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Ixorene, a New Dammarane Triterpene from the Leaves of *Ixora coccinea* Linn.

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Abstract: A new triterpene ixorene (**1**) with dammarane skeleton was isolated from the leaves of *Ixora coccinea*, along with the three known constituents *β*-sitosterol (**2**), lupeol (**3**) and D-mannitol (**4**). The structure was elucidated on the basis of extensive 1D and 2D-NMR studies and mass spectrometry as 17*β*-dammara-12, 20-diene-3*β*-ol.

Keywords: *Ixora coccinea*; Rubiaceae; Ixorene; Terpenoid; Dammarane.

1. Introduction

Dammarane is a group of triterpenoids isolated from various plants and exhibited valuable biological activities such as antitumoral [1], cytotoxic [2], anti-inflammatory [3], immunosuppressive [4] and antitubercular [5]. Ixorene (**1**) investigated the first example of dammarane skeleton triterpene and first record from the leaves of *I. coccinea* Linn. Recently we have reported a new tirucallane triterpene from the flower of this plant [6] and also evaluated the effect of flower extract and its fraction on cardiovascular system, a first report of this effect on *Ixora coccinea* [7]. In the course of our continuing interest for the discovery of active metabolites from the *I coccinea* we isolated four compounds, ixorene (**1**), *β*-sitosterol (**2**) [8], lupeol (**3**) [8] and D-mannitol (**4**) [9]. Ixorene (**1**) found to be a new compound and its structure was elucidated through 1D and 2D NMR studies and mass spectrometry as 17*β*-dammara-12, 20-diene-3*β*-ol. The whole plant of *I. coccinea*, having a broad spectrum of biological activities, in the present communication is to be focusing only the leaves of this plant which was reported as anti-inflammatory [10,11], antimicrobial [12], antidiarrheal [13], antiulcer [14], antioxidant [15,16] and antinociceptive [17]. The isolated compound *β*-sitosterol (**2**) and lupeol (**3**) are also active metabolites which possess anti-inflammatory, antimitotic [11,18], antioxidant [19,20], antitumor, anticancer, chemoprotective and cardio-protective [19] activities, so it presumed that these compounds probably responsible for much of this plant activities. D-mannitol (**4**) also medicinally important compound which is a potent diuretic [21,22] and used as vaccine for diuretic purposes many years ago. All the history of this plant leads to the emergence of important active metabolites which ultimately may be evolved into drugs and therapeutic potential of promising new agents.

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2. Materials and Methods

2.1. General experimental procedures

UV-visible and IR-spectra were recorded on Thermoelectron ($Vision_{pro}$ Software V4.10) and $Vector_{22}$ spectrometers, respectively, while EIMS and HREIMS were obtained on Jeol MSR_{oute} and JMS HX-110. The ^{1}H NMR, ^{13}C NMR, HSQC, HMBC, COSY-45° and NOESY spectra were obtained on a Bruker Avance AV500 spectrophotometer operating at 500 MHz for ^{1}H- and 125 MHz for ^{13}C-NMR in $CDCl_3$. All TLC analyses were performed at ambient temperature using analytical silica gel $60F_{254}$ precoated cards (0.2 mm thickness) and visualized under UV light (254 and 366 nm) and iodine spray.

2.2. Plant material

The leaves of *I. coccinea* were collected in the gardens of the University of Karachi. The plant was authenticated by Dr. Sahar Zaidi, Department of Botany, University of Karachi, and a voucher specimen (No. KUH 68501) was deposited in the herbarium of the same department.

2.3. *Extraction and isolation of compounds*

The fresh and uncrushed leaves of *I. coccinea* (2.2 kg) were sequentially extracted with MeOH and the pooled extract was concentrated under reduced pressure. The crude extract *IXLM* (213 g) was fractionated into seven fractions by using petroleum ether (*IXLM1*), dichloromethane (*IXLM2*), dichloromethane:ethylacetate (1:1) (*IXLM3*), ethylacetate (*IXLM4*), ethylacetate:methanol (1:1) (*IXLM5*), methanol (*IXLM6*) and an insoluble fraction (*IXLM7*). The fraction *IXLM1* was charcoaled and evaporated under reduced pressure which gave thick residue (3.01 g), of which (1.04 g) was purified through preparative TLC (silica gel, Hexane: ethylacetate, 6.5 : 3.5) and obtained nine bands. The bands *B2* (10 mg), *B4* (10 mg) and *B5* (6 mg) were found to be pure and identified as ixorene (**1**), *β*-sitosterol (**2**) and lupeol (**3**) respectively. D-mannitol was obtained as white crystals (**4**, 30 mg) from the ethyl acetate fraction *IXLM4*.

17β-dammara-12,20-diene-3β-ol (**1**).Yellow solid, $[\alpha]_D^{24}$: +57.6 (*c* 0.0052, $CDCl_3$)
UV λ_{max} (MeOH) nm ($\log_{10}\varepsilon$): 230(4.0), 244(4.1). IR ν_{max} (KBr) cm^{-1}: 3406, 2927, 2857, 1675, 1550, 1463, 1380, 915.
HR EIMS *m/z* (rel. intensity, %): 426.3864 (M^+, calcd. for $C_{30}H_{50}O$, 426.3862, 8), 408.3780 (M^+ -H_2O, calcd. for $C_{30}H_{48}$, 408.3756, 61), 394.3582 (M^+–CH_3,-OH, calcd. for $C_{29}H_{46}$, 394.3600, 10), 207.1774 (calcd. for $C_{14}H_{23}O$, 207.1749, 90), 190.1775 (calcd. for , $C_{14}H_{22}$, 190.1772, 32), 189.1657 (calcd. for $C_{14}H_{21}$, 189.1643, 100). EIMS *m/z* (rel. intensity, %): 426 (M^+, $C_{30}H_{50}O$, 79), 218 ($C_{16}H_{26}$, 58), 207 ($C_{14}H_{23}O$, 90), 189 ($C_{14}H_{21}$, 100), 147 ($C_{11}H_{15}$, 36), 133 ($C_{10}H_{13}$, 32), 107 (C_8H_{11}, 51), 71(C_5H_{11},10).
^{1}H and ^{13}C NMR: see Table 1.

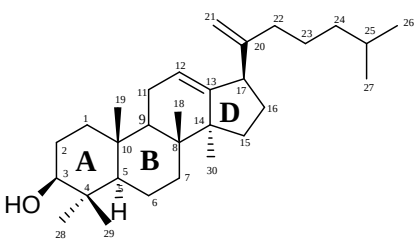

Figure 1. Ixorene (**1**), 17β-dammara-12,20-diene-3β-ol

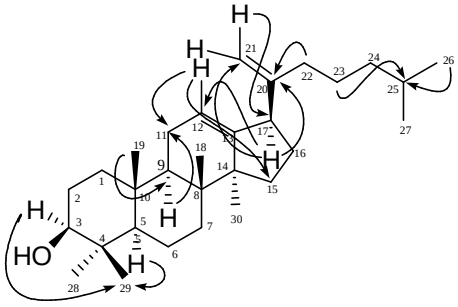

Figure 2. Important HMBC correlation of ixorene (**2**)

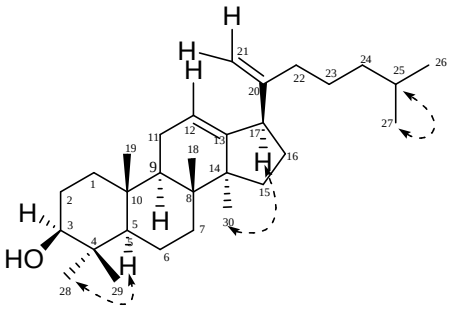

Figure 3. Important NOESY correlations of ixorene (**1)**

3. Results and Discussion

Ixorene (**1),** a yellow solid, $[\alpha]_D^{24}$: +57.6 (*c* 0.0052, CDCl3), showed a molecular ion peak in high resolution electron impact mass spectrum (HREIMS) at *m/z* 426.3864 (M^+, 8%, cal. 426.3862) corresponding to the molecular formula $C_{30}H_{50}O$ which showed six degree of unsaturation as four ring and two olefinic bond. Its IR spectrum revealed the presence of hydroxyl group at 3406 cm^{-1}, CH stretching at 2927 cm^{-1} and 2857, >C=C< at 1675 cm^{-1} with geminal methyls at 1380 cm^{-1}[23] where as the terminal methylene showed vibration at 915 cm^{-1}[24]. The ^{1}H-NMR spectrum of compound (**1)** in $CDCl_3$ (Table 1) showed a downfield signal at δ 5.39 (td, *J*=7.0, 1.5, H-12) along with the two doublets of terminal olefinic protons at δ 4.69 (d, J_{gem}=2, H-21a) and δ 4.54 (d, J_{gem}=2, H-21b) [25]. A one oxymethine proton resonates in doublet of doublet at δ 3.16 (H-3α) with a coupling constant 11.5Hz and 5Hz which indicated that ixorene exhibited β-oriented hydroxyl group [26].

Ixorene (**1)** belongs to dammarane type triterpene which showed base peak at *m/z* 189.1657 (cal 189.1643, $C_{14}H_{21}$) arises due to the removal of water from the fragment of 207.1774 (cal. 207.1749, $C_{14}H_{23}O$), a fragment was formed with high percent intensity during the retro Diels Alder fragmentation pattern. This fragmentation result indicated that compound exhibited unsaturation in the ring at C-12 and the olefinic proton (δ 5.39 td) showed one bond correlation with δc123.15 in HSQC along with strong vicinal correlation in COSY-45° with the H-11. The two terminal olefinic protons (H-21a and H21b) showed the long range correlation in HMBC with C-20 and C-17 whereas the important correlation ^2J and ^3J of H-17α (δ 2.35m) with C-22, C-21, C-20, C-15 and C-12 confirmed the connectivity of terminal olefinic side chain with the ring and which also displayed fragment ion at *m/z* 111(C_8H_{15}).

The side chain containing gem methyls group appear in IR spectrum at 1380cm^{-1} and showing two doublet at δ 0.85 (*J*=6.5, H-26) and δ 0.82 (*J* 6.5, H-27) in NMR with the one bond correlations with δc 22.70 and δc 19.75 in HSQC plot. The HMBC spectrum was also supported by showing correlation of H-26 and H-27 with C-25 (δc 32.81) (Figure 2). COSY- 45° reaffirmed the connectivity of methyls by showing the vicinal correlation with H-25.

The spectral data of (**1)** for ring A, B, C and D completely agree with those reported dammarane teriterpene [4,23]. Ixorene (**1)** differed from other dammarane skeleton due to the presence of unsaturation at C-12 with the terminal olefinic side chain. The 17β substituted configuration of the side chain in (**1)** is based on downfield chemical shift at δ 2.35m (H-17) [2-4,23,27] and NOESY, whereas a crucial NOE is observed between H-17 with H-30 which confirmed that H-17 is in α-orientation (Figure 3). All of above data led to fully identified the compound (**1)** as 17*β*-dammara-12,20-diene-3*β*-ol (Figure 1) which was corroborated by important fragment ions in the HR EIMS (see Experimental).

Table 1. NMR spectral data for Ixorene (**1)** (at 500MHz in $CDCl_3$, δ in ppm, *J* in Hz).

Position	δc	δ_H (*J* in Hz)	HMBC
1	37.38	1.23 m,1.23 m	C-19
2	27.46	1.50 m,1.57 m	C-3, 4
3	79.03	3.16 dd(11.5,5)	C-2,4,29
4	38.89	-	-
5	55.36	0.65 m	C-3,7, 29
6	20.98	1.23 m,1.37 m	C-7,8,29
7	37.45	1.04 m,1.04 m	C-6,18
8	42.88	-	-
9	50.51	1.24m	C-7, 11
10	28.01	-	-
11	25.22	1.37 m, 1.63 m	C-8,12,13
12	123.15	5.39 td (7,1.5)	C-15,17, C-11
13	140.31	-	-
14	43.03	-	-
15	39.88	1.97 m, 1.97 m	C-12,13, C-8
16	34.34	1.37 m, 1.37 m	C-14,17
17	48.01	2.35 m	C-22,21, C-20,15, C-12
18	14.57	0.92 s	C-7, 14
19	15.37	1.01 s	C-1,8,9
20	150.97	-	-
21	109.31	4.69 d(2), 4.54 d(2)	C-17
22	29.69	1.29m,1.29m	C-20,23
23	29.90	1.90m,1.23 m	C-22, 25, C-27
24	24.48	1.23m,1.29m	C-22
25	32.81	1.35 m	C-23
26	22.70	0.85 d(6.5)	C-25
27	19.75	0.82 d(6.5)	C-25
28	27.99	0.94 s	C-3,5
29	18.02	0.75 s	C-3,5,10
30	16.01	1.01s	C-9,14,15

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