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COUMARINS, ACRIDONE ALKALOIDS AND A FLAVONE FROM *CITRUS GRANDIS*

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Key Word Index—*Citrus grandis*; Rutaceae; acridone alkaloids; flavone; coumarins; antimicrobial activity; structure elucidation; honyucitrin; honyudisin.

Abstract—An acetone extract of root bark of *Citrus grandis Osbeck* gave a new flavone, honyucitrin, and a new coumarin, honyudisin, together with nine known coumarins and 11 acridone alkaloids. Their structures were determined by spectral methods and some chemical transformations. The antimicrobial activity of the compounds was also examined.

INTRODUCTION

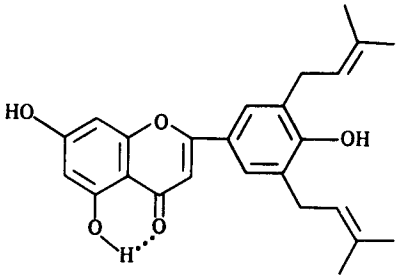
The peel of the fruit of *Citrus grandis Osbeck* (Chinese name: Honyu) is used in Taiwan as a folk medicine for the treatment of stomach ache. Recently, we reported [1] the isolation of a new linear pyranoacridone alkaloid, honyumine (**17**), from the root bark of this plant. We now describe the isolation, characterization, and antimicrobial activity of two new compounds, honyucitrin (**1**) and honyudisin (**2**), nine known coumarins and 11 known alkaloids from the same source.

RESULTS AND DISCUSSION

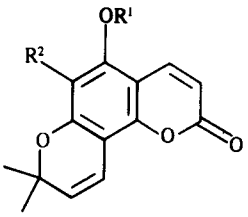
Honyucitrin (**1**), molecular formulae $C_{25}H_{26}O_5$ (HRMS), gave a position ferric chloride test for phenolic hydroxyl groups. The presence of which was supported by the presence of a strong hydroxyl-absorption band at 3400 cm^{-1} in the IR spectrum. The UV spectrum of **1** coupled with a pale magenta colour reaction in Mg–HCl solution suggested a flavone derivative [2]. The ^1H NMR spectrum showed signals at δ1.76 (12H, *s*, 4 × Me), 3.46 (4H, *d*, $J = 7$ Hz, 2 × CH_2) and 5.40 (2H, *m*) which together with the fragmentation ions at *m/z* 351 $[M - CH=C(Me)_2]^+$, 337 $[M - CH_2CH=C(Me)_2]^+$, and 267 $\{M - 2 \times [CH_2CH=C(Me)_2] + H^\bullet\}^+$ established the presence of two C-linked 3,3-dimethylallyl (prenyl) side chains. The *meta* coupling doublets at δ6.25 and 6.51 (each 1H, $J = 2$ Hz) were characteristic for H-6 and H-8 and indicated a 5,7-substituted flavonoid. The presence of a chelated and two non-chelated hydroxyl groups in honyucitrin were indicated by the signals at δ13.03, 7.94 and 9.58 (each 1H, disappeared in D_2O). One of these had to be at C-5, because it was the only one to form a chelate. Two other hydroxyl groups were at C-4′ and C-7 as shown by the characteristic bathochromic shift of Band I and Band II in the UV spectra of **1** upon addition of NaOMe (421.5 nm) and NaOAc (276.9 nm), respectively [2]. A singlet signal at δ6.60 was due to H-3. The aromatic two-proton singlet signals at δ7.69 (2H) were attributable to the 2′,6′-protons of ring B [3]. These spectral results suggested the structure **1** for honyucitrin.

Honyudisin (**2**), $C_{19}H_{20}O_4$ (M^+ 312), had UV spectral features similar to those of 5-methoxyseselin (**8**) [4]. This together with the IR spectrum (1700, 1630, 1620 and 1600 cm^{-1}) and the ^1H NMR spectrum (δ6.10 and 8.13, pyranone-ring protons) established the presence of a coumarin nucleus with a 5,7-dioxygenated pattern [5]. The presence of a phenolic hydroxyl group was clear from the deep green ferric chloride test, a bathochromic shift of the UV band with NaOMe, the presence of an IR band at 3360 cm^{-1} and a ^1H NMR signal at δ8.56 (disappeared in D_2O). A six-protons singlet at δ1.46 coupled with AB-type signals at δ5.72 and 6.73 (each 1H, *d*, $J = 10$ Hz) revealed the presence of a 2,2-dimethylpyran ring. In addition, the presence of a phenyl group in the molecule was inferred from the ^1H NMR signals at δ1.65 (3H, *s*), 1.79 (3H, *s*), 3.41 (2H, *d*, $J = 7$ Hz) and 5.17 (1H, *m*), and the mass spectral fragments at *m/z* 243 $[M - 68]^+$. The above data were in excellent accord with structure **2**, **4** or **5** for honyudisin. To confirm the structure of honyudisin, it was cyclized in 10% HCl solution to give **7** which indicated the *ortho*-location of the hydroxyl and prenyl groups in honyudisin. Its ^1H NMR, MS and IR spectra, mixed mp and TLC were different from those of trachyphyllin (**4**) [6]. Thus structure **4** could be discounted. Methylation of honyudisin with diazomethane afforded the *o*-methyl derivative **3**, which was different from dipetalin (**6**) [7] (direct comparison with authentic sample). Therefore, structure **5** for honyudisin could also be excluded. On the basis of the above results we were led to assign structure **2** to honyudisin.

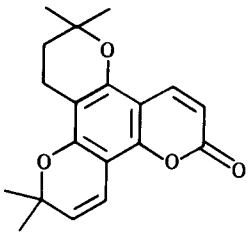
The presence of 5-methoxyseselin (**8**) [4], clausarin (**9**) [4], xanthyletin (**10**) [4, 8], xanthoxyletin (**11**) [4, 8], nordentatin (**12**) [8], scopoletin (**14**) [9], umbelliferone (**15**) [10], honyumine (**17**) [1], citracridone-I (**18**) [4, 8], -II (**19**) [4], 5-hydroxynoracronycine (**20**) [4], grandisinine (**21**) [4], glycocitrine-I (**22**) [4], citpressine-I (**23**) [4], -II (**24**) [4], grandisine-I (**25**) [4], -II (**26**) [4] and the natsucitrine-II (**27**) [11] were confirmed by direct comparison with authentic samples. The physical constants



1



2 R^1 = H, R^2 = $CH_2CH{=}C(Me)_2$
3 R^1 = Me, R^2 = $CH_2CH{=}C(Me)_2$
8 R^1 = Me, R^2 = H



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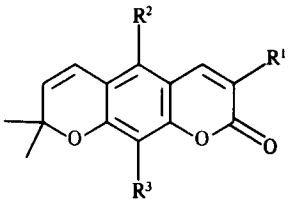
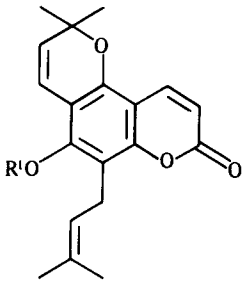


4 R^1 = H, R^2 = OH, R^3 = $CH_2CH{=}C(Me)_2$
9 R^1 = R^3 = $C(Me)_2CH{=}CH_2$, R^2 = OH
10 R^1 = R^2 = R^3 = H
11 R^1 = R^3 = H, R^2 = OMe
12 R^1 = H, R^2 = OH, R^3 = $C(Me)_2CH{=}CH_2$



5 R^1 = H
6 R^1 = Me

and spectroscopic data (UV, IR and ^{1}H NMR) of **13** and **16** were in agreement with those described in the literature for cedrelopsin [12] and thamnosin [13].

The antimicrobial activities of the seven coumarins and eight acridone alkaloids isolated from the root bark of *C. grandis* are shown in Table 1. Nordentatin (**12**) showed 100% inhibition of the growth of *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus* at $\leqslant 10$ μg/ml. Xanthyletin (**10**) brought about the complete inhibition of growth of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhi* at $\leqslant 100$ μg/ml and *Bordetella bronchiseptica* at $\leqslant 50$ μg/ml. *Bordetella bronchiseptica* was distinctly inhibited by scopoletin (**14**) and glycocitrine-I (**22**) at $\leqslant 100$ μg/ml.

EXPERIMENTAL

Mps: uncorr; ^{1}H NMR: 100 MHz, $CDCl_3$, TMS as int. standard; MS: direct inlet;. UV: EtOH; IR: KBr.

Plant material. Citrus grandis was collected in Taiwan, and identified by Professor C.-S. Kuoh. The specimen is deposited in the Herbarium of Chia-Nan Junior College of Pharmacy, Tainan, Taiwan.

Extraction and separation. The dried root bark (1.3 kg) of *C. grandis* was extracted with Me_2CO and concd to afford a brown syrup (30 g), 25 g of which was chromatographed on a silica gel column with C_6H_6–Me_2CO (9:1) as the eluant to yield five fractions. Fraction 1 (7.98 g) was rechromatographed on silica gel with *n*-hexane–EtOAc (5:1) to afford successively **9** (17 mg), **8** (515 mg), **10** (3 g), **11** (2 g), **19** (12 mg), and **21** (0.7 g). Fraction 2 (6 g) was also rechromatographed on silica gel and eluted with *n*-hexane–EtOAc (4:1) to give **2** (17 mg), **22** (184 mg), **17** (8 mg), **20** (0.75 g), **18** (0.55 g) and **24** (130 mg) successively. Fraction 3 (5 g) was subjected to silica gel column chromatography and elution with C_6H_6–Me_2CO (9:1) yielded successively **1** (26 mg), **16** (2.1 mg), **12** (10 mg), **25** (3 mg), **13** (2.0 mg), **27** (20 mg) and **23** (50 mg). Fraction 4 (3 g) was applied to a silica gel column and eluted C_6H_6–Me_2CO (19:1) affording unknown **a** and **b**, **15** (6 mg) and **26** (0.5 mg). Fraction 5 (1.8 g) was treated similarly to give **14** (50 mg).

Honyucitrin (**1**). Pale yellow powder from *i*-Pr_2O, mp 199.5–201°, pale pink colour with Mg–HCl, dark green colour reaction with $FeCl_3$. HRMS: for $C_{25}H_{26}O_5$, 406.1798 (obs.), 406.1778 (calcd). UV λ_{max}nm (log ε): 212 (sh, 4.58), 225 (sh, 4.40), 245 (sh, 4.23), 269.4 (4.19), 300 (sh, 4.09) and 343.2 (4.25); $\lambda_{max}^{+AlCl_3}$ nm: 217 (sh), 253 (sh), 278.9, 304.1 (sh), 353.5, 385

Table 1. Antimicrobial activities of coumarins and acridone alkaloids from *C. grandis*

Organism	10		12				14		22		24	
	100*	50	10	100	50	10	5	100	50	100	50	100	50
Gram positive bacteria													
Staphylococcus aureus ATCC 6538p	−	−	−	+	+	+	−	−	−	−	−	−	−
Staphylococcus epidermidis ATCC 12228	−	−	−	−	−	−	−	−	−	−	−	−	−
Bacillus subtilis ATCC 6633	−	−	−	+	+	+	−	−	−	−	−	−	−
Staphylococcus faecium ATCC 10541	−	−	−	−	−	−	−	−	−	−	−	−	−
Gram negative bacteria													
Micrococcus luteus ATCC 9341	−	−	−	+	+	+	−	−	−	−	−	−	−
Escherichia coli ATCC 10536	−	−	−	−	−	−	−	−	−	−	−	−	−
Klebsiella pneumoniae ATCC 10031	+	−	−	−	−	−	−	−	−	−	−	−	−
Pseudomonas aeruginosa ATCC 25619	+	−	−	−	−	−	−	−	−	−	−	−	−
Bordetella bronchiseptica ATCC 4617	+	+	−	−	−	−	−	±	−	+	−	±	−
Salmonella typhi ATCC 6539	+	−	−	−	−	−	−	−	−	−	−	−	−

Compounds **2**, **8**, **9**, **15**, **18**–**21** and **27** were inactive against all organisms at 100 μg/ml.

* μg/ml.

+, Complete growth inhibition, measured after 48 hr.

−, Ineffective, normal growth occurred after 24 hr.

±, Not completely effective, faint growth occurred after 24 to 48 hr.

(sh); $\lambda_{max}^{+AlCl_3+HCl}$ nm: 217 (sh), 230 (sh), 253 (sh), 278.9, 304.1 (sh), 351.5, 385 (sh); λ_{max}^{+NaOMe} nm: 235 (sh), 265.9, 275 (sh), 322.3, 421.5; λ_{max}^{+NaOAc} nm: 276.9, 306.7 and 354.9. IR ν_{max} cm^{-1}: 3400, 1650, 1610, 1500. MS *m/z*: 406 $[M]^+$, 389, 351,337, 335, 307, 295, 267, 153, 69, 59 (100%).

Honyudisin (**2**). Yellowish green granules from *n*-hexane–EtOAc (6:1), mp 179–180°. HRMS: for $C_{19}H_{20}O_4$, 312.1341 (obs.), 312.1360 (calcd). UV λ_{max} nm (log ε): 229.6 (4.22), 245.3 (sh, 4.03), 273.7 (sh, 3.70). 285.5 (sh, 3.84), 295.4 (3.89) and 334 (3.87); λ_{max}^{+NaOMe} nm: 235 (sh), 255.6, 297 (sh), 310.5, 325 (sh), 350 (sh) and 423.9. IR ν_{max} cm^{-1}: 3360, 1700, 1630, 1620, 1600. MS *m/z*: 312 $[M]^+$ (12%), 297, 295, 256, 253, 243, 242, 241 (100%), 225 and 223.

Cyclization of honyudisin (**2**). **2** (2 mg) was heated with 10% HCl (1 ml) on a water bath for 30 min and then the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ extract was purified by prep. TLC (silica gel, *n*-hexane–EtOAc 4:1) to afford **7** as a yellow oil. UV λ_{max} nm: 202.3, 215 (sh), 229.8, 245 (sh), 295.5 and 331.8; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2930, 2850, 1730 and 1600; ^1H NMR: δ1.36 (6H, *s*, 2 × Me), 1.46 (6H, *s*, 2 × Me), 1.81 (2H, *t*, *J* = 7 Hz, H-2″), 2.63 (2H, *t*, *J* = 7 Hz, H-1″), 5.54 (1H, *d*, *J* = 10 Hz, H-2′), 6.09 (1H, *d*, *J* = 9 Hz, H-3), 6.81 (1H, *d*, *J* = 10 Hz, H-1′) and 7.96 (1H, *d*, *J* = 9 Hz, H-4); MS *m/z*: 312 $[M]^+$ (100%), 297, 274, 241, 186, 171 and 143.

Methylation of honyudisin (**2**). **2** (3 mg) was suspended in Et_2O (5 ml), treated with excess CH_2N_2 and left overnight. The soln was evapd to a colourless syrup which was purified by prep. TLC (silica gel, *n*-hexane–EtOAc 4:1) to give **3** as colourless needles (2.3 mg), mp 93–94°. UV λ_{max} nm (log ε): 226.8 (3.79), 284 (3.33), 294.7 (3.37) and 338 (3.42); IR ν_{max} cm^{-1}: 1720, 1645, 1620, 1600; ^1H NMR: δ1.46 (6H, *s*, 2 × Me), 1.69 (3H, *s*, Me), 1.79 (3H, *s*, Me), 3.33 (2H, *d*, *J* = 7 Hz, H-1″), 3.82 (3H, *s*, OMe), 5.16 (1H, *m*, H-2″), 5.65 (1H, *d*, *J* = 10 Hz, H-2′), 6.20 (1H, *d*, *J* = 9.5 Hz, H-3), 6.84 (1H, *d*, *J* = 10 Hz, H-1′), 7.86 (1H, *d*, *J* = 9.5 Hz, H-4); MS *m/z*: 326 $[M]^+$, 311 (100%), 301, 258, 241, 225, 213.

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