

Full Paper

Spirowallichione: A Rearranged Multiflorane from *Euphorbia wallichii* Hook F. (Euphorbiaceae)

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Abstract: *Euphorbia wallichii* of the family Euphorbiaceae yielded a new rearranged pentacyclic triterpene of the multiflorane class which we have named spirowallichione. The structure of this natural spirocompound was elucidated with the aid of modern spectroscopic techniques, including 2D-NMR.

Keywords: *Euphorbia wallichii*; Euphorbiaceae; rearranged multiflorane; spirowallichione; characterization

Introduction

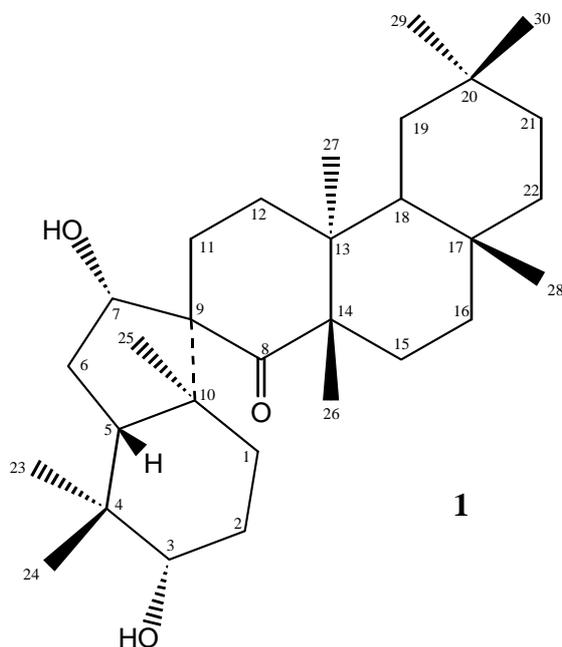
The Euphorbiaceae constitute one of the largest families of higher plants, comprising about 300 genera and 7,500 species. The genus *Euphorbia* is one of the six largest genera of flowering plants, having approximately 2,160 species. The Euphorbias are supposedly named after Euphorbus, a Greek physician to Juba II, the Romanized king of Numidia, who is said to have used the plant's milky latex as an ingredient in his medicinal preparations. Some of the plants belonging to the genus *Euphorbia* have been used in folk medicine for hundreds of years. They have been used for the treatment of cancers, tumors, migraine, skin diseases, gonorrhea, intestinal parasites and warts [1-2]. *E.*

antisiphilitica is a popular herbal remedy in India, where it is used for the treatment of liver ailments [3]. *E. prostrata* is also applied in Indian folk medicines as an anti-inflammatory and blood purifier [4]. The extract of *E. fischeriana* is used in the manufacture of an ointment for psoriasis (a chronic skin disease in which red scaly pustules and patches appear) [5]. Latex of *E. lateriflora* is used as a treatment for ringworm and in dilute aqueous solution, as a purgative [6]. It is also considered as a remedy for enlargement of the liver and spleen [7]. Roots of *E. wallichii* have been traditionally used in Tibetan folk medicines for the treatment of edema and skin disease such as furuncle, exanthema and cutaneous anthrax [8].

Members of *Euphorbia* are rich in phenolics [9], aromatic esters [9], steroids [10], diterpenoids [11-16], tetracyclic triterpenoids [10], pentacyclic triterpenoids [9,11], essential oils [17] and several bioactive constituents [18-19]. A chemical literature survey of *E. wallichii*, an endemic plant of Pakistan, revealed mentions of the presence of abietane [20] and rearranged diterpenoids [21]. This prompted us to investigate *Euphorbia wallichii* for its chemical constituents. We report herein the identification of a new rearranged triterpenoid of the multiflorane class isolated from this source.

Results and Discussion

Compound **1**, named spirowallichione, was obtained as a white powder (mp: 210-212°C) by elution of a silica gel column loaded with methanol soluble part of *E. wallichii* with 10% ethyl acetate in hexane.



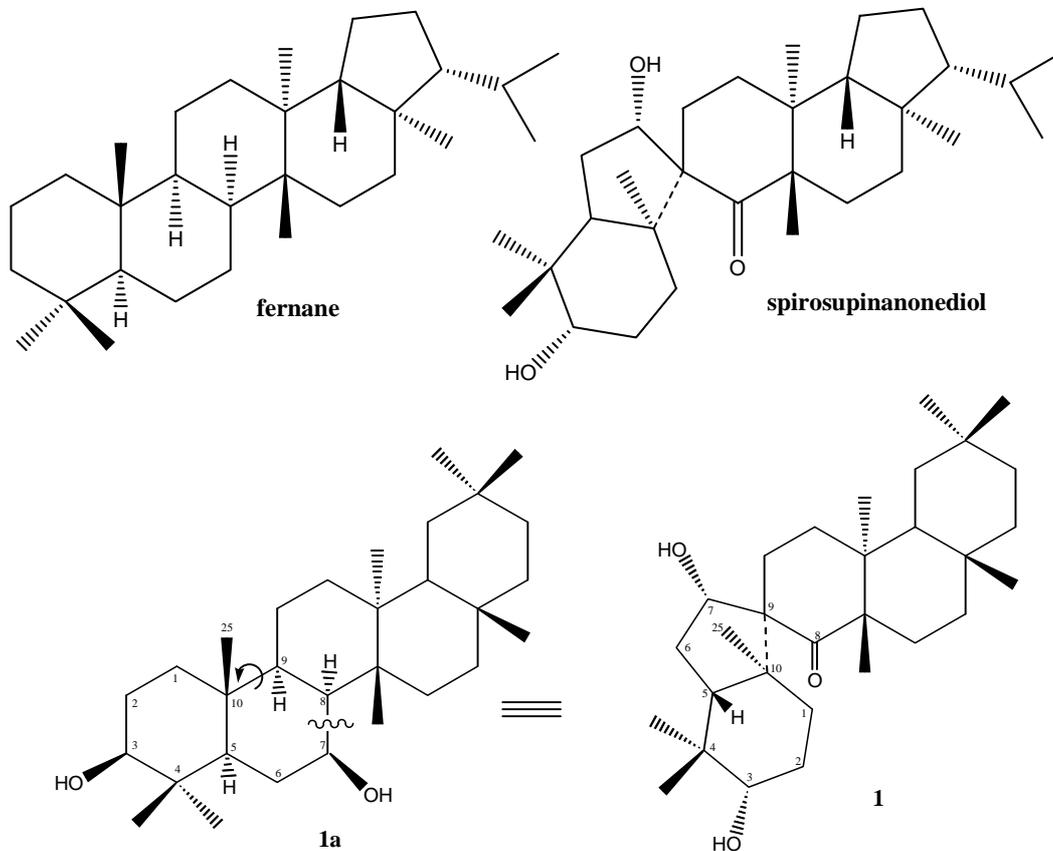
Its infrared spectrum displayed two prominent absorptions at 3416 and 1641 cm^{-1} , attributed to the presence of hydroxyl and ketone functionalities, respectively. The molecular mass of **1** was determined from the peak at m/z 458 obtained by Field Desorption Mass Spectrometry and the formula of corresponding peak was confirmed as $\text{C}_{30}\text{H}_{50}\text{O}_3$ via a High Resolution Electron Impact Mass Spectrum. The proton NMR spectrum of **1** exhibited eight methyl singlets between δ 0.86-1.22, suggesting its triterpenic nature. In addition to these methyl singlets, the spectrum showed two significant doublet of

doublets signals at δ 3.47 ($J = 9.8, 6.1$ Hz) and 4.20 ($J = 7.1, 7.1$ Hz), attributable to the H-3 and H-7 carbonylic protons, respectively. The remaining signals are described in the Experimental section.

The ^{13}C -NMR (broad-band) spectrum of **1** displayed altogether 29 carbon signals, while the formula obtained from HR-EIMS indicated a C_{30} molecule. DEPT experiments resolved these signals into eight methyls, ten methylenes, four methines and the remaining eight signals as quaternary carbons. A signal at δ 21.8 in the broad-band spectrum was resolved into two signals as a methyl and a methylene in the DEPT experiments. Thus, the triterpenic nature was reconfirmed. The carbonylic carbons resonated at δ 79.3 (C-3) and 77.9 (C-7). A downfield signal at δ 220.1 was due to the ketone function in the molecule. The detailed ^{13}C -NMR data are given in the Experimental section.

When the NMR data of **1** were compared with that of the various classes of pentacyclic triterpenoids, it was observed that compound **1** is very close to the multiflorane class **1a**. During the comparison of NMR spectral data, it was noticed that some of the NMR signals due to rings A, B and C in a reported rearranged natural triterpenoid of the fernane class obtained from *E. supina* named spiro-supinanonediol are comparable with the data of **1** [22]. Thus, it was concluded that **1** is a rearranged-multiflorane. This rearrangement can be explained as shown in Figure 1, where bond breaking between C-7 and C-8 and bond rotation about C-9 and C-10 can account for the structure of spirocompound **1**.

Figure 1. Fernane/spiro-supinanonediol relationship and possible multiflorane (**1a**) to spiro-wallichione (**1**) rearrangement.



The structure of the rearranged part (rings-A to C) of the molecule in **1** was deduced from the HMBC connectivities (see Figure 2) and cross-checked *via* a COSY experiment (see Figure 3). The stereochemistry of the hydroxyl functions in the molecule was determined as α at both C-7 and C-3. In the case of the C-7 hydroxyl, this fact was established by NOESY experiment (Figure 3), while at C-3 the magnitude of coupling constants was the determining factor for this conclusion. In nature, most of pentacyclic triterpenoids having a hydroxyl function at C-3 are equatorial with a β orientation. In compound **1**, the magnitude of coupling constant ($J = 9.8, 6.1$ Hz) due to the H-3 carbinylic proton suggested an equatorial orientation of the hydroxyl but facing towards the α -face due to the fact that ring-B has twisted after bond rotation along C-9 and C-10 (fig. 1).

Figure 2. Important HMBC connectivities in **1**.

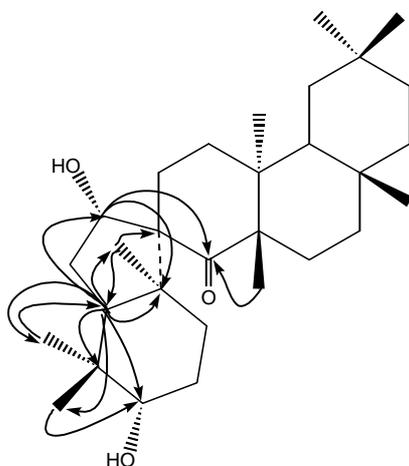
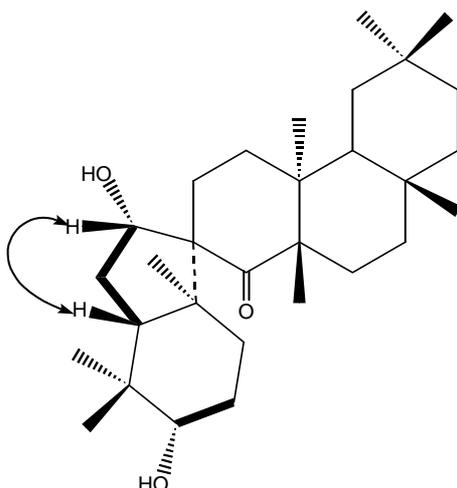


Figure 3. Important COSY (—) and NOESY (↔) connectivities.



To the best of our knowledge spirowallichione (**1**) has not been reported until now from any natural source, and thus it represents a new addition in the existing list of natural spirocompounds. The rearrangement observed in ring-B of spirowallichione (**1**) and spirocupinanediol, suggests the need to explore which enzyme is responsible for this process and whether this phenomenon is a characteristic feature of only *Euphorbia* species.

Conclusions

Isolation of a rearranged pentacyclic triterpenoid of multiflorane class from *E. wallichii* containing a spiro center named spirowallichione has been achieved and the structure elucidated with the aid of modern spectroscopic techniques including 2D-NMR.

Experimental

General

The melting point was recorded in glass capillary tubes using a Buchi 535 melting point apparatus and is uncorrected. Optical rotation was measured on a JASCO DIP-360 (Japan Spectroscopic Co. Ltd., Tokyo, Japan) digital polarimeter. The IR spectrum was recorded on a Shimadzu IR-460 instrument. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were recorded at 400 and 100 MHz, respectively, on a Bruker AM 400 spectrometer using TMS as an internal standard. The Electron Impact, Field Desorption and High Resolution Electron Impact mass spectra were scanned on a Jeol-JMS HX-110 mass spectrometer.

Collection and Identification

The plant material (all parts) was collected in August 2003, from Malumjabbah (a northern hilly area of Pakistan) and identified by Prof. Dr. Khalida Khatoun, Department of Botany, University of Karachi (Pakistan) where a voucher specimen is deposited (# KK-08-03-19).

Extraction and Isolation

The collected plant material was dried under shade for a week. The dried material (5.5 kg) was then soaked in methanol (10 L) for eight days. The resulting extract was concentrated (308 g) by means of evaporation under vacuum and subjected to silica gel column chromatography using hexane, hexane-ethyl acetate, ethyl acetate and ethyl acetate-methanol as mobile phases. A white powder **1** (6.0 mg) was obtained with 10 % ethyl acetate in hexane after washing with methanol.

Spirowallichione (**1**): $\text{C}_{30}\text{H}_{50}\text{O}_3$; white powder; mp 210-12 °C; $[\alpha]_{\text{D}}^{28}$: -35 (c 1.03, CHCl_3); IR_{max} (CHCl_3) cm^{-1} : 3416 (OH), 1641 (C=O); $^1\text{H-NMR}$ (CDCl_3) δ : 4.20 (1H, dd, $J = 7.1, 7.1$, Hz, H-7), 3.47 (1H, dd, $J = 9.8, 6.1$ Hz, H-3), 2.71 (1H, dd, $J = 14.3, 6.0$ Hz, H-5), 2.03 (1H, m, H-6a), 1.59 (2H, m, H-2), 1.53 (1H, m, H-6b), 1.22 (3H, s, H-26), 1.05 (3H, s, H-28), 0.99 (3H, s, H-30), 0.94 (3H, s, H-23), 0.93 (3H, s, H-29), 0.88 (6H, s, H-25, H-27), 0.86 (3H, s, H-24); $^{13}\text{C-NMR}$ (CDCl_3) δ : 34.3 (C-1), 28.4 (C-2), 79.3 (C-3), 37.9 (C-4), 47.9 (C-5), 21.8 (C-6), 77.9 (C-7), 220.1 (C-8), 60.0 (C-9), 49.7 (C-10), 30.0 (C-11), 31.4 (C-12), 38.7 (C-13), 54.4 (C-14), 25.5 (C-15), 35.9 (C-16), 30.6 (C-17), 43.7 (C-18), 34.9 (C-19), 28.2 (C-20), 33.9 (C-21), 36.4 (C-22), 29.8 (C-23), 16.7 (C-24), 17.4 (C-25), 22.4 (C-26), 21.8 (C-27), 31.5 (C-28), 34.4 (C-29), 32.9 (C-30); HMBC: see Figure 2; COSY-45: see Figure 3; NOESY: see Figure 3; EIMS: m/z 458 (M^+), 440 ($\text{M-H}_2\text{O}^+$), 425 ($\text{M-CH}_3\text{-H}_2\text{O}^+$), 305 (100%), 205; HREIMS: 458.3787 (calcd. for $\text{C}_{30}\text{H}_{50}\text{O}_3$, 458.3762), 440.3668 (calcd. for $\text{C}_{30}\text{H}_{48}\text{O}_2$, 440.3656),

425.3317 (calcd. for C₂₉H₄₅O₂, 425.3320), 305.2440 (calcd. for C₂₀H₃₃O₂, 305.2482), 205.1942 (calcd. for C₁₅H₂₅, 205.1956); FDMS: *m/z* 458.

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Sample Availability: Samples of the compounds are available from the corresponding author.

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