This reagent consists of Ceric sulphate, trichloroacetic acid, and conc. H_2SO_4, and reacts with the substances on TLC plates to produce visible spots. Finally, all chemicals and enzymes used in the determination of enzyme inhibition activities, including antimicrobial, lipoxygenase, and Brine shrimp tests, were sourced from Sigma, a well-known supplier of laboratory chemicals and equipment. These tests were likely performed to determine the efficacy of various substances as inhibitors of certain enzymes or as antimicrobial agents.

2.1.1. Plant Material

Tamarix indica was collected in May from Attock and recognized by Botany Department, University of Karachi where voucher specimen (H.No : 68370) have been kept in herbarium center.

2.1.2. Isolation

6 Kg whole dried powder form plant was used, it refluxed with ethanol (95 %). The crude extract (0.5 Kg) was obtained by evaporating under reduced pressure, separated between *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and water soluble fractions. The chloroform soluble part (150g) were recolumn chromatographed and eluting with *n*-hexane- chloroform and chloroform-methanol, 11 major fractions obtained labeled as T^1-T^{11} (T=*Tamarix*).

The fraction T^5 (*n*-hexane-chloroform, 5:5) revealed many spots on silica gel TLC after re-chromatographed over PTLC using n-hexane-acetone (8.0:2.0) the solvent system gave compound **5** (8 mg). The fraction T^7 (n-hexane-chloroform, 4:6) exhibited five spots on TLC, re-chromatographed over silica gel column chromatography, eluting with *n*-hexane-ethyl acetate (6.0-3.0) solvent system afforded compound **1** (28 mg) and 4(20mg). The fraction T^{10} (*n*-hexane-chloroform, 2:8) showed six major spots on TLC were rechromatographed and finally Preparative silica gel TLC system *n*-hexane-acetone (5.5 : 4.5) to give compound **2** (13 mg) and compound **3** (18 mg).

*2.1.3. (3β, 22E)-stigmasta-7, 9(11), 22-trien-3-yl dodecanoate (new compound) **(1)***

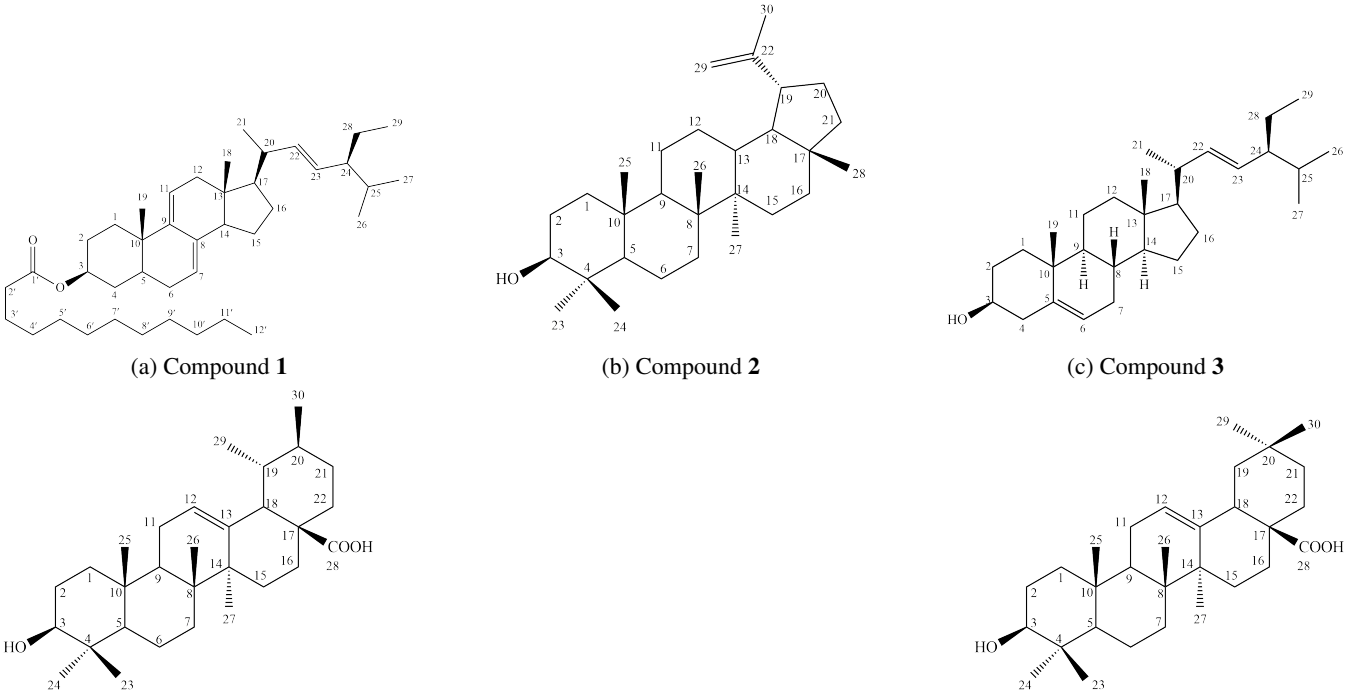
The compound is a colorless, amorphous solid with a specific rotation of -23.0 (c = 1.0, MeOH) at 22°C. Its FT-IR spectrum shows characteristic peaks at 1715 cm^{-1} (carbonyl moiety), 1650-1600 cm^{-1} (olefin group), and 1227 cm^{-1} (C−O). The ^{1}H-NMR spectrum (recorded at 500 MHz in $CDCl_3$) exhibits signals at δ 1.21-1.25 (m, 18H, H-3´-11´), 2.34 (t, 2H, J = 7.5 Hz, H-2´), 0.85 (t, 3H, J = 6.5 Hz, H-12´), 4.24 (dt, 1H, J = 10.8, 8.9 Hz, H-3), 5.35 (t, 1H, J = 7.8 Hz, H-11), 5.13 (dd, 1H, J = 15.2, 7.6 Hz, H-22), 5.03 (dd, 1H, J = 15.1, 7.3 Hz, H-23), 0.98 (s, 3H, Me-19), 6.04 (t, 1H, J = 7.3 Hz, H-7), 0.5 (s, 3H, Me-18), 0.82 (d, 3H, J= 6.4 Hz, Me-21), 0.81 (d, 3H, J = 6.2 Hz, Me-27), 0.52 (t, 3H, J = 7.2 Hz, Me-29), 0.86 (d, 3H, J = 6.2 Hz, Me-26)

The ^{13}C-NMR spectrum (recorded at 125 MHz in $CDCl_3$) shows signals at δ 174.7 (C-1´), 21.1 (C-2´), 28.1-29.2 (C-3´-C-11´), 54.3 (C-12´), 34.5 (C-1), 27.1 (C-2), 72.9 (C-3), 33.1 (C-4), 39.2 (C-5), 29.5 (C-6), 117.5 (C-7), 136.3 (C-8), 144.4 (C-9), 32.5 (C-10), 118.2 (C-11), 41.8 (C-12), 40.4 (C-13), 50.7 (C-14), 23.2 (C-15), 27.7 (C-16), 54.2 (C-17), 11.6 (C-18), 19.1 (C-19), 37.2 (C-20), 18.4 (C-21), 33.4 (C-22), 26.3 (C-23), 45.6 (C-24), 28.7 (C-25), 18.2 (C-26), 19.7 (C-27), 23.1 (C-28), and 12.2 (C-29)

3. Results and Discussion

By subjecting the chloroform-soluble fraction of the *T. indica* plant to column chromatography, a total of five compounds were separated and identified, including four previously known ones and a newly discovered compound. It (1) was purified colorless amorphous solid compound from chloroform soluble extract of plant. **Salkowski and Lieberman Burchard** reaction showed steroidal nature of compound. In HREIMS data molecular ion peak appeared at m/z 592.5201, determined molecular formula (Calculated for $C_{41}H_{68}O_2$, 592.5254) and exhibited eight degrees of unsaturation in compound.

The IR (ν_{max} cm^{-1}) spectral data displayed peaks at 1715 cm^{-1}, 1227 cm^{-1} and 1650 cm^{-1} indicated carbonyl carbon, ester linkage and olefinic moiety respectively. EIMS data exhibited a major fragment peak at m/z 452 ($C_{31}H_{49}O_2$) after the loss of side chain, peak at m/z 140 ($C_{10}H_{19}$) is distinctive feature of steroid molecules. Another major peak appeared at m/z 548 [M−CO_2] showed presence of two oxygen atoms in molecule. Presence of two olefinic moieties in steroid ring B and C, C=O. is indicated by Peak appeared at m/z 411 ($C_{28}H_{44}O_2$) after fission of D ring.



(a) Compound **1** (b) Compound **2** (c) Compound **3**

(d) Compound **4** (e) Compound **5**

Figure 1: Structures of compounds **1-5**

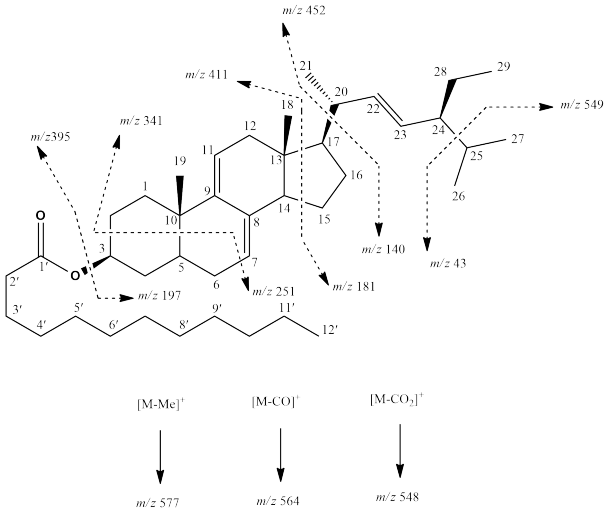


Figure 2: Important mass fragmentation of compound **1**

The presence of a steroid was indicated in the ^{1}H-NMR spectrum by downfield signals at δ 4.24 (H-3), δ 6.04 (H-7), and 5.35 (H-11), which revealed an oxymethine functional group connected to four neighboring proton atoms, a tri-substituted C=C bond, and Δ7,9(11) unsaturation, respectively. Signals at δ 5.13 and 5.03 showed a 1,2-disubstituted olefinic unsaturated bond. Upfield signals at δ 0.98 and 0.51 were due to an angular methyl group and a steroidal methyl group at secondary carbon atoms, respectively. Doublets appeared at δ 0.82, 0.86, and 0.81, indicating a steroidal methyl group. A triplet signal at δ 0.85 showed a dodecanoyl moiety, and a multiplet signal between δ 1.21-1.25 indicated nine methyl groups. A signal at δ 2.34 indicated the presence of a methylene along with a carbonyl carbon. In the ^{13}C-NMR spectrum, the presence of an ester linkage (C=O) was indicated by a signal at δ174.7. The compound structure was further interpreted using proton-proton COSY and HMBC correlation.

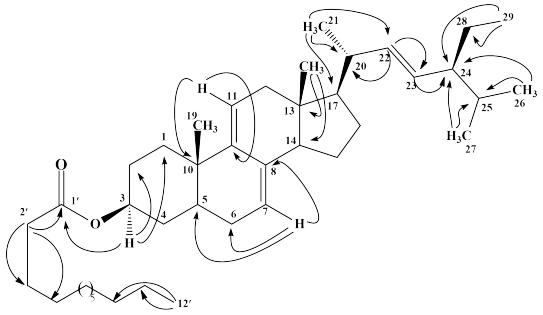


Figure 3: Important HMBC correlation in compound **1**

The HMBC data showed a signal at δ 4.61, indicating the oxymethine proton of C-3, which is a carbon atom with a hydroxyl (-OH) group attached to it. The J2 correlation observed with C-2(δ 27.1) and C-4(δ 33.3) indicates that these carbon atoms are adjacent to C-3, while the J3 correlations with C-1' signal at δ 170.1, C-1, at δ 34.4 and C-5 at δ 39.3 indicate that they are also connected to other parts of the molecule. The β and equatorial configuration of the acetate group is also noted, which is a typical feature of steroids. Olefinic proton at C-7, which is a carbon atom with a double bond (=) attached to it.

The J2 correlation with C-9 at δ 144.5 and C-12 at δ 41.9 indicates that these carbon atoms are adjacent to C-7, while the J3 correlations with C-8 at δ 136.2, C-10 at δ 32.6, and C-13 at δ 40.5 indicate that they are also connected to other parts of the molecule. The equatorial 3β configuration of the molecule is also noted, which indicates the orientation of the functional groups attached to the carbon atoms. All these correlations was confirmed position of the olefin moieties at C-7 and C-9 in the molecule and equatorial 3β configuration

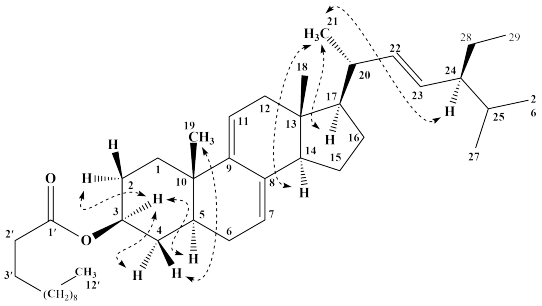


Figure 4: Important NOESY correlation in compound **1**

The compound 1 produced a methyl dodecanoate and a free alcohol when subjected to basic hydrolysis. In HR EI-MS the alcoholic moiety showed peak at m/z 410.5010 for molecular formula $C_{29}H_{48}O$ (Calcd. 410.3548), showing m.p 160 - 161° C, was identified as stigmasta-7, 9 (11), 22- triene-3-ol [12, 13]. The indication of methyl dodecanoate moiety in compound 1 was determined by following comparison those data reported in the literature, by relative TLC comparison of compound with an authentic liquid sample, by boiling point which showed at 260-2614°C and by ^{1}H-NMR and IR spectral data [14]. The compound 1 was identified as (3β, 22E)-stigmasta-7, 9 (11), 22-trien-3-yl dodecanoate in all of these investigations

4. Conclusion

Comparison of spectral and physical data of known compounds (Figure 1) with literature these compound were recognized as Lupeol **(2)** Stigmasterol **(3)** Ursolic acid **(4)** , Oleanolic acid **(5)**

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