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ALKALOIDS AND COUMARINS OF *CITRUS GRANDIS*

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Key Word Index—*Citrus grandis*, Rutaceae, Acridone alkaloids, buntanine; coumarins, citrubuntin

Abstract—Buntanine, a new prenylated acridone alkaloid and citrubuntin, a new coumarin together with twenty-four known compounds have been isolated from the root bark of *Citrus grandis*. The structures of buntanine and citrubuntin were established by spectral methods and chemical evidence.

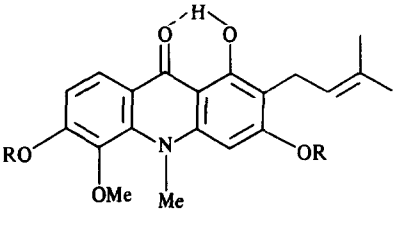
INTRODUCTION

In the course of a continuing search of chemical constituents of plants of the genus *Citrus* in Taiwan, we reported the isolation of two novel skeletal homoacridone alkaloids, citropone-A (**5**) and -B (**6**) from the root bark of *Citrus grandis* Osb. f. buntan Hayata [1] Further examination of the same source has now resulted in the isolation of another two new compounds, designated as buntanine and citrubuntin from the root bark of *C. grandis* f. buntan which, in addition, has also yielded 22 known compounds. The structures of buntanine and citrubuntin were established as **1** and **3** from the following evidence

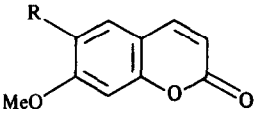
RESULTS AND DISCUSSION

Buntanine (**1**), mp 247–249° was isolated as yellow plates The molecular formula of **1** was determined as $C_{20}H_{21}NO_5$ on the basis of microanalysis and the $[M]^+$ at *m/z* 355 in the mass spectrum. The UV spectrum at 206, 220, 262, 300 (*sh*), 334 and 388 nm showed the absorptions typical of a 9-acridone nucleus [2–4]. The bathochromic shifts of UV band with aluminium trichloride or sodium methoxide and IR bands at 3400, 3100 and 1630 cm^{-1} and ^1H NMR signals at δ14.96 (1H) and 9.14 (2H) [disappeared on D_2O], indicated the presence of three phenolic hydroxyl groups in buntanine, and at least one of the hydroxyl groups was hydrogen bonded. AB-type signals in the ^1H NMR of **1** appeared at δ6.92 and 8.05 (each 1H, *d*, $J = 9$ Hz) which were attributed to mutually *ortho*-located H-7 and H-8, respectively. The two methyl signals at δ1.64, 1.79, a benzylic methylene doublet at δ3.36 ($J = 7$ Hz) together with the triplet for a vinylic proton at δ5.31 and mass fragmentation ions at *m/z* 300 $[M-CH=C(Me)_2]^+$ and 287 $[M-CH_2CH=C(Me)_2]^+$, suggested the presence of an isopentenyl group in buntanine Two sharp singlets at δ3.76 and 3.99 (each 3H) were assigned to methoxy and/or *N*-methyl groups while a singlet at δ6.47 could be attributed to a lone aromatic proton at H-4 (or H-2). In the ^{13}C NMR spectrum of **1**, an *N*-methyl carbon signal at δ39.49 and a benzylic carbon ($C_{1'}$) of isopentenyl group at δ21.12 indicated the location of a isopentenyl group at C_2 [5]. In order to confirm the relative location of hydroxyl and methoxy group, then, a NOE experiment was carried out for the *O*-methoxymethyl derivative (**2**) which was prepared by treatment of buntanine (**1**) with chloromethylmethyl ether and sodium hydroxide in the presence of a phase-transfer catalyst. Irradiation of the methylene protons of the methoxymethylether moiety of **2** at δ5.39 (2 × 2H, *s*) exhibited 10.2 and 11.1% enhancements of the H-4 at δ6.58 and H-7 at δ7.16, respectively On the other hand, irradiation of the *N*-methyl signal at δ4.02 gave a 18.6% increase of the H-4 at δ6.58. However, on irradiation of the methoxy group signal at δ3.83, no NOE was observed at any proton signal. All these data are in agreement with the structure **1** for buntanine.

Citrubuntin (**3**), $C_{15}H_{14}O_3$, mp 115–117° showed a UV spectrum characteristic of a 7-oxycoumarin [6]. The ^1H NMR spectrum supported the 6-alkyl-7-oxy substituted coumarin [7]. The spectral data and physical



1 R = H
2 R = CH_2OMe



3 R = $CH\overset{E}{=}CHC(Me)=CH_2$
4 R = $CH_2CH_2CH(Me)_2$

constants of the hydrogenation product of **3** was superimposable on those of **4** which was obtained by catalytic hydrogenation of suberosin (**9**) According to the above spectral data and chemical transformation, citrubuntin could be formulated as 6-(3′-methylbut-1′, 3′-dienyl)-7-methoxy coumarin (**3**).

In addition to the two new compounds, osthol (**7**) [8], clausarin (**8**) [9], suberosin (**9**) [9, 10], xanthoxyletin (**10**) [10], xanthyletin (**11**) [9, 10], crenulatin (**12**) [10], nordentatin (**13**) [10], umbelliferone (**14**) [8], balyumine-A (**15**) [11], balyumine-B (**16**) [11], glycocitrine-I (**17**) [12], grandisinine (**18**) [13], citpressine-I (**19**) [9, 13], citpressine-II (**20**) [9, 13], citracridone-I (**21**) [9, 13], citracridone-II (**22**) [9, 13], prenylcitpressine (**23**) [9, 13], 5-hydroxynoracronycine (**24**) [9, 13], grandisine-I (**25**) [13], grandisine-II (**26**) [13], *p*-hydroquinone and *β*-sitosterol were also isolated and characterized from the root bark of this plant

EXPERIMENTAL

Mps uncorr ^{1}H NMR $CDCl_3$, except where noted, TMS as int standard, MS direct inlet UV MeOH, IR $CHCl_3$, unless otherwise stated

Plant material Citrus grandis f buntan was collected from Tainan Hsien, Taiwan, in August 1984 and verified by Prof C-S Kuoh A specimen is deposited in the Herbarium of Chia-Nan Junior College of Pharmacy, Tainan, Taiwan, Republic of China

Extraction and separation Air-dried and powdered root bark (1 45 kg) of *C grandis* f buntan was extracted with Me_2CO at room temp The Me_2CO extract was subjected to silica gel column chromatography and eluted with C_6H_6 and C_6H_6–Me_2CO (4 1) successively to afford 16 fractions Fraction 1–10, on repeated C C over silica gel and prep TLC (silica gel) (solvent appropriate combinations of *n*-C_6H_{12}, C_6H_6, Me_2CO, *i*-Pr_2O, EtOAc) gave **15** (5 mg), **3** (6 mg), **7** (7 mg), **16** (4 mg), **8** (21 mg), **9** (0 2 g), *β*-sitosterol (0 2 g), **10** (6 2 g), **11** (4 7 g), *p*-hydroquinone (25 mg), **18** (3 5 g), **22** (50 mg), **20** (35 mg), **6** (3 mg), **5** (10 mg) and **12** (4 mg), respectively Fraction 11–16 was rechromatographed on a silica gel column with C_6H_6–Me_2CO (9 1) as eluant to yield successively **18** (2 8 g), **17** (0 7 g), **21** (1 6 g), **19** (70 mg), **23** (22 mg), **13** (42 mg), **24** (25 mg), **25** (90 mg), **26** (52 mg), **1** (0 2 g) and **14** (30 mg).

Buntanine (**1**) Yellow plates from Me_2CO, mp 247–249° Calc for $C_{20}H_{21}NO_5$ C, 67 59, H, 5 96, Found C, 67 23, H, 5 95%. UV λ_{max} nm (log ε) 206 (4 18), 220 (4 18), 262 (4 62), 300 (sh, 4.08), 334 (4 09) and 388 (3 63), $\lambda_{max}^{AlCl_3}$ nm (log ε) 207 (4 28), 230 (4.23), 263 (4 52), 282 (4 72), 353 (4 31), 425 (3 67), λ_{max}^{NaOMe} nm (log ε) 207 (4 28), 224 (sh, 4 16), 262 (4 73), 295 (sh, 4 35) and 370 (4 33) IR ν_{max}^{KBr} cm^{-1} 3400, 3100, 1630, 1585, 1530 MS *m/z* (rel int) 355 (M^+, 78), 340 (24), 312 (88), 300 (100), 297 (37), 287 (16), 285 (31), 282 (7), 272 (10), 268 (6), 256 (6), 254 (6), 242 (7) and 230 (7) ^{13}C NMR ($CDCl_3$ + DMSO-d_6) δ 179 31 (*s*), 162 35 (*s*), 161 47 (*s*), 156 00 (*s*), 145 09 (*s*), 138 53 (*s*), 135 14 (*s*), 130 17 (*s*), 123 09 (*d*), 122 39 (*d*), 116.01 (*s*), 112 33 (*d*), 108 00 (*s*), 103 61 (*s*), 91 03 (*d*), 60 78 (*q*), 39 49 (*q*), 25 68 (*q*), 21 12 (*t*) and 17 78 (*q*)

Methoxymethylation of **1** A mixture of **1** (30 mg), 0 1% NaOH aq (15 ml), a phase-transfer catalyst (Adogen 464) (8 mg) and CH_2Cl_2 (25 ml) was stirred at room temp for 30 min, and then excess chloro-dimethyl ether was added After 1 hr, the reaction mixture was extracted with H_2O, dried and evapd The residue was chromatographed on silica gel and eluted with C_6H_6–Me_2CO (9 1) to afford **2** as yellow needles, mp 110–112° UV λ_{max} nm 205, 221, 260 (sh), 276, 300 (sh), 329 and 400 IR ν_{max} cm^{-1} 1620, 1605, 1580 and 1550 MS *m/z* 443 (M^+), 428, 400, 388, 372, 356, 354, 344 and 338 ^{1}H NMR· δ 1.68 (3H, *s*, Me), 1 83 (3H, *s*, Me), 3 42 (2H, *d*, *J* = 7 Hz, H-1′), 3 55 (3H, *s*, OMe), 3 59 (3H, *s*, OMe), 3 83 (3H, *s*, 5-OMe), 4 02 (3H, *s*, N-Me), 5 29 (1H, *t*, *J* = 7 Hz, H-2′), 5 39 (4H, *s*, 2 × –OCH_2O–), 6 58 (1H, *s*, H-4), 7 16 (1H, *d*, *J* = 8 Hz, H-7), 8 20 (1H, *d*, *J* = 8 Hz, H-8), 14 51 (1H, *s*, 1–OH), δ (acetone-d_6) 1 65 (3H, *s*, Me), 1 81 (3H, *s*, Me), 3 37 (2H, *d*, *J* = 7 Hz, H-1′), 3 53 (3H, *s*, OMe), 3 56 (3H, *s*, OMe), 3 87 (3H, *s*, 5-OMe), 4 04 (3H, *s*, N-Me), 5 28 (1H, *t*, *J* = 7 Hz, H-2′), 5 42 (2H, *s*, OCH_2O-), 5 46 (2H, *s*, –OCH_2O–), 6 69 (1H, *s*, H-4), 7 21 (1H, *d*, *J* = 8 Hz, H-7), 8 11 (1H, *d*, *J* = 8 Hz, H-8) and 14 75 (1H, *s*, 1–OH)

Citrubuntin (**3**) Colourless powder from ether, mp 115–117° $C_{15}H_{14}O_3$ UV λ_{max} nm (log ε) 208 (4 21), 278 (4 41), 303 (sh, 4 13) and 345 (3 98) IR ν_{max} cm^{-1} 1710, 1610, 1560 MS *m/z* (rel int) 242 (M^+, 77), 227 (100), 211 (37), 199 (32), 183 (24), 171 (29), 155 (40), 141 (19), 128 (39), and 115 (27) ^{1}H NMR δ 1 96 (3H, *s*, Me), 3 86 (3H, *s*, 7–OMe), 5 05 (2H, *br s*, =CH_2), 6 17 (1H, *d*, *J* = 9 5 Hz, H-3), 6 68 (1H, *s*, H-8), 6 74 (2H, *s*, H-1′, 2′), 7 43 (1H, *s*, H-5), 7 53 (1H, *s*, H-4), δ ($CDCl_3$ + acetone-d_6) 1 96 (3H, *s*, Me), 3 99 (3H, *s*, 7–OMe), 5 11 (2H, *s*, =CH_2), 6 20 (1H, *d*, *J* = 9 5 Hz, H-3), 6 77 (1H, *d*, *J* = 16 Hz, H-2′), 6 99 (1H, *d*, *J* = 16 Hz, H-1′), 7 75 (1H, *s*, H-5), 7 83 (1H, *d*, *J* = 9 5 Hz, H-4)

Hydrogenation of **3** A solution of **3** (5 mg) in THF (10 ml) was stirred under H_2 in the presence of 5% Pd/C (5 mg) at room temp for 1 hr The soln was filtered and the conc filtrate recryst from *n*-C_6H_{12} gave **4** (4 5 mg) as colourless needles, mp 64–65° UV λ_{max} nm 223 4, 242 (sh), 252 (sh), 295 (sh), 331 IR ν_{max} cm^{-1} 1735, 1612, 1565, 1505 MS *m/z* 246 (M^+), 189 (100%), 159, 131, 103 ^{1}H NMR δ 0 95 (6H, *d*, *J* = 6 5 Hz, 2 × Me), 1 33 –1 76 (3H, *m*, H-2′, 3′), 2 62 (2H, *t*, *J* = 7 7 Hz, H-1′), 3 88 (3H, *s*, 7–OMe), 6 22 (1H, *d*, *J* = 9 4 Hz, H-3), 6 76 (1H, *s*, H-8), 7 18 (1H, *s*, H-5), and 7 61 (1H, *d*, *J* = 9 4 Hz, H-4)

Hydrogenation of suberosin (**9**) 50 mg of **9** was treated as described for **3** to yield a colourless needles which were identified as **4** by the comparison of mp, IR, ^{1}H NMR, MS and TLC

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