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Research Article

Biologically Active Long-chain Aliphatic Alcohols and Esters from the Bark of *Symplocos racemosa*

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ABSTRACT

The phytochemical investigation of the petroleum ether and chloroform extract from the bark of stem of *Symplocos racemosa* Roxb. yielded four long chain alcohols, n- hexacosanol (1), n-octacosanol (2), nonaeicosanol (3) and n-hentriacontanol (4) and two long chain esters, methyl triacontanoate (5) and tricontyl palmitate (6) were isolated from this plant. These structures were elucidated by spectroscopic methods.

Keywords: *Symplocos racemosa*, Symplocaceae, long chain alcohols and esters.

INTRODUCTION

Symplocos racemosa Roxb., colloquially known as Lodh or Lodhra, belongs to family Symplocaceae. In Ayurveda, the bark of *S. racemosa* is frequently used for gynecological disorders such as menorrhagia, leucorrhea, vaginal fibroids and itching[1,2]. It is also recommended for the treatment of diarrhea and dysentery as an astringent[3]. Lodhrasava, a fermented polyherbal ayurvedic ancient preparation, containing bark of *Symplocos racemosa* as a major ingredient, is used as uterine tonic[4], digestive and laxative as well as for the treatment of urinary tract disorders, liver ailments, anemia, irritable bowel syndrome and ulcers[5]. Decoction of its bark is used as gargle for improving the health of inflamed and bleeding gums[6]. Its paste is topically applied for treating acne and suppressing the malodor of the body due to its antibacterial and antimicrobial properties[7,8]. Studies conducted on animals showed that the aqueous extract of the bark of this plant significantly increased the FSH and LH levels and ethanolic extract showed antifibrinolytic activity[6]. These studies have validated its traditional use as a medicine for gynecological disorders. Phenolic glycosides, glycosides, substituted glycosides, triterpenoids and long chain aliphatic alcohols and esters were previously isolated from this plant. Phenolic glycosides, substituted glycoside, and glycosides are reported for their inhibitory activity against snake venom phosphodiesteraseI, thymidine phosphorylase, human nucleotide pyrophosphatase phosphodiesterase, plasma cell antigen 1(PC-1), lipoxygenase, alpha-glucosidase and urease[9-18]. Inhibitors of PC-1 are being studied for treatment for some forms of arthritis suggesting that Lodhra may be a potential source of anti-arthritic medicine. In view of our interest in axial malodor suppressant natural products we have reinvestigated this plant.

RESULTS AND DISCUSION

Recently the use of long-chain aliphatic alcohols and esters as nutraceuticals and cosmetics ingredients respectively has been phenomenally increased. Long-chain aliphatic alcohols collectively known as policosanol, are being used for the treatment of various chronic diseases including diabetes and hypercholesterolemia[19-21]. Long chain aliphatic esters especially are used in cosmetic products formulations[22]. In view of the above cited commercial importance of these compounds, we have separated fat from petroleum ether and chloroform extracts of the bark of *Symplocos racemosa*. This fatty portion on chromatographic separation afforded biologically active long chain alcohols (**1-4**) and esters (**5-6**).

The mass spectrum of compound **1** showed the molecular ion peak at m/z 382 corresponding to molecular formula $C_{26}H_{54}O$. Its IR spectrum displayed the presence of a hydroxyl group (3440 cm^{-1}). A three proton triplets at δ 0.86 and a two proton triplet at δ 3.61 suggested the presence of a terminal methyl group and a methylene attached to hydroxyl group respectively. A quintet integrated for two protons appeared at δ 1.55 was assignable to methylene group attached to hydroxyl methyl group and a broad singlet at δ 1.24 integrated for 46 protons suggested the presence of 23 methylene groups. The presence of long aliphatic chain in compound **1** was further confirmed from its mass spectrum which showed a loss of 14 amu units (-CH_2 units) between a number of ion peaks. These spectral data were similar to those reported in literature for n- hexacosanol[23]. Thus compound **1** was identified as n- hexacosanol.

The mass spectrum of compound **2**, **3** and **4** showed the molecular ion peak at m/z 410, m/z 424 and m/z 452 corresponding to $C_{28}H_{58}O$, $C_{29}H_{60}O$ and $C_{31}H_{64}O$ respectively. The IR and ^1H NMR of these compounds were similar to those of compound **1** suggesting that these compounds are also long chain alcohols. Compounds 3, 4 and 5 were identified as n-octacosanol[24,25],

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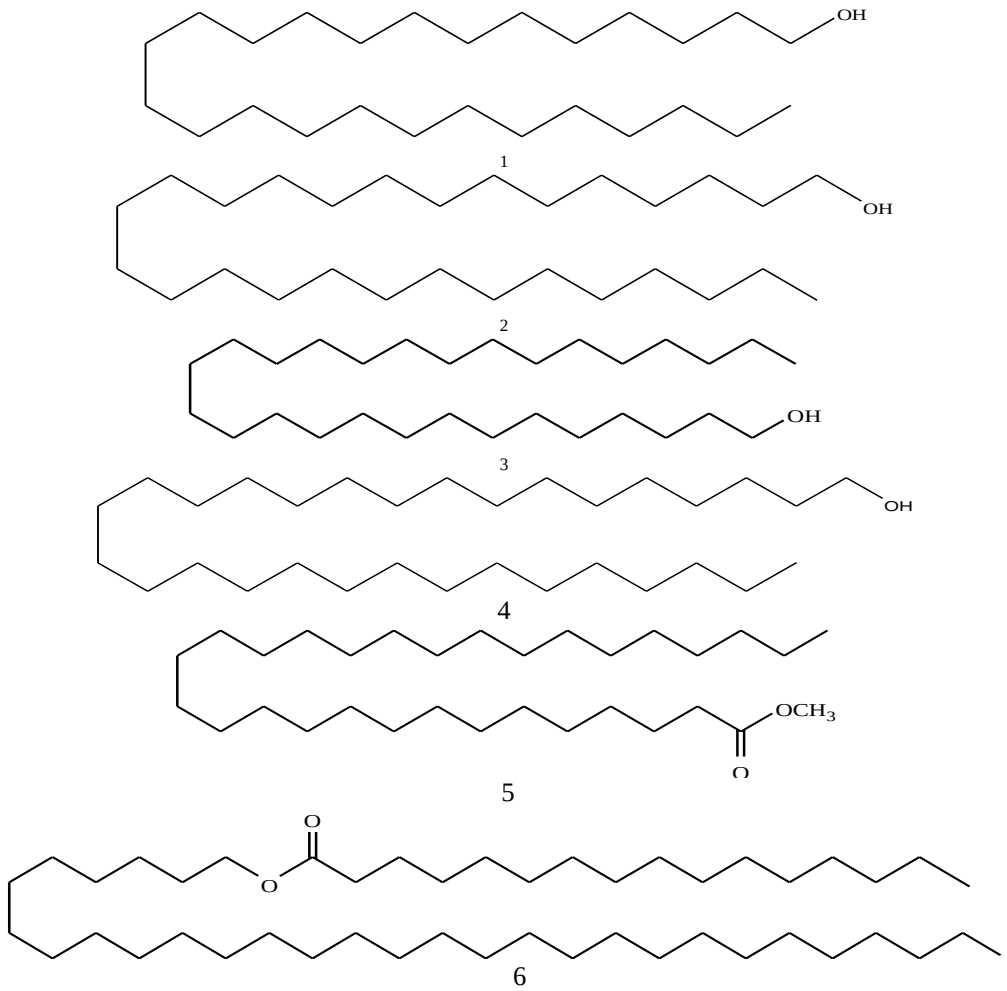

nonaeicosanol[26] and n-hentriacontanol[26] respectively by comparing their spectral data with those reported in literature.

The mass spectrum of compound **5** showed a molecular ion peak at m/z 466 which was in agreement with molecular formula $C_{31}H_{62}O_2$. Its IR spectrum showed absorption at 1741 cm^{-1}(C=O, ester). The ^1H NMR data of this compound suggested that its long chain was identical to compound **3** however, the peak for hydroxyl methyl group was not observed. The presence of a methoxyl group at δ 3.61(s) and a CH_2 attached to carbonyl carbon at δ 2.25 in its ^1H NMR spectrum revealed the presence of a methyl ester group in the molecule**.** The functionalities noted above were further supported by the mass spectrum which gave characteristic fragmentation of methyl ester group at m/z 73$[CH_3OOCCH_2]^+$ and 59$[CH_3OC{=}O]^+$. These spectral data which were similar to those reported for methyl triacontanoate led to assignment of structure 5 as methyl triacontanoate [27].

Compound **6** was assigned the molecular formula $C_{46}H_{92}O_2$ from its mass spectrum which had $[M]^+$ at m/z 676. Compound **6** was confirmed to be a long chain fatty ester by comparing its spectral data with those of compound **5**. In its ^1H NMR spectrum, the absence of a signal for methoxyl group at δ 3.61(s) and the presence of a triplet at δ 4.01 characteristics for a ($COOCH_2$) group suggested that in its molecule ester group is the part of long chain . A six proton triplet observed at δ 0.82 was assigned to two terminal methyl groups. Compound 6 was identified as tricontyl palmitate[28] by comparing its spectral data with those reported in the literature.

Experimental

Silica Gel (Si, 60-120 mesh, E.Merck) was used as adsorbents for column chromatography. Qualitative and quantitative thin layer chromatography were conducted on TLC aluminum sheets kieselgel 60 F_{254} pre-coated ((20×20cm)), layer thickness 0.25 mm (Merck) and glass plates of uniform thickness (approx. 0.5 mm) size(20×20cm) coated with silica gel GF-254 respectively. Spots were visualized by heating silica gel plates at 80ºC in UV chamber/ iodine chamber.

Spray Reagent: Ceric sulphate reagent and 10%H_2SO_4 were used for the detection of compounds.

Ceric sulphate Reagent: Ceric sulphate (0.1 g) and trichloroacetic acid (1g) were dissolved in 4 ml distilled water. The solution was boiled and conc. H_2SO_4 was added drop wise until the disappearance of turbidity.

The IR spectra were recorded on Shimadzu FTIR-8400S, spectrophotometer with KBr pellets. NMR spectra were obtained on Bruker DRX- 300 FT NMR spectrometer for ^1H at 300 MHz. EIMS data were generated on Joel SX-102 mass spectrometer instrument in the positive ion mode.

Plant Material: The air dried bark of *Symplocos racemosa* (Symplocaceae) was purchased from a local vendor of traditional medicine and its genuineness was established by Pharmacognosy studies.

Extraction and Isolation: The air-dried and grounded bark of *S. racemosa* (6 kg) was extracted successively with Pet –ether (60-80°C) and chloroform on water bath (60-80°C).The petroleum ether and chloroform extracts were filtered hot, concentrated under reduced pressure and yielded 35 g and 30 g semi solid mass respectively.

Precipitation of Fat from Petroleum ether and Chloform extracts

The petroleum ether extract was treated with acetonitrile and aliquot of the acetonitrile phase was transferred into a centrifuge tube and stored in a freezer for 2 hours, where-with the major part of fat precipitated. The precipitate was separated by decantation and dried (10gm). Acetonitrile phase was also combined, filtered and evaporated to dryness. Chloroform extract was also treated similarly and afforded 8.0 gm fat.

The fat obtained from both the extracts were combined and re-dissolved in n-hexane and loaded into a silica gel column, chromatographed and following fractions were collected; 1(Pet ether: chloroform 3:1), n- Hexacosanol, n- Octacosanol, 2(Pet ether: chloroform 1:1) n-Hentriacontanol, nonaeicosanol, 3(Pet ether: chloroform 1:3) methyl triacontanoate, tricontyl palmitate.

Compound 1: White crystals (15 mg,), $C_{26}H_{54}O$, m.p. 78-80 °C; IR (KBr): ν_{max} (cm^{-1}) 3440, 2895, 1451, 1056 cm^{-1}. ^1H NMR [300 MHz, $CDCl_3$]: (δ ppm) 3.61(2H, t, *J* = 6.7 Hz), 1.55 (2H, quint), 1.24 (46 H, sbr,), 0.86 (3H, t, *J* = 6.8 Hz, H-26). EIMS (rel.int. %): *m/z* 382 $[M]^+$ (3.9), 364 $[M\text{-}H_2O]^+$ (100).

Compound 2: Light yellow colour (8 mg), $C_{28}H_{58}O$, m.p. 83°C; IR (KBr) υ_{max} (cm^{-1}): 3440(OH), 2850 (C-H stretching), 1059(C-O stretching), 730 and 720 $(CH_2)_n$. ^1H NMR [300 MHz, $CDCl_3$] : (δ ppm) 3.61 (2H, t, J=6Hz), 1.55 (2H, quint), 1.23 (52 H ,sbr,), 0.84 (3H, t, J=6.6 Hz,) EIMS (m/z): 410 $[M]^+$, 392 $[M\text{- }18]^+$, 377, 363

Compound 3: Amorphous powder(9 mg) , $C_{29}H_{60}O$, m.p. 83°C; IR (KBr) υ_{max} (cm^{-1}): 3442 (OH), 2914, 2850 (C–H), 1460 (OH, def.), 1032 (C–O, str.), 756, 669 cm^{-1}; ^1H-NMR [300 MHz, $CDCl_3$]: (δ ppm) 3.64 (2H, t, J= 6.62 Hz, H-1), 1.55 (2H ,quint.) ,1.24 (52H, sbr), 0.87 (3H, t, J= 6.50 Hz). EIMS (rel.int.%) m/z 424 $[M]^+$ (11.4), 409 (3), 393 (17.4), 381 (2.1), 364 (14.1), 337 (3.7), 280 (13), 279 (11.2), 211 (28), 196 (3.4), 195 (6.4), 183 (3.2), 167 (18), 155 (10.3), 85 (19.1), 83 $[CH{=}CH(CH_2)_3CH_3]^+$ (92.6), 57 $[C_4H_9]$ (100).

Compound 4: White opaque waxy crystals (11 mg), $C_{31}H_{64}O$; m.p. 67-69°C; IR (KBr) ν_{max} (cm^{-1}): 3401-3152, 2950, 2850, 1480, 1460, 1080, 1050, 730-720, 710 cm^{-1} . ^1HNMR [300 MHz, $CDCl_3$]: (δ ppm) 0.88 (3H, t, J=7.5Hz), 1.23 (58H, sbr), 3.63 (2H, t, j=7.5 Hz); EIMS (rel. int.,%); m/z 452 (M^+; 0.12), 434(0.15), 420(4.8), 392(6.2), 364(0.2), 335(0.1), 307(2.8), 279(2), 251(3), 223(4), 195(8), 167(8), 139(14.1), 111(34), 97(64.2), 57(100), 55(61.9).

Compound 5: Colourless gummy solid (12 mg): $C_{31}H_{62}O_2$; IR (KBr) υ_{max} (cm^{-1}): 2920, 2850 (C–H), 1742 (C = O, ester), 1170 (C-O, str.), 880 720, 651 cm^{-1}. ^1H-NMR [300 MHz, $CDCl_3$] (δ ppm) 3.62 (3H, s), 2.26 (2H, t, J = 7.5 Hz), 1.59 (2H, quint., J = 7.5 Hz), 1.26 (52H, sbr), 0.86 (t, 3H, J = 6.90 Hz). EIMS m/z (rel. int. %): 466 $[M]^+$ (2.3), 438 (8.8), 424 (1.9), 410 (20.6), 396 (3.9), 382 (19.3), 368 (5.3), 354 (13.2), 340 (1.8), 326 (3.9), 312 (1.2), 298 (11.6), 284 (2.2), 270 (7.4), 101 $[CH_3O\text{-}C = (O^+H)\text{–}CH_2CH = CH_2]^+$ (37.3), 87 $[HOOC\text{–}(CH_2)^3]^+$ (89.1), 73 $[CH_3OOCCH_2]^+$ (61.3), 71 $[C_5H_{11}]^+$ (50.8), 59 $[CH_3OC = O]^+$ (83.6), 57 $[C_4H_9]^+$ (100).

Compound 6: White powder(11 mg), $C_{46}H_{92}O_2$, m.p. 72-74°C; IR (KBr) υ_{max} (cm^{-1}): 2918, 2850 (C–H), 1734 (C= O, ester), 1170 (C–O, str.), 760, 729, 719, 669cm^{-1}; ^1H-NMR [300 MHz, $CDCl_3$] (δ ppm): 4.03 (t, 2H, J= 6.7 Hz), 2.25 (2H,t, J= 7.5 Hz,), 1.57 (4H, quint., J= 7.01 Hz), 1.28 (78H, sbr,), 0.83 (6H, t, J= 6.90 Hz,). EIMS m/z (rel. int. %): 676 $[M]^+$ (4.2), 648 (19.4), 634 (5.2), 620 (27.5), 606 (8.1), 592 (45), 578 (16.3), 564 (56.9), 550 (2.4), 485 (1.8), 381 (2.6), 369 (6.8), 364 (5.4), 270 $[{\cdot}CH_2\text{-}^+OH\text{-}CO\text{-}(CH_2)_{14}CH_3]^+$ (6.8), 271 $[CH_3\text{-}^+OH\text{-}CO\text{-}(CH_2)_{14}CH_3]^+$ (13.5), 256 $[{\cdot}O\text{-}C{=}(O^+H)\text{–}(CH_2)_{14}CH_3]^{\cdot+}$ (27.7), 257 $[HO\text{-}C = (O^+H)\text{-}(CH_2)_{14}CH_3]^+$ (72.1), 239 $[O = C\text{-}(CH_2)_7CH_3]^+$ (23.5), 71 $[C_5H_{11}]^+$ (69.4), 57$[C_4H_9]^+$ (100).

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REFERENCES

1. Nighantu B, In: Pandey GS (Ed). Edn 10, Chaukhambha Publications, 1998, 128.
2. Nadkarni KM. Indian Materia Medica, Edn10, Popular prakashan, Mumbai, 1996, 1186.
3. Ayurveda Sar Sangrah, Baidyanath Prakashan, 1998**,** 661.
4. Ali M, Bhutani KK, Srivastava TN. Triterpenoids from *Symplocos racemosa* bark. *Phytochemistry* 1990; 29: 3601-3604.
5. Dhaon R, Jain GK, Sarin JPS, Khanna NM *Indian Journal of Chemistry, Section B* 1989; 28: 982.
6. Bhutani KK, Jadhav AN, Kalia V. Effect of *Symplocos racemosa* Roxb. on gonadotropin release in immature female rats and ovarian histology. *Journal of Ethnopharmacology* 2004; 94: 197.
7. Khan MR, Kihara M, Omoloso AD Antimicrobial activity of *Symplocos cochinensis*. *Fitoterapia* 2001; 72: 825.
8. Acebey-Castellon IL, Voutquenne-Nazabadioko L, Doan Thi Mai H, Roseau N, Bouthagane N, Muhammad D, Le Magrex Debar E, Gangloff SC, Litaudon M, Sevenet T, Hung NV , Lavaud CJ. Triterpenoid saponins from *Symplocos lancifolia*. Journal of Natural Product 2011; 74: 163.
9. Abbasi MA, Ahmad VU, Zubair M, Fatima N, Farooq U, Hussain S, Lodhi MA, Choudhary MI. Phosphodiesterase and thymidine phosphorylase-

inhibiting salirepin derivatives from *Symplocos racemosa*. *Planta Medica* 2004; 70: 1189.
10. Badoni R, Semwal DK, Kothiyal SK, Rawat U. Chemical constituents and biological applications of the genus *Symplocos*. *Journal of Asian Natural Product Research* 2010; 12: 1069.
11. Todd MJ, Hausinger RP. *Journal of Biological Chemistry* 1989; 264: 15835.
12. Ahmad VU, Zubair M, Abbasi MA, Rashid MA, Rasool N, Khan SN, Choudhary MI, Kousar F. Structure determination of bioactive galloyl derivatives by NMR spectroscopy. *Magnetic Resonance in Chemistry* 2005; 43: 486.
13. Ahmad VU, Rashid MA, Abbasi MA, Rasool N, Zubair M. New salirepin derivatives from *Symplocos racemosa*. *Journal of Asian Natural Product Research* 2007; 9: 209.
14. Ahmad VU, Abbasi MA, Hussain H, Akhtar MN, Farooq U, Fatima N, Choudhary MI. Phenolic glycosides from *Symplocos racemosa*: natural inhibitors of phosphodiesterase I. *Phytochemistry* 2003; 63: 217.
15. Choudhary MI, Fatima N, Abbasi MA, Jalil S, Ahmad VU, Atta-ur-Rahman. Phenolic glyscosides, a new class of human recombinant nucleotide pyrophosphatase phosphodiesterase-1 inhibitors. *Bioorganic & Medicinal Chemistry* 2004; 12: 5793.
16. Lodhi MA, Abbasi MA, Choudhary MI, Ahmad VU. Kinetics studies on triacontanyl palmitate: a urease inhibitor. *Natural Product Research* 2007; 21: 721.
17. Ahmad VU, Lodhi MA, Abbasi MA, Choudhary MI. Kinetics study on a novel natural inhibitor of alpha-chymotrypsin. *Fitoterapia* 2008; 79: 505.
18. Miszczak-Zaborska E, Smolarek M, Bartkowiak J. Influence of the thymidine phosphorylase (platelet-derived endothelial cell growth factor) on tumor angiogenesis. Catalytic activity of enzyme inhibitors. *Postepy Biochemii*, 2010; 56: 61.
19. Yatcilla M. Policosanols: cholesterol solution or controversial problem? *Nutraceuticals World* 2002; 5: 28.
20. Taylor JC, Rapport L, Lockwood GB. Octacosanol in human health, *Nutrition* 2003; 19: 192.
21. Damgé C, Hillaire-Buys D, Koenig M, Gross R, Hoeltzel A, Chapal J, Balboni G, Borg J, Ribes G. *European Journal of Pharmacology* 1995; 274: 133.
22. http://www.epa.gov/hpv/pubs/summaries/alipestr/c13466rt7.pdf.
23. Ali AA, Sayed HM, Ibrahim SRM, Zaher AM**.** *Phytopharmacology* 2013; 4: 69.
24. Beyon JH. Mass Spectrometry and its Application to Organic Chemistry, Elsevier Publishing Company, Amsterdam, 1960, 346.
25. Biemann K. Mass Spectrometry, Organic Chemical Applications, McGraw Hill, Book Co. Inc., New York, 1962, 109.
26. Manorajani M, Korta S, Mehta BK. *Indian Journal of Chemistry* 1999; 38: 1148.
27. Cocker W, Shaw SJ. *Journal of the Chemical Society* 1962; 5: 194.
28. Abbasi MA, Ahmad VU, Zubair M, Nawaz SA, Lodhi MA, Farooq U, Choudhary MI. Lipoxygenase inhibiting ethyl substituted glycoside from Symplocos racemosa. *Natural Product Research* 2005; 19: 509-515.