INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

A NEW OXOTIRUCALLOIC ACID FROM THE STEM BARK OF *MANGIFERA INDICA* VAR. "*LANGRA*"

Ansari S. H.*, Ali Mohd., Naquvi Kamran Javed
Phytochemical Research Laboratory, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi 110062, India

Article Received on: 05/11/11 Revised on: 20/01/12 Approved for publication: 06/02/12

*Prof. S. H. Ansari, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi - 110 062, India
Email: shansari189@rediffmail.com

ABSTRACT
Phytochemical investigation of the ethanolic extract of the stem bark of *Mangifera indica* variety "*Langra*" yielded three new phytoconstituents characterized as n-docosan-18′-one-22′-ol-1′-yl hexanoate, 20(R), 24(R)-3-oxotirucalla-cis-1,24- dien-26-oic acid and *n*-dotriacont-7α-ol-3,13-dione along with β-sitosterol. The structures of these phytoconstituents have been elucidated on the basis of spectral data analysis and chemical reactions.
KEYWORDS: *Mangiferaindica* var. *Langra*, stem bark, hydroxydocasanyl heptanoate, tirucalloic acid, hydroxyl dotriacontane dione.

INTRODUCTION

Mangifera indica L. (Anacardiaceae), commonly known as Am or mango, is a large evergreen tree with a heavy dome shaped crown and straight, stout bole. It occurs throughout India, other parts of temperate Asia, southern Europe and America[1]. It is the prominent fruit crop and over 1,000 mango types are grown in various parts of India, each having its own peculiar taste, flavour and consistency of pulp. The mango stem bark is astringent, anthelmintic and used to treat haemoptysis, haemorrhage, nasal catarrh, diarrhoea, ulcers, diphtheria, rheumatism and for lumbrici[2]. The stem bark stops vomiting[3]. Aliphatic constituents, coumarin, mangiferine[4-6], sequiterpinenoids[6,7], triterpenoids[8,9] and phenolics[10,11] have been reported from the stem barks of different cultivars of *M. indica*. This paper describes the isolation and characterization of phytoconstituents from the bark of *Mangifera indica* var. *Langra*.

MATERIAL AND METHODS

General

The melting points were determined on a Perfit apparatus and are uncorrected. The IR spectra were recorded in KBr pellet on Win IR FTS 135 instrument (Biorad, USA). ^{1}H (300 MHz) and ^{13}C (75 MHz) spectra were recorded by Bruker spectrospin NMR instrument in $CDCl_3$ using TMS as internal standard. EIMS were scanned at 70 eV on a Jeol D-300 instrument (Jeol, USA). Column chromatography was performed on silica gel (Merck, 60–120 mesh) and thin layer chromatography on silica gel G-coated TLC plates (Merck).

Plant material

Stem bark of *Mangifera indica* variety "*Langra*" was collected from Laharpur, Sitapur (U.P.) and identified by Prof. M. P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard (Hamdard University). A voucher specimen (No. PRL/JH/08/44) was deposited in the herbarium of the Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi, India.

Extraction and isolation of compounds

The dried and powdered stem bark (4.0 kg) was extracted with ethanol (95%) in a Soxhlet apparatus. The extracts were combined and the solvent evaporated under reduced pressure to obtain a dark brown viscous mass (480 g). The dried alcoholic extract was dissolved in minimum amount of methanol and adsorbed on silica gel to form slurry. The slurry was air-dried and chromatographed over silica gel column prepared in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol to isolate the following compounds:

Hydroxydocasanyl heptanoate (1)

Elution of the column with petroleum ether-chloroform (3:1) gave colourless crystals of **1**, recrystallized from chloroform-methanol (1:1), 8.0 g (0.2 %yield), R_f = 0.25 (petroleum ether-chloroform,1:3), m.p. 80-81° C; UV λ_{max} (MeOH): 210 nm (log ε 4.5); IR γ_{max} (KBr): 3410, 2920, 1735, 1710, 1465, 1375, 1260, 1180, 1030, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.10 (2H, t, *J*=7.0 Hz, H_2-1), 3.60 (1H, d, *J*=5.5 Hz, H_2-22′a), 3.53 (1H, d, *J*=6.0 Hz, H_2-22b), 2.20 (2H, m, H_2-2), 2.11 (2H, m, H_2-17′), 2.06 (2H, m, H_2-19′), 1.46 (4H, m, 2 × H_2) 1.20 (36H, brs, 18 × H_2), 0.93 (3H, t, *J*=6.0 Hz, Me-6); ^{13}C NMR ($CDCl_3$): 203.93 (C-18′), 171.47 (C-1), 65.80 (C-1′), 61.36 (C-22′), 51.24 (C-2), 36.76 (C-17′), 34.51 (C-19′), 29.81 (CH_2), 29.37 (CH_2), 29.12 (16 × CH_2), 24.94 (CH_2), 22.19 (CH_2), 14.33 (Me-6); EIMS *m/z* (rel.int): 454 $[M]^+$ ($C_{28}H_{54}O_4$) (2.4), 423 (2.9), 381 (14.2), 355 (17.5), 353 (9.7), 339 (21.1), 155 (14.1),143 (9.0), 141 (16.8),129 (11.2), 127 (24.1), 115 (23.6), 101 (12.5), 99 (24.3), 73 (48.2), 69 (65.2), 57 (100).

Mangtirucalloic acid (2)

Elution of the column with petroleum ether-chloroform (1:1) yielded a colourless amorphous mass of **2**, recrystallized from chloroform-methanol (1:1), 2.20 g (0.055% yield), R_f=0.55 (benzene-chloroform), m.p. 170-172°; $[\alpha]_D^{25}$=O° (C 1.0260, MeOH); UV λ_{max}: 227 nm (log ε 6.5); IR γ max (KBr): 3100, 2970, 2900, 1705, 1680, 1630, 1440, 1420, 1365, 1260, 935 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 6.03 (1H, d, *J*=6.0 Hz, H-1), 5.95 (1H, d, *J*=6.0 Hz, H-2), 5.16 (1H, d, *J*=3.5 Hz, H-24), 1.80 (3H, brs, Me-27), 1.01 (3H, brs, Me-19), 0.97 (3H, brs, Me-29), 0.95 (3H, d, *J*=6.0 Hz, Me-21), 0.90 (3H, brs, Me-28), 0.85 (3H, brs, Me-30); ^{13}C NMR ($CDCl_3$): 128.12 (C-1), 139.06 (C-2), 202.38 (C-3), 47.41 (C-4), 50.73 (C-5), 18.84 (C-5), 18.84 (C-6), 24.16 (C-7), 39.79 (C-8), 46.25 (C-9), 38.19 (C-10), 21.28 (C-11), 28.40 (C-12), 42.33 (C-13), 50.82 (C-14), 29.75 (C-15), 29.14 (C-16), 49.62 (C-17), 13.28 (C-18), 19.76 (C-19), 20.56 (C-20), 18.33 (C-21), 35.43 (C-22), 25.46 (C-23), 125.28 (C-24), 135.06 (C-25) 181.13 (C-26), 27.64 (C-27), 24.45 (C-28), 27.35 (C-29), 21.52 (C-30); EIMS *m/z* (rel.int): 454 $[M]^+$ ($C_{30}H_{62}O_3$)

(14.5), 439 (100), 410 (21.5), 395 (17.3), 313 (11.7), 290 (17.2), 236 (16.7), 218 (13.2), 190 (11.2), 164 (23.5), 141 (27.1), 136 (42.8), 121 (24.3).

Hydroxytriacontane dione (3)

Elution of the column with chloroform afforded colourless amorphous powder of **3**, recrystallized from chloroform-methanol (1:1), 3.2 g (0.08% yield), R_f=0.33 ($CHCl_3$), m.p. 100-102º; $[\alpha]^{D}_{25}$ = -18.2. (C 1.86, MeOH); UV λ_{max} (MeOH): 221 nm (log ε 5.6); IR γ_{max} (KBr): 3410, 2900, 2825, 1710, 1702, 1445, 1400, 1345, 1155, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.20 (1H, dd, *J*=6.5, 5.5, Hz, H-7), 2.60 (2H, m, H_2-12), 2.47 (2H, m, H_2-14), 2.01 (2H, m, H_2-4), 1.97 (2H, m, H_2-2), 1.62 (4H, m, 2 × CH_2), 1.52 (4H, m, 2 × CH_2), 1.29 (18H, brs, 9 × CH_2), 1.25 (20H, brs, 10 × CH_2), 0.95 (3H, t, *J*=6.5 Hz, Me-32), 0.73 (3H, t, *J*=6.6 Hz, Me-1); ^{13}C NMR ($CDCl_3$): δ 204.81 (C-13), 201.43 (C-3), 69.96 (C-7), 54.84 (C-12), 50.37 (C-14), 47.23 (CH_2), 35.03 (CH_2), 32.71 (CH_2), 29.65 (8 × CH_2), 29.43 (7 × CH_2), 28.97 (5 × CH_2), 24.21 (CH_2), 22.13 (CH_2), 14.26 (Me-32), 14.08 (Me-1); EIMS *m/z* (rel.int): 494 $[M]^+$ ($C_{32}H_{62}O_3$) (3.9), 479 (12.1), 476 (21.5), 465 (4.1), 437 (6.2), 395 (17.2), 365 (18.4), 295 (16.8), 267 (12.5), 227 (3.6), 199 (10.6), 129 (7.9), 127 (4.6), 113 (14.1),111 (19.3), 99 (9.2), 85 (45.3), 83 (81.3), 71 (79.2), 69 (76.1), 57 (100).

β-sitosterol (4)

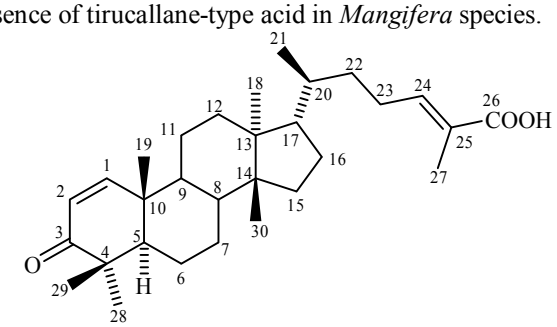
Further elution of the column with chloroform furnished colourless crystalline compound **4**, m.p.139-140º, 4.0 g (yield 0.1%); EIMS *m/z* (rel. int.): 414 $[M]^+$ ($C_{29}H_{50}O$) (16.1); acetate m.p. 127-128º .

RESULTS AND DISCUSSION

Compound **1**, named hydroxydocosanyl heptanoate, was obtained as a colourless crystalline mass from petroleum ether-chloroform (3:1) eluents. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3410 cm^{-1}), ester group (1735 cm^{-1}), keto function (1710 cm^{-1}) and long chain aliphatic chain (725 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **1** was established at *m/z* 454 consistent to the molecular formula of a fatty acid ester, $C_{28}H_{54}O_4$. The ion peaks arising at *m/z* 423 $[M\text{-}CH_2OH]^+$, 73, 381 $[C_{18}\text{-}C_{19}$ fission$]^+$, and 101, 353 $[C_{17}\text{-}C_{18}$ fission$]^+$, indicated the existence of the hydroxyl group at the terminal C-22′ carbon and carbonyl fuction at C-18′. The ion fragment generating at *m/z* 339, 115 $[O\text{-}C_1$, fission$]^+$ and 355, 99 [CO-O fission]$^+$ suggested that hexanoic acid was esterified with C_{22} alcohol. The ^{1}H NMR spectrum of **1** showed a two-proton triplet at δ 4.10 (*J*=7.0 Hz) and two one-proton doublets at δ 3.60 (*J*=5.5 Hz) and 3.53 (*J*=6.0 Hz) were assigned to oxygenated methylene H_2-1′ and to hydroxymethylene H_2-22′ protons. Three two-proton multiplets at δ 2.20, 2.11 and 2.06 were ascribed to methylene H_2-2 proton adjacent to the ester function and methylene H_2-17′ and H_2 -19′ protons nearby to carbonyl group, respectively. The other methylene protons appeared at δ 1.46 (4H) and 1.20 (36H). A three-proton triplet at δ 0.93 (*J*=6.0 Hz) was accounted to terminal C-6 primary methyl protons. The ^{13}C NMR spectrum of **1** exhibited signals for carbonyl carbon at δ 203.93 (C-18′), ester carbon at δ 171.47 (C-1), oxygenated methylene carbon at δ 65.80 (C-1′), hydroxymethylene carbon at δ 61.36 (C-22), other methylene carbons between δ 51.24-22.19 and methyl carbon at δ 14.33 (C-6). On the basis of these evidences, the structure of **1** has been characterised as *n*-docosan-18′-one-22′-ol-1′-yl n-hexanoate. This is a new fatty acid ester.

1

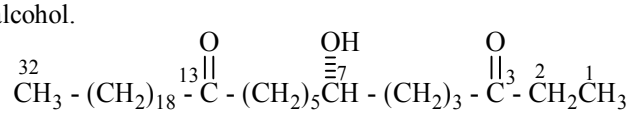
Compound **2**, designated as mangtirucalloic acid, was obtained as a colourless amorphous mass from petroleum ether-chloroform (1:1) eluents. It yielded effervescences with sodium bicarbonate solution indicating the presence of carboxylic group and gave positive Zimmermann test[11] for 3-keto triterpenoids. Its IR spectrum displayed characteristic absorption bands for carboxylic group (3100, 1680 cm^{-1}), carbonyl function (1705 cm^{-1}) and unsaturation (1630 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular weight of **2** was determined at *m/z* 454 consistent with the molecular formula of a tetracyclic triterpene, $C_{30}H_{46}O_2$. The ion fragments appearing at *m/z* 439 $[M\text{-}Me]^+$, 410 $[M\text{-}CO_2]^+$ and 395 $[410\text{-}Me]^+$ supported the existence of the carboxylic function in the molecule. Elimination of side chain $C_8H_{13}O_2$ (mass unit 141) from the molecular ion gave an ion peak at *m/z* 313 indicating the presence of C_8 unsaturated side chain with a carboxylic group. The ion peaks generating at *m/z* at 136 $[C_{5,6}\text{-}C_{9,10}$ fission$]^+$ and 164, 90 $[C_{7,8}\text{-}C_{9,10}$ fission$]^+$ indicated the saturated nature of ring B and existence of a vinylic linkage and carbonyl group in ring A placed at C-3 on the basis of biological analogy. The ion peaks appearing at *m/z* 190 $[C_{8,14}\text{-}C_{9,11}$ fission$]^+$ and 218, 236 $[C_{8,14}\text{-}C_{12,13}$ fission$]^+$ suggested the saturated nature of ring C. The ^{1}H NMR spectrum of **2** showed three one-proton doublets at δ 6.03 (*J*=6.0 Hz), 5.95 (*J*=6.00 Hz) and 5.16 (*J*=3.5 Hz) assigned to *cis*-oriented vinylic H-1, H-2 and H-24 protons, respectively. A three-proton broad signal at δ 1.80 was ascribed to C-27 methyl proton located on the vinylic carbon. The other methyl protons appeared from δ 1.01 to 0.70 similar to tirucallane-type triterpenoids[11]. The ^{13}C NMR spectrum of **2** exhibited important signals for carbonyl carbon at δ 202.38 (C-3), vinylic carbons at δ 128.12 (C-1), 139.06 (C-2), 125.28 (C-24) and 135.06 (C-25), carboxylic carbon at δ 181.13 (C-26) and methyl carbons between δ 27.64-13.28. The 1H and ^{13}C NMR values of **2** were compared with the reported data of tirucallane-type triterpenoids[11-14]. On the basis of these observations the structure of **2** was formulated as 20(R), 24(R)-3-oxotirucalla-*cis*-1,24-dien-26-oic acid. This is a new triterpene and constitutes the first report of the presence of tirucallane-type acid in *Mangifera* species.



2

Compound **3**, named hydroxydotriacontane dione, was obtained as an amorphous powder from chloroform eluents. Its IR spectrum exhibited distinctive absorption bands for hydroxyl group (3410 cm^{-1}), carbonyl groups (1710, 1702 cm^{-1}) and long aliphatic chain (700 cm^{-1}). On the basis of mass and ^{13}C NMR spectra the molecular ion peak of **3** was determined at *m/z* 494 corresponding to a molecular formula of a diketo aliphatic alcohol, $C_{32}H_{62}O_3$. The peaks arising at *m/z* 479 $[M\text{-}Me]^+$, 476 $[M\text{-}H_2O]^+$, 465 $[M\text{-}C_2H_5]^+$ and 437, 57 $[C_3\text{-}C_4$ fission]+ indicated the presence of one hydroxyl group and location of one of the keto group at C-3. The ion

fragments generating at *m/z* 99, 395 $[C_6\text{-}C_7$ fission$]^+$, 129, 365 $[C_7\text{-}C_8$ fission]+, 199, 295 $[C_{12}\text{-}C_{14}$ fission$]^+$, suggested the existence of the hydroxyl group at C-7 and other carbonyl function at C-13. ^{1}H NMR spectrum of **3** displayed a one-proton doublet at δ 4.20 with coupling interaction of 6.5 and 5.5 Hz assigned to *α*-oriented carbinol H-7 proton. Four two-proton multiplets protons at δ 2.60, 2.47, 2.01 and 1.97 were associated with the methylene protons adjacent to C-13 and C-3 carbonyl carbons. The other methylene carbons resonated between δ 1.62-1.25. Two three-proton triplets at δ 0.95 (*J*=6.5 Hz) and 0.73 (*J*=6.6 Hz) were due to terminal C-32 and C-1 primary methyl protons. The ^{13}C NMR spectrum of **3** exhibited important signals for carbonyl carbons at δ 204.81 (C-13) and 201.43 (C-3), hydroxymethine carbon at δ 69.96 (C-7) and methyl carbons at δ 14.26 (C-32) and 14.08 (C-1). The absence of any signal beyond *δ* 4.20 in the ^{1}H NMR spectrum and between *δ* 201.43-69.96 in the ^{13}C NMR spectrum suggested saturated nature of the molecule. These data led to formulate the structure of this natural product **3** as *n*-dotriacont-7α-ol-3,13-dione. This is a new aliphatic dione alcohol.



3

Compound **4** is a known phytoconstituents identified as β-sitosterol.

CONCLUSION

The phytochemical investigation of the stem bark of *M. indica* var. *langra* furnished a 3-oxotirucalla-*cis*-1,24-dien-26-oic acid which might be useful as a chromatographic marker for quality control of this drug.

AKNOWLEDGEMENT

The authors are thankful to the Head, Sophisticated Analytical Instrumentation Facility (SAIF), Central Drug Research Institute, Lucknow, for recording mass spectrum of the compounds.

REFERENCES

1. Anonymous. The Wealth of India, Raw Material, National Institute of Science Communication and Information Resources, CSIR, New Delhi, 2003; 6(4): 265-285.
2. Sharma SK, Ali M. Evaluation of chemical constituents of *Mangifera indica* (Mango). Hamdard Medicus, 1992; 35(3): 110-133.
3. Mhaskar KS, Blatter E, Caius JF. Kirtikar and Basu′s Illustrated medicinal Plants, Sri Satguru Publication, 2000; 3: 916-922.
4. Sharma SK, Ali M. Chemical constituents of stem bark of *Mangifera indica* cultivar *dusehri*. Indian Drugs, 1993; 30: 446-449.
5. Sharma SK, Ali M. Chemical constituents of stem bark of *Mangifera indica* cultivar *dusehri*. Indian J Pharmaceut Sci, 1994; 56: 53-56.
6. Sharma SK, Ali M. Chemical constituents of stem bark of *Mangifera indica* cultivar *deshi*. J Indian Chem Soc, 1995; 72: 339-342.
7. Sharma SK, Ali M. Sesquiterpenic constituents of stem bark of *Mangifera indica* cultivar *chausa*. Indian J Nat Prod, 1993a; 9: 3-5.
8. Anjaneyulu V, Babu JS, Krishna MM, Connolly JD. 3-Oxo-*20S*, 24*R*-epoxy-dammarane-25ξ, 26-diol from *Mangifera indica*. Phytochemistry, 1993; 32(2): 469-471.
9. Anjaneyulu V, Babu JS, Connolly JD. 29-hydroxymangiferonic acid from *Mangifera indica*. Phytochemistry, 1994; 35(5): 1301-1303.
10. Ghosal S, Chakrabarti DK. Differences in phenolic and steroidal constituents between healthy and infected florets of *Mangifera indica*. Phytochemistry, 1988; 27(5): 1339-1343.
11. Ali M. Techniques in Terpinoid Identification. Birla Publications, Delhi, 2001; p. 366-384.
12. Gewali MB, Hattori M, Tezuka Y, Kikuchi T, Namba T. Constituents of the Latex of *Euphorbia antiquorum* L. Phytochemistry, 1990; 29(5): 1625-1628.
13. Da Silva MFDGF, Francisco RHP, Gray AI, Lechat JR, Waterman PG. Lanost-7-en triterpenes from stem bark of *Santiria trimera*. Phytochemistry, 1990; 29(5): 1629-1632.
14. Tanaka R, Matsunaga S. Triterpene lactones from the stem bark of *Abies firma*. Phytochemistry, 1991; 30(6): 1983-1987.

Source of support: Nil, Conflict of interest: None Declared