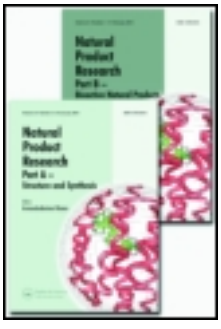

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Phytochemical investigation of the seeds of Althea officinalis L.

Sunita Rani [a], Suroor A. Khan [a] & M. Ali [a]
[a] Department of Pharmaceutical Chemistry, Jamia Hamdard (Hamdard University), New Delhi 110062, India
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Phytochemical investigation of the seeds of *Althea officinalis* L.

Sunita Rani, Suroor A. Khan* and M. Ali

Department of Pharmaceutical Chemistry, Jamia Hamdard (Hamdard University), New Delhi 110062, India

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Phytochemical investigation of the seeds of *Althea officinalis* L. (Malvaceae) led to the isolation of three new phytoconstituents, identified as *n*-hexacos-2-enyl-1,5-olide (altheahexacosanyl lactone), 2β-hydroxycalamene (altheacalamene) and 5,6-dihydroxycoumarin-5-dodecanoate-6β-D-glucopyranoside (altheacoumarin glucoside), along with the known phytoconstituents lauric acid, β-sitosterol and lanosterol. The structures of these compounds were established on the basis of spectral analysis and chemical reactions.

Keywords: *Althea officinalis* L.; altheahexacosanyl lactone; 2β-hydroxycalamene; coumarin glucoside

1. Introduction

Althea officinalis L. (Malvaceae), commonly known as gulkhairo or marshmallow, is a downy, perennial herb, distributed in the Himalayas from Kashmir to Himachal Pardesh (National Institute of Science Communication and Information Resources, 2003). The seeds of this plant are demulcent, diuretic and febrifugal (Mhaskar, Blatter, & Caius, 2000). In the Unani medicinal system, *A. officinalis* is prescribed as an expectorant and is known to relieve bronchitic and bronchial catarrh (Indian Council of Medical Research, 2004).

Malvalic acid and some fatty acids were previously extracted from the seeds of this plant (Karawaya, Balbana, & Afifi, 1982; Mishina, Kornievskii, Shkurupii, & Dolia, 1975). This article describes the isolation of hexadecanyl δ-lactone, hydroxycalamene and a coumarin glucoside along with the known compounds lauric acid, β-sitosterol and lanosterol from the seeds of *A. officinalis* L.

2. Results and discussion

Compounds **2**, **4** and **5** are the known phytoconstituents identified as lauric acid, β-sitosterol (Chung, Ali, Chun, Lee, & Ahmad, 2007; Haba et al., 2007) and lanostanol (Ali, 2001; Nes, Norton, Parish, Meenan, & Popjak, 1989), respectively.

Compound **1**, designated as altheahexacosanyl lactone, was obtained as a colourless mass from the petroleum ether–chloroform (9 : 1) eluants. Its IR spectrum

*Corresponding author. Email: sakhan_pchem@yahoo.com

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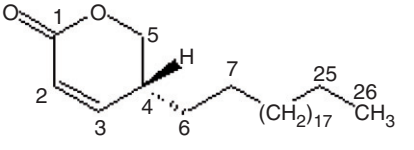
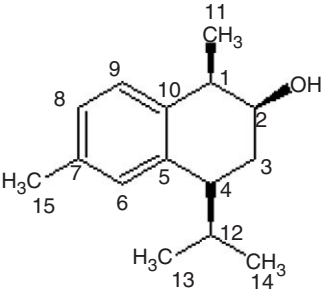
exhibited absorption bands due to a δ-lactone ring at 1720 cm^{-1} and conjugated C=C at 1640 cm^{-1}. Its positive FAB-MS exhibited a molecular ion peak at m/z 392, corresponding to the molecular formula $C_{26}H_{48}O_2$. It indicated three double bond equivalents, which were adjusted in the unsaturated lactone ring. The mass spectrum showed C_nH_{2n}, C_nH_{2n+2} and C_nH_{2n-1} ion peaks separated by 14 mass units, and the intensities of the fragments decreased on increasing molecular weight, suggesting a long aliphatic chain (Misra, Singh, Pandey, & Sharma, 1989; Stoianova-Ivanova, Hadjieva, & Papow, 1969). There was a sudden increase in the ion peak at m/z 295 $[(CH_2)_{20}CH_3]^+$ due to the expulsion of the δ-lactone having a mass unit of m/z 97 $[C_5H_5O_2]^+$. The ^{1}H-NMR spectrum of **1** displayed a double doublet at δ 5.63 ($J = 6.3$, 5.7 Hz), integrated for one proton, assigned to vinylic H-3. A doublet at δ 5.45 ($J = 6.3$ Hz) was ascribed to *cis*-oriented vinylic H-2. Two doublets at δ 4.16 ($J = 3.6$ Hz) and 4.13 ($J = 3.9$ Hz) were attributed to oxygenated methylene protons H_2-5. Broad multiplets at δ 1.61 with half widths of 8.6 Hz were accounted to a β-oriented H-4 methine proton. The remaining methylene protons appeared at δ 1.31 ($1 \times CH_2$) and δ 1.18 ($19 \times CH_2$). The primary methyl protons Me-26 appeared as a three-proton triplet at δ 0.84 ($J = 7.2$ Hz). The ^{13}C-NMR spectrum exhibited important carbon signals at δ 173.61 assigned for a C-1 ester carbon and at δ 130.85 and 128.78 for C-2 and C-3 vinylic carbons, respectively. The methylene carbons appeared in the range between δ 37.09 and 22.65. The terminal methyl carbon signal appeared at δ 14.09. The ^{1}H–^{1}H COSY spectrum of **1** showed correlations of H_2-5 with H-4 and H-3. The HMBC spectrum of **1** exhibited the interaction of C-1 with H-2 and C-5 with H-4 and H-3. Based on this evidence, the structure of **1** has been elucidated as *n*-hexacos-2-enyl-1,5-olide. This is a new δ-lactone isolated from a plant source for the first time.



δ-Lactone type compounds have also been isolated from *Malvastrum coromandalianum* (Alam, Chopra, Ali, & Niwa, 1996), *Phyllanthus fraternus* (Gupta & Ali, 2000), *Desmotrichum fimbrianum* (Roy, Ali, Sharma, & Ramachandran, 2002) and *Oryza sativa* (Chung et. al., 2007).

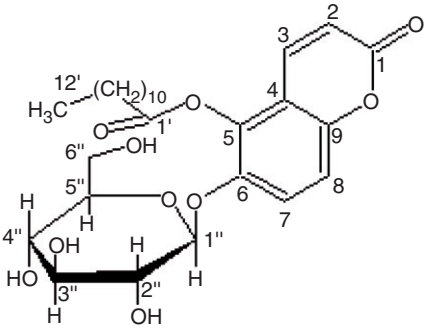
Compound **3**, designated as altheacalamene, was obtained as a brown amorphous powder from chloroform–methanol (99 : 1). Its IR spectrum showed absorption bands for a hydroxyl group (3470 cm^{-1}) and an aromatic ring (1525, 1081 cm^{-1}). Its FAB-MS displayed a molecular ion peak at m/z 218 corresponding to the molecular formula of a hydroxycalamene type sesquiterpene, $C_{15}H_{22}O$. The important ion fragments arising at m/z 203 $[M-ME]^+$, 175 $[M-C_3H_7]^+$, 160 $[175-Me]^+$ and 145 $[160-Me]^+$ indicated the location of one hydroxyl, one isopropyl and two methyl functions in the molecule. The ^{1}H-NMR spectrum of **3** showed two doublets at δ 7.50 ($J = 9.2$ Hz) and 6.92 ($J = 3.0$ Hz) assigned to ortho-coupled H-9 and meta-coupled H-6, respectively. A double doublet at δ 6.53 with coupling interactions of 9.2, 3.0 Hz was attributed to an ortho, meta-coupled H-3 proton. A broad multiplet at δ 4.35 with a half width of 16.7 Hz was ascribed to an α-oriented carbinol H-2 proton. A broad signal at δ 2.50 was accounted to C-15 methyl protons

attached to a C-7 aromatic carbon. Three doublets at 1.36 ($J = 6.3$ Hz), 1.24 ($J = 6.1$ Hz) and 0.86 ($J = 6.9$ Hz), all integrated for three protons each, were associated to C-11, C-13 and C-14 secondary methyl protons, respectively. Two multiplets at δ 2.27 ($w_{1/2} = 14.5$ Hz) and 2.09 ($w_{1/2} = 14.1$ Hz) and a multiplet at δ 1.89 were assigned to methine H-4, H-1 and methylene H_2-3 protons, respectively. The presence of the half width of 14.5 Hz of the signal at δ 2.27 and of 14.1 Hz of the signal at δ 2.09 suggested α-orientation of the methine H-4 and H-1 protons, respectively. The ^{13}C-NMR spectrum of **3** showed the presence of one carbinol carbon at δ 67.53 (C-2), aromatic carbons between δ 115.28 and 131.64, methyl carbons between δ 15.61 and 28.40, a methylene carbon at δ 23.33 (C-3) and methine carbons at δ 29.86 (C-1) and 28.40 (C-4). The DEPT spectrum of **3** exhibited the presence of four methyl, one methylene, seven methine and three quaternary carbons. The HMBC spectrum of **3** showed correlations of C-2 with H-1, H_2-3 and H_3-11, and C-7 with H_3-15, H-6 and H-8. On the basis of the above mentioned data, the structure of **3** has been elucidated as 2β-hydroxycalamene.



Compound **6**, named altheaecoumaryl glucoside, was obtained as a colourless mass from chloroform–methanol (4 : 1) eluants. Its UV spectrum revealed absorption maxima at 255, 301 and 345 nm, indicating the coumarin nature of the molecule. Its IR spectrum showed characteristic absorption bands for a hydroxyl group at 3350 and 3210 cm^{-1}, an ester group at 1726 cm^{-1}, conjugated C=C at 1620 cm^{-1} and an aromatic ring at 1056 cm^{-1}. The FAB-MS of compound **6** displayed a molecular ion peak at m/z 522, consistent with the molecular formula of coumarin glycoside combined with C-12 ester $C_{27}H_{38}O_{10}$. The important ion peaks arising at m/z 359 $[M-C_6H_{11}O_5]^+$, 343 $[M-C_6H_{11}O_5]^+$, 163 $[C_6H_{11}O_5]^+$, 183 $[CO(CH_2)_{10}CH_3]^+$, 339 $[M-183]^+$ and 176 $[359-183]^+$ suggested the location of one sugar unit and C_{12}-ester linkage attached to the coumarin. The ^{1}H-NMR spectrum of **6** displayed two sets of ortho-coupled doublets, one-proton each, at δ 7.51 ($J = 9.2$ Hz) and 6.87 ($J = 9.2$ Hz) assigned correspondingly to aromatic H-7 and H-8 protons. Two doublets at δ 6.63 ($J = 7.5$ Hz) and 5.34 ($J = 7.5$ Hz) were attributed to *cis*-oriented vinylic H-3 and H-2 protons, respectively. The anomeric proton H-1″ of the sugar molecule appeared as a doublets at δ 5.06 ($J = 7.1$ Hz). The other sugar protons resonated between δ 4.13 and 3.66. The methylene H_2-2′ proton adjacent to an ester group appeared as two one-proton doublets at δ 2.30 ($J = 6.5$ Hz) and 2.27 ($J = 7.5$ Hz). The remaining methylene protons resonated at δ 1.59 (CH_2-3′) and 1.25 (8 × CH_2). A triplet at δ 0.87 ($J = 6.6$ Hz) integrating for three protons was accounted to primary Me-12′ methyl protons. The ^{13}C-NMR spectrum of **6** displayed a carbon signal at δ 177.55 assigned to the carbonyl carbon C-1. The aromatic carbons resonated between δ 146.70 and

112.30. The ester carbon C-1′ appeared at δ 174.01, whereas the adjacent carbon C-2′ and methyl carbon C-12′ resonated at δ 55.97 and 14.06, respectively. A signal at δ 102.74 was accounted to anomeric C-1″ protons. The remaining signals between δ 73.87 and 61.74 were accounted to other carbons of the sugar unit. The HMBC spectrum of **6** exhibited the correlation of C-6 with H-7 and H-1″; C-1 with H-2 and H-3; and C-1′ with H_2-2′. Acid hydrolysis of **6** yielded lauric acid and D-glucose. On the basis of this evidence, the structure of **6** has been established as 5,6-dihydroxycoumarin-5-dodecanoate-6-β-D-glucopyranoside. It is an unknown coumarin derivative isolated from a natural or synthetic source for the first time.



3. Experimental

3.1. *General experimental procedures*

Melting points were determined on a Perfit melting point apparatus and are uncorrected. IR spectra were recorderd on KBr pellets using a Jasco FT-IR-5000 instrument. UV spectra were scanned in methanol on a Lamda Bio 20 spectrophotometer. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded on an Advance Dry 400, Bruker Spectrospin in $CDCl_3$. Mass spectra were measured in FAB ionisation mode with a JEOL-JMS-DX 303.

Silica gel G (Qualigens, 60–120 mesh, Mumbai, India) was used for column chromatography. Silica gel G (Qualigens, Mumbai) was used for analytical thin-layer chromatography (TLC). Spots were visualised by exposure to iodine vapour, UV radiation and by spraying reagents.

3.2. *Plant material*

The seeds of *A. officinalis* were purchased from Khari Baoli market, Delhi, and identified by Dr M.P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. A voucher specimen (no. PRL/JH/07/06) has been deposited in the Herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

3.3. *Extraction*

The air dried defatted powdered seeds (3 kg) of *A. officinalis* were extracted with ethanol in a Soxhlet apparatus for 72 h. The ethanolic extract was concentrated to

obtain a dark brown viscous mass (475 g). A small portion (2 g) of the extract was analysed chemically to determine the presence of different chemical constituents.

3.4. *Isolation*

The viscous extracted mass was adsorbed on silica gel (60–120 mesh) to form slurry. The slurry was air dried and chromatographed over a silica gel column packed in petroleum ether. The column was eluted with petroleum ether, a mixture of petroleum ether and chloroform (9 : 1, 3 : 1, 1 : 1 and 1 : 3), pure chloroform and finally a mixture of chloroform and methanol (99.5 : 0.5, 99 : 1, 49 : 1, 19 : 1, 9 : 1, 4 : 1, 3 : 2 and 1 : 1). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions (having the same R_f values) were combined and crystallised. The isolated compounds were recrystallised to obtain the pure compounds. The following compounds were isolated from the *A. officinalis* seed extract.

3.4.1. *Altheahexacosanyl lactone (**1**)*

Elution of the column with a petroleum ether–chloroform (9 : 1) mixture furnished a colourless mass of **1**, recrystallised from acetone. R_f: 0.23 (*n*-hexane); m.p. = 68–69°C; 98.5 mg = (0.0003% yield); UV (MeOH) $\lambda_{max} = 201$ nm (log ε 3.6); IR (KBr) $\nu_{max} = 2923, 2843, 1720, 1640, 1470, 1280, 1120, 702\,cm^{-1}$; ^{1}H-NMR ($CDCl_3$) = 5.63 (H, dd, $J = 6.3$, 5.7 Hz, H-3), 5.45 (1H, $J = 6.3$ Hz, H-2), 4.16 (1H, d, $J = 3.6$ Hz, H_2-5a), 4.13 (1H, d, $J = 3.9$ Hz, H_2-5b), 1.61 (1H, brm, $w_{1/2} = 8.6$ Hz, H-4b), 1.31 (2H, brs, CH_2), 1.18 (38H, brs, 19 × CH_2), 0.84 (3H, t, $J = 7.2$ Hz, Me-26); ^{13}C-NMR ($CDCl_3$) = δ 173.61(C-1), 130.85 (C-2), 128.78 (C-3), 68.13 (C-5), 38.72 (C-4), 37.09 (CH_2), 34.05 (CH_2), 32.75 (CH_2), 31.92 (CH_2), 31.59 (CH_2), 30.34 (CH_2), 29.69 (11 × CH_2), 28.93 (CH_2), 24.92 (CH_2), 22.65 (CH_2), 14.09 (CH_3); FAB-MS $m/z = 392$ $[M]^+(C_{26}H_{48}O_2)$ (5.9), 349 (1.1), 321 (1.2), 295 (23.6), 281 (5.3), 239 (5.7), 169 (7.2), 155 (8.9), 141 (10.2), 123 (11.5), 113 (12.0), 97 (100), 85 (41.5).

3.4.2. *Lauric acid (**2**)*

Elution of the column with chloroform afforded colourless crystals of **2**, recrystallised from methanol. m.p. = 45–46°C; lit. m.p. = 44–48°C (O'Neil, 2001); 100 mg (0.00033% yield); UV (MeOH) $\lambda_{max} = 207$ nm; IR (KBr) $\nu_{max} = 3305, 2975, 2854, 1700, 1272, 795\,cm^{-1}$; ^{1}H-NMR (DMSO-$d_6$) = δ 2.20 (1H, d, $J = 7.2$, 5.7 Hz, H_2-2a), 2.17(1H, d, $J = 7.2$ Hz, H_2-2b), 1.47 (2H, brs, H_2-3), 1.27 (16H, brs, 8 × CH_2), 0.85 (3H, t, $J = 6.2$ Hz, Me-12); ^{13}C-NMR ($CDCl_3$) = δ 183.12 (C-1), 39.96 (CH_2), 33.97 (CH_2), 31.84 (CH_2); 29.62 (5 × CH_2), 24.84 (CH_2), 22.60 (CH_2), 14.04 (CH_3); FAB-MS $m/z = 201[M + H]^+(C_{12}H_{24}O_2)$.

3.4.3. *Altheacalamene (**3**)*

Elution of the column with chloroform–methanol (99 : 1) yielded a brown amorphous powder of **3**, recrystallised from chloroform–ethyl acetate (1 : 5); $R_f = 0.68$ (petroleum ether, chloroform, methanol, 1 : 4 : 1); m.p. = 270°C; 120 mg (0.0004% yield); UV (MeOH) $\lambda_{max} = 217, 285, 303$ nm (log ε 4.3, 5.1, 3.1, 2.1); IR (KBr) $\nu_{max} = 3470, 2936, 2850, 1651, 1525, 1400, 1081\,cm^{-1}$; ^{1}H-NMR (DMSO-$d_6$) = δ 7.50 (1H, d, $J = 9.2$ Hz,

H-9), 6.92 (1H, d, $J = 3.0$ Hz, H-6), 6.53 (1H, dd, $J = 9.2$, 3.0 Hz, H-8), 4.35 (1H, brm, $w_{1/2} = 16.7$ Hz, H-2α), 2.50 (3H, brs, Me-15), 2.27 (1H, m, $w_{1/2} = 14.5$ Hz, H-4), 2.09 (1H, m, $w_{1/2} = 14.1$ Hz, H-1), 1.89 (2H, m, H_2-3), 1.36 (3H, d, $J = 6.3$ Hz, Me-11), 1.24 (3H, d, $J = 6.1$ Hz, Me-13), 0.86 (3H, d, $J = 6.9$ Hz, Me-14); ^{13}C-NMR (DMSO-d_6) = δ 29.86 (C-1), 67.53 (C-2), 23.33 (C-3), 28.40 (C-4), 128.13 (C-5), 127.42 (C-6), 131.64 (C-7), 115.28 (C-8), 125.62 (C-9), 128.69 (C-10), 15.61 (C-11), 29.86 (C-12), 18.26 (C-13), 23.33 (C-14), 28.40 (C-15); FAB-MS $m/z = 218$ $[M]^+(C_{15}H_{22}0)$ (11.7), 203 (15.8), 201 (9.6), 175 (42.3), 160 (18.1), 145 (63.2).

3.4.4. *β-Sitosterol (**4**)*

Elution of the column with chloroform–methanol (19 : 1) gave colourless crystals of **4**, recrystallised from methanol. $R_f = 0.30$ (chloroform : methanol, 1 : 1); m.p. = 138–139°C; lit. m.p. 140°C (O'Neil, 2001); 120 mg (0.0004% yield); UV (MeOH) $\lambda_{max} = 205$ nm; IR (KBr) $\nu_{max} = 3465$, 2955, 2845, 1640, 1475, 1365, 1210, 1105 cm^{-1}; ^{1}H-NMR ($CDCl_3$) = δ 5.30 (d, $J = 5.5$ Hz, H-6), 3.51(1H, brs, $w_{1/2} = 16.5$ Hz, H-3α), 1.01(3H, brs, Me-19), 0.97 (3H, d, $J = 6.5$ Hz, Me-21), 0.86 (3H, d, $J = 6.0$ Hz, Me-26), 0.83 (3H, d, $J = 6.03$ Hz, Me-29), 0.81 (3H, d, $J = 6.0$ Hz, Me-27), 0.67 (3H, brs, Me-18). FAB-MS $m/z = 414$ $[M]^+$ ($C_{29}H_{50}O$).

3.4.5. *Lanostanol (**5**)*

Elution of the column with chloroform–methanol (9 : 1) afforded a colourless crystalline mass of **5**, recrystallised from methanol; $R_f = 0.86$ (*n*-butanol, acetic acid, water, 5 : 1 : 2); m.p. = 138–140°C; lit. m.p. 138–140°C (Nes et al., 1989); 100 mg (0.00033% yield); UV (MeOH) $\lambda_{max} = 209$ nm (log Є 5.3).; IR (KBr) $\nu_{max} = 3404$, 2919, 2850, 2851, 1607, 1401, 1262, 1198, 129, 1095, 1025, 803 cm^{-1}; ^{1}H-NMR (DMSO-d_6) = δ 3.67 (1H, dd, $J = 5.5$, 9.1 Hz, H-3α), 1.23 (9H, brs, Me-28, Me- 29, Me-30), 1.16 (3H, brs, Me-19), 1.08 (3H, d, 71, $J = 6.7$ Hz, Me-21), 1.06 (3H, brs, Me-18), 0.85 (3H, d, $J = 6.8$ Hz, Me-26), 0.83 (3H, d, $J = 6.8$ Hz, Me-27); FAB-MS $m/z = 430[M]^+(C_{30}H_{54}0)$.

3.4.6. *Althaeacoumaryl glucoside (**6**)*

Elution of the column with a chloroform–methanol (4 : 1) mixture furnished a colourless mass of **6**, recrystallised from methanol. $R_f = 0.82$ (chloroform–ethyl acetate; 4 : 1); m.p. = 93°C; 150 mg (0.0005% yield); UV (MeOH) $\lambda_{max} = 229$, 255, 301, 345 nm (log ε 4.1, 3.6, 3.5, 3.9); IR (KBr) $\nu_{max} = 3350$, 3210, 2926, 2850, 1726, 1620, 1460, 1056 cm^{-1}; ^{1}H-NMR ($CDCl_3$) = δ 7.51(1H, d, $J = 9.2$ Hz, H-7), 6.87(1H, d, $J = 9.2$ Hz, H-8), 6.63 (1H, d, $J = 7.5$ Hz, H-3), 5.34 (1H, d, $J = 7.5$ Hz, H-2), 5.06 (1H, d, $J = 7.1$ Hz, H-1″), 4.13 (1H, m, H-5″), 3.86 (1H, dd, $J = 6.5$, 7.1 Hz, H-3), 3.84 (1H, brs, H-3″), 3.81 (1H, brs, H-4″), 3.66 (2H, brs, H_2-6″), 2.30 (1H, d, $J = 7.5$ Hz, H_2-2′a), 2.27 (1H, d, $J = 7.5$ Hz, H_2-2′b), 1.59 (2H, brs, CH_2-3′), 1.25 (16H, brs, $8 \times CH_2$), 0.87 (3H, t, $J = 6.5$ Hz, Me-12′);^{13}C-NMR ($CDCl_3$): δ 177.55 (C-1), 112.30 (C-2), 114.2 (C-3), 119.25 (C-4), 146.70 (C-5), 143.31 (C-6), 129.24 (C-7), 128.55 (C-8), 127.04 (C-9), 174.01(C-1′), 55.97 (C-2′), 36.96 (C-3′), 33.97 (C-4′), 31.85 (C-5′), 29.63 (C-6′), 29.63 (C-7′), 27.17 (C-8′), 24.71 (C-9′), 22.61 (C-10′), 20.75 (C-11′), 14.06 (C-12′), 102.74 (C-1″), 71.85 (C-2″), 70.11 (C-3″), 68.62 (C-4″), 73.87 (C-5″),

61.74 (C-6″); FAB-MS $m/z = 522[M]^+$ ($C_{27}H_{38}O_{10}$) (7.8), 359 (18.3), 343 (9.5), 339 (22.7), 183 (16.4), 176 (12.5), 163 (5.3), 155 (33.2), 113 (27.1), 97 (67.8).

3.4.6.1. *Hydrolysis of* ***6***. Compound **6** (40 mg) was dissolved in ethanol (5 mL), to which conc. HCl (2 mL) was added and the reaction mixture heated for 1 h on a steam bath. It was cooled and extracted with petroleum ether (3×5 mL) to separate lauric acid (co-TLC comparable). The reaction mixture was dried under reduced pressure and the residue dissolved in chloroform to isolate 5,6-dihydroxycoumarin, IR $\nu_{max} = 1721$, 3410, 3250 cm^{-1}, $[M]^+$ 178 $[C_9H_6O_4]^+$. The residue was dissolved in a minimum amount of water and chromatographed on silica gel TLC with a standard sample of D-glucose using EtOAc–HOAc–H_2O–MeOH (6 : 1 : 1 : 2), $R_f = 0.4$.

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