

Pyrotechnoic Acid: A Glycol-oleanolic Acid Conjugate from *Leptadenia pyrotechnica* (Asclepiadaceae)

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Summary: The butanol soluble part of the ethanolic extract of *Leptadenia pyrotechnica* belonging to the family Asclepiadaceae yielded a new glycerol-oleanolic acid conjugate named pyrotechnoic acid (1). The structure of 1 was established with the aid of 1D and 2D NMR spectroscopy.

Introduction

The family Asclepiadaceae is comprised of 175-180 genera and 2200 species distributed mainly in the tropical and sub-tropical regions. In Pakistan, this family is represented by 23 genera and 41 species. Only two species of the genus *Leptadenia* of the family Asclepiadaceae are found in Indo-Pak region. *Leptadenia pyrotechnica*, found chiefly in dry and sandy places in Punjab, Western U.P., Rajistan and Northern parts of Pakistan along the sea coast. The plant yields a fiber used for rope and paper manufacture. The plant provides fodder for cattle. The tuberous roots are consumed as vegetable.

Chemical literature survey of *Leptadenia pyrotechnica* shows that this plant is rich with triterpenoids [1]. Latex of *L. hastata* is found to be rich with only triterpenoids which were separated by TLC technique [2].

Results and Discussion

The butanol soluble part of the ethanolic extract of *Leptadenia pyrotechnica* yielded 1 as a white powder. The IR spectrum of 1 showed the broad absorption which covered the range from 3500-2400 cm^{-1} showing the presence of hydroxyl of carboxyl function in the molecule. The carbonyl absorption of the carboxyl function appeared at 1715 cm^{-1} . Adjacent to the 1715 cm^{-1} , a weak absorption band at 1640 cm^{-1} was observed which was due to the olefinic function in the molecule. The EI mass spectrum of 1 showed the molecular ion peak at m/z 500 which was further confirmed through FDMS. The other prominent peak in the same spectrum (EI) was observed at 455, due to the loss of carboxyl function from the molecular ion peak. The molecular formula of 1 was depicted as $\text{C}_{32}\text{H}_{52}\text{O}_4$ with the aid of HRMS (observed m/z 500. 3901; calcd. m/z 500. 3865).

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The $^1\text{H-NMR}$ spectrum of **1** showed the presence of seven methyl singlets at δ 1.30, 1.24, 1.06, 1.04, 1.02, 0.98 and 0.93. The olefinic moiety was confirmed in $^1\text{H-NMR}$ spectrum through the appearance of the signal at δ 5.53 as a broad singlet. Two more triplets having the coupling constants 6.8 Hz indicating their direct connection to each other appeared in the $^1\text{H-NMR}$ spectrum at δ 3.78 and 3.98 having the integration of two protons each. When these two triplets were correlated with the carbon signals through hetero-COSY technique it was found that the signals at δ 3.98 was connected with carbon at δ 62.2 (C-1') and the second triplet at δ 3.78 was connected to carbon appearing at δ 73.1 (C-2'). When the multiplicities of the signals at δ 73.1 and δ 62.2 were determined through DEPT experiments they were found as methylenes. Through the same technique (DEPT) all thirty-two signals were resolved into seven methyls, twelve CH_2 , five CH, the remaining eight quaternary carbons. The most downfield quaternary carbon appeared at δ 181.2 was due to the carboxylic function. Another downfield quaternary carbon signal at δ 145.0 and a methine signal appearing at δ 121.5 (C-12) were due to the olefinic moiety in the molecule. Another methine signal at δ 78.0 was due to the C-3 at which glycol moiety was attached. The orientation of glycol moiety as β can be justified by the coupling constant of H-3 ($J=11.3$ and 6.1Hz).

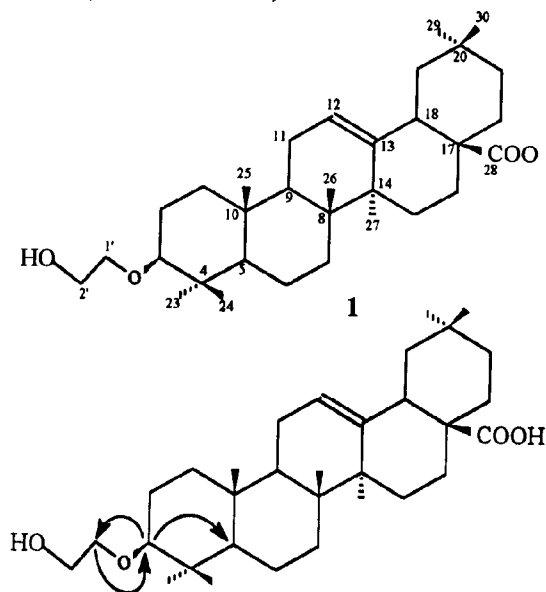


Fig.1 Important HMBC connectivities.

The formula of **1** showed that it contains seven degrees of unsaturation. Out of seven, two could be due to an olefinic and a carboxyl functions. The remaining five must be in the form of rings. Thus **1** is pentacyclic in nature with seven methyls, a carboxylic function, a double bond and a glycol moiety. The position of glycol moiety at C-3 was determined through selective HMBC technique (Fig.1). The various C-H connectivities in the molecule were determined through hetero-COSY technique. With the aid of the above discussed spectral data and comparison with various pentacyclic triterpenoids, [3,4] it has been concluded that **1** belongs to the olean series of triterpenoid and named 3-glycol-oleanolic acid (pyrothechnoic acid) which is a new addition in pentacyclic series of triterpenoids.

Experimental

General experimental:

The ^1H and $^{13}\text{C-NMR}$ spectra were recorded at 300 and 75 MHz, respectively, on Bruker AM-300 in $\text{C}_5\text{D}_5\text{N}$.

Collection and extraction

The leaves of *Leptadenia pyrotechnica* (fresh wt., 30 kg) were collected from Bakkhar (Pakistan) and identified by Dr. Qaisar, Department of Botany, University of Karachi, where the voucher specimen is deposited in the herbarium. The leaves were dried under shade for a week and then soaked in EtOH (25 L) for ten days. The EtOH was removed by evaporation under reduced pressure and the gummy mass thus obtained, was diluted with H_2O . Chlorophyll was separated out by shaking it with n-hexane. The same H_2O layer was then mixed with EtOAc and then the EtOAc soluble portion was separated out. Again the same H_2O layer was treated with BuOH. The BuOH soluble portion was concentrated and then divided into various fractions by means of vacuum liquid chromatography (VLC). The fraction eluted with 30% chloroform in methanol from VLC was subjected to column chromatography using CHCl_3 - MeOH and finally, pure MeOH as mobile phase. As a result of this, a fraction was obtained which on washing with acetone gave colorless powdery material (13.5mg).

IR (KBr) ν_{max} : 3500-2400 (OH), 1715 (C=O) and 1640 (C=C) cm^{-1} ; EIMS m/z : 500 (M^+), 455 (M-COOH , 100%) $^+$, 440, 207, 203 and 189; HRMS:

m/z 500.3901 (calcd. m/z 500.3865 for $C_{32}H_{52}O_4$);
FDMS: m/z 500; 1H -NMR (C_5D_5N): δ 5.53 (1H, br.s., H-12), 3.98 (2H, t, $J = 6.8$ Hz, H-1'), 3.78 (2H, t, $J = 6.8$ Hz, H-2'), 3.45 (1H, dd, 11.3, 6.1 Hz, H-3), 2.71 (1H, br.s., H-5), 1.30, 1.24, 1.06, 1.04, 1.02, 0.98 and 0.93 (each 3H, s, 7 x CH_3); ^{13}C -NMR (C_5D_5N): δ 181.2 (C-28), 145.0 (C-13), 121.5 (C-12), 78.0 (C-3), 73.1 (C-2'), 62.2 (C-1'), 55.8 (C-5), 48.1 (C-9), 46.7 (C-19, 17), 42.0 (C-18, 4), 39.8^a (C-8), 39.4^a (C-4), 38.9 (C-1), 37.4 (C-10), 34.3 (C-21), 33.3 (C-29, 22, 7), 31.0 (C-20), 28.7^b (C-23), 28.3^b (C-15), 28.2^b (C-2), 26.2 (C-27), 23.8 (C-30, 16, 11), 18.8 (C-6), 17.5

(C-26), 16.5 (C-24) and 15.5 (C-25); HMBC: see Fig.1.

a,b: assignments may be interchanged.

References

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