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New triterpenoids from Morus alba L. stem bark

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New triterpenoids from *Morus alba* L. stem bark

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Two lupeol-type pentacyclic triterpenoids characterised as lup-20(29)-en-3β-ol-27-oic acid (moruslupenoic acid A) and lup-12, 20(29)-dien-3β-ol-26-oic acid (moruslupenoic acid B) and lanst-5, 24-dien-3β-yl acetate (moruslanosteryl acetate) along with the known triterpenoidal phytoconstituents α-amyrin acetate, β-amyrin-β-D-glucopyranoside and betulinic acid have been isolated from the stem bark of *Morus alba* L. (Moraceae). The structures of the isolated phytoconstituents were established on the basis of spectral data analysis and chemical means.

Keywords: *Morus alba*; Moraceae; stem bark; triterpenoids; moruslupenoic acid A; moruslupenoic acid B; moruslanosteryl acetate

1. Introduction

Morus alba L. (Moraceae) is a monoecious or dioecious shrub or moderate-sized tree commonly known as mulberry. The genus *Morus* consists of over 150 species; among them, *M. alba* is dominant (Anonymous, 2001; Butt, Nazir, Sultan, & Schroen, 2008). Mulberry is found from temperate to subtropical regions of the Northern hemisphere to the tropics of the Southern hemisphere and grows in a wide range of climatic, topographical and soil conditions from sea level to altitudes as high as 4000 m. The plant is indigenous to China and commonly cultivated in Baluchistan, Afghanistan, northern part of the trans-Indus territory and N.W. Himalaya. The bark of the large stem is brown, rough, fissures mostly vertical, considered as vermifuge and purgative. Mulberry is used for its foliage to feed the silkworm (Anonymous, 2001; Ercisli & Orhan, 2007; Mhaskar, Blatter, & Caius, 2000). Mulberry is a promising nature's functional tonic and has a unique nutritional profile containing proteins, phenolics, flavonoids and anthocyanins, which enhance its significance. Phytol, β-sitosterol, lanost-7-en-3-on, α-amyrin, β-amyrin, lupeol, mulberrin, cyclomulberrin, mulberrochomene, cyclomulberrochromene, mulberranol, albanol A and B have been reported from the stem bark. β-Sitosterol has inhibitory effect on 5-α-reductase, which has a key role in steroid biosynthesis, whereas α-amyrin, β-amyrin and lupeol have strong antiinflammatory effect and are a potent inhibitor of protein kinase in rat liver cells. (Boszormenyi et al., 2009; Butt et al., 2008; Deshpande, Parthasarathy, & Venkataraman, 1968; Rama Rao, Deshpande, & Shastri, 1983). This article describes the isolation and characterisation of triterpenoids from the stem bark of *M. alba* of Delhi region.

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2. Results and discussion

Six compounds were isolated (Figure 1). Compounds **1**, **3** and **6** are the known triterpenoids identified as α-amyrin acetate (Ali, Ravinder, & Ranachandram, 2000; Sirat, Susanti, Ahmad, Takayama, & Kitajima, 2010; Wang, Liu, & Xu, 2009), β-amyrin-β-D-glucopyranoside (Ali, 2001; Mahato & Kundu, 1994) and betulinic acid (Ghosh, Mandal, A. Chakraborty, Rasul, M.G. Chakraborty, & Saha, 2010; Li et al., 2009; Malik, Ahmad, Anjum, & Basha, 2010), respectively.

Compound **2**, named moruslanosteryl acetate, was obtained as colourless needle shaped crystals from petroleum ether-chloroform (1:1) eluents. It responded positively to triterpenoid tests. Its infrared (IR) spectrum exhibited characteristic absorption bands for ester group at 1730 cm^{-1} and unsaturation at 1646 cm^{-1}. On the basis of FAB MS and ^{13}C NMR spectra, its molecular weight was established at m/z 468 consistent of a molecular formula of a triterpenic ester, $C_{32}H_{52}O_2$. It had seven degrees of double bond equivalents; four of them were adjusted in the tetracyclic carbon framework of the triterpene, two in the vinylic linkage and the remaining one in the ester function. The mass spectrum of **2** exhibited the prominent ion peaks generated at m/z 453 $[M-Me]^+$, 410 $[453-CH_3CO]^+$, 408 $[M-CH_3COOH]^+$, 393 $[408-Me]^+$ and 378 $[393-Me]^+$ indicating the presence of the acetate group in the molecule. The ion peaks arising at m/z 111 $[C_8H_{15}$, side chain$]^+$ 357 [M–side chain]$^+$, 315 $[357-$ring C$]^+$, 342 $[357-Me]^+$, 297 $[357-CH_3COOH]^+$, 282 $[297-Me]^+$ and 267 $[282-Me]^+$ supported the existence of one of the vinylic linkage in the side chain and another one in the triterpenic nucleus and the acetate group. The ion fragments produced due to fragmentation of the mass unit 297 at m/z 174 [$C_{8,14}$–$C_{9,11}$ fission, $C_{13}H_{18}]^+$, 188 [$C_{8,14}$–$C_{11,12}$ fission, $C_{14}H_{20}]^+$ and 202 [$C_{8,14}$–$C_{12,13}$ fission, $C_{15}H_{22}]^+$ suggested saturated nature of the ring C. The ion peak arising due to fragmentation of the rings A and B at m/z 142 $[C_8H_{14}O_2]^+$, 194 $[C_{12}H_{18}O_2]^+$, 134 $[194-CH_3COOH]^+$, 119 $[134-Me]^+$, 208 [$C_{7,8}$–$C_{9,10}$ fission; $C_{13}H_{20}O_2]^+$, 148 $[208-CH_3COOH]^+$ and 133 $[148-Me]^+$ indicated the existence of one of the vinylic linkage at C-5 and the hydroxyl group in the ring A, which was placed at C-3 by considering biogenetic pathway. The ^1H NMR spectrum of **2** displayed two one-proton multiplets at δ 5.22 and 5.07 assigned to vinylic protons H-6 and H-24, respectively. A one-proton doublet at δ 4.51 with coupling interaction of 5.5 and 9.5 Hz was ascribed α-oriented H-3 carbinol proton. Three broad signals at δ 2.01, 1.65 and 1.57, all integrating for three protons each, were attributed corresponding to acetyl methyl and to C-26 and C-27 methyl protons attached to C-25 olefinic carbon. A three-proton doublet at δ 0.96 ($J = 6.3$ Hz) was accounted to C-21 secondary methyl proton. The C-18, C-19, C-28, C-29 and C-30 tertiary methyl protons appeared as three-proton broad signals at δ 0.73, 1.10, 0.90, 0.82 and 0.77, respectively. The remaining methine and methylene proton resonated in the range of δ 2.1–1.02. The ^{13}C NMR spectrum of **2** showed important signals for acetyl carbons at δ 170.81 and 21.26, vinylic carbons at δ 145.94 (C-5), 125.09 (C-6), 117.55 (C-24) and 130.86 (C-25), oxygenated methine carbon at δ 81.08 (C-3) and methyl carbons from δ 13.09 to 25.82. The ^1H and ^{13}C NMR spectral data of **2** were compared with lanosterol-type triterpenoids (Ali, 2001; Ansari, Ali, & Qadary, 1993; Sharma & Ali, 1996). The ^1H-^1H COSY spectrum of **2** showed correlation of H-3 with H_2-2, $COCH_3$ and H_3-28; H-6 with H_2-7 and H-8; and H-24 with H_2-23, H_3-26 and H_3-27. The HMBC spectrum of **2** exhibited interactions of AcO with H-3; C-5 with H-6, H_2-7 and H_3-29; and C-25 with H_2-23, H-24, H_3-26 and H_3-27. Alkaline hydrolysis of **2** yielded lup-5, 24-dien-3β-ol. On the basis of the above-mentioned discussion, the structure of **2** has been formulated as *lanst-5, 24-dien-3β-yl acetate*. This is a new tetracyclic triterpenoid.

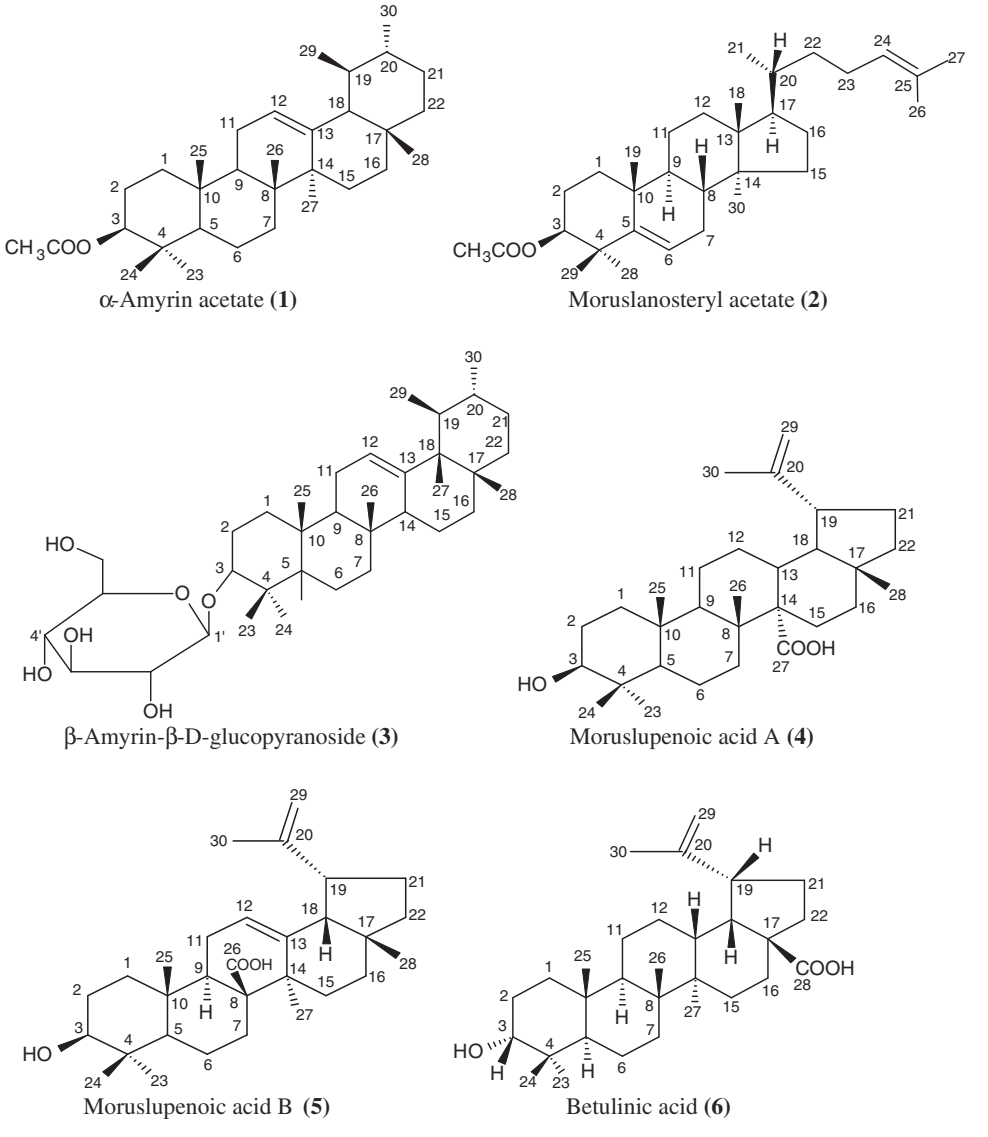

Figure 1. Structure of compounds **1**–**6** isolated from the methanolic extract *Morus alba* L. stem bark.

Compound **4**, designated as moruslupenoic acid A, was obtained as a colourless amorphous powder from chloroform-methanol (93 : 7) eluents. It responded positively to Liebermann-Burchardt test for triterpenoid and yielded effervescences with sodium bicarbonate solution because of the presence of carboxylic group. Its IR spectrum exhibited distinctive absorption bands for hydroxyl group (3460 cm^{-1}), carboxylic function (1686 cm^{-1}) and unsaturation (1642 cm^{-1}). On the basis of FAB MS and ^{13}C NMR spectra, its molecular weight was established at m/z 456 consistent with the molecular formula of triterpenic acid, $C_{30}H_{48}O_3$. It indicated seven double bond equivalents; five of them were adjusted in the pentacyclic skeleton of the pentacyclic triterpenoid and one each in the vinylic linkage and carboxylic function. The mass spectrum of **4** showed prominent ion peaks generated at m/z 438 $[M-H_2O]^+$, 397

$[438-C_3H_5]^+$, 411 $[M-COOH]^+$, 393 $[411-H_2O]^+$, 378 $[393-CH_3]^+$, 441 $[M-CH_3]^+$ and 426 $[441-CH_3]^+$ supporting the existence of the one each of carboxylic group, hydroxyl function and isopropenyl group in the molecule. The ion fragments arising at *m/z* 140 [$C_{5,6}$–$C_{9,10}$ fission, $C_9H_{16}O]^+$, 154 [$C_{6,7}$–$C_{9,10}$ fission, $C_{10}H_{18}O]^+$ and 168 [$C_{7,8}$–$C_{9,10}$ fission, $C_{11}H_{20}O]^+$ indicated saturated nature of the ring A and B and the presence of the hydroxyl group, which was placed at C-3 on biogenetic anomaly. The ion fragments formed at *m/z* 208, 248 [$C_{8,14}$–$C_{9,11}$ fission, $C_{14}H_{24}O$ and $C_{16}H_{24}O_2]^+$, 234 [$C_{8,14}$–$C_{11,12}$ fission, $C_{15}H_{22}O_2]^+$ and 220 [$C_{8,14}$-$C_{12,13}$ fission, $C_{14}H_{20}O_2]^+$ suggested the existence of carboxylic function at C_{27}/C_{28} and saturated nature of the ring C. The ion peaks appearing at *m/z* 150, 306 [$C_{14,15}$–$C_{13,18}$ fission, $C_{11}H_{18}$ and $C_{19}H_{30}O_3]^+$, 136 [$C_{13,18}$–$C_{15,16}$ fission, $C_{10}H_{16}]^+$ and 122 [$C_{13,18}$–$C_{16,17}$ fission, $C_9H_{14}]^+$ indicated the location of carboxylic group at C-27 and isopropenyl chain in ring E at C-19. The ^{1}H NMR spectrum of **4** displayed two one-proton broad signals at δ 4.68 and 4.56 assigned to unsaturated methylene protons H_2-29. A one-proton double doublet at δ 3.50 ($J = 5.5$, 9.3 Hz) was attributed to α-oriented carbinol proton H-3. A three-proton broad signal at δ 1.65 was accounted C-30 methyl proton attached at the vinylic carbon C-20. The other methyl signals appeared between δ 1.24 and 0.65. The remaining methine and methylene protons resonated in the range of δ 2.99–1.17. The ^{13}C NMR spectrum of **4** displayed important signals for carboxylic carbon at δ 177.65 (C-27), vinylic carbons at δ 150.67 (C-20) and 109.93 (C-29), carbinol carbon at δ 77.38 (C-3) and methyl carbons in the range of δ 28.47–14.79. The ^{1}H and ^{13}C NMR spectral values were compared with betulinic acid and other related lupene-type terpenoids (Ali, 2001; Mahato & Kundu, 1994). The DEPT spectrum of **4** showed the presence of 6 methyl, 11 methylene, 6 methine and 7 quaternary carbons. The HMBC spectrum of **4** exhibited correlation of C-3 with H_2-2, H_2-1 and H_3-24; C-27 with H-13 and H_2-15; and C-20 with H_2-29, H_3-30 and H-19. On the basis of foregoing discussion, the structure of **4** has been elucidated as *lup-20(29)-en-3β-ol-27-oic acid*. This is a new pentacyclic triterpenoid.

Compound **5**, named moruslupenoic acid B, was obtained as colourless flakes from chloroform-methanol (93 : 7) eluents. It responded positively to Liebermann-Burchardt test and produced effervescences with sodium bicarbonate solution. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3436 cm^{-1}), carboxylic function (3390, 1686 cm^{-1}) and unsaturation (1638 cm^{-1}). On the basis of FAB MS and ^{13}C NMR spectra, its molecular weight was established at *m/z* 454 consistent with the molecular formula of pentacyclic triterpenoic acid, $C_{30}H_{46}O_3$. Its molecular formula indicated eight double bond equivalents; five of them were adjusted in the pentacyclic carbon framework of a triterpenoid, two in vinylic linkages and one in the carboxylic group. The mass spectrum of **5** showed prominent ion peaks generated at *m/z* 409 $[M-COOH]^+$, 394 $[409-CH_3]^+$, 379 $[304-CH_3]^+$ and 364 $[379-CH_3]^+$ supporting the existence of the carboxylic function in the molecule. The ion peaks arising at *m/z* 238 $[C_{14}H_{22}O_3]^+$ and 216 $[C_{16}H_{24}]^+$ because of retro-Diels-Alder fragmentation indicated the location of one of the vinylic linkage at C-12 and carboxylic function in ring A/B. The ion fragments appearing at *m/z* 220 $[238-H_2O]^+$, 205 $[220-CH_3]^+$, 190 $[205-CH_3]^+$, 193 $[238-COOH]^+$, 175 $[193-H_2O]^+$, 160 $[175-CH_3]^+$ and 145 $[160-CH_3]^+$ also supported the presence of the hydroxyl function in the ring A/B, which was placed at C-3 on the basis of biogenetic consideration. The ion peaks formed at *m/z* 175 $[216-C_3H_5]^+$, 189 $[216-C_2H_3]^+$, 201 $[216-CH_3]^+$, 186 $[201-CH_3]^+$, 159 $[186-C_2H_3]^+$ and 118 $[159-C_3H_5]^+$ indicated lupene-type triterpenoid. The ^{1}H NMR of **5** displayed a one-proton double doublet at δ 5.33 ($J = 5.4$, 6.0 Hz) assigned to vinylic H-12 proton. Two one-proton broad signals at δ 4.71 and 4.67 were attributed to methylene H_2-29. A one-proton double doublet at δ 3.27 with coupling interaction of 5.4 and 10.4 Hz was ascribed to α-oriented

carbinol H-3 proton. A three-proton broad signal at δ 1.67 was accounted to C-30 methyl protons attached to C-20 vinylic carbon. Six broad signals between δ 1.20 and 0.74, all located on the saturated carbons were associated with the tertiary methyl protons. The remaining methylene and methane protons resonated in the range of 2.91–1.15. The ^{13}C NMR spectrum of **5** showed important signals for carboxylic carbon at δ 177.97 (C-26), vinylic carbons at δ 132.51 (C-12), 146.76 (C-13), 150.32 (C-20) and 108.89 (C-29), carbinol carbon at δ 77.98 (C-3), methyl carbons between δ 28.62 and 14.21 and the remaining methylene and methine carbons in the range of δ 55.61 to 20.42. The ^{1}H and ^{13}C NMR spectral data with the lupene type compounds (Ali, 2001; Mahato & Kundu, 1994). The multiplicity of each carbon was determined by DEPT spectrum of **5**. The HMBC spectrum of **5** exhibited interaction of C-4 with H-3, H_2-2, H-5 and H_3-24; C-8 with H-9 and H_2-7; C-13 with H-12, H_2-11, H_3-27 and H-18; and C-20 with H_2-29, H_3-30 and H-19. On the basis of spectral data analysis and chemical reactions, the structure of **5** has been established as *lup-12, 20(29)-dien-3β-ol-26-oic acid*. This is an unreported lupenic acid. Cyathadonic acid and epistriatic acid are the lupene-type triterpenoids from *Cyathus striatus* Willd. ex, Pers., which possess carboxylic function at C-8 (Ayer, Flanagan, & Reffstrup, 1984).

3. Experimental

3.1. *General*

Melting points were determined on a Perfit apparatus without correction. The IR spectra were measured in KBr pellet on a Bio-Red FT-IR spectrometer. Ultraviolet (UV) spectra were obtained in methanol with a Lambda Bio 20 spectrometer. ^{1}H (300 MHz), ^{13}C (75 MHz), COSY and HMBC NMR spectra were recorded on Bruker spectrospin spectrometer. $CDCl_3$ (Sigma-Aldrich, Bangalore, India) was used as solvent and TMS as an internal standard. FAB MS analyses were performed on a JEOL SX 102/Da-600 instrument equipped with direct inlet probe system. Column chromatography separations were carried out on silica gel (Merck, 60–120 mesh, Mumbai, India). Precoated silica gel plates (Merck, Silica gel 60 F_{254}) were used for analytical thin layer chromatography visualised by exposure to iodine and UV radiations.

3.2. *Plant material*

The stem bark of *M. alba* L. was collected from the campus of Jamia Hamdard, New Delhi, and identified by Dr. H.B. Singh, Scientist F and Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A voucher specimen of drug was deposited in the herbarium of NISCAIR with a reference number NISCAIR/RHMD/Consult/-2008-09/1059/90.

3.3. *Extraction and isolation*

The air-dried bark (2 kg) of *M. alba* were coarsely powdered and extracted exhaustively with methanol using Soxhlet apparatus for 72 h. The extract was concentrated under reduced pressure to get dark brown mass (260 g). Small portion of the extract was analysed chemically to determine the presence of different chemical constituents. The extract was dissolved in minimum amount of methanol and adsorbed on silica gel (60–120 mesh) for preparation of slurry. The slurry was dried and subjected to silica gel column chromatography.

3.3.1. *α-Amyrin acetate (**1**)*

Elution of the column with petroleum ether-chloroform (1:1) yielded colourless crystals of **1**, recrystallised from acetone, (497 mg, 0.62% yield). R_f 0.69 (petroleum ether-chloroform

(1:1), melting point (m.p.) 225–226°C; +ve ion FAB MS *m/z* (rel. int.): 468 $[M]^+$ ($C_{32}H_{52}O_2$) (88.3).

3.3.2. *Moruslanosteryl acetate (**2**)*

Further elution of the column with petroleum ether-chloroform (1:1) furnished colourless needles of **2**, recrystallised from acetone, (122 mg, 0.15% yield). R_f 0.82 (petroleum ether-chloroform, 1:1); m.p. 124–125°C; UV λ_{max} (MeOH): 257 nm (log ε 4.2); IR ν_{max} (KBr): 2950, 2884, 1730, 1646, 1454, 1365, 1245, 1024 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.22 (1H, m, H-6), 5.07 (1H, m, H-24), 4.51 (1H, dd, *J*=5.5, 9.5 Hz, H-3a), 2.01 (3H, brs, OCH_3), 1.65 (3H, brs, Me-26), 1.57 (3H, brs, Me-27), 1.10 (3H, brs, Me-19), 0.96 (3H, d, *J*=6.3 Hz, Me-21), 0.90 (3H, brs, Me-28), 0.82 (3H, brs, Me-29), 0.77 (3H, brs, Me-30), 0.73 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): δ 37.79 (C-1), 29.66 (C-2), 81.08 (C-3), 43.48 (C-4), 145.94 (C-5), 125.09 (C-6), 33.73 (C-7), 33.90 (C-8), 50.73 (C-9), 36.79 (C-10), 22.01 (C-11), 35.73 (C-12), 48.77 (C-13), 51.24 (C-14), 24.16 (C-15), 27.53 (C-16), 53.20 (C-17), 13.09 (C-18), 18.54 (C-19), 35.12 (C-20), 18.11 (C-21), 24.77 (C-22), 27.28 (C-23), 117.55 (C-24), 130.86 (C-25), 25.68 (C-26), 25.31 (C-27), 25.82 (C-28), 23.72 (C-29), 19.51 (C-30), 170.81, 21.26 ($OCOCH_3$); +ve ion FAB MS *m/z* (rel. int.): 468 $[M]^+$ ($C_{32}H_{52}O_2$) (69.5), 453 (86.3), 410 (71.8), 408 (53.8), 393 (33.1), 378 (10.2), 357 (12.6), 342 (21.0), 315 (11.6), 297 (29.8), 282 (13.2), 267 (15.3), 208 (26.7), 202 (26.3), 194 (31.5), 188 (73.2), 174 (65.2), 148 (78.2), 144 (84.1), 142 (65.3), 134 (89.8), 119 (83.8), 111 (26.3), 95 (100).

3.3.3. *β-Amyrin-β-D-glucopyranoside (**3**)*

Elution of the column with chloroform-methanol (97:3) afforded colourless crystals of **3**, recrystallised from methanol, (705 mg, 0.88% yield). R_f 0.38 (chloroform-methanol, 24 : 1); m.p. 210–215°C; +ve ion FAB MS *m/z* (rel. int.): 588 $[M]^+$ ($C_{36}H_{60}O_6$) (2.6).

3.3.4. *Moruslupenoic acid A (**4**)*

Further elution of the column with chloroform-methanol (97:3) gave colourless amorphous powder of **4**, recrystallised from methanol, (171 mg, 0.21% yield). R_f 0.56 (chloroform-methanol, 24:1); m.p. 275–276°C; UV λ_{max} (MeOH): 253 nm (log ε 4.1); IR ν_{max} (KBr): 3460, 2940, 2868, 1686, 1642, 1455, 1272, 1236, 1083 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.68 (1H, brs, H_2-29a), 4.56 (1H, brs, H_2-29b), 3.50 (1H, dd, *J*=5.5, 9.3 Hz, H-3a), 1.65 (3H, brs, Me-30), 1.24 (3H, brs, Me-23), 0.93 (3H, brs, Me-24), 0.88 (3H, brs, Me-25), 0.76 (3H, brs, Me-26), 0.65 (3H, brs, Me-28); ^{13}C NMR ($CDCl_3$): δ 38.06 (C-1), 27.50 (C-2), 77.38 (C-3), 39.26 (C-4), 55.86 (C-5), 18.39 (C-6), 34.40 (C-7), 40.65 (C-8), 50.54 (C-9), 36.17 (C-10), 19.11 (C-11), 25.53 (C-12), 38.97 (C-13), 55.38 (C-14), 30.57 (C-15), 32.19 (C-16), 42.41 (C-17), 47.02 (C-18), 49.05 (C-19), 150.67 (C-20), 29.63 (C-21), 36.84 (C-22), 28.47 (C-23), 14.79 (C-24), 16.14 (C-25), 16.31 (C-26), 177.65 (C-27), 16.89 (C-28), 109.93 (C-29), 19.36 (C-30); +ve ion FAB MS *m/z* (rel. int.): 456 $[M]^+$ ($C_{30}H_{48}O_3$) (18.3), 441 (23.1), 438 (39.0), 426 (11.2), 411 (19.6), 397 (20.5), 393 (13.5), 378 (15.3), 306 (23.0), 248 (18.2), 234 (21.6), 220 (19.1), 208 (21.3), 168 (18.2), 154 (93.6), 150 (40.1), 140 (39.8), 136 (65.7), 122 (68.5).

3.3.5. *Moruslupenoic acid B (**5**)*

Further elution of the column with chloroform-methanol (97:3) gave colourless flakes of **5**, recrystallised from methanol, (445 mg, 0.55% yield). R_f 0.56 (chloroform-methanol, 24:1); m.p. 275–276°C; UV λ_{max} (MeOH): 253, 280 nm (log ε 5.3, 1.2); IR ν_{max} (KBr): 3436, 3390, 2927, 2855, 1686, 1638, 1457, 1375, 1237, 1106, 1032, 883 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.33 (1H, dd, *J*=5.4, 6.0 Hz, H-12), 4.71 (1H, brs, H_2-29a), 4.67 (3H, brs, H_2-29b), 3.27 (1H, dd,

J=5.4, 10.4 Hz, H-3a), 1.67 (3H, brs, Me-30), 1.20 (3H, brs, Me-23), 0.96 (3H, brs, Me-24), 0.91 (3H, brs, Me-25), 0.79 (3H, brs, Me-27), 0.74 (3H, brs, Me-28); ^{13}C NMR ($CDCl_3$): δ 38.37 (C-1), 27.62 (C-2), 77.98 (C-3), 38.89 (C-4), 55.61 (C-5), 20.42 (C-6), 33.90 (C-7), 54.92 (C-8), 50.07 (C-9), 37.74 (C-10), 26.88 (C-11), 132.51 (C-12), 146.76 (C-13), 40.27 (C-14), 30.12 (C-15), 31.85 (C-16), 41.95 (C-17), 48.74 (C-18), 46.43 (C-19), 150.32 (C-20), 29.14 (C-21), 36.69 (C-22), 28.62 (C-23), 15.62 (C-24), 17.85 (C-25), 177.97 (C-26), 15.08 (C-27), 14.21 (C-28), 108.89 (C-29), 19.23 (C-30); +ve ion FAB MS m/z (rel. int.): 454 $[M]^+$ ($C_{30}H_{46}O_3$) (15.3), 409 (38.7), 394 (31.5), 379 (18.6), 364 (18.3), 238 (25.0), 220 (21.6), 216 (31.9), 205 (26.3), 201 (29.8), 193 (16.1), 190 (18.3), 189 (39.0), 186 (30.5), 180 (21.7), 175 (36.2), 160 (45.8), 159 (37.2), 145 (63.8), 118 (81.2).

3.3.6. *Betulinic acid (**6**)*

Elution of the column with chloroform-methanol (89:1) produced colourless crystalline mass of **6**, recrystallised from chloroform-methanol (1:1), (346 mg, 0.43% yield). R_f 0.89 (chloroform-methanol, 7.3:1); m.p 113–115°C; +ve ion FAB MS m/z (*rel. int.*): 457 $[M]^+$ ($C_{30}H_{49}O_3$) (64.8).

4. Conclusions

This work characterised chemical compounds in the stem bark of *M. alba* that can be of great help for its standardisation, as it is a drug of controversial identity in the traditional system of medicine in India.

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