



A Phytochemical Examination of Flavone from *Citrus limettiodes* Tanaka

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Abstract: A phytochemical study of the stems of *Citrus Limettiodes* Tanaka afforded a novel flavonoid, characterized as 5,4'-dihydroxy, 3'-(3''-methyl but-2''-enyl) 3,5',6-trimethoxy flavone 7-O- β -D-glucopyranoside.

Key words: *Citrus limettiodes* Tanaka, 5,4'-dihydroxy 3'-(3''-methyl but-2''-enyl) 3,5',6-trimethoxy flavone 7-O- β -D-glucopyranoside.

I. Introduction

Citrus limettiodes Tanaka (N.O. Rutaceae) (1, 2) commonly known as 'Mitha Nibu' is widely cultivated in all subtropical regions. The fruits of the plant are useful as refrigerant in fever and jaundice, biliousness, brain troubles and have tendency to haemorrhage. The rind is antheimintic, astringent to the bowels, cooling and strengthen the gums and teeth (3). Systematic examination of the metabolites present in *Citrus Limettiodes* Tanaka has revealed the presence of d-limonene, sterol esters (4), Plictran (5), flavanone glycosides (6) and ascorbic acid (7). The isolation and identification of a 3-methoxy flavone: 5,4'-dihydroxy 3'-(3''-methyl but-2''-enyl) 3,5',6-trimethoxy flavone 7-O- β -D-glucopyranoside is given.

II. Materials and Methods

The plant material was collected from United chemicals and Allied products, 10 Clive Row, Calcutta-1, India.

Instruments

Melting points : Buchi melting apparatus, uncorrected; UV : Hitachi 320 spectrophotometer; MS : Jeol-D-300 (EI/CI) 60 eV ; IR : Perkin Elmer 881 spectrometer, $^1\text{H-NMR}$: Varian FT - 20 (270 MHz) ; $^{13}\text{C-NMR}$ Bruker WM-400 (400 MHz, FT-NMR) : Chemical shifts in δ value (ppm) with TMS as an internal standard.

Isolation of 1

Ground and dried stems were extracted with aq EtOH, The extract was evaporated under reduced pressure. The concentrated gummy mass obtained was partitioned with hexane, C_6H_6 , CHCl_3 , EtOAc and Me_2CO . The concentrated EtOAc soluble fraction was chromatographed over Si-gel column using $\text{Me}_2\text{CO-MeOH}$ as eluant. The fractions collected from $\text{Me}_2\text{CO-MeOH}$ (9:1) gave compound 1, crystallized from MeOH as light yellow needles, which showed a single homogeneous spot on TLC over Si-gel ($\text{MeOH-H}_2\text{O-AcOH}$ 40:60:1).

m.p. 286-87 °C M^+ 590; analysed for $\text{C}_{29}\text{H}_{34}\text{O}_{13}$ (%C-58.59, %H-5.76); UV $\lambda_{\text{max}}^{\text{MeOH}}$ 270, 347; $\lambda_{\text{max}}^{\text{NaOMe}}$ 282, 395; $\lambda_{\text{max}}^{\text{AlCl}_3}$ 278, 389, 405; $\lambda_{\text{max}}^{\text{AlCl}_3 + \text{HCl}}$ 277, 389, 405; $\lambda_{\text{max}}^{\text{NaOAc}}$ 272, 348; IR (KBr) ν_{max} cm^{-1} : 3455(OH), 1660 ($\alpha - \beta$ unsaturated C=O), 1535 (C=C) 2835, 1210, 1120, 1015 (C-O), 830; $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ PPM : 1.34 (6H, br, s, 2CH_3), 5.17 (1H, brt, $J=7$ Hz, $=\text{C}_2''$ H), 3.67 (1H, m, C_1'' H), 3.70 (1H, m, $-\text{C}_1''$ Hb), 3.98 (3H, s, OMe), 4.02 (3H, s, OMe), 3.82(3H, s, OMe), 7.77 (1H, d, $J=2.5$ Hz, H-2'), 7.50 (1H, d, $J=2.5$ Hz, H-6'), 6.87 (1H, s, H-8), 5.71 (1H, d, $J=7$ Hz, $\text{C}-1''$ H), 4.08-5.71 (7H, m), EIMS (m/e) (M^+) absent, 428 (M^+ - sugar), 373(Aglycone $-\text{C}_4\text{H}_7$), 183 ($\text{A}_1^+ + \text{H}$), 155 ($\text{A}_1^+ + \text{H}$) $-\text{CO}$ 204 (B_1^+), 219 (B_2^+), 398 (Aglycone $-\text{OCH}_3$), 270 (Aglycone $-\text{OCH}_3-\text{CO}$), $^{13}\text{C-NMR}$: 163.60 (C-2), 134.62 (C-3), 182.10 (C-4), 152.55 (C-5), 131.34 (C-O-6), 160.52 (C-7), 94.52 (C-8), 157.30 (C-9), 106.90 (C-10), 121.42 (C-1'), 110.15 (C-2'), 104.82 (C-3'), 150.92 (C-4'), 147.92 (C-5'), 120.35 (C-6'), 23.2 (C-1''), 123.8 (C-2''), 131.32 (C-3''), 26.7 (C-4''), 19.8 (C-5''), 101.92 (C-1''), 74.20(C-2''), 76.50 (C-3''), 70.55 (C-4''), 76.60 (C-5'') 60.52 (C-6''), 60.94, 59.67, 60.87 (3OMe).

Acid hydrolysis of 1

A sample of 1 was refluxed with 7% alc H_2SO_4 for 6 hours. Work up in the usual way afforded an aglycone 1a, crystallized from MeOH, yellowish, morphous powder, $\text{C}_{23}\text{H}_{24}\text{O}_8$, m.p. 171-72 °C M^+ 428 (found : C, 64.47; H, 5.59 Calcd : C, 64.48; H, 5.60%); UV $\lambda_{\text{max}}^{\text{MeOH}}$ 270, 346; $\lambda_{\text{max}}^{\text{NaOMe}}$ 281, 394, 332; $\lambda_{\text{max}}^{\text{AlCl}_3}$ 272, 390, 402; $\lambda_{\text{max}}^{\text{AlCl}_3 + \text{HCl}}$ 274, 390, 405; $\lambda_{\text{max}}^{\text{NaOAc}}$ 287, 344; $\lambda_{\text{max}}^{\text{NaOAc} + \text{H}_3\text{BO}_3}$ 289, 350; IR $\nu_{\text{max}}^{\text{KBr}}$ 3350 (OH), 1610 ($\alpha - \beta$ unsaturated C=O), 2825, 1320, 1230, 1105, 822 cm^{-1} ; $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ ppm 1.34 (6H, br, s, 2CH_3), 5.12 (1H, br, t, $J=7$, Hz=CH), 3.65 (1H, m, $-\text{C}_1''$ H), 3.69 (1H, m, $-\text{C}_1''$ Hb), 3.90 (3H, s, OMe), 4.0 (3H, s, OMe), 3.84 (3H, s, OMe), 7.75 (1H, d, $J=2.5$ Hz, H-2'), 7.50(1H, d, $J=2.5$ Hz, H-6') 6.82 (1H, s, H-8), 12.15, 11.45, 9.80 (3-OH groups, s) : Ms (m/e) : 428 (M^+), 373 ($\text{M}^+ - \text{C}_4\text{H}_7$), 183 ($\text{A}_1^+ + \text{H}$), 155 ($\text{A}_1^+ + \text{H} - \text{CO}$), 216 (B_1^+), 219(B_2^+).

The aqueous layer was worked up for the identification of sugar and on PC examination in BAW (n-Butanol: Acetic acid: Water 4: 1: 5) showed the presence of D – glucose (PC and Co-PC).

Permethylation of 1

A sample of 1 was treated with MeI and Ag₂O in dimethyl formamide and left for two days at room temperature. On usual Work up it gave nona methyl ether, dark yellow needle m.p. 152-53°C M⁺ 674 (Found: C, 62. 27) H. 6.78; Calcd: C. 62. 31; H, 6.82%).

Enzymatic hydrolysis of 1

The compd 1 was dissolved in EtOH and mixed with Almond Emulsin and left for 72 hours and filtered, The aglycone and hydrolysate were examined separately. Aglycone C₂₃H₂₄O₈ m.p. 171-72°C M⁺ 428. The hydrolysate on concentration was examined for sugar moiety by PC using whatman No. 1 and 3 (n-Butanol-Acetic acid – water 4: 1: 5). The sugar was identified as D-glucose with authentic sample.

Periodate oxidation of 1

The glycoside 1 was dissolved in MeOH and treated with sodium Meta periodate (0. 1 N). It consumes 2.02 moles of periodate and liberated 1.08 moles of formic acid suggested that D-glucose was present in pyranose form.

Cyclisation

Reaction of 1 on refluxing with 98% HCOOH acid yielded a chromane derivative 1b (M⁺ = 590).

III. Result and discussion

The compound 1 was isolated as light yellow needles, m.p. 286-87°C M⁺ 590 and analysed for C₂₉H₃₄O₁₃; It gave positive Molish test, reduces tollens reagent, FeCl₃ test and colour reactions of flavonols (8, 9). The IR spectrum of the 1 showed the absorption bands at 3455 (OH), 1658 (α – β unsaturated C=O), 1535 (C=C), 2835 (–OCH₃), 830, 1015 (C–O).. The UV spectrum showed absorption maxima at 270 and 347 nm and changes in the presence of diagnostic shift reagents (10) suggested free hydroxyl groups at C-4', C-5 positions with blocked C-7 and C-3 positions.

The ¹H-NMR spectrum of 1 showed a set of signals that could be attributed as side chain of prenyl group –CH₂ – CH = C<^{CH₃}_{CH₃}. Two one-proton mutliplets at δ 3.67 and 3.70, a one-proton broad triplet at δ 5.17

(J= 7Hz) and one singlet integrating for 6H protons at δ 1.34 assigned the presence of prenyl group. The aromatic B-ring signals at δ 7.77 (1H, d, J =2.5 Hz, H-2') and 7.50 (1H, d, J =2.5 Hz, H-6') (two meta-coupled doublets deshielded by C=O) were assigned to H-2' and H-6' respectively. The presence of three methoxyl groups were confirmed by shift at δ 3.98, 4.02 and 3.82 appearing as singlets, A singlet at δ 6.87 for one proton intensity was assigned to H-8. Two sharp and board singlets at δ 12.35 and 11.06 each for one proton were assigned protons of 5-OH and 4' –OH respectively, The spectrum of 1 also revealed the sugar protons at δ 5.71 as doublet (J=7.5 Hz) assigned to H-1 of glucose (B-configuration), ¹³C-NMR spectrum of 1 revealed the presence of 29 carbon atoms in it and agreed with the structure of glycoside as 1.

The EI mass spectrum of 1 was in full agreement with the assigned structure, The molecular ion peak as expected was not observed, The MS showed a fragment ion at m/z 428 which corresponds to the loss of monosaccharide from the molecular ion. The loss of Isobutenyl radical from aglycone gave fragment at m/z 378. A Retro Diel's Alder fragmentation was observed at m/z 183 and m/z 204 leading to [A₁⁺ +H] and [B₁⁺] fragments which indicated the presence of two hydroxyl and one methoxy in ring A and B prenyl group at C-3' in ring B of the aglycone. Compd, 1 gave single chromane derivative 1b [M⁺ = 590], thus confirming that prenyl group was neighboured by a single hydroxy group. Acid hydrolysis of the 1 with 7% HCl gave D-glucose (PC and GLC) and a aglycone C₂₃H₂₄O₈ m.p.171-172°C M⁺ 428. The aglycone gave a bathochromic shift of 17 nm in Band II with NaOAc indicating that the sugar was linked to C-7 position of the aglycone. Aglycone was identified as 7, 5, 4'-trihydroxy, 3'-prenyl, 3, 5', 6-trimethoxy flavone by its UV, IR, ¹H-NMR spectral analysis, Kuhn methylation (MeI/DMF/Ag₂O) of 1 followed by acid hydrolysis yielded 2, 3, 4, 6,-tetra-o-methyl-D- glucose confirmed that the sugar was attached via its C₁ –OH to the C₇-OH of the aglycone. 1 on enzymatic hydrolysis with Almond emulsion (11) indicated β- linkage between the sugar and alycone the sugar was confirmed as D-Glucose (by PC and Co-TLC). The sodium Meta periodate oxidation (12) of the 1 indicated that sugar was in the pyranose form. Thus the compound 1 is assigned the structure 5, 4' –dihydroxy, 3' – (3'' –methyl, but-2'' – enyl) ,3,5',6 – trimethoxy flavone 7-O β-D-glucopyranoside.

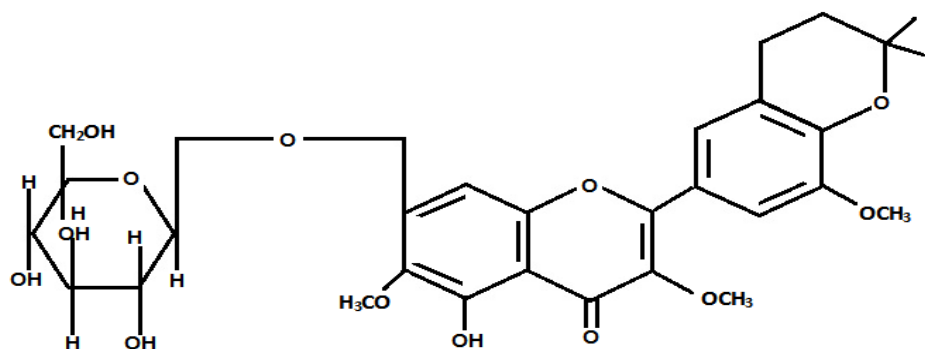
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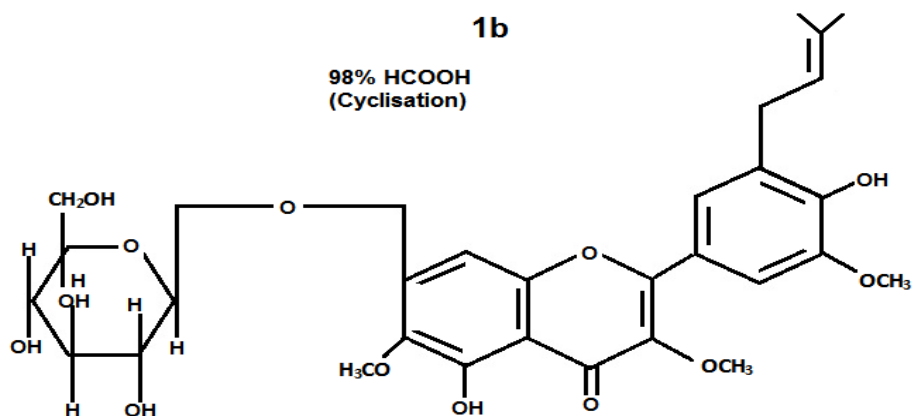
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1b

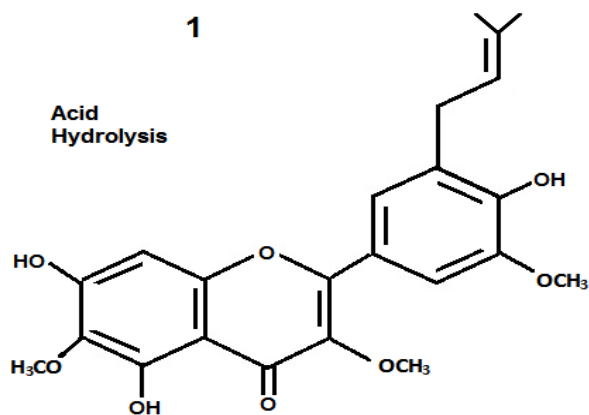
**98% HCOOH
(Cyclisation)**



1

**Acid
Hydrolysis**

Sugar +



1a