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Chemical constituents from the rhizomes of *Curcuma aromatica* Salisb

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ABSTRACT

Curcuma aromatica Salisb. (Zingiberaceae) is a perennial herb found throughout India. Its rhizomes are substituted for turmeric and used externally to treat bruises, sprains, skin eruptions, infections and to improve complexion. Phytochemical investigation of the methanolic extract of the rhizomes yielded three new homosesterterpenoids characterized as 5, 9, 13, 17, 20-pentamethyl-n-heneicos-cis-3-en-6β, 7β, 8β-triol (curcusesterterpene A); 5, 9, 13, 17, 20-pentamethyl-n- heneicos-cis-6-en-2β,4β,5α-triol (curcusesterterpene B); 5, 9, 13, 17, 20-pentamethyl-n-heneicos-cis-3-en-6β, 7β, 9α-triol (curcusesterterpene C) along with the known compounds n-nonacosan-1-ol and curcumin. The structures of these phytoconstituents have been elucidated on the basis of structural data analysis and chemical reactions.

Keywords: *Curcuma aromatica*, Zingiberaceae, rhizomes, homosesterterpenoids.

INTRODUCTION

Curcuma aromatica Salisb. (Zingiberaceae), commonly known as jangli haldi or yellow zedoary, is an erect perennial herb scattered throughout India and cultivated in West Bengal and Kerala. The rhizomes are tuberous, large, orange-red and aromatic; substituted for turmeric and applied externally to cure bruises, sprains, skin eruptions, infections and to improve complexion [1]. The rhizomes contained zederone [2], curdione, neocurdione, curcumol, tetramethyl pyrazine, 1,2-hexadecanediol [3], 9-oxo-neoprocurcumenol [4] and volatile oil mainly composed of β-curcumene, *ar*-curcumene, xanthorrhizol, germacrone, camphor, curzerenone, 7-methanoazulene, 1,8-cineole, β-elemene and linalool [5-8]. The present paper describes the isolation of three new phytoconstituents from of the rhizomes of *C. aromatica* procured from Delhi.

MATERIALS AND METHODS

General experimental procedure

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, Mumbai,60-120 mesh) and thin layer chromatography on silica gel G-coated TLC plates (Merck, Mumbai). Spots were visualized by exposure to iodine vapours, UV radiation and by spraying reagents.

Plant Material

The rhizomes of *C. aromatica* (3 kg) were obtained from Khari Baoli market, Delhi and identified by Prof. M. P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard (Hamdard University). A voucher specimen No.PRL/JH/08/36 was deposited in the herbarium of the Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi, India.

Extraction and isolation

The air-dried defatted rhizomes (3 kg) of *C. aromatica* were coarsely powdered and extracted with methanol in a Soxhlet apparatus for 72 hours. The methanolic extract was concentrated to obtain dark viscous mass (475 g). It was dissolved in minimum amount of methanol and adsorbed on silica gel (60-120 mesh) to form slurry. The slurry was air-dried and chromatographed over silica gel columns packed in petroleum ether. The column was eluted with petroleum ether, mixture of petroleum ether and chloroform (9:1, 3:1, 1:1 and 1:3), pure chloroform and finally mixture of chloroform and methanol (99.5:0.5, 99:1, 49:1, 19:1, 9:1).Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions (having same R_f values) were combined and crystallized. The isolated compounds were recrystallized to get the pure compound(s).

Curcusesterterpene A (1)

Elution of the column with petroleum ether yielded colourless crystals of **1**, recrystallized from methanol, 110 mg (0.0037 % yield); R_f : 0.83 (*n*-hexane); m.p. 72-73^0 C; $[\alpha]^{30}_{D:}$ +21^0 ($CHCl_3$, 0.1); UV λ_{max}: 208 nm (log ε 3.1); IR υ_{max} (KBr): 3450, 3360, 2955, 2845, 2360, 1640, 1475, 1360, 1210, 795 cm^{-1}; ^{1}H NMR : Table- 1; ^{13}C NMR : Table- 2; EIMS *m/z* (rel. int.): 412 $[M]^+$ ($C_{26}H_{52}O_3$) (92.1), 397 (33.2), 394 (100), 382 (29.1), 379 (39.2), 369 (9.6), 367 (12.8), 341 (6.1), 329 (30.1), 313 (7.1), 299 (11.3), 271 (19.7), 269 (23.5), 239 (22.0), 229 (18.4), 211 (30.4), 199 (15.3), 183 (9.3), 173 (6.9), 169 (7.2), 160 (17.1), 143 (12.4), 141 (12.5), 113 (11.2), 99 (21.3), 97 (56.1), 85 (57.2), 83 (59.2), 71 (60.1), 57 (83.7), 55 (24.2), 43 (84.1).

Curcusesterterpene B (2)

Further elution of the column with petroleum ether afforded colourless mass of **2**, recrystallized from methanol, 285 mg (0.0095 %yield); R_f : 0.32 (benzene); m.p. 90-91^0 C; $[\alpha]^{30}_{D:}$ +12.0^0 ($CHCl_3$, 0.1); IR υ_{max} (KBr): 3450, 3360, 2955, 2845, 2360, 1630, 1470, 1365, 1210 cm^{-1}; ^{1}H NMR : Table-1; ^{13} C NMR : Table-2; EIMS *m/z* (rel. int.): 412 $[M]^+$ ($C_{26}H_{52}O_3$) (100), 397 (32.8), 367 (33.5), 353 (8.8), 329 (23.4), 271 (16.2), 253 (20.8), 239 (8.0), 211 (23.9), 173 (11.3), 169 (10.1), 159 (24.4), 133 (21.7), 99 (18.1), 71 (60.1), 59 (33.6), 57 (76.5), 43 (98.1).

n-Nonacosan-1-ol (3)

Elution of the column with petroleum ether-chloroform (3:1) afforded colourless amorphous powder of **3**, recrystallized from methanol : diethyl ether (1:1), 620 mg (0.0207 % yield); R_f : 0.41 (toluene); m.p. 88-89°C; IR υ_{max}: 3500, 2950, 2845, 1508, 1470, 1355, 1205, 1050, 795, 715 cm^{-1}; ^1H NMR: 3.57 (1H, d, *J*=6.56 Hz, H_2-1a), 3.53 (1H, d, *J*=6.57 Hz, H_2-1b), 1.53 (2H, m, H_2-2), 1.42 (2H, brs, CH_2), 1.19 (50H, brs, 25 × CH_2), 1.06 (2H, brs, CH_2), 0.83 (3H, t, *J*=6.23 Hz, Me-29); ^{13}C NMR: δ 60.81 (C-1), 37.78 (CH_2), 37.11 (CH_2), 34.42 (CH_2), 32.11 (CH_2), 30.88 (CH_2), 28.71 (CH_2), 25.98 (19 × CH_2), 21.59 (CH_2), 12.98 (CH_3); EIMS *m/z* (rel. int.): 424 $[M]^+$ $C_{29}H_{60}O$ (9.3), 307 (8.8), 283 (11.1), 262 (7.6), 256 (12.7), 227 (22), 213 (21.2), 199 (24.3), 185 (19.7), 127 (22.8), 113 (25.1), 99 (74.1), 85 (43.2), 71 (45), 69 (51.7), 57 (62.5).

Curcusesterterpene C (4)

Elution of the column with petroleum ether-chloroform (1:1) furnished colourless crystalline mass of **4,** recrystallized from methanol, 130 mg (0.0043 % yield); R_f : 0.39 (chloroform); m.p. 110-111^0 C; $[\alpha]^{30}_{D:}$ +16.5 ^{0} ($CHCl_3$, 0.1); IR υ_{max} (KBr): 3485, 3365, 2918, 2840, 2360, 1635, 1470, 1360, 1210 cm^{-1}; ^1H NMR : Table- 1; ^{13}C NMR : Table- 2; EIMS *m/z* (rel. int.): 412 $[M]^+$ ($C_{26}H_{52}O_3$) (100), 397 (28.3), 394 (69.8), 379 (32.6), 369 (9.2), 329 (30.1), 302 (36.0), 288 (15.9), 271 (21.9), 269 (12.8), 255 (39.7), 211 (25.4), 201 (8.2), 157 (22.2), 143 (33.1), 132 (23.9), 120 (21.1), 108 (25.3), 95 (32.8), 83 (15.2), 71 (19.3), 57 (34.2), 55 (12.3), 43 (36.1).

Curcumin (5)

Elution of the column with chloroform-methanol (99:1) afforded yellow crystalline mass of **5**, recrystallized from methanol, 630 mg (0.021 % yield); R_f: 0.86 (chloroform: methanol:: 9:1); m.p. 180-183^{0}C; UV λ_{max}: 240 nm (log ε 3.2); IR υ_{max} (KBr): 3450, 3400, 2945, 2860, 2360, 1690, 1650, 1570, 1310, 980 cm^{-1}; ^1H NMR : δ 7.58 (1H, d, *J*=15.7 Hz, H-8), 7.53 (1H, d, *J*=15.7 Hz, H-12), 7.06 (4H, brs, H-2, H-5, H-15, H-18), 7.04 (1H, brs, D_2O exchangeable, OH), 6.91 (2H, d, *J*=8.5 Hz, H-6, H-19), 6.50 (1H, d, *J*=15.7 Hz, H-7), 6.45 (1H, d, *J*=15.7 Hz, H-13), 5.82 (1H, brs, D_2O exchangeable, OH), 3.90 (8H, brs, OMe-3; OMe-17, H_2-11); ^{13}C NMR : δ 126.44 (C-1), 120.67 (C-2), 140.18 (C-3), 148.48 (C-4), 115.11 (C-5), 109.82 (C-6), 115.11 (C-7), 122.38 (C-8), 182.75 (C-9), 55.38 (C-10), 182.75 (C-11), 122.38 (C-12), 115.11 (C-13), 122.38 (C-14), 120.67 (C-15), 140.18 (C-16), 147.26 (C-17), 120.67 (C-18), 100.62 (C-19), 55.38 (2 × OMe); EIMS *m/z* (rel. int.): 368 $[M]^+$ ($C_{21}H_{20}O_6$) (41.6), 350 (43.5), 271 (15.8), 230 (11.3), 216 (11.5), 191 (27.4), 188 (37.4), 177 (18.2), 176 (100), 149 (26.3), 146 (7.2), 144 (32.1), 134 (12.5), 129 (11.2), 123 (17.1), 118 (10.2), 108 (9.2).

RESULTS AND DISCUSSION

The structures of the known compounds **3** and **5** have been characterized as *n*-nonacosan-1-ol [9] and 1, 7-bis-(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione (curcumin) [10], respectively, on the basis of spectral data analysis.

Compound **1**, named curcusesterterpene A, was obtained as a colourless crystalline mass from petroleum ether eluants. It decolourized bromine water indicating unsaturated nature of the molecule. Its IR spectrum exhibited characteristic absorption bands for hydroxyl groups (3450, 3360 cm^{-1}) and unsaturation (1640 cm^{-1}). On the basis of electron impact mass and ^{13}C NMR spectra of **1**, its molecular weight was established at *m/z* 412 related to molecular formula of an

acyclic homosesterterpenic molecule, $C_{26}H_{52}O_3$. It indicated one double bond equivalent which was adjusted to the vinylic linkage. The prominent ion peaks of diagnostic importance at *m/z* 43, 369 [C_{19}-C_{20} fission]$^+$, 71, 341 [C_{17}-C_{18} fission]$^+$, 99, 313 [C_{16}-C_{17} fission]$^+$, 141, 271 [C_{13}-C_{14} fission]$^+$, 169 [C_{12}-C_{13} fission]$^+$, 183, 229 [C_{11}-C_{12} fission]$^+$, 211 [C_9-C_{10} fission]$^+$ and 239, 173 [C_8-C_9 fission]$^+$ indicated saturated nature of the carbon chain from C_8 to C_{22} and the presence of the methyl functionalities at C-9, C-13, C-17 and C-20. The ion peaks at *m/z* 269, 143 [C_7-C_8 fission]$^+$, 299, 113 [C_6-C_7 fission]$^+$, 329, 83 [C_5-C_6 fission]$^+$ and 55 [C_4-C_5 fission]$^+$ supported the location of the hydroxyl group at C-6, C-7 and C-8 and olefinic linkage at C-3 (4). The ^{1}H NMR spectrum of **1** showed a one-proton multiplet at δ 5.13 with half-width of 11.2 Hz and a one-proton double doublet at δ 5.36 (J = 4.8, 6.3 Hz) assigned to cis- oriented vinylic H-3 and H-4, respectively. Three one-proton double doublets at δ 3.64 (J = 8.9, 5.3 Hz), 3.52 (J = 5.3, 5.1 Hz) and 3.47 (J = 5.1, 6.3 Hz) were attributed correspondingly to H-6α, H-7α and H-8α carbinol protons, respectively. A three-proton triplet at δ 0.84 (*J*=7.05 Hz) was accounted to C-1 primary methyl protons. A six-proton broad signal at δ 1.01 was ascribed to C-21 and C-26 secondary methyl protons. The C-22, C-23, C-24 and C-25 secondary methyl protons appeared as three-proton doublets at δ 0.82 (*J*=6.3 Hz), 0.68 (*J*=5.6 Hz), 0.88 (*J*=6.0 Hz) and 0.91 (*J*=6.6 Hz), respectively. The remaining methylene and methine protons resonated between δ 2.27 - 1.25 (Table 1). The presence of methyl signals between δ 0.84–1.01 suggested that all the methyl functionalities were attached to saturated carbons. The ^{13}C NMR spectrum of **1** displayed two desheilded signals at δ 120.75 and 123.03 assigned to C-3 and C-4 vinylic carbons, respectively. Three signals at δ 67.89, 65.62 and 68.94 were accounted correspondingly to C-6, C-7 and C-8 carbinol carbons. The methyl carbons appeared at δ 14.12 (Me-1), 24.91 (Me-21, Me-23, Me-26), 22.70 (Me-22), 22.67 (Me-24) and 26.07 (Me-25). The remaining methine and methylene carbons resonated in the range δ 55.97-29.32 (Table 2).The ^{1}H and ^{13}C NMR spectral data of **1** with the linear sesterterpenoids [11]. The HMBC spectrum of **1** showed correlations of C-3 with H-4, H_2-2 and H_3-1; and C-7 with H-6 and H-8. Based on these evidences, the structure of **1** has been elucidated as 5, 9, 13, 17, 20-pentamethyl-*n*-heneicos-cis-3-en-6β, 7β, 8β-triol. This is a new homosesterterpene and the existence of such phytoconstituent is reported for the first time in *C. aromatica*.

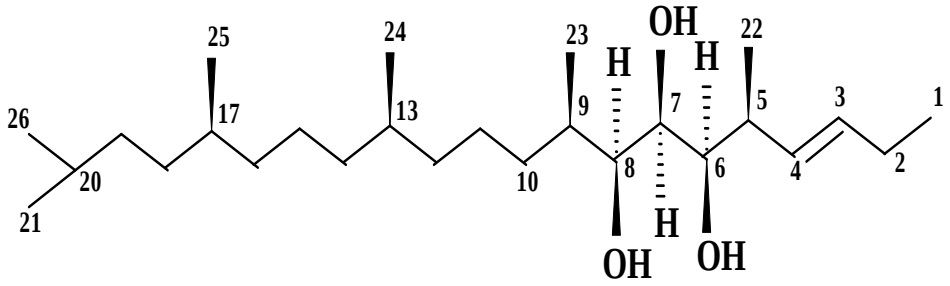
Compound **2**, designated as curcusesterterpene B, was obtained as a colourless mass from petroleum ether eluants. It decolourized bromine water indicating unsaturated nature of the compound. Its IR spectrum exhibited characteristic absorption bands for hydroxyl group (3450, 3360 cm^{-1}) and unsaturation (1630 cm^{-1}). Its molecular weight was established as *m/z* 412 on the basis of EI mass and ^{13}C NMR spectra which corresponded to the molecular formula of an acyclic homosesterterpene, $C_{26}H_{52}O_3$. It had only one degree of unsaturation adjustable to the olefinic linkage. The ion fragments of diagnostic importance appeared at *m/z* 99 [C_{16}-C_{17} fission]$^+$, 271 [C_{13}-C_{14} fission]$^+$, 211 [C_9-C_{10} fission]$^+$, 169 [211-C_3H_6]$^+$, 239, 173 [C_8-C_9 fission]$^+$, 159 [173-CH_2]$^+$, 133 [159-CH=CH]$^+$ and 253 [C_5-C_6 fission]$^+$ suggested the location of the olefinic linkage at $\Delta^{6(7)}$ and methyl functionalities at C-9, C-13, C-17 and C-20. The ion peaks at *m/z* 353, 59 [C_3-C_4 fission]$^+$ and 367 [C_2-C_3 fission]$^+$ supported the existence of the hydroxyl groups at C-5, C-4 and C-2 positions. The ^{1}H NMR spectrum of **2** exhibited a one-proton doublet at δ 5.36 (*J*=5.1 Hz) and a one-proton triple doublet at δ 5.11 (J = 5.1, 5.5, 9.5 Hz) assigned to cis oriented vinylic H-6 and H-7, respectively. A one-proton multiplet at δ 3.51 ($w½$ = 13.6 Hz) and a one-proton double doublet at δ 3.66 (J= 4.9, 8.3 Hz) were attributed to α-

oriented H-2 and H-4 carbinol protons, respectively. A six-proton broad signal at δ 1.03 was ascribed to Me-21 and Me-26. Three doublets at δ 0.69 (*J*=5.7 Hz), 0.87 (*J*=6.1 Hz) and 1.00 (*J*=6.5 Hz) integrating for three protons each were accounted to C-23, C-24 and C-25 secondary methyl protons, respectively.

A three-proton broad signal at δ 1.25 was ascribed to C-22 methyl proton attached to hydroxyl bearing C-5 position. The remaining methyl, methylene and methine protons appeared in the range δ 2.31–1.16 (Table 1). The methyl proton resonances in the range δ 1.25-0.69 indicated the location of all methyl groups at saturated carbons. The ^{13}C NMR spectrum of **2** showed vinylic carbon signals at δ 121.73 (C-6) and 132.51 (C-7), carbinol carbon signals at δ 65.33 (C-2), 67.53 (C-4) and 71.84 (C-5) and methyl signals at δ 11.98 (C-1), 23.08 (C-21), 26.09 (C-22), 24.31 (C-23), 23.08 (C-24), 21.09 (C-25) and 19.02 (C-26) (Table 2). The HMBC spectrum of **2** showed correlations of C-6 with H-7 and H-4; and C-2 with H_3-1, H_2-3 and H-4. On the basis of the foregoing account the structure of **2** has been formulated as 5, 9, 13, 17, 20-pentamethyl-*n*-heneicos-cis-6-en-2β,4β,5α-triol. This is a new homosesterterpene isolated from the natural or synthetic source for the first time.

Compound **4**, named curcusesterterpene C, was obtained as a colourless crystalline mass from the petroleum ether-chloroform (1:1) eluants. It decolourized bromine water and showed characteristic IR absorption bands for hydroxyl groups (3485, 3365 cm^{-1}) and unsaturation (1635 cm^{-1}). On the basis of EI impact mass and ^{13}C NMR spectra, its molecular ion peak was determined at *m/z* 412 corresponding to the molecular formula of an acyclic homosesterterpene $C_{26}H_{52}O_3$. It had one degree of unsaturation adjustable to the vinylic linkage. The prominent ion fragments appearing at *m/z* 71 [C_{17}-C_{18} fission]$^+$, 271[C_{13}-C_{14} fission]$^+$ and 211[C_9-C_{10} fission]$^+$ suggested saturated nature of the carbon chain from C_9 to C_{21} and location of the methyl functionalities at C_9, C_{13}, C_{17} and C_{21}. The ion peaks at *m/z* 255, 157 [C_8-C_9 fission]$^+$, 269 [C_7-C_8 fission]$^+$, 369 [M-C_3H_7]$^+$, 329, 83[C_5-C_6 fission]$^+$, 55 [C_4-C_5 fission]$^+$, 397 [M-Me]$^+$ and 394 [M-H_2O]$^+$ indicated the location of the hydroxyl groups at C_9, C_7 and C_6 and vinylic linkage at $\Delta^{3(4)}$. The ^{1}H NMR spectrum of **4** displayed a one-proton multiplet at δ 5.13 with half-width of 10.5 Hz and a one-proton double doublet at δ 5.34 (*J*=4.8, 4.8 Hz) assigned to cis-oriented vinylic H-3 and H-4 protons, respectively. A one-proton double doublet at δ 3.54 (*J*=8.3, 5.5 Hz) and a one-proton triple doublets at δ 3.50 (J= 5.3, 4.8, 9.6 Hz) were attributed correspondingly to α-oriented H-6 and H-7 carbinol protons, respectively. A six-proton broad signal at δ 1.00 was associated with C-21 and C-26 methyl protons. A three-proton broad signal at δ 1.21 was ascribed to C-23 methyl protons attached to hydroxyl bearing C-9 position. Three doublets at δ 0.92 (*J*=6.3 Hz), 0.86 (*J*=6.9 Hz) and 0.92 (*J*=6.3 Hz), integrating for three protons each, were accounted to C-22, C-24 and C-25 secondary methyl protons, respectively. A three-proton triplet at δ 0.61 (*J*=6.5 Hz) attested the presence of C-1 primary methyl protons. The appearance of all the methyl resonances in the range from δ 1.21 to 0.61 supported their location on the saturated carbons. The remaining methylene and methine carbons appeared between δ 2.34-1.08 (Table 1). The ^{13}C NMR spectrum of **4** exhibited important vinylic and carbinol carbon signals at δ 120.68 (C-3), 121.70 (C-4), 67.31 (C-6), 68.90 (C-7) and 71.79 (C-9). The methyl carbon signals resonated at δ 11.95 (C-1), 24.05 (C-21), 21.06 (C-22, C-26), 19.80 (C-23), 19.38 (C-24) and 18.76 (C-25) (Table 2). The HMBC spectrum of **4** showed correlations of C-3 with H_2-2, H_3-1 and H-4; C-6 with H-5 and H-7; and C-9 with H_2-8, H-7, H_3-23 and H_2-10. On the basis of the

foregoing discussion, the structure of **4** has been elucidated as 5, 9, 13, 17, 20-pentamethyl-*n*-heneicos-cis-3-en-6β, 7β, 9α-triol. This is a new homosesterterpenic constituent reported from a synthetic or natural source for the first time.



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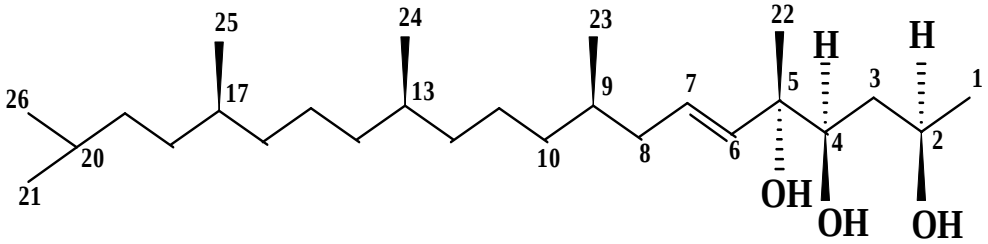

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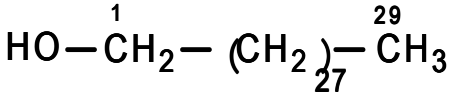

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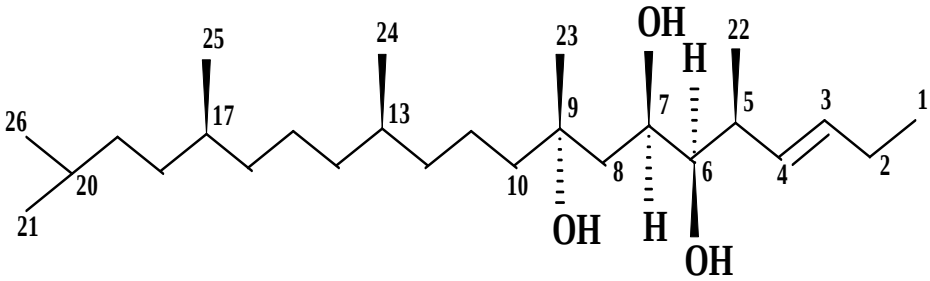

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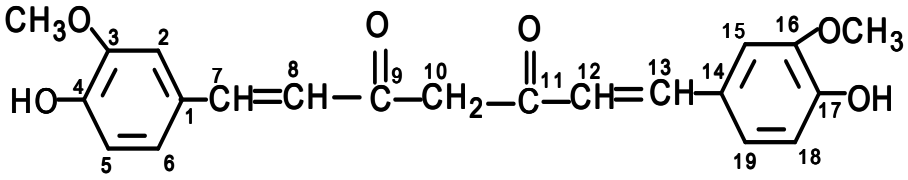

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CONCLUSION

Phytochemical investigation of the rhizomes of *Curcuma aromatica* led to the isolation of three new homosesterterpenoids along with n-nonacosan-1-ol and curcumin. This is the first report of occurrence of homosesterterpenoids in the *Curcuma* species

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