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FLAVONOIDS FROM ROOT BARK OF *CITRUS SINENSIS* AND *C. NOBILIS*

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Key Word Index—*Citrus sinensis* var. *brasiliensis*; *C. nobilis* var. *sunki*; Rutaceae; root bark; flavanone; chalcone; citrunobin.

Abstract—A new chalcone, citrunobin together with citflavanone and lonchocarpol-A(senegalensein) were isolated from the root bark of *Citrus sinensis* var. *brasiliensis*. Citrunobin and citflavanone were also isolated from the corresponding bark of *C. nobilis* var. *sunki*. The structure of citrunobin was elucidated as (*E*)-1-[7′-hydroxy-5′-methoxy-2′,2′-dimeth-yl-2′H-chromen-8′-yl]-3-(4-hydroxyphenyl)prop-2-enone from spectroscopic and chemical evidence. The absolute configurations of citflavanone and lonchocarpol-A were determined as 2*S* from their CD spectral data.

INTRODUCTION

In previous papers [1, 2], we have reported the isolation of several acridone alkaloids, coumarins, flavonoid, sesquiterpene and unidentified compounds, **a**–**d** from the root bark of *Citrus sinensis* var. *brasiliensis*. Compounds **a** and **b** have also been isolated from the root bark of *C. nobilis* var. *sunki*. The present paper deals with the structural elucidation of the unidentified compounds **a**–**c**.

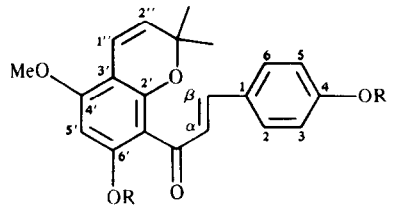
RESULTS AND DISCUSSION

Compound **a** (**1**), red plates, gave a mass spectrum which showed a $[M]^+$ peak at *m/z* 352 and a ^{13}C NMR spectrum (21 carbons) consistent with the formula $C_{21}H_{20}O_5$. Its IR spectrum exhibited a strong absorption band at 1630 cm^{-1} characteristic of a conjugated ketone with intramolecular hydrogen bonding. The UV spectrum showed absorption maxima at 229, 290 and 310 nm. These data are consistent with the compound having a chalcone skeleton [3]. It formed a diacetate derivative **2** with IR bands at 3590 and 3300 cm^{-1} indicating the presence of two hydroxyl groups. The bathochromic shifts of **1** with $AlCl_3$ (+96 nm), NaOMe (+120 nm) and NaOAc (100 nm) in the UV spectrum indicated the presence of 4,6-dihydroxyl groups in the molecule [3]. This feature was also supported by two singlet signals at δ5.27 and 14.34 (each 1H, disappeared with D_2O) in the ^1H NMR spectrum. The lower field singlet signal (δ14.34) was due to a strongly hydrogen bonded hydroxyl group.

Table 1. ^1H NMR spectra (100 MHz) of *Citrus* flavonoids

	1	2	5	6	7
2,6-H (2′,6′-H)	7.52 (2H, *d*, 8.5)	7.50 (2H, *d*, 7)	7.32 (2H, *d*, 8.4)	7.47 (2H, *d*, 8.5)	7.25 (2H, *d*, 8)
3.5-H (3′,5′-H)	6.88 (2H, *d*, 8.5)	7.07 (2H, *d*, 7)	6.89 (2H, *d*, 8.4)	7.15 (2H, *d*, 8.5)	6.82 (2H, *d*, 8)
α-H	7.75 (1H, *d*, 15.6)	6.94 (1H, *d*, 14)	—	- -	—
β-H	8.02 (1H, *d*, 15.6)	7.37 (1H, *d*, 14)	—	-	-
5′-H (6-H)	6.06 (1H, *s*)	6.18 (1H, *s*)	6.00 (1H, *s*)	6.18 (1H, *s*)	—
1″-H	6.59 (1H, *d*, 10)	6.58 (1H, *d*, 10)	6.52 (1H, *d*, 10)	6.58 (1H, *d*, 10)	5.15 (2H, *m*)
2‴-H	5.47 (1H, *d*, 10)	5.51 (1H, *d*, 10)	5.46 (1H, *d*, 10)	5.55 (1H, *d*, 10)	3.29 (4H, *t*, 7)
4-OH (4′-OH)	5.27 (1H, *s*)	—	6.00 (1H, *s*)	- -	6.35 (1H, *s*)*
6′-OH (5-OH)	14.34 (1H, *s*)	—	12.10 (1H, *s*)	—	12.24 (1H, *s*)
7-OH	—	—	—	- -	5.69 (1H, *br s*)*
2-Hax	—	—	5.36 (1H, *dd*, 3 & 12.9)	5.46 (1H, *dd*, 3 & 13.4)	5.27 (1H, *dd*, 4 & 12)
3-Hax	—	—	3.07 (1H, *dd*, 12.9 & 17.1)	2.97 (1H, *dd*, 13.4 & 16.7)	3.02 (1H, *dd*, 12 & 17)
3-Heq	—	—	2.79 (1H, *dd*, 3 & 17.1)	2.73 (1H, *dd*, 3 & 16.7)	2.72 (1H, *dd*, 4 & 17)
4-OMe	3.86 (3H, *s*)	3.81 (3H, *s*)	—	-	—
Me	1.55 (6H, *s*)	1.39 (6H, *s*)	1.42 (3H, *s*)	1.45 (3H, *s*)	1.69 (6H, *s*)
			1.44 (3H, *s*)	1.46 (3H, *s*)	1.73 (3H, *s*)
					1.79 (3H, *s*)
OAc	—	2.27 (3H, *s*)	—	2.33 (3H, *s*)	—
		2.17 (3H, *s*)		2.37 (3H, *s*)	

Values are in ppm. Figures in parentheses are coupling constants in Hz.
*Values can be interchanged.



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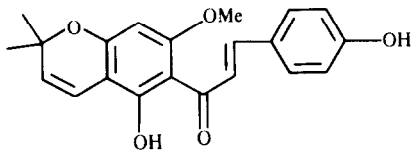

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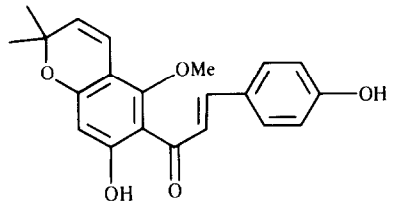

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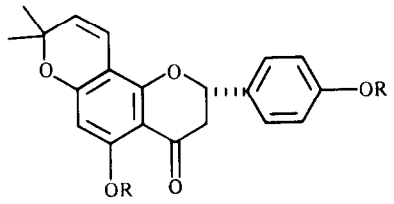

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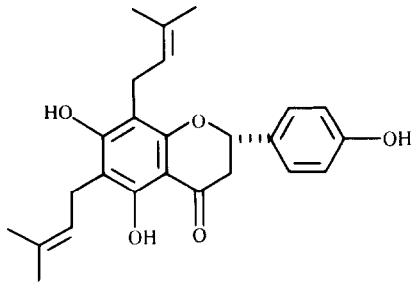

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In the ^{1}H NMR spectrum of **1** (Table 1), the (*E*)-vinylic protons of a chalcone moiety appeared at δ7.75 and 8.02 as an AB quartet ($J = 15.6$ Hz). The AB pattern at δ5.47 and 6.59 ($J = 10$ Hz) coupled with a six proton singlet at δ1.55 indicated the presence of a 2,2-dimethyl-pyran ring in the molecule. A singlet signal at δ3.86 (3H) was assigned to the methoxyl protons. Two *ortho* coupled doublets centred at δ6.88 and 7.52 integrating for four protons of an AB system were due to the protons on a *para* disubstituted benzene ring. A singlet signal at δ6.06 was assigned to a proton on the second aromatic ring. The mass spectrum of **1** showed a $[M]^+$ peak at *m/z* 352, a base peak at *m/z* 217 and a prominent peak at *m/z* 337. According to the above data, three possible structures **1**, **3** and **4** were thus considered for compound **a**. Because compound **a** gave a negative Gibb's test, structure **3** could be excluded. In the diacetate (**2**), the singlet signal occurred at δ6.18, a downfield shift (0.12 ppm) which is consistent only with the proton being at C-5′. The doublet signal at δ6.88 shifted appreciably (0.19 ppm) to lower field (δ7.07) on acetylation of the chalcone pointed to the hydroxyl group being at C-4. On the other hand, a NOE experiment involving irradiation of the methoxyl group resonances while examining the integrated intensity of the signals from the aromatic protons was carried out. An enhancement of 16.8% of the integral for the signal due to H-5′ at δ6.06 together with the absence of any long-range coupling between the signal aromatic proton and the proton of the dimethylpyran moiety [4, 5] suggested that structure **4** was unlikely, and as a result, structure **1** was favoured for compound **a**. It is a new compound which we have named citrunobin.

Compound **b** (**5**), colourless plates, mp 169–171° [lit. 98–100°] [6], $[\alpha]_D$ −2.5° ($CHCl_3$). The spectral data of **5** and its derivative **6** was in agreement with that of citflavanone which has previously been isolated from *Citrus* plants by Furukawa *et al.* [6]. However, the absolute configuration of citflavanone has not been determined. The CD spectrum of **5** exhibited a positive Cotton effect at 316 nm and a negative one at 295 nm due to a transition which is characteristic of 2S flavanones [7]. Furthermore, the large coupling constant ($J_{2Hax,\ 3Hax} = 12.9$ Hz) between the C-2 and C-3 protons suggest that the 2-phenyl ring exists in the equatorial position which is thermodynamically favourable [8]. Therefore, citflavanone (**5**) has the 2*S* configuration at C-2.

Compound **c** (**7**), amorphous powder, mp 90–95°, $[\alpha]_D$ −4.5° ($CHCl_3$). Its spectral data (UV, IR, ^{1}H NMR and mass spectrum) closely resembled those of lonchocarpol-A (segalensein) (**7**) [9, 10]; however, the stereochemistry is not at present known. The CD spectrum of **7** showed a positive Cotton effect at 333 nm and a negative one at 291 nm which was similar to citflavanone (**5**). Therefore, the absolute configuration of **7** was determined as 2*S*.

EXPERIMENTAL

Mps: uncorr. ^{1}H NMR (250 MHz) were recorded in $CDCl_3$ except where noted. Chemical shifts are shown in ppm (δ) with TMS as int. std. MS were recorded using a direct inlet system. UV were determined in MeOH and IR recorded in $CHCl_3$ soln.

Extraction and separation. Procedures for extn and sepn of compounds from root barks of *C. sinensis* Osbeck var. *brasiliensis* Tanaka and *C. nobilis* Lour. var. *sunki* Hort. were as described in refs [1, 2].

Citrunobin (**1**). Red plates, mp 182–184°, (Et_2O). Found: $[M]^+$ 352.1272; $C_{21}H_{20}O_5$, requires 352.1309. UV λ_{max} nm (log ε): 229 (4.45), 290 (4.36) and 316 (4.57); λ^{+AlCl_3} nm: 225, 262, 295, 322 (sh), 345 (sh); λ^{+NaOMe} nm: 225, 291, 306 (sh) and 436; λ^{+NaOAc} nm: 290, 416. IR ν_{max} cm^{-1}: 3590, 3300, 1630, 1605, 1585, 1545 and 1510. MS *m/z*: 352 $[M]^+$, 337, 323, 232, 217 (100%), 202, 120 and 91. ^{13}C NMR: δ193.1 (*s*), 167.4 (*s*), 161.2 (*s*), 158.1 (*s*), 155.8 (*s*), 142.6 (*d*), 130.3 (*d*), 128.4 (*s*), 125.1 (*d*), 124.6 (*d*), 116.8 (*d*), 116.1 (*d*), 106.4 (*s*), 103.3 (*s*), 92.7 (*d*), 78.3 (*s*), 55.8 (*q*), 28.0 (*q*).

Acetylation of citrunobin (**1**). Compound **1** (2 mg) was treated with Ac_2O (0.3 ml) and NaOAc (6 mg), the mixt. allowed to stand overnight and poured into ice H_2O. The mixt. was extracted × 3 with Et_2O. The Et_2O extract was washed with 2% $NaHCO_3$ and dried (Na_2SO_4). The Et_2O soln was filtered and evapd to afford a yellowish oil **2** (2 mg). UV λ_{max} nm: 226, 287. IR ν_{max} cm^{-1}: 1750, 1640, 1590. MS *m/z*: 436 $[M]^+$, 410, 394, 379 (100%), 256 and 217.

Citflavanone (**5**). Colourless plates, mp 169–171° (Et_2O), $[\alpha]_D$ −2.5° ($CHCl_3$; *c* 0.6). Found: $[M]^+$, 338.1145; $C_{20}H_{18}O_5$, requires 338.1153. UV λ_{max} nm: 229, 265 (sh), 271, 296 (sh), 309 and 337. IR ν_{max} cm^{-1}: 3280, 1635, 1610 and 1590. MS *m/z* (rel. int.): 338 $[M]^+$ (31), 323 (58), 260 (7), 217 (4), 203 (100), 149 (15), 120 (11). ^{13}C NMR: δ196. 1 (*s*), 163.7 (*s*), 162 (*s*), 156.9 (*s*), 156.2 (*s*), 130.4 (*s*), 127.8 (*d*), 126.5 (*d*), 115.6 (*d*), 115.5 (*d*), 102.9 (*s*), 102.0 (*s*), 97.6 (*d*), 78.8 (*d*), 78.3 (*s*), 43.0 (*t*), 28.5 (*q*) and 28.2 (*q*). CD (0.8 mg in 25 ml MeOH) $[\theta]$ λ (nm): $[\theta]_{275}0$, $[\theta]_{295}$ −9506, $[\theta]_{310}0$, $[\theta]_{316}$ +3697, $[\theta]_{400}0$.

Acetylation of citflavanone (**5**). Compound **5** (5 mg) was treated as described for **1** to yield colourless plates of **6**, mp 172–174° (Et_2O). UV λ_{max} nm: 211, 236 (sh), 265, 297. IR ν_{max} cm^{-1}: 1745, 1640, 1600 and 1570. MS *m/z*: 422 $[M]^+$, 380, 365 (100%), 337, 323, 322, 236, 218, 217, 203 and 120.

Lonchocarpol-A (**7**). Amorphous powder, mp 90–95° (Et_2O), $[\alpha]_D$ −4.5° ($CHCl_3$; *c* 0.2). UV λ_{max} nm: 227, 298, 340. IR ν_{max} cm^{-1}: 3950, 3350, 1630, 1615 and 1515. MS *m/z*: 408 $[M]^+$ (100%), 393, 365, 354, 353, 337, 309, 297, 273, 260, 245, 233, 232, 231, 217, 204, 203, 189, 177, 147, 135 and 120. CD (1.4 mg in 25 ml MeOH) $[\theta]_{\lambda\,(nm)}$: $[\theta]_{265}0$, $[\theta]_{291}$ −30600, $[\theta]_{310}0$, $[\theta]_{333}$ +5100, $[\theta]_{390}0$.

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