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3-Methoxy Flavones: The Potential Antiviral Agents from *Citrus Recticulata*, Blanco

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Phytochemical study of the fruits of *Citrus reticulata* Blanco afforded kaempferide trimethyl ether 1 and quercetin 3,7,3′,4′-tetramethyl ether 2 which were characterised by various chemical degradations and spectral studies.

INTRODUCTION

Citrus reticulata Blanco (N.O. Rutaceae) is commonly known as '*Santara*' and cultivated in all subtropical regions of the world[1]. Its fruits are laxative, anthelmintic and aphrodisiac[2]. Systematic examination of the metabolites present in *Citrus reticulata* Blanco has revealed the presence of limonoid glycosides[3], flavonone diglycosides[4], steroids[5], and a few flavones[6]. The benzene soluble fraction of the methanolic extract of the fruits of *Citrus reticulata* on column chromatography over silica-gel afforded two known flavonoidal compounds in successive eluants $CHCl_3$:EtOAc (4:1, 1:1) which have been identified as kaempferide trimethyl ether and quercetin 3,7,3′,4′-tetramethyl ether which were firstly isolated from this plant.

EXPERIMENTAL

Plant Material: The fruits of *Citrus reticulata* Blanco were procured from M/s United Chemicals and Allied Products.

Isolation of the Flavonoids: The air-dried and crushed fruits of *Citrus reticulata* Blanco were extracted with aq-MeOH. The extract was evaporated under reduced pressure. The concentrated brown gummy syrup obtained was partitioned with petroleum ether, C_6H_6, $CHCl_3$, EtOAc and Me_2CO. The benzene fraction was chromatographed over a silica-gel column using solvent with their increasing polarity. Elution with $CHCl_3$:EtOAc (4:1) gave compound 1 and $CHCl_3$:EtOAc (1:1) gave compound 2. Crystallisation of 1 and 2 with EtOH gave pale yellow needles, which showed a single homogeneous spot on TLC over Silica-gel using C_6H_6:MeCOEt:MeOH:H_2O (70:20:8:2) respectively.

Kaempferide trimethyl ether 1: Yellow needles, 153–154°C, IR $\nu_{max}(cm^{-1})$ 3345 (OH), 3723 (—OCH_3), 1625 (α-β unsaturated C=O system); UV λ_{max} nm:

258, 356 (MeOH); 258, 250, 354 ($AlCl_3$); 258, 249, 356 ($AlCl_3$ + HCl); 254, 320, 378 (NaOMe); 254, 356 (NaOAc); 255, 356 (NaOAc/H_3BO_3); ^{1}H-NMR (90 MHz, $CDCl_3$); δ7.89 (2H, dd, J = 1.5 Hz, 7.5 Hz, H-2′, 6′), 6.84 (2H, dd, J = 1.5 Hz, 7.5 Hz, H-3′, 5′), 6.46 (1H, d, J = 2 Hz, H-8), 6.12 (1H, d, J = 2 Hz, H-6), 3.90 (3H, S, OMe), 3.82 (3H, S, OMe), 3.79 (3H, S, OMe), 4.02 (3H, S, OMe); EIMS m/z 342 ($C_{19}H_{18}O_6$), 327 ($C_{19}H_{18}O_6$–CH_3), 314 ($C_{19}H_{18}O_6$–CO), 180 (A_1^+), 181 (A_1 + H)$^+$, 153 (A_1 + H$^+$–CO), 132 (B_1^+), 135 (B_2)$^+$.

Quercetin 3,7,3′,4′ tetramethyl ether 2: Pale yellow needles, 159–60°, IR ν_{max}(cm^{-1}) (KBr): 3420 (OH), 3775 (—OCH_3), 1620 (α-β unsaturated C=O system); UV λ_{max} nm: 254, 356, 332 (MeOH); 282, 362, 305 (NaOMe); 272, 402, 300 ($AlCl_3$); 273, 399, 300 ($AlCl_3$ + HCl); 256, 272, 356 (NaOAc); 252, 356, 306 (NaOAc + H_3BO_3); ^{1}H-NMR (90 MHz, $CDCl_3$); δ7.76. (1H, J = 2.0 Hz, d, H-2′), 7.58 (1H, d, J = 2.0 Hz, 9.0 Hz H-6′), 6.34 (1H, d, J = 9.0 Hz, H-5′), 6.45 (1H, d, J = 2.5 Hz, H-8), 6.12 (1H, d, J = 2.5 Hz, H-6), 3.98 (3H, S, OMe), 3.48 (3H, S, OMe), 4.03 (3H, S, OMe), 3.88 (3H, S, OMe). EIMS m/z: 357 ($C_{19}H_{17}O_7$), 329 ($C_{19}H_{17}O_7$—CO), 342 ($C_{19}H_{17}O_7$—CH_3), 166 (A_1^+), 167 (A_1 + H$^+$), 138 (A_1 + H$^+$—CO), 162 (B_1^+), 165 (B_2^+).

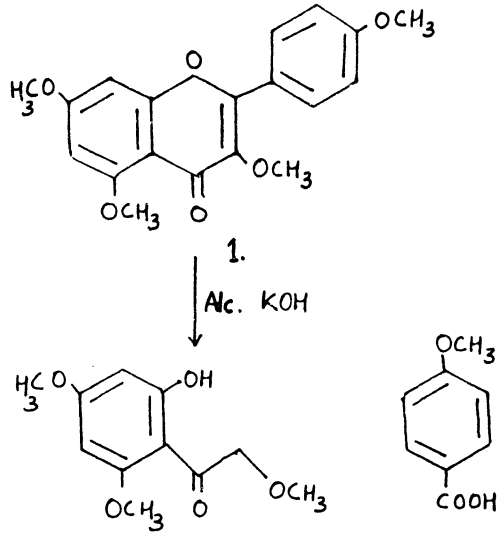
RESULTS AND DISCUSSION

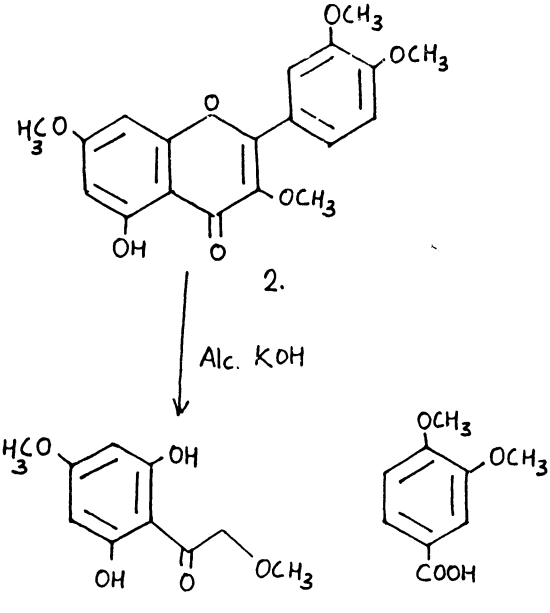
The compound 1 $C_{19}H_{18}O_6$ 153–54°C and compound 2 $C_{19}H_{17}O_7$ 159–60°C gave all positive colour reactions for flavonoids (7, 8). The UV spectrum of 1 in methanol showed strong absorption bands at 258 and 356 nm and did not change in the presence of diagnostic shift reagents suggesting that free hydroxyl groups were not attached in flavone system. The ^{1}H-NMR spectrum (90 MHz, $CDCl_3$) of 1 showed four sharp singlets for four methoxyl group at δ3.90, 3.82, 3.79 and 4.02. Two doublets each of one proton intensity were observed at δ6.46 (1H, d, J = 2Hz, H-8) and 6.12 (1H, d, J = 2 Hz, H-6). Two double doublets of two proton intensity were observed at δ7.89 (2H, dd, J = 1.5 Hz, 7.5 Hz, H-2′, 6′). and 6.84 (2H, dd, J = 1.5 Hz, 7.5 Hz, H-3′, 5′). In EIMS of 1 Retro Diel's Alder fragmentation were observed at m/z 181 and m/z 132 leading to fragments (A_1 + H)$^+$ and (B_1)$^+$ which indicated the presence of two methoxy group in ring C and one methoxy group in ring B at C-4′ position.

The UV spectrum of 2 in methanol showed strong absorption bands at 254 and 356 nm with a shoulder at 332 nm. Presence of C-5 hydroxy group is indicated by the fact that $AlCl_3$ induced bathochromic shift of 46 nm is band I.[8] The ^{1}H-NMR spectrum (90 MHz, $CDCl_3$) showed four singlets for 4 methoxy groups at δ3.98, 3.48, 4.03 and 3.88 (4 × 3H, 4S, 40 Me). Two doublets were assigned at δ6.45 and δ6.12 for H-8 (1H, d, J = 2.5 Hz) and H-6 (1H, d, J = 2.5 Hz). The relative positions of methoxyl and hydroxyl groups as shown in 2 followed from the mass spectrum which showed RDA fragments at m/e 167 and 162 were in conformity with the structure 2. The molecular peak at M$^+$ 357 ($C_{19}H_{17}O_7$) along with other important fragmentation peaks at m/e 342 ($C_{19}H_{17}O_7$—CH_3), 165 (B_2^+).

The structure of compounds 1 and 2 were mainly confirmed by its alkaline hydrolysis, in which both yielded two products. Alkaline degradation of 1 yielded

2-hydroxy, 4,6-dimethoxy (1′-methoxy) acetophenone, $C_{11}H_{14}O_5$, M^+ 226 and *p*-methoxy benzoic acid, $C_8H_8O_3$, m.p. 215–17°C $M^+ = 152$, but compound 2 gave 2,6-dihydroxy 4-methoxy (1′-methoxy) acetophenone, $C_{10}H_{12}O_5$, M^+-212 and 3,4-dimethoxy benzoic acid, $C_9H_{10}O_4$, m.p. 178°, M^+-182.





On the basis of these findings compound, 1 and 2 were identified as kaempferide trimethyl ether and quercetin 3,7,3′,4′-tetramethyl ether.

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