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FLAVONOIDS OF THE CITRUS CULTIVAR CALAMONDIN AND SYNTHETIC 2′,β-DIHYDROXYCHALCONES

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Key Word Index—*Citrus*; Rutaceae; calamondin; methoxyflavones; 5,6,7,8,3′,4′-hexamethoxyflavanone; 2′, β-dihydroxy-3′,4′,5′,6′,3,4-hexamethoxychalcone; 2′, β-dihydroxy-3′,4′,5′,6′,4-pentamethoxychalcone.

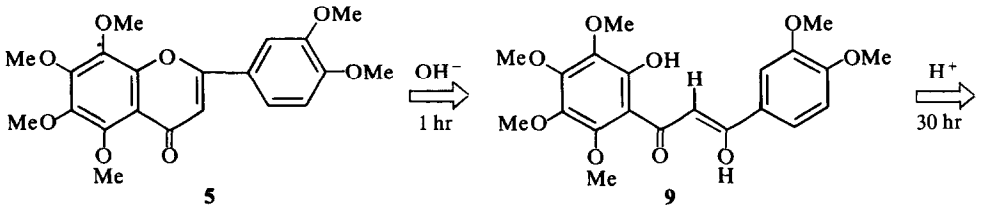
Abstract—Eight methoxyflavones were isolated and identified from the peel of calamondin. Citromitin and 5-*O*-desmethylcitromitin are actually nobiletin and 5-*O*-desmethylnobiletin, respectively. 5,6,7,8,3′,4′-Hexamethoxyflavanone and 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavanone are not constituents of calamondin, although previously reported.

INTRODUCTION

The calamondin, grown primarily as an ornamental, produces small, sour, loose-skinned mandarin-like fruit. The most recent data compiled by Barrett and Rhodes [1] suggest that the characteristics of calamondin may be derived from *Citrus reticulata* and *Fortunella margarita*. The identities of the parents of this plant are of taxonomic interest. Sastry and Row [2, 3] reported the isolation and identification of two methoxyflavanones from the calamondin not previously reported in any type of *Citrus*. Later Chaliha *et al.* [4] reported the presence of these same compounds in *C. reticulata*. We examined the peel of calamondin to determine what methoxyflavonoids were present and whether there was a possible chemical link between calamondin and *C. reticulata*.

RESULTS AND DISCUSSION

Eight methoxyflavonoids (**1**–**8**) were isolated and identified from the peels of calamondin (Table 1). We had previously isolated seven of these compounds from Valencia orange (*C. sinensis*) and seven from a *C. reticulata* × (*C. paradisi* × *C. reticulata*) hybrid [5]. The major flavonoid isolated was 5,6,7,8,3′,4′-hexamethoxyflavone (nobiletin) (**5**). A close examination of the data (MP and UV) reported by Sastry and Row for 5,6,7,8,3′,4′-hexamethoxyflavanone and 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavanone revealed they were virtually identical to the two flavone analogs we had isolated (Table 2). Alkaline hydrolysis [2] of flavone (**5**) produced **9**, 2′,β-dihydroxy-3′,4′,5′,6′,3,4-hexamethoxychalcone. The NMR spectrum of **9** in three solvents showed the compound to be primarily in the β-enol



5 **9**

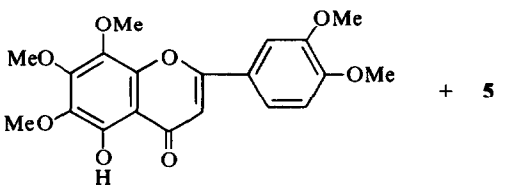


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Table 1. Flavonoids isolated from calamondin and synthetic intermediates

	Compound	Found in calamondin mg of pure compound*
1	5-Hydroxy-6,7,8,3',4'-pentamethoxyflavone	1
2	5,6,7,8,4'-Pentamethoxyflavone	8
3	3,5,6,7,8,3',4'-Heptamethoxyflavone	1
4	5,6,7,4'-Tetramethoxyflavone	1
5	5,6,7,8,3',4'-Hexamethoxyflavone	18
6	5,6,7,3',4'-Pentamethoxyflavone	3
7	5,7,8,4'-Tetramethoxyflavone	1
8	5,7,8,3',4'-Pentamethoxyflavone	1
9	2',β-Dihydroxy-3',4',5',6',3,4-hexamethoxychalcone†	
10	2',β-Dihydroxy-3',4',5',6',4-pentamethoxychalcone†	
11	2'-Hydroxy-3',4',5',6',3,4-hexamethoxychalcone†	
12	5,6,7,8,3',4'-Hexamethoxyflavanone†	

*Isolated from 1.3 kg of calamondin peel.
†Synthetic.

form (97% in CS_2 and 50% in DMSO-d_6). Both enol forms were observed when run in $CDCl_3$. The dicarbonyl form was highest (50%) in DMSO-d_6. When **9** was refluxed 30 hr with acid **5** was the major product plus **1** which is an expected product from **5** under prolonged reflux [5]. Alkaline hydrolysis of **2**, 5,6,7,8,4'-pentamethoxyflavone, gave **10**, 2',β-dihydroxy-3',4',5',6',4-pentamethoxychalcone. Ring closure of **10** gave **2**. We synthesized 2'-hydroxy-3',4',5',6',3,4-hexamethoxychalcone (**11**). Ring closure of **11** gave **12**, 5,6,7,8,3',4'-hexamethoxyflavanone, which Sastry and Row reported they had isolated. The UV, MS and NMR spectra of **12** confirmed the identity of the flavanone we synthesized. TLC of **12** as a reference compared with an extract of calamondin peel in three solvents gave no evidence that **12** was present in calamondin. These studies indicate that the flavanone citromitin reported by Sastry and Row was actually the flavone **5**, and the alkaline hydrolysis product they isolated must have been **9** instead of **11** as reported (see Table 2). The UV spectrum of **9** had λ_{max} of 384, 294 nm and for **11**, 373 and inflections at 310 and 264 nm. Sastry and Row reported 382 and 288 nm for their alkaline hydrolysis product. They reported the synthesis of 5-*O*-desmethylcitromitin from citromitin but actually prepared **1** (5-*O*-desmethylnobiletin). The names citromitin and 5-*O*-desmethylcitromitin should be dropped from usage. Oliverio and Casinovi [7] have apparently synthesized **11** and **12**. They reported mp 119° for **12** while ours had mp 101–102.5° and they reported mp 97–98° for **11** while ours was 92.5–93°. Two attempts to prepare 2',6',β-trihydroxy-3',4',5',3,4-pentamethoxychalcone from **1** failed. The first attempt was a 30 min alkaline reflux and the major product was starting material. The second attempt was a 20 hr reflux which gave degradation products. The desired reaction apparently does not proceed because of hydrogen bonding between the 5-hydroxyl group and the carbonyl.

The methoxyflavonoids (34 mg) isolated from calamondin (Table 1) when converted to percentages of each compound and compared to those isolated from a mandarin hybrid and Valencia orange [5] indicate that one of the parents of calamondin was a mandarin. When compared to the quantities isolated from Dancy (*C. reticulata*) and Valencia (*C. sinensis*) leaves (unpublished data) the same relationship was apparent.

EXPERIMENTAL

Preparation of peel extract. Fr. peel (1.3 kg) of calamondin was ground in a blender and extracted on a counter rotating mixer with a total of 8 l. C_6H_6. The C_6H_6 extract was dried, evapd and the oily residue distilled at 50° and 0.1 mm Hg. The residue was then separated by prep-TLC [5].

Prep-TLC and solvent systems. Si gel GF 1 mm plates were used with the following solvent systems prepared from C_6H_6 and Me_2CO v/v. (A) = (9:1); (B) = (4:1); (C) = 17:3)

Flavanone and chalcone synthesis. The procedure of ref. [8] was used to synthesize gram quantities of 2-hydroxy-3,4,5,6-tetramethoxyacetophenone, 2'-OH-3',4',5',6'-3,4-hexamethoxychalcone and 5,6,7,8,3',4'-hexamethoxyflavone. All mps are uncorr. NMR spectra were recorded at 60 MHz with TMS as an int. stand.

2'-Hydroxy-3',4',5',6',3,4-hexamethoxychalcone (**11**) [2]. To a soln of 2-hydroxy-3,4,5,6-tetramethoxyacetophenone (200 mg) and 3,4-dimethoxybenzaldehyde (1.3 g) in EtOH (10 ml) was added aq. NaOH (3 g in 3 ml) at 0°. After 2 days at room temp. the reaction mixture was taken up in H_2O (125 ml) and the unreacted starting materials were removed by extraction with C_6H_6 (2 × 100 ml) and Et_2O (2 × 100 ml). The soln was then acidified (HCl) and extracted with Et_2O (2 × 100 ml). The Et_2O extract was washed with 2.5% $NaHCO_3$ (3 × 30 ml) to remove acids. The extract was dried and the solvent removed. After 3 crystallizations from EtOH, (26.4 mg) of product **11**, mp 92–93°, was obtained. Prep-TLC of the mother liquor in solvent system A yielded an additional 12 mg of **11**. UV λ_{max}^{EtOH} nm (log ε: 373 (4.27), 310 *sh* (3.98), 264 *sh* (3.86). MS (*m/e*): 404M^+. NMR $CDCl_3$ (δ). 13.25 (*s*, 2-OH), 7.78 (*s*, H-α, H-β), 7.15 (*m*, H-2, H-6), 6.83 (*d*, *J* = 8, H-5), 3.94 (*m*-6MeO). Large scale synthesis of **11** by the procedure of ref [8] gave 2.5 g of **11** and 0.19 g of **12** [7].

5,6,7,8,3',4'-Hexamethoxyflavanone (**12**) [2]. After refluxing 42 mg of **11** with 3% alc H_2SO_4 (10 ml) for two hr, the solvent was removed *in vacuo* and 100 ml H_2O added. The aq. mixture was extracted with 4 × 100 ml portions C_6H_6. The extract was dried and the solvent removed. The oil was separated by prep-TLC (solvent system B). Five mg of **11** and 8.4 mg of **12**, mp 101–102.5° were isolated. Prep-scale synthesis of **12** gave 0.625 g. UV λ_{max}^{EtOH} nm (log ε): 338 (3.46), 280 (4.16). MS (*m/e*). 404M^+. NMR $CDCl_3$ (δ): 6.9 (*m*, H-2',5',6'), 5.30 (*q*, H-2), 3.90 (*m*-6MeO), 2.95 (unsym *t*, H-3 *cis*, H-3-*trans*).

Table 2. Comparison of citromitin and 5-hydroxycitromitin to a known flavone 5,6,7,8,3',4'-hexamethoxyflavone (nobiletin)

	mp	UV(nm)	log ε
Citromitin*	134–136	335, 272, 250	4.38, 4.23, 4.32
Nobiletin	133–134	334, 272, 251	4.38, 4.22, 4.27
5-*O*-Desmethylcitromitin*	146–147	344, 283, 255	4.37, 4.28, 4.19
5-*O*-Desmethylnobiletin	147–148	340, 283, 253	4.34, 4.25, 4.16
Citromitin hydrolysis product*	116–117	382, 288	4.30, 4.09
2'-β-Dihydroxy-3',4',5',6',3,4-hexamethoxychalcone	110–110.5	384, 294	4.27, 4.04
5,6,7,8,3',4'-Hexamethoxyflavanone	101–102.5	338, 280	3.46, 4.16

*See ref. [2].

2′-β-Dihydroxy-3′,4′,5′,6′,3,4-hexamethoxychalcone (**9**). A soln of **5**, 1 g in 46 ml EtOH was refluxed with 4 g KOH for 1 hr and the EtOH removed. Water (100 ml) was then added and acidified with HCl. The soln was extracted with C_6H_6 (4 × 100 ml). After C_6H_6 was dried and removed, the product was crystallized ×4 from MeOH mp 109–110.5°. Purification by prep-TLC (in C_6H_6) gave **9** mp 110–110.5, UV λ_{max}^{EtOH} nm (log ε): 384(4.27), 294(4.04), MS (*m/e*): 420M$^+$, 388[M-Me-OH]$^+$, NMR, CS_2 (δ): 15.60 (*s*-OH-β), 12.20 (*s*-OH-2′), 7.36 (*m*, H-2, H-6), 7.16 (0.93H, *s*, H-α), 6.76 (*d*, *J* = 8, H-5), 4.33 (0.07 H_2, *s*, CH_2), 3.80 (*m*, 6 × MeO), NMR, $CDCl_3$ (δ): 15.70 (*s*, OH-β), 12.96 (*s*, OH-β), 12.36 (*s*, OH-2′), 7.51 (*m*, H-2, H-6), 7.30 (0.8H, *s*, H-α), 6.91 (*d*, *J* = 8, H-5), 4.60 (0.2 H_2, *s*, CH_2), 3.88 (m, 6 × MeO), NMR, DMSO-d_6, (δ): 11.73 (*s*, OH-β), 9.65 (*s*, OH-2′), 7.56 (*m*, H-2, H-6), 7.07 (*d*, *J* = 8, H-5), 6.70 (0.5H, *s*, H-α), 4.56 (0.5H_2, *s*, CH_2), 3.75 (*m*, 6 × MeO).

5,6,7,8,3′,4′-Hexamethoxyflavone (**5**). A soln of 100 mg **9** in 48.5 ml EtOH and 1.5 ml H_2SO_4 was refluxed for 30 hr. The solvent was removed *in vacuo* and 100 ml H_2O added. The H_2O soln was extracted with C_6H_6 (5 × 50 ml). The C_6H_6 extract was dried and the solvent removed. The product was crystallized from MEOH, mp 126–134°. Prep-TLC, solvent system C, of the product gave 48 mg **5** mp 133–134°, UV λ_{max}^{EtOH} nm (log ε): 334 (4.38), 272 (4.22), 251 (4.27). NMR $CDCl_3$ (δ): 7.46 (*m*, H-2′, H-6′), 6.92 (*d*, *J* = 8, H-5), 6.56 (*s*, H-3), 3.96 (*m*, 6 × MeO), and 7 mg of **1** mp 144–144.5°, IR and MS confirmed **5** and **1**.

2′-β-Dihydroxy-3′,4′,5′,6′,4-pentamethoxychalcone (**10**). A soln of **2** (1 g) was prepared as above except for refluxed 30 min, yielding 0.5 g of **10** mp 75.5–77°. UV λ_{max}^{EtOH} nm (log ε): 376 (4.29), 288 (4.08), MS (*m/e*): 390M$^+$, 358 (M-Me-OH)$^+$, NMR $CDCl_3$ (δ): 15.65 (*s*, OH-β), 12.39 (*s*, OH-2′), 7.86 (*d*, *J* = 8, H-2, H-5), 7.28 (*s*, H-α), 6.93 (*d*, *J* = 8, H-3, H-5), 4.05 (*s*, MeO), 3.85 (*s*-4MeO).

5,6,7,8,4′-Pentamethoxyflavone (**2**). A soln of **10** (100 mg) was prepared as above but refluxed only 3 hr, yielding **2** (74 mg) mp 149.5–151°. IR and MS confirmed **2**.

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