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Chemical constituents of leaves and stem bark of *Plumeria obtusa*

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Abstract

The continued studies on the constituents of the fresh leaves and stem bark of *Plumeria obtusa* Linn. have led to the isolation and characterization of four new triterpenoids, dammara-12,20(22)*Z*-dien-3-one (**1**), dammara-12,20(22)*Z*-dien-3β-ol (**2**), olean-12-en-3β,27-diol (**3**), and 27-hydroxyolean-12-en-3-one (**4**) and 12 known compounds, which included eight triterpenoids; dammara-3β,20(*S*),25-triol (**5**), urs-12-en-3β-hydroxy-27-*Z*-feruloyloxy-28-oic acid (**6**), 3β-hydroxyolean-12-en-28-oic acid (**7**), 3β,27-dihydroxylupan-29-ene (**8**), 3β-hydroxylupan-29-en-28-oic acid (**9**), 3β-hydroxyursan-12-en-28-oic acid (**11**), 3β-hydroxy-27-*p*-coumaroyloxy-olea-12-en-28-oic acid (**12**) and urs-12-en-3-one (**15**); an iridoid 1α-plumieride (**10**); a cardenolide 3α,14β-dihydroxy-17β-card-20(22)-enolide (**13**); a fatty acid ester methyl *n*-octadecanoate (**14**) and a steroid 3β-hydroxy-Δ^5-stigmastane (**16**). The new constituents were characterized through spectroscopic studies including 1D (^{1}H and ^{31}C NMR) and 2D (COSY-45, NOESY, *J*-resolved, HMQC and HMBC) NMR and chemical transformations. This is the first report on the isolation of dammarane tri-terpenoids from *P. obtusa.* Compounds **5** and **6** are hitherto unreported from *P. obtusa.* The known compounds were identified by comparison of their spectral data with those reported in the literature.

Keywords: *Plumeria obtusa*; Apocynaceae; Triterpenes; Dammara-12,20(22)*Z*-dien-3-one; Dammara-12,20(22)*Z*-dien-3β-ol; Olean-12-en-3β,27-diol; 27-Hydroxyolean-12-en-3-one

1. Introduction

Plants of genus *Plumeria* (Apocynaceae), had their origin from Central America. Different species are now found widely distributed in the warmer regions of the world (Krishnamurthi, 1969) and reputed for their medicinal properties, e.g., antifouling (Coppen et al., 1983), anticancer (Fujimoto and Made, 1988), algicidal (Coppen, 1983). Keeping these properties in view work on *Plumeria obtusa* was started in our group several years ago (Siddiqui et al., 1999). The present paper deals with a continuation of these studies on the stem bark and leaves of *Plumeria obtusa* leading to the isolation and structure elucidation of four new triterpenoids, champalin A, champalinol, champalinone, and champalin B, characterized as dammara-12,20(22)*Z*-dien-3-one (**1**), dammara-12,20(22)*Z*-dien-3β-ol (**2**), olean-12-en-3β,27-diol (**3**) and 27-hydroxyolean-12-en-3-one (**4**), respectively, along with 12 known compounds. The known compounds included eight triterpenoids dammara-3β,20(*S*),25-triol (**5**), 3β-hydroxy-27*Z*-feruloyloxy-urs-12-en-28-oic acid (**6**), 3β-hydroxyolean-12-en-28-oic acid (oleanolic acid, **7**), 3β,27-dihydroxylupan-29-ene (betulin, **8**), 3β-hydroxylupan-29-en-28-oic acid (betulinic acid, **9**), 3β-hydroxyursan-12-en-28-oic acid (ursolic acid, **11**), 3β-hydroxy-27-*p*-coumaroyloxy-olea-12-en-28-oic acid (obtusilinin, **12**) and urs-12-en 3-one (α-amyrenone, **15**); an iridoid, 1α-plumieride (**10**); a fatty acid ester, methyl *n*-octadecanoate (methyl stearate, **14**); a cardenolide, 3α,14β-dihydroxy-17β-card-20(22)-enolide (3-epidigitoxigenin, **13**); and a steroid, 3β-hydroxy-Δ^5-stigmastane (β-sitosterol, **16**). Compounds **5** (Toshiya and Koichiro, 1974) and **6** (Li et al., 2000) are isolated for first time from this source. Triterpenoids with dammarane skeleton are hitherto unreported from *P. obtusa* and **1**, **2** and **5** are the first such examples. Structures of the new compounds were established by spectral studies and chemical transformations while the

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known were identified by comparing their physical and spectral data published in the literature (Siddiqui et al., 1987, 1988, 1999; Mahato et al., 1994; Chakraborti and Barua, 1963; Robinson and Martel, 1970; William et al., 1964; Janiak et al., 1963; Krasso et al., 1972; Ardenne et al., 1964).

2. Results and discussion

The HREIMS of champalin A (**1**) showed the molecular ion peak at m/z 424.3692 corresponding to the molecular formula $C_{30}H_{48}O$ (calc. for $C_{30}H_{48}O$, m/z 424.3705). The IR spectrum exhibited absorbance at 1710 (cyclic ketone), 1610 (C=C), 1384 and 1359 cm^{-1} (geminal methyls). The ^1H NMR spectrum (Table 1) showed the signals for five quaternary methyls as singlets at δ 0.82, 0.84, 0.86, 0.87 and 0.88 for H-18, H-19, H-28, H-29 and H-30, two secondary methyls as doublets at δ 0.83 ($J_{25,26} = 6.5$) and 0.85 ($J_{25,27} = 6.9$ Hz) for H-26 and H-27, indicating its triterpenoidal nature. These spectral features indicated the presence of a dammarane skeleton (Sharma and Tandon, 1982). Beside these, the ^1H NMR spectrum also displayed resonances for two olefinic protons, one as a quartet of triplet at δ 5.40 ($J_{22,23} = 6.9$, $J_{22,21} = 1.3$ Hz) and other as a triplet at δ 5.17 ($J_{12,11a} = J_{12,11b} = 3.8$ Hz) indicating the presence of two trisubstituted double bonds in the molecule. Their corresponding carbons were recognized at δ 129.7 (C-22) and δ 118.1 (C-12), respectively, in the HMQC plot. One of the double bond (δ_H 5.17) was placed at C-12 on the basis of splitting pattern in the ^1H NMR spectrum and fragments in the HREIMS (vide structure) at m/z 218.1990 ($C_{16}H_{26}$) and at m/z 205.1594 ($C_{14}H_{21}O$) arising from retro-Diels Alder cleavage around ring C. Furthermore, a broad triplet with J values of 1.3 Hz indicated a vinylic methyl (δ_C 22.68) and favored a $\Delta^{20(22)}$ double bond justifying the quartet of triplet at δ_H 5.40 for H-22. The *cis* stereochemistry of this double bond was determined through nOe interaction between H-21 and H-22 and comparison of ^{31}C NMR values (Table 3) of **1** with related *cis* and *trans* isomers (Warren et al., 1976). A carbonyl group as indicated by the IR (ν_{max} 1710 cm^{-1}) and ^{31}C NMR spectra (δ 216.5) was placed at C-3 on biogenetic grounds (Siddiqui et al., 1989) and was supported by the appearance of mass fragment in HREIMS at m/z 205.1594 (vide structure) instead of a typical mass fragment appearing at m/z 207 in MS of 3-hydroxydammar-12-ene, as well as by comparison of spectral data with those of similar compounds (Sharma and Tandon, 1982, Asakawa et al., 1977). Thus seven de-

Table 1
NMR spectral data of triterpene **1** (400 MHz), **2**–**4** (300 MHz), ($CDCl_3$, δ in ppm, J in Hz)

H No.	**1**	**2**	**3**			**4**
	δ (^1H NMR)	δ (^1H NMR)	δ (^1H NMR)	COSY correlations	HMBC correlations	δ (^1H NMR)
H-1			1.60 (m)			
H-2a	2.48 (m)		1.21 (m)	H-3α		2.36 (m)
H-2b	2.32 (m)		1.21 (m)	H-3α		2.27 (m)
H-3α		3.21 (dd, J = 10.5, 4.9)	3.20 (dd, J = 10.5, 4.8)			
H-5			0.50 (m)			
H-6			1.41 (m)			
H-7a/b			1.34 (m)			
H-9			1.49 (m)	H-11a, H-11b		
H-11α			1.89 (m)	H-9, H-11b, H-12		
H-11b			2.01 (m)	H-11a, H-12	C-12	
H-12	5.17 (t, J = 3.8)	5.10 (t, J = 3.6)	5.09 (t, 3.5)	H-11a, H-11b	C-13	5.40 (t, J = 3.3)
H-15			1.21 (m)			
H-16			1.85 (m)			
H-18	0.82 (s)[b]	0.78 (s)	1.83 (dd, J = 14.0, 3.7)		C-12	1.87 (dd, J = 12.8, 4.8)
H-19a/b	0.84 (s)[b]	0.82 (s)	1.49 (m)			
H-21a/b	1.62 (d, J = 1.2)	1.67 (t, J = 1.2)	1.35 (m)			
H-22a/b	5.40 (qt, J = 6.9, 1.2)	5.39 (qt, J = 7.0, 1.2)	1.51 (m)			
H-23	2.27 (m)		0.96 (s)			0.96 (s)
H-24			0.78 (s)			0.84 (s)
H-25			0.72 (s)			0.79 (s)
H-26	0.83[a] (d, J = 6.9)	0.83 (d, J = 7.2)	0.89 (s)			0.86 (s)
H-27a	0.85[a] (d, J = 6.5)	0.93 (d, J = 7.2)	3.52 (d, J = 11.0)	H-27b	C-13	3.38 (d, J = 11.0)
H-27b			3.15 (d, J = 11.0)	H-27a		3.26 (d, J = 11.0)
H-28	0.86 (s)[b]	0.98 (s)	1.06 (s)			1.08 (s)
H-29	0.87 (s)[b]	0.88 (s)	0.98 (s)			0.98 (s)
H-30	0.88 (s)[b]	0.85 (s)	0.91 (s)			0.93 (s)

[a,b] Assignments interchangeable.

grees of unsaturation were accounted for by a carbonyl function at C-3, two trisubstituted double bonds and four rings of the basic tetracyclic skeleton. In the light of these observations, the structure of **1** was elucidated as dammara-12,20(22)*Z*-dien-3-one.

The HREIMS of champalin B (**2**) showed molecular ion peak at m/z 426.3790 corresponding to the molecular formula $C_{30}H_{50}O$ exhibiting an increment of 2 a.m.u. in the mass of **2** from that of **1**. This could be justified by a hydroxyl group instead of a carbonyl function at C-3 since the IR spectrum displayed absorption for a hydroxyl (ν_{max} 3650 cm^{-1}) instead of a carbonyl group. Further, the ^1H NMR spectrum showed a proton geminal to secondary hydroxyl group at δ 3.21 as a double doublet ($J = 10.5$ and $J = 4.9$ Hz) attributable to H-3 with axial disposition. It was placed at C-3 on biogenetic grounds (Siddiqui et al., 1989) and its β-disposition was evident from the chemical shift and coupling constants of H-3α (Mahato et al., 1994; Williams and Fleming, 1973). These data led to characterize champalin B as dammara-12,20(22)*Z*-dien-3β-ol (**2**). Finally, the structure was confirmed by its transformation to **1** through reaction with PCC/CH_2Cl_2 (vide experimental).

The HREIMS of champalinol (**3**) showed a molecular ion peak (M^+) at m/z 442.3802 corresponding to the molecular formula $C_{30}H_{50}O_2$ (calc. for $C_{30}H_{50}O_2$, 442.3810). The IR spectrum exhibited absorbance at 3250 (OH), 2950 (C–H aliphatic), 1625 (C=C), 1130 (C–O), 960–940 (C=C–H) cm^{-1}. The ^1H NMR spectrum displayed resonances for seven methyls located on quaternary carbons (δ 1.06, 0.98, 0.96, 0.91, 0.89, 0.78 and 0.72), a one-proton double doublet at δ 1.83 with $J_{18\beta,19\alpha} = 14.0$ and $J_{18\beta,19\beta} = 3.7$ Hz for H-18β and a triplet at 5.09 ($J_{12,11a} = J_{12,11b} = 3.5$ Hz) for H-12, demonstrating that the compound is of olean-12-ene type (Siddiqui et al., 1989; Begum et al., 1994), which was further confirmed by the significant mass fragments at m/z 207.1750 ($C_{14}H_{23}O$) and 234.1973 ($C_{16}H_{26}O$) derived from characteristic retro-Diels Alder cleavage around ring C and the fragments at m/z 203.1791 ($C_{15}H_{23}$) and 133.1019 ($C_{10}H_{13}$) originating from m/z 234.1973 (vide structure). The fragment ion at m/z 207.1750 led to place a hydroxyl group at C-3 on biogenetic reasons (Nes and Varkey, 1976) and its β-configuration was evident from the chemical shift values (δ 3.20), coupling constants (dd, $J_{3\alpha,2\beta} = 10.5$ and $J_{3\alpha,2\alpha} = 4.8$ Hz) of H-3α (Table 2) (Honda and Komori, 1986) and from the nOe cross peak between H-3α and H-23. Apart from this, the ^1H NMR exhibited two oxymethylene protons at δ 3.52 and 3.15 each as doublet with $J_{gem} = 11.0$ showing their linkage with a quaternary carbon. In the HMBC plot, these protons showed their interaction with C-13. These observations favored the second hydroxyl group at C-27. The presence of the hydroxyl group at more labile position (C-27) was also evident from the base peak at m/z 203.1791 $[234.1973 - CH_2OH]^+$ instead of at m/z 234 (Siddiqui et al., 1979) and another ion at m/z 411.3632 $[M - CH_2OH]^+$ as well as from the ^{31}C NMR spectrum (Table 3) in which C-13 suffered an upfield shift (δ 138.7) while C-12 shifted downfield (δ 125.0) (Mahato et al., 1994). The NOESY plot showed interactions of oxymethylene protons (H-27a/b) with one another as well as with H-9, confirming its α-orientation. On acetylation (Ac_2O/Pyr) **3** afforded the diacetyl derivative (**3a**) in the ^1H NMR of which the signals of oxymethylene protons shifted to δ 4.40 and 3.60 (each d with $J_{gem} = 11.0$) and that of the oxymethine proton to δ 4.48 (dd $J_{3\alpha,2\beta} = 11.0$ and $J_{3\alpha,2\alpha} = 5.0$ Hz). On oxidation with pyridinium chlorochromate **3** formed the 3-oxo-27-aldehyde (**3b**), in which the signal for H-3 disappeared and signals for the C-27 hydroxymethyl protons were replaced by that of a formyl proton resonating as a singlet at δ 9.31 in the ^1H NMR spectrum (Table 2). The assignments of all the

Table 2
^1H NMR spectral data of **3** and **3a**–**3d** (400 MHz, $CDCl_3$, δ in ppm, J in Hz)

H No.	3a	3b	3c	3d
H-2a	u.i.	2.53 (m)	u.i.	2.54 (m)
H-2b	u.i.	2.36 (m)	u.i.	2.43 (m)
H-3α	4.47 (dd, $J = 11.0$, 5.0)	–	3.18 (dd, $J = 10.8$, 4.8)	–
H-12	5.11 (t, $J = 3.5$)	5.23 (t, $J = 3.4$)	5.15 (t, $J = 3.5$)	5.34 (t, $J = 3.5$)
H-18	1.91 (dd, $J = 14.1$, 3.9)	1.90 (dd, $J = 14.0$, 4.1)	1.94 (dd, $J = 14.1$, 3.7)	2.35 (dd, $J = 14.0$, 4.8)
H-27a	4.04 (d, $J = 11.0$)	9.31 (s)	4.14 (d, $J = 10.9$)	4.14 (d, $J = 10.5$)
H-27b	3.65 (d, $J = 11.0$)	–	3.69 (d, $J = 10.9$)	3.74 (d, $J = 10.5$)
CH_3	1.07 (s)	1.27 (s)	1.28 (s)	1.09 (s)
	0.99 (s)	1.11 (s)	1.12 (s)	1.06 (s)
	0.96 (s)	1.09 (s)	1.00 (s)	0.99 (s)
	0.94 (s)	1.03 (s)	0.96 (s)	0.96 (s)
	0.90 (s)	0.90 (s)	0.95 (s)	0.90 (s)
	0.85 (s)	0.89 (s)	0.94 (s)	0.89 (s)
	0.84 (s)	0.82 (s)	0.79 (s)	0.79 (s)
$OCOCH_3$	2.02	–	2.04	2.01
	2.01	–	–	–

u.i. Unidentified.

Table 3
^{31}C NMR chemical shifts for **1** and **3** (75 MHz, $CDCl_3$, δ in ppm)

C No.	1	3
C-1	39.8	38.8
C-2	34.1	29.6
C-3	216.5	79.1
C-4	47.3	38.7
C-5	55.3	55.2
C-6	22.7	18.3
C-7	31.9	38.7
C-8	38.8	39.4
C-9	46.0	47.6
C-10	36.6	36.8
C-11	24.4	25.9
C-12	118.1	125.0
C-13	158.0	138.7
C-14	55.4	37.9
C-15	24.7	30.6
C-16	29.1	23.3
C-17	51.21	42.0
C-18	14.1	47.6
C-19	15.9	46.4
C-20	142.5	31.9
C-21	22.68	34.0
C-22	129.7	35.2
C-23	23.7	28.1
C-24	39.3	15.6
C-25	27.9	15.6
C-26	22.61	16.7
C-27	22.5	69.8
C-28	32.6	23.3
C-29	19.73	33.2
C-30	16.8	21.3

Assignments of carbons are based on BB and DEPT and comparison with the values reported in the literature for similar partial structures (Mahato et al., 1994; Siddiqui et al., 1987, 1989; Sharma and Tandon, 1982; Asakawa et al., 1977; Warren et al., 1976).

carbons and protons were made by ^{31}C NMR (broadband decoupled, DEPT) and various 2D NMR experiments including, HMQC, HMBC, ^{1}H–^{1}H COSY-45 and NOESY spectra (Table 1) and by comparison with the data of similar compounds (Siddiqui et al., 1989; Begum et al., 1994). Based on these data, the structure of **3** has been assigned as olean-12-en-3β,27-diol. This compound has been obtained earlier (Budzikiewicz et al., 1963) during transformation of β-amyrin into oleanolic acid and its 3α epimer is reported as a natural product from *Plumeria rubra* (Akhtar and Malik, 1993). However, this is the first instance of isolation of 3β epimer as a natural product.

The molecular formula of champalinone (**4**) was deduced as $C_{30}H_{48}O_2$ by HREIMS showing molecular ion peak at m/z 440.3652 (calc. for $C_{30}H_{48}O_2$, 440.3654). It showed IR absorptions at 3250 (OH), 3000 (aliphatic C–H), 1710 (C=O) and 1150 cm^{-1} (C–O). The UV displayed no maxima up to 210 nm. The spectral data (vide experimental) of **4** showed its close resemblance with **3**. The only difference observed was the absence of signal for H-3α in the ^{1}H NMR spectrum (Table 1) and presence of a retro-Diels Alder mass fragment at m/z 205.1598 instead of 207 in the HREIMS of **4** (vide structure), which indicated the presence of a carbonyl group at C-3 (ν_{max} 1710 cm^{-1}). It was further confirmed by the appearance of an ion at m/z 409.3480 corresponding to $[M - CH_2OH]^+$ in comparison to m/z 411.3632 in **3**. The structure was finally confirmed by chemical transformation of **3**–**4** (Scheme 1). Attempts were made to selectively oxidize the C-3 hydroxyl group of **3** using a mild oxidizing agent PCC but both hydroxyl groups were oxidized and **3b** was obtained. Therefore, in order to protect the primary hydroxyl group **3** was treated with triphenylmethyl chloride in pyridine at room temperature as well as under reflux but no change was observed which might be attributed to the steric hindrance around C-27 hydroxyl group. Attempts to selectively acetylate the primary hydroxyl group with Ac_2O/Pyridine were tried under varying conditions with no success. Finally, acetylation at 10 °C for 1 h afforded a 1:1 mixture of **3a** and the monoacetyl derivative (**3c**). After separation through prep. TLC (vide experimental) **3c** was treated with PCC in CH_2Cl_2 to obtain the keto-ester **3d**. Hydrolysis of the ester group at C-27 of **3d** using K_2CO_3/MeOH ultimately furnished **4**. Thus spectral features and the chemical transformation along with comparison of partial structures with those in the literature (Begum et al., 1994; Boar et al., 1977) resulted in assigning the structure of **4** as 27-hydroxyolean-12-en-3-one.

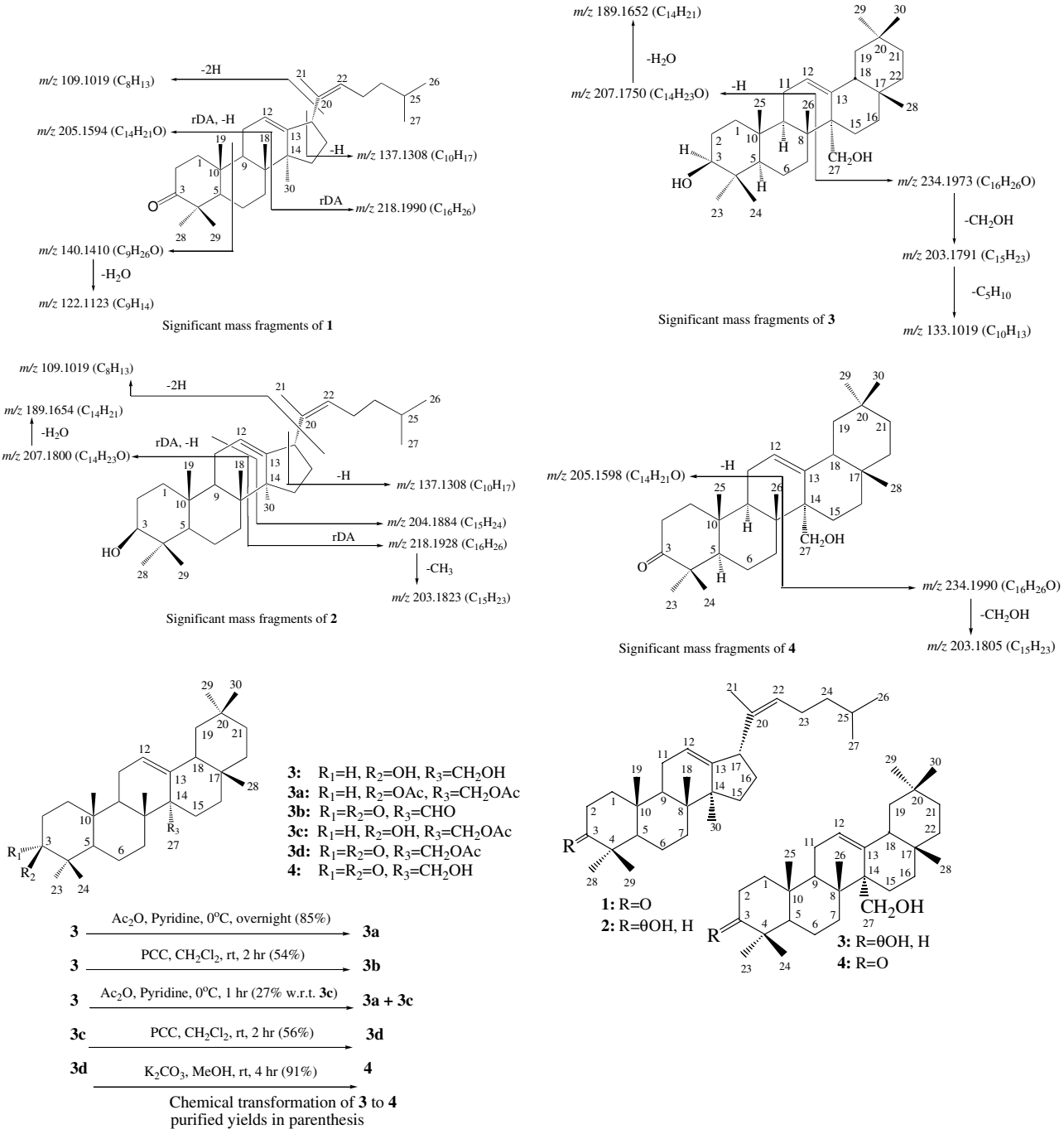
3. Experimental

3.1. General experimental procedures

Prep. TLC was carried out on 20 × 20 cm silica gel GF_{254} plates, prepared in-house; bands were observed under JÜRGENS UV lamps at 254 and 366 nm; vacuum liquid chromatography (VLC) was carried out on silica gel GF_{254}, E. Merck; column chromatography was carried out on silica gel 60, E. Merck, mesh size 70–230; ^{1}H and ^{31}C NMR (BB and DEPT) were recorded in $CDCl_3$ on a Bruker Aspect AM 300 and Avance 400 spectrometers operating at 300 and 400 MHz (for ^{1}H NMR), respectively, and 75 MHz (for ^{31}C NMR). Chemical shifts are reported in ppm (δ) referenced to the signals relative to TMS specified (δ C*H*Cl$_3$, δ *C*DCl$_3$) for ^{1}H and ^{31}C, respectively. EIMS and FABMS were recorded on Varian MAT-311A and JEOL JMS-HX110 instruments, respectively. UV and IR spectra were recorded on Hitachi UV-3200 and Jasco A-302 spectrophotmeter, respectively.

3.2. Plant material

Leaves and stem bark of *P. obtusa* Linn. were collected from Karachi, Pakistan, in the month of April and identified by Dr. S.I. Ali, Department of Botany



Scheme 1. Chemical transformation of **3** to **4** purified yields in parenthesis.

University of Karachi. A voucher specimen [No. 9317 KHU] has been deposited in the herbarium of the same department.

3.3. Extraction and isolation

The fresh, undried and uncrushed leaves (12 kg) were repeatedly extracted with methanol (five times) at room temperature. The solvent from the combined extract was evaporated in vacuo and the concentrate was partitioned between EtOAc and H_2O. The EtOAc phase was treated with 4% aq. Na_2CO_3 to separate the acidic and the neutral compounds. The EtOAc phase containing the neutral fraction was washed, dried and charcoaled. The charcoal bed was successively washed with EtOAc and MeOH–benzene (1:1). The EtOAc filtrate and washings were combined and their solvent removed in vacuo to give a neutral fraction (N), which was divided into petroleum ether soluble (N-1) and petroleum ether insoluble (N-2) fractions. N-2 was successively

treated with petroleum ether–EtOAc (7:3), petroleum ether–EtOAc (1:1) and EtOAc to give fractions N-3, N-4 and N-5, respectively. N-3 (29 g) was subjected to VLC ($CHCl_3$, $CHCl_3$–MeOH in order of increasing polarity). The VLC fraction no. 2, which eluted with $CHCl_3$ was further purified by preparative TLC (silica gel plates, $CHCl_3$–MeOH, 9.5:0.5) to afford four compounds champalinol (**3**, 90 mg), oleanolic acid (**7**, 80 mg), betulin (**8**, 110 mg) and betulinic acid (**9**, 90 mg). The VLC fraction nos. 3 and 4, which also eluted with $CHCl_3$–MeOH (9.5:0.5) afforded 1α-plumieride (**10**) after preparative thick layer chromatography (petroleum ether–EtOAc; 7:3).

In another working, the fresh undried stem bark (10 kg) of *P. obtusa* was repeatedly extracted with MeOH (five times) at room temperature. The methanolic extracts were combined and freed of the solvent under reduced pressure to give a thick syrup, which was partitioned between EtOAc and H_2O. The EtOAc phase was treated with 4% aq. Na_2CO_3 solution to separate the neutral from acidic constituents. After usual work-up, the EtOAc phase (N′) containing neutral components was divided into petroleum ether soluble (PES) and insoluble (PEI) fractions.

Fraction PEI (5.7 g) was subjected to column chromatography (silica gel, petroleum ether, petroleum ether–EtOAc and EtOAc, in order of increasing polarity) to obtain 79 fractions. Fractions 6–13, which eluted with petroleum ether–EtOAc (8:2) yielded pure ursolic acid (**11**; 200 mg). Fraction no. 18, which eluted with petrol–EtOAc (6:4), on subjecting to column chromatography (silica gel, petroleum ether, petroleum ether–EtOAc and EtOAc, in order of increasing polarity) and purification over thick layer (petroleum ether–EtOAc, 8:2) afforded further quantities of ursolic acid (**11**; 104 mg) along with obtusilinin (**12**; 7.1 mg). Fractions 63–65, which eluted with petroleum ether–EtOAc (6:4 and 1:1) were combined and subjected to TLC (silica plates, petroleum ether–EtOAc, 9.7:0.3; re-run twice) affording 3β-hydroxy-27*Z*-feruloyloxy-urs-12-en-28-oic acid (**6**; 8.3 mg) and 3-epidigitoxigenin (**13**; 3.4 mg).

Fraction PES (60 g) was subjected to VLC (petroleum ether, petroleum ether–EtOAc, EtOAc). The VLC fraction nos. PES-3–PES-4 (7.5 g), which eluted with petrol were subjected to column chromatography (petroleum ether, petroleum ether–EtOAc and EtOAc). Column fraction no. 9 (1 g), which eluted with petroleum ether–EtOAc (9.5:0.5), was further subjected to flash column chromatography (FCC) (petroleum ether, petroleum ether–EtOAc, EtOAc in order of increasing polarity). The fractions no. 10 (1 g) of FCC, which eluted with petroleum ether–EtOAc (9.9:0.1) afforded champalin A (**1**; 53.4 mg), methyl stearate (**14**; 60 mg) and α-amyrenone (**15**; 3.5 mg) on separation over prep. TLC (petroleum ether, eluted for three times). The petrol–EtOAc (9:1) eluates of FCC furnished a crystalline solid on evaporation of the solvent which on recrystallization from ether afforded fine needles of β-sitosterol (**16**; 97 mg). The mother liquor of β-sitosterol was subjected to column chromatography (petrol, petrol–EtOAc, EtOAc, in order of increasing polarity). The petrol–EtOAc (9.9:0.1 to 9.5:0.5) eluates were combined and afforded betulin (**8**; 5.2 mg) and 3β,20(*S*),25-trihydroxydammarane (**5**; 3.2 mg) along with a new compound champalin B (**2**; 2.1 mg) on separation over prep. TLC (petrol–EtOAc 9.5:0.5). Fraction PEI (30 g) was subjected to column chromatography ($CHCl_3$, $CHCl_3$–MeOH, MeOH, in order of increasing polarity). The fraction no. PEI-11 and PEI-12 of the column, which eluted with $CHCl_3$–MeOH (9.9:0.1) afforded champalinone (**4**; 5 mg) as a pure compound.

*3.4. Characterization of champalin A (dammar-12,20(22)(Z)-dien-3-one; **1**)*

Amorphous powder (53.4 mg); $[\alpha]_D^{27} + 43.1°$ ($CHCl_3$ *c* 0.58); λ_{max}^{MeOH} nm: 204.2; $\nu_{max}^{CHCl_3}$ cm^{-1}; 3100 (C–H), 1710 (cyclic ketone), 1610 (C=C), 1384 and 1359 cm^{-1} (geminal methyls); ^1H NMR $CDCl_3$ (400 MHz) solvent as IS, Table 1; ^{31}C NMR $CDCl_3$ (400 MHz) referenced to the signals relative to TMS specified (δ C*H*Cl_3, *C*DCl_3) for ^1H and ^{13}C, respectively, Table 3: HREIMS 70 eV, *m/z* (rel. int.): 424.3692 $[M]^+$ (required for $C_{30}H_{48}O$, 424.3705) (6), 409.3477 $[M - CH_3, C_{29}H_{45}O]^+$ (22), 220.1835 $[C_{15}H_{24}O]^+$ (24), 218.1990 $[C_{16}H_{26}]^+$ (100), 205.1594 $[C_{14}H_{21}O]^+$ (25), 109.1019 $[C_8H_{13}]^+$ (37).

*3.5. Characterization of champalin B (dammara-12,20(22)Z-dien-3-ol; **2**)*

Colourless oil (2.1 mg), $[\alpha]_D^{27} + 44.2°$ ($CHCl_3$ *c* 0.04); λ_{max}^{MeOH} nm: 204; $\nu_{max}^{CHCl_3}$ cm^{-1}; 3650 br s (OH), 1710 vs (C=O), 1600 s (C=C); ^1H NMR $CDCl_3$ (300 MHz) referenced to the signals relative to TMS specified (δ C*H*Cl_3, *C*DCl_3) for ^1H and ^{13}C, respectively, Table 3; HREIMS 70 eV, *m/z* (rel. int.); 426.3790 $[M]^+$ (required for $C_{30}H_{50}O$, 426.3801) (13), 411.3594 $[M - Me, C_{29}H_{47}O]^+$ (4), 218.1928 $[C_{16}H_{26}]^+$ (100), 207.1800 $[C_{14}H_{23}O]^+$ (19), 204.1884 $[C_{15}H_{24}]^+$ (45), 203.1823 $[C_{15}H_{23}]^+$ (21), 189.1654 $[C_{14}H_{21}]^+$ (19), 137.1308 $[C_{10}H_{17}]^+$ (8).

*3.6. Characterization of champalinol (3β,27-dihydroxy-olea-12-ene; **3**)*

Amorphous powder (90 mg), $[\alpha]_D^{27} + 42.1°$ ($CHCl_3$ *c* 0.80); λ_{max}^{MeOH} nm: 202.2; $\nu_{max}^{CHCl_3}$ cm^{-1}; 3250 (OH), 2950 (C–H aliphatic), 1625 (C=C), 1130 (C–O), 960–940 (C=C–H); ^1H NMR $CDCl_3$ (300 MHz) referenced to the signals relative to TMS specified (δ C*H*Cl_3, *C*DCl_3) for ^1H and ^{13}C, respectively, Table 2; ^{31}C NMR $CDCl_3$

(75 MHz) residual solvent as IS, Table 3: HREIMS 70 eV, m/z (rel. int.); 442.3802 $[M]^+$, (required for $C_{30}H_{50}O_2$ 442.3810) (31), 411.3632 $[C_{29}H_{47}O]_+$ (12), 234.1973 $[C_{16}H_{26}O]^+$ (20), 207.1750 $[C_{14}H_{23}O]^+$ (14), 203.1791 $[C_{15}H_{23}]^+$ (100), 189.1652 $[C_{14}H_{21}]^+$ (11), 133.1019 $[C_{10}H_{13}]^+$ (35).

3.7. Characterization of champalinone (27-hydroxy-olea-12-en-3-one; 4)

Colourless amorphous powder (5 mg), λ_{max}^{MeOH} nm: 203.5; $\nu_{max}^{CHCl_3}$ cm^{-1}; 3250 br s (OH), 3000 m (CH aliphatic), 1710 vs (C=O), 1150 s (C–O); ^1H NMR $CDCl_3$ (300 MHz) referenced to the signals relative to TMS specified (δ C*H*Cl$_3$, *C*DCl$_3$) for ^1H and ^{13}C, respectively, Table 5; ^{31}C NMR $CDCl_3$ (75 MHz) residual solvent as IS, Table 1: HREIMS 70 eV, m/z (rel. int.); 440.3652 $[M]^+$, (required for $C_{30}H_{48}O_2$ 440.3654) (43), 409.3480 $[M-CH_2OH, C_{29}H_{45}O]^+$ (5), 234.1990 $[C_{16}H_{26}O]^+$ 219.1756 $[C_{15}H_{23}O]^+$ (14), 205.1598 $[C_{14}H_{21}O]^+$ (20), 203.1805 $[C_{15}H_{23}]^+$ (100), 133.1021 $[C_{10}H_{13}]^+$ (39).

3.8. Acetylation of 3 to 3a

To a solution of **3** (10.0 mg) in pyridine (1 ml), Ac_2O (1 ml) was added and the reaction mixture kept overnight at room temperature. On usual work up diacetate (**3a**) was obtained. ^1H NMR ($CDCl_3$, 400 MHz), Table 2; EIMS 70 eV, m/z 526 $[M]^+$ (15), 466 $[M-AcOH]^+$ (31), 451 (19), 423 (18), 406 $[M-2AcOH]^+$ (52), 276 (43), 249 (10), 203 (100), 189 (13), 133 (39).

3.9. Oxidation of 3 to 3b

To a solution of **3** (15.0 mg) in CH_2Cl_2 (1.5 ml), a solution of pyridinium chlorochromate (PCC, 20 mg) in CH_2Cl_2 (3.5 ml) was added and the contents stirred vigorously at room temperature for 2 h. Reaction mixture was taken in Et_2O and filtered. The filtrate was evaporated and purified on column (silica gel, petrol–EtOAc, 8:2) to obtain olea-12-en-3-one-27-al (**3b**; 8 mg). ^1H NMR ($CDCl_3$, 400 MHz), Table 2; EIMS 70 eV, m/z 438 $[M]^+$ (12), 408 $[M-CHO]^+$ (32), 232 (28) and 205 (49), 203 (100), 133 (31).

3.10. Conversion of 3 into 4

To a cold solution of **3** (30 mg) in pyridine (1 ml), Ac_2O (1 ml) was added and the reaction mixture stirred for 1 h at 10 °C. On usual work up a mixture of **3a** and **3c** (3β-hydroxy-27-acetoxy-olea-12-ene) was obtained in a ratio of almost 1:1. The mixture was separated on TLC (petroleum ether–EtOAc, 8:2) to afford pure **3a** (8.5 mg) and **3c** (9 mg). ^1H NMR ($CDCl_3$, 400 MHz), Table 2; EIMS 70 eV, m/z 484 $[M^+]$. Compound **3c** was treated with PCC in CH_2Cl_2 at room temperature for 2 h and the product (**3d**), purified through column chromatography (silica gel; petroleum ether–EtOAc, 9:1). ^1H NMR ($CDCl_3$, 400 MHz), Table 2; EIMS 70 eV, m/z 482 $[M^+]$. Compound **3d** (5 mg) on treatment with methanolic K_2CO_3 at room temperature with constant stirring for 4 h and usual work up yielded **4** (3 mg). The TLC and spectral data of the product coincided well with those of the isolated champalinone (**4**).

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