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Original Article

Cinnamic acid and lanostenoic acid derivatives from the leaves of Pyracantha *crenulata* (D. Don) M. Roem

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ABSTRACT

Pyracantha crenulata (D. Don) M. Roem. (Rosaceae) is distributed in the northern Himalayas and China as a perennial shrub. The leaves are used to prepare herbal teas, sun burn creams and facial creams and taken as a nervine tonic. An air-dried leaf powder of P. *crenulata* was exhaustively extracted with methanol. The concentrated leaf extract was adsorbed on silica gel (60-120 mesh) for the preparation of a slurry. The dried slurry was chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol successively in order of increasing polarity to isolate β-sitosterol, *cis*-3,4-dioxyethylene-5-methoxycinnamic acid and lanost-54-en-2β,3β-diol 26-oic acid (cranulanostenoic acid). The structures of all the isolated phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

Keywords: *Pyracantha crenulata*, leaves, chemical constituents, isolation, characterization.

INTRODUCTION

Pyracantha crenulata (D. Don) M. Roem., syn. *Crataegus crenulata* Roxb., *Cotoneaster crenulatus* (D. Don) K. Koch, *Mespilus crenulata* D .Don (Rosaceae), known as the Nepalese firethorn or ghingaru, is distributed in the Himalayas from Sutlaj to Bhutan between 800 – 2,500 m and in China as a perennial shrub. It is a 1 to 3 m highly ramified and prickly bush with alternate, oblong leaves, white flowers and orange-red fruits. The fruits are antispasmodic**,** appetizer, cardiotonic**,** diuretic, coronary vasodilator, hypotensive, nutritious and sedative; used to treat burns, cardiac failure, myocardial debility, paroxysmal tachycardia, hypertension, arteriosclerosis and Buerger's disease. The fruits are consumed by aged people for rejuvenation and to reduce joint pains. A beverage of the berries is nutritious and taken to relieve anxiety, anorexia, insomnia and neurasthenia. The fruit powder combined with yoghurt is given to cure bloody dysentery. The pome fruit is edible and eaten by birds[1-6] . A root decoction is used in baths to alleviate body pain[7]. The leaves possess antioxidant, immune modulatory and anti-inflammatory activities and are used to prepare herbal teas, sun burn creams and facial creams. The stem bark is used to relieve heavy bleeding during menstruation and malarial fever[8]. A combination of *Ginkgo biloba* and *P. crenulata* leaves is taken as a nervine tonic. The plant makes an excellent hedge and is grown as an ornamental plant[9].

The fruits contained proteins, vitamins, sugars, flavonoids, oligomeric proanthocyanidins, tannins, polyphenols, β-sitosterol, esculatin and quercetin[10-12]. The flowers yielded phenyl ethylamine, O-methoxyphenyl ethylamine and tyamine. The plant possessed 2-phenylchromones and chlorogenic acid[2]. Pyracrenic acid was isolated from the bark[13]. The major fatty acids of seed oil were linoleic, oleic and palmitic acids[14]. The fruits and other plant parts showed anti-inflammatory[13,15], antioxidant[16], antiurolithiatic[17], diuretic[18] and antimicrobial[6] activities. Owing to traditional medicinal uses of P. *crenulata*, it was thought worthwhile to study chemical composition of the plant. The manuscript describes isolation and characterization of chemical constituents from the leaves of *P. crenulata*.

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MATERIALS AND METHODS

General procedures: Melting points were determined on a Perfit melting apparatus (Ambala, Haryana, India) and are uncorrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer in methanol. Infrared spectra were recorded on a Bio-Rad FTIR 5000 spectrophotometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were scanned on Advance DRX Bruker spectrospin 400 and 100 MHz, respectively, instruments using TMS as an internal standard. Mass spectra were obtained by effecting FAB ionization at 70 eV on a JEOL-JMS DX 303 spectrometer equipped with direct inlet probe system. Column chromatography was performed on a silica gel (60-120 mesh, Qualigen, Mumbai, India) column. TLC was run on silica gel G (Qualigen) coated plates. Spots were visualized by exposing to iodine vapors and UV radiation and spraying with ceric sulfate solution.

Plant material: The leaves of *P. crenulata* were collected from local areas of Dehradun, Uttarakhand, India and authenticated at the Botanical Survey of India (BSI), Dehradun, India. A voucher specimen of the plant was deposited in the Botanical Survey of India herbarium.

Extraction and isolation: The air-dried leaves (500 g) were coarsely powdered and extracted with methanol exhaustively in a Soxhlet apparatus. The combined extracts were filtered and concentrated under reduced pressure to get a dark brown viscous mass (51.2 g, 10.2%). The dried extract was dissolved in minimum quantity of methanol and adsorbed on silica gel (60-120 mesh) for preparation of a slurry. It was dried in air and chromatographed over a silica gel column (1.6 m x 16 mm x 2 mm) packed in petroleum ether. The column was eluted successively with different solvents in increasing order of polarity in various combinations of petroleum ether, chloroform and methanol in order of increasing polarity. The fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized. The isolated compounds were recrystallized to get pure compounds. The following compounds were isolated from the methanolic extract of the leaves of P. *crenulata*:

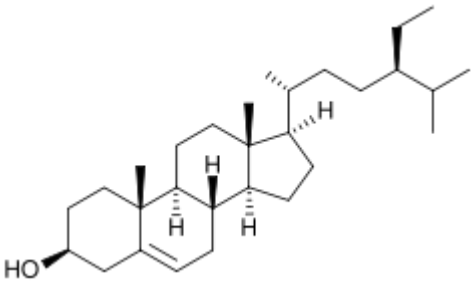
β-Sitosterol (1): Elution of the column with petroleum ether - chloroform (1 : 3) yielded colourless amorphous powder of **1**, yield 127 mg, m. p. 137-138 ^{o}C; UV λ_{max} (MeOH): 211 nm (log ε 2.9); IR υ_{max} (KBr): 3401, 1654 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.34 (1H, m, H- 6), 3.54 (1H, brs, $w_{½}$ = 18.5 Hz, H- 3), 1.01 (3H, brs, M- 19), 0.94 (3H, d, J = 6.2 Hz, Me- 21), 0.87 (3H, d, J = 6.5 Hz, Me-27), 0.84 (3H, J = 6.3 Hz, Me- 26), 0.82 (3H, t, J = 6.1 Hz, Me-29), 0.67 (3H, brs, Me-18), 2.28 – 1.05 (29H, 11 x CH_2, 7 x CH); ^{13}C NMR ($CDCl_3$): δ 71.81 (C- 3), 42.34 (C- 4), 140.78 (C- 5), 121.68 (C- 6), 50.21 (C- 9), 36.14 (C- 10), 39.81 (C- 13), 56.80 (C- 14), 56.11 (C- 17), 11.85 (C- 18), 19.33 (C- 19), 36.73 (C- 20), 19.03 (C- 21), 45.90 (C-24), 29.68 (C- 25), 21.07 (C- 26), 19.78 (C- 27), 24.94 (C- 28), 11.97 (C- 29); +ve ion FAB MS *m/z* (rel.int.): 415 $[M+H]^+$ ($C_{29}H_{51}O$) (15.1), 398 (100), 383 (13.5).

3,4-Dioxyethylene-5-methoxycinnamic acid (2): Elution of the column with chloroform yielded yellow crystals of **2**, recrystallized from acetone, 73 mg, m. p. 277 – 279 ° C, UV λ max (MeOH): 237, 323 nm (log ε 4.2, 3.9); IR υ_{max} (KBr): 3185, 2935, 2837, 1687, 1647, 1545, 1487, 1455, 1372, 1317, 1251, 1183, 1029, 930 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.11 (1H, d, J = 6.3 Hz, H- 8), 6.37 (1H, d, J = 2.1 Hz, H-2), 6.23 (1H, J = 6.3 Hz, H-7), 6.19 (1H, d, J = 2.1 Hz, H-6), 3.42 (2H, t, J = 5.5 Hz, H_2-1′), 3.39 (2H, t, J = 5.5 Hz, H_2-2′), 3.35 (3H, s, OMe); ^{13}C NMR ($CDCl_3$): δ 157.71 (C- 1), 112.89 (C- 2), 164.92 (C- 3), 161.98 (C- 4), 158.33 (C- 5), 105.38 (C- 6), 94.91 (C- 7), 99.37 (C- 8), 180.47 (C- 9), 72.73 (C- 1′, C-2′), 60.77 (OMe); +ve ion FAB MS *m/z* (rel.int.): 237 $[M+H]^+$ ($C_{12}H_{13}O_5$) (25.1), 191 (12.4), 165 (9.2).

Cranulanostenoic acid (**3**): Elution of the column with chloroform – methanol (48:1) afforded a colourless amorphous powder of **3**, yield 98 mg, m.p. 240-242 ^{o}C; UV λ_{max} (MeOH): 209 nm (log ε 3.7); IR υ_{max} (KBr): 3448, 3247, 2927, 2851, 1698, 1636, 1455, 1380, 1273, 1180, 1095, 1007 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.31 (1H, d, J = 5.6 Hz, H-6), 3.93 (1H, d, J = 9.5 Hz, H- 3α), 3.31 (1H, brm, $w_{1/2}$ = 16.5 Hz, H-2α), 2.17 (1H, m, H-25), 1.29 (3H, brs, M- 28), 1.14 (3H, brs, Me-19), 1.06 (3H, brs, Me-30), 0.99 (3H, d, J = 6.3 Hz, Me-27), 0.93 (3H, d, J = 6.6 Hz, Me- 21), 0.84 (3H, brs, Me-29), 0.77 (3H, brs, Me-18), 2.08 – 1.32 (22H, 9 x CH_2, 4 x CH); ^{13}C NMR ($CDCI_3$): δ 35.71 (C-1), 71.69 (C-2), 81.38 (C-3), 39.75 (C-4), 139.51 (C-5), 118.98 (C-6), 28.72 (C-7), 38.19 (C-8), 49.85 (C-9), 36.51 (C-10), 22.43 (C-11), 37.06 (C-12), 47.72 (C-13), 53.45 (C-14), 29.96 (C-15), 32.55 (C-16), 52.38 (C-17), 14.56 (C-18), 17.46 (C-19), 34.36 (C-20), 18.82 (C-21), 35.13 (C-22), 25.11 (C-23), 30.37 (C-24), 31.27 (C-25), 177.48 (C-26), 26.03 (C-27), 28.57 (C-28), 26.82 (C-29), 16.11 (C-30); +ve ion FAB MS *m/z* (rel.int.): 475 $[M+H]^+$ ($C_{30}H_{51}O_4$) (5.1), 316 (10.6), 295 (35.5), 292 (43.6), 289 (13.5), 252 (16.6), 238 (21.5), 236 (15.1), 184 (14.6), 182 (23.5), 168 (28.6), 143 (33.5), 123 (26.4).

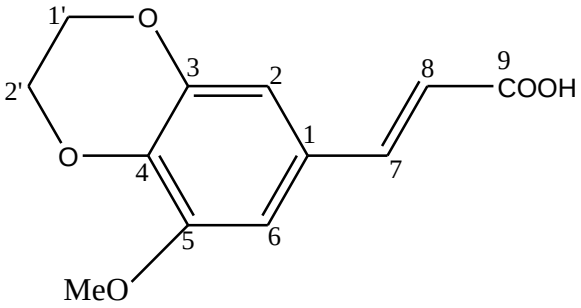
RESULTS AND DISCUSSION

The compound **1**, 415 $[M+H]^+$ ($C_{29}H_{51}O$), responded to steroidal tests positively and showed IR absorption bands for hydroxyl groups (3401cm^{-1}) and unsaturation (1654 cm^{-1}). On the basis of analysis of the ^1H and ^{13}C NMR spectra and comparison of the spectral data with the reported values the structure of **1** was determined as β-sitosterol[19-21] .



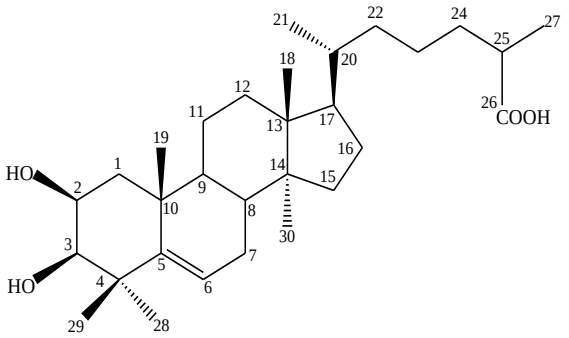
β- Sitosterol (**1**)

Compound **2** gave effervescences with sodium bicarbonate solution and showed IR absorption bands for carboxylic group (3185, 1687 cm^{-1}), unsaturation (1647 cm^{-1}) and aromatic ring (1545, 1029 cm^{-1}). Its molecular ion peak was determined at *m/z* 237 $[M+H]^+$ on the basis of mass and ^{13}C NMR spectra consistent with the molecular formula of an aromatic acid $C_{12}H_{13}O_5$. The ion peaks arising at *m/z* 191 $[M - COOH]^+$ and 165 [M – CH=CH-COOH, mass unit 71]$^+$ indicated that it was a derivative of cinnamic acid. The ^1H NMR spectrum of **2** displayed four one-proton doublets at δ 8.11 (J = 6.3 Hz) and 6.23 (J = 6.3 Hz) assigned to *cis*-oriented vinylic H-8 and H-7 protons, respectively, and at δ 6.37 (J = 2.1 Hz) and 6.19 (J = 2.1 Hz) ascribed correspondingly to meta-coupled aromatic H-2 and H-6 protons. Two two-proton triplets at δ 3.42 and 3.39 with coupling interactions of 5.5 Hz each were accounted to oxygenated methylene H_2-1′ and H_2-2′ protons, respectively. A three-proton singlet at δ 3.35 was due to the methoxy protons. The ^{13}C NMR spectrum of **2** exhibited signals for aromatic and vinylic carbons between δ 161.98 - 105.38, carboxylic carbon at δ 180.47 (C- 9), oxymethylene carbons at δ 72.73 (C- 1′, C-2′) and methoxy carbon at δ 60.77 (OMe). The absence of any ^1H NMR signal below δ 3.35 and ^{13}C NMR signal beyond δ 60.77 ruled out the existence of any saturated carbon in the molecule. These evidences led to establish structure of **2** as *cis*-3,4-dioxyethylene-5-methoxycinnamic acid. This is a new aromatic acid.



(*cis*)-3,4-Dioxyethylene-5-methoxycinnamic acid (**2**)

Compound **3**, named cranulanostenoic acid, yielded effervescences with sodium bicarbonate solution and showed IR absorption bands for hydroxyl groups (3448 cm^{-1}), carboxylic function (3247, 1698 cm^{-1}) and unsaturation (1636 cm^{-1}). Its molecular ion peak was determined at *m/z* 475 $[M+H]^+$ on the basis of FAB mass and ^{13}C NMR spectra relating to a triterpenic acid, $C_{30}H_{51}O_4$. The ion fragments generating at *m/z* 306, 168 [$C_{6,7}$ – $C_{9,10}$ fission]$^+$ and 292, 182 [$C_{7,8}$ – $C_{9,10}$ fission]$^+$ suggested the presence of the vinylic linkage at C_5 and two hydroxyl groups in ring A/B . The ion peaks appearing at *m/z* 266 [$C_{8,14}$ – $C_{9,11}$ fission]$^+$, 252, 222 [$C_{8,14}$ – $C_{11,12}$ fission]$^+$ and 236, 238 [$C_{8,14}$ – $C_{12,13}$ fission]$^+$ indicated saturated nature of the ring C. The ion fragments produced at *m/z* 184 [$C_{14,15}$ – $C_{13,170}$ fission]$^+$ and 143 [C_{17} – C_{20} fission, side chain C_8H_{15}]$^+$ supported the existence of the carboxylic group in the saturated side chain. The ^1H NMR spectrum of **3** exhibited a one-proton doublet at δ 5.31 (J=5.6 Hz) assigned to vinylic H-6, a one-proton doublet at δ 3.93 (J = 9.5 Hz, H-3α) and a one-proton multiplet at δ 3.31 with half width of 16.5 Hz ascribed to α-oriented oxygenated methine H-3α and H-2α protons, respectively, five three-proton broad singlets at δ 1.29, 1.14, 1.06, 0.84 and 0.77 due to tertiary C-28, C-19, C-30, C-29 and C-18 methyl protons and as two three-proton doublets at δ 0.99 (J = 6.3 Hz) and 0.93 (J = 6.6 Hz) associated with secondary C-27 and C-21 methyl protons. The remaining methylene and methine protons appeared between δ 2.17 – 1.32. The ^{13}C NMR spectrum of **3** displayed signals for vinylic carbons at δ 139.51 (C-5) and 118.98 (C-6), carbinol carbons at δ 71.69 (C-2) and 81.38 (C-3), carboxylic carbon at δ 177.48 (C-26) and methyl carbons from δ 28.57 to 15.56. The ^1H and ^{13}C NMR spectral data of the triterpenic unit of **3** were compared with the reported spectral data of lanostene-type triterpenoids [22-25]. On the basis of these evidences the structure of **3** was established as lanost-5-en-2β,3β-diol 26-oic acid, a new lanostenoic acid.



Cranulanostenoic acid (**3**)

CONCLUSION

Phytochemical investigation of a methanolic extract of the leaves of *P. crenulata* resulted in the isolation of β-sitosterol, *cis*-3,4-dioxyethylene-5-methoxycinnamic acid and lanost-54-en-2β,3β-diol 26-oic acid. This work has enhanced understanding about the phytoconstituents of the plant. These compounds may be used as chromatographic markers for standardization of the leaves.

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Conflicts of interests: We declare that we have no conflict of interest.

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