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Chemical Examination of the Fruits of *Citrus reticulata* Blanco

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The present paper reports the isolation and identification of non-flavonoidic and flavoniodic constituents from the fruits of *Citrus reticulata.*

Citrus reticulata Blanco[1,2] (family-Rutaceae), which is commonly known as Santara in Hindi, is widely cultivated in India and all subtropical regions of the world. The ripe fruit is used as laxative, aphrodisiac and removes *vata*, causes *kapha* and *pitta* (Ayurveda). Earlier workers[3,4] have reported limonoid glycosides and flavonone glycosides in the furit juice of the plant and because of its significant importance, it was thought worthwhile to investigate it further phytochemically.

The present paper deals with the isolation and structural elucidation of two non-flavonoidal and one flavonoidal constituents from the fruits of *citrus reticulata* Blanco. The constituents were β-sitosterol, β-amyrin and Naringenin, which were identified by chemical degradations and spectroscopic techniques.

The dried and powdered fruits of *Citrus reticulata* (procured from Allied and United Chemicals, Calcutta) were exhaustively extracted with methanol. The methanol extract was concentrated under reduced pressure. The brown gummy mass so obtained was fractionated by extracting it successively with pet-ether, benzene, chloroform, ethyl acetate and acetone. The pet-ether and benzene fractions on TLC (Si-gel) showed identical results; they were therefore combined. The combined fractions were then subjected to column chromatography over Si-gel and eluted with different solvents in the order of their increasing polarity. The pet-ether-benzene (6 : 4) eluents showed the presence of two spots on TLC over Si-gel (pet-ether benzene 5 : 5). They were, therefore, separated by TLC and assigned compounds CR-I and CR-2. The benzene-chloroform (8 : 2) eluates on crystallization with EtOAc gave yellow needle-shaped crystals which showed homogeneity on TLC over Si-gel (ben-ether 5 : 5) and assigned compound CR-3.

CR-1

It was obtained as white amorphous powder, which on crystallization with $CHCl_3$ gave white needle-shaped crystals, m.pt. 136–37°C (α_D) 32.1° ($CDCl_3$). It gave a monoacetylated derivative, m.pt 114–115°C (α_D)-48.4° ($CHCl_3$).

The IR spectrum showed bands at 1375 cm^{-1} indicating the presence of *gem*-dimethyl group, 840 cm^{-1} showing olefinic double bond, 3340 cm^{-1} hydroxyl group. ^{1}H-NMR (90 MHz, $CDCl_3$) ppm (TMS as internal reference): 0.70 (S, 3H, 18–Me), 0.80 (d, J = 6.8 Hz, 3H, 28 Me), 0.88 (d, H = 6.5 Hz, 6H, 26, 27 Me),

0.92 (d, J = 6.5 Hz, 3H, 21 Me), 1.02 (s, 3H, 19 Me), 3.56 (m, H, 3–a–H), 5.36 (m, 1H, olefinic proton), 1.07–2.34 (–CH_2, –CH proton of cyclic and side chains) and MS data: m/z M^+ 414.

On the basis of these data and by direct comparision of spectral data and co-chromatography with authentic sample[5], CR-I was identified as β-sitosterol.

CR-2

It was obtained as white needles, m.p. 198°C. It gave Liebermann-Burchard test[7]. The IR spectrum showed bands at 3360 cm^{-1} (OH), 2960 cm^{-1} (C–H), 1650 cm^{-1}, 1040–980 cm^{-1} (C=C). ^{1}H-NMR ($CDCl_3$) ppm: 0.78 (s, 3H, OMe), 0.83 (s, 3H, Me), 0.88 (s, 6H, 2XMe), 0.95 (s, 3H, Me), 0.98 (s, 3H, Me), 1.0 (s, 3H, Me), 1.14 (S, 3H, Me), 1.08, 2.01, 3.01 (dd, J = 9 Hz and 7Hz; 1Hz, –CH_2, –CH protons of cyclic and side chains), 4.88 (^{1}H–S, br, OH), 5.21 (^{1}H, m, olefinic f proton) and MS data; m/z M^+ 426.

Thus compound CR-2 was identified as β-amyrin[6]. It was further identified as β-amyrin by m.m.pt. and Co-TLC with an authentic sample.

CR-3

The fractions eluted from column with benzene-$CHCl_3$ (8 : 2) mixture on crystallization with EtOAc gave yellow needle-shaped crystals, m.pt. 248–50°C. It was characterized as Naringenin by direct comparison with an authentic sample of Naringenin (R_f-value m.pt., m.m.pt., co-chromatography and PMR). UV_{max} MeOH 265, 290 sh, 340 sh, NaOAc 276, 298 sh, 340 sh, NaOMe 298, 290, IR_{max}^{KBr} 1667 (C=O), 3550 (OH), 2940 (C–H), 850 (C=C) aromatic. ^{1}H-NMR ($CDCl_3$) ppm: 7.40 (2H, d, J = 8.5 Hz, H–2′,6′), 6.96 (2H, d, J = 8.5 Hz, H–3′,5′), 6.64 (^{1}H, d, J = 2.5 Hz, H–6), 6.86 (1H, d, J = 2.5 Hz, H–8), 2.79–2.98 (2H, M, J = 12 Hz, 4H, 17 Hz, H–3.3′), 5.20 (1H, q, J_1 = 12 Hz, J = 4 Hz; H–2), MS data: m/z M^+ 288.

Thus on the basis of these spectral data and co-chromatography with authentic sample, CR-3 was characterized as Naringenin[8].

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