^{1}H NMR: δ11.98 (1H, *s*, OH), 11.43 (1H, *s*, OH), 7.25 (1H, *d*, J = 9.2 Hz, H-6), 6.73 (1H, *d*, J = 9.2 Hz, H-7), 6.52 (1H, *d*, J = 2.47 Hz, H-4), 6.39 (1H, *d*, J = 2.47 Hz, H-2), 3.93 (3H, *s*, OMe), 3.87 (3H, *s*, OMe); EIMS *m/z*: 288 $[M]^+$ ($C_{15}H_{12}O_6$), 273 $[M-Me]^+$, 245 $[M-C_2H_3O]^+$, 230 $[M-C_3H_6O]^+$.

Acknowledgements—The authors are deeply indebted to Mr. Shafi-Uddin and Mr. Wasim Ahmed (Research Fellows, H.E.J. Research Institute of Chemistry, University of Karachi) for their valuable suggestions concerning the manuscript.

REFERENCES

1. Barbara, C., Jolanta, K., Henryk, S., Janina, M. and Ryszard, C. (1976) *Herba Pol.* **22**, 154.
2. Manucharyan, M. A., Musaelyan, M. S. and Davtyan, Ts. M. (1981) *Biol. Zh. Arm.* **34**, 94.
3. Yukhananov, D. Kh. and Sapunova, L. A. (1976) *Rastit. Resur.* **12**, 244.
4. Geller, (1965) *Acta Phytother. Amst.* **12**, 154.
5. Abubakirov, N. K., Kambulin, N. A., Kryzhenkov, A. N., Sultanov, T. G. and El'Kind, L. A. (1960) *Sbornik Nauch. Trudov Tashkent. Med. Inst.* **16**, 222.
6. Baeva, R. T., Karryev, M. O. and Abubakirov, N. K. (1975) *Izv. Akad. Nauk Turkm. SSR. Ser. Fiz.-Tekh., Khim. Geol. Nauk.* **4**, 83.
7. Baeva, R. T., Karryev, M. O. and Abubakirov, N. K. (1974) *Khim. Prir. Soedin* **6**, 804.
8. Baeva, R. T., Karryev, M. O., Litvinenko, V. I. and Abubakirov, N. K. (1974) *Khim. Prir. Soedin* **10**, 171.
9. Abubakirov, N. K. and Amanmuradov, K. (1964) *Zh. Obshch. Khim.* **34**, 1661.
10. Sluis, W. G. and Labadie, R. P. (1985) *Phytochemistry* **24**, 2601.
11. Markham, R. K. (1964) *Tetrahedron* **20**, 991.
12. Ghosal, S., Sharma, P. V., Chaudhuri, R. K. and Bhattacharya, S. K. (1973) *J. Pharm. Sci.* **62**, 926.
13. Takagi, S. and Yamaki, M. (1982) *Yakugaku Zasshi* **102**, 546.
14. Idris, M. S. H., Jefferson, A. and Scheinmann, F. (1977) *J. Chem. Soc. Perkin I* (19) 2158.
15. Toda, F. (1981) *Handbook of ^{13}C-NMR Spectra*, p. 249. Sankyo, Japan.
16. Johnson, L. F. and Jankowski, W. C. (1978) *Carbon-^{13}C NMR Spectra*, p. 323, 412. Robert E. Krieger, New York.

Phytochemistry, Vol. 28, No. 12, pp. 3574–3576, 1989.
Printed in Great Britain.

0031 9422/89 $3.00 + 0.00

ALKALOIDS AND COUMARINS FROM STEM BARK OF *CITRUS GRANDIS*

SHIOW-CHYN HUANG, MING-TYAN CHEN and TIAN-SHUNG WU*†

Department of Applied Chemistry, Chia-Nan Junior College of Pharmacy, Tainan, Taiwan, Republic of China; †Department of Applied Chemistry, Providence University, Shalu 43309, Taichung Hsien, Taiwan, Republic of China

(*Received in revised form* 25 *April* 1989)

Key Word Index—*Citrus grandis f. buntan*; Rutaceae; acridone alkaloids; buntanmine-A; coumarins; buntansin.

Abstract—An acetone extract of the stem bark of *Citrus grandis. f. buntan* afforded a new acridone alkaloid, buntanmine-A and a new coumarin, buntansin, together with six known acridone alkaloids and 10 known coumarins. Structures were elucidated by spectral methods and chemical transformations.

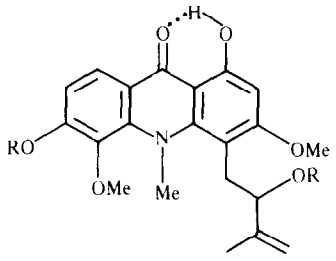
INTRODUCTION

Citrus grandis f. buntan (Chinese name: Buntan) is a well known fruit belonging to the Rutaceae in Taiwan. In continuation of our chemical investigation of the genus *Citrus* [1], we were interested in the constituents of this species. Recently, we have reported on the isolation of novel skeletal homoacridone alkaloids, acridone alkaloids and coumarins from the root bark of the plant [1, 2]. Further examination of the stem bark has now resulted in the isolation of 18 compounds, two (**1** and **3**) of which are a new acridone alkaloid and a new coumarin, respectively. In this paper, we describe the structural determination of these compounds.

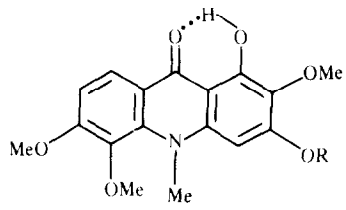
*Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

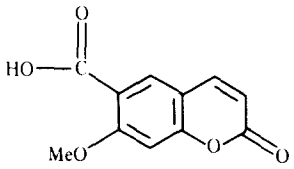
Buntanmine-A (**1**) was obtained as yellowish granules, mp 201–202°, $[\alpha]_D$ −116.7° (MeOH). The molecular formula was determined as $C_{21}H_{23}NO_6$ by HR mass spectrometry. The UV spectrum of **1** showed similar absorptions to that of grandisinine (**8**), indicating the presence of a 9-acridone nucleus [3–6]. The bathochromic shifts of UV bands with shift reagents and IR bands at 3407 and 1614 cm^{-1} together with signals at δ14.28 and 6.40 (each 1H, disappearing with D_2O) in the ^{1}H NMR spectrum, indicated the presence of hydroxyl groups in **1** with at least one of them being strongly hydrogen bonded. The AB type signals in the ^{1}H NMR spectrum at δ6.95 and 8.01 (each 1H, *d*, J = 8.8 Hz) were attributed to mutually *ortho*-located aromatic protons, the lower field signal being assigned to H-8 in the



1 R = H
2 R = Ac



9 R = H
9a R = Ac



3

acridone nucleus. Signals at δ3.59 (3H) and 3.93 (6H) were due to an N-Me and two methoxy groups. A lone aromatic proton appeared at δ6.40 as singlet. The presence of the remaining 2′-hydroxy-3′-methylbut-3′-enyl side chain in the molecule was inferred by the signals at δ4.81, 4.91 (each 1 H, *s*, C=CH_2), 4.33 (1 H, *m*, H-2′), 3.12 (2H, *m*, H-1′), 1.81 (3H, *s*, Me), 1.59 (1H, *s*, OH) in the ^{1}H NMR spectrum, and ^{13}C NMR signals at δ17.2 (*q*), 32.9 (*t*), 73.9 (*d*), 109.8 (*t*), 159.5 (*s*) together with the mass fragment ion peak at *m/z* 314 $[M-C_4H_7O]^+$. In the ^{13}C NMR spectrum of **1**, an *N*-methyl carbon signal at δ48.4 and an aromatic carbon (C-2) at δ93.6 (*d*) indicated the location of the C_5 unit side chain at C-4 [6, 7]. On the other hand, the doublet signal of **1** at δ6.95 was shifted appreciably (0.1 ppm) to lower field (δ7.05) on acetylation together with a signal for an aromatic carbon (C-7) at δ112.6 which was similar to that found in grandisinine (**8**) [6, 7], indicating the presence of a phenolic hydroxyl group at C-6. On the basis of these results structure **1** was assigned to buntanmine-A.

Buntansin (**3**) was isolated as colourless granules, mp 216–217°. The molecular formula was established as $C_{11}H_8O$ by HR mass spectrometry, ($[M]^+$, *m/z* 220). It showed UV absorption maxima at 223, 231 239, 298 (sh) and 323.4 nm. The IR bands at 1742, 1620 cm^{-1} were similar to those found in crenulatin (**15**) [1] being characteristic of a typical 6-alkyl-7-alkoxycoumarin nucleus. The presence of a carboxylic acid group in the molecule was inferred from the IR bands at 3345 and 1695 cm^{-1} and the mass fragment ion peak at *m/z* 175 $[M-CO_2H]^+$. In the ^{1}H NMR spectrum of **3** signals for H-3 and H-4 appeared at δ6.36 and 8.06 (each 1H, *d*, J = 10 Hz), respectively. The spectrum also showed a methoxy group at δ3.91 and two singlet aromatic protons at δ7.15 and 8.09. The lower field signal at δ8.09 was assigned to H-5 due to the anisotropic effect of the carboxylic group. From the above spectral data, we propose structure **3** for buntansin.

The known acridone alkaloids citpressine-I (**4**) [6], -II (**5**) [6], citracridone-I (**6**) [6], 5-hydroxynoracronycine (**7**) [6], grandisinine (**8**) [6], and the known coumarins 5-methoxyseselin (**10**) [6], xanthyletin (**11**) [6], xanthoxyletin (**12**) [6], umbelliferone (**13**) [8], scopoletin (**14**) [8], crenulatin (**15**) [1], citrubuntin (**16**) [1], suberenone (**17**) [9], ulopterol (**18**) [10] and (+)-5-demethyltoddanol (**19**)* [11] were also isolated from the stem bark of *C. grandis*. These compounds were identified by comparison of their spectral data (UV, IR, ^{1}H NMR and mass spectra) and/or mmp with the corresponding authentic samples. According to spectral data and chemical evidence, compound **9** was assigned to atalafoline [12, 13]; however, direct comparison with an authentic compound was not made.

EXPERIMENTAL

Mps: uncorr. ^{1}H (100 MHz) and ^{13}C NMR (25 and 62.5 MHz) were recorded in $CDCl_3$ except where noted. Chemical shifts are shown in ppm (δ) with TMS as int. std. MS were measured using a direct inlet system. UV were determined in MeOH and IR recorded in KBr except where stated.

Plant material. Citrus grandis Osbeck *f. buntan* Hayata was collected from Tainan Hsien, Taiwan in August 1984 and identified 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. Dried stem bark (2 kg) was extracted with Me_2CO and concd to afford a brown syrup (165 g). The Me_2CO extract (160 g) was chromatographed on a silica gel column and eluted with C_6H_6–Me_2CO (9:1) to give 32 frs. Fr. 1 (26.6 g) was rechromatographed on silica gel with *n*-hexane–EtOAc (19:1) to afford successively **10** (0.6 g), **12** (6.1 g), **8** (3.2 g), and **5** (0.4 g). Fr. 2 (7.3 g) was also rechromatographed on silica gel and eluted with C_6H_6–Me_2CO (25:1) to give **12** (2.1 g), **11** (1.2 g), **6** (25 mg), **5** (18 mg), **8** (0.3 g), **7** (0.1 g), **4** (15 mg), **19** (6 mg), compound **a** (1 mg) and **1** (12 mg). Fr. 3 (2 g) was treated similarly to give **5** (0.1 g), **8** (0.3 g) and **15** (25 mg), respectively. Fr. 4 (7.3 g) was repeatedly chromatographed on silica gel with C_6H_6–Me_2CO (19:1) to afford successively, **6** (5 mg), **5** (14 mg), **15** (25 mg) and **8** (0.1 g). Fr. 5 (3.9 g) was

* We give a trivial name for the convenience of use.

rechromatographed on silica gel using C_6H_6–Me_2CO to give **7** (7 mg), **17** (2 mg), **4** (22 mg) and compound **b** (10 mg). Fr. 6 (3.5 g) was also subjected to rechromatography on silica gel using $CHCl_3$–MeOH (25:1) and each fr. was monitored by TLC to yield **6** (1.6 mg), **9** (2 mg) and **4** (5 mg), respectively. Fr. 7 (8.5 g) was subjected to silica gel CC and eluted with $CHCl_3$–Me_2CO (25:1) to obtain **16** (13 mg), **15** (5 mg), **17** (4 mg), **14** (16 mg), and **13** (5 mg). Compound **18** (35 mg) and **3** (6 mg) were sepd from frs 13 and 16, respectively.

Buntanmine-A (**1**). Yellowish granules (Me_2CO), mp 201–202°, $[\alpha]_D$ −116.7° (MeOH; *c* 0.12). Found: $[M]^+$, 385.1488; $C_{21}H_{23}NO_6$, requires, 385.1523. UV λ_{max} nm (log ε): 223.3 (4.2), 261.5 (sh, 4.62), 268 (4.64), 333.4 (4.26), 391.6 (3.78); $\lambda_{max}^{+AlCl_3}$ nm: 237.9, 266.0 (sh), 278.2, 358.6, 442.9; λ_{max}^{+NaOMe} nm: 266.3, 295.2 (sh), 332 (sh), 377.8. IR ν_{max} cm^{-1}: 3407, 1614, 1590, 1556, 1069, 894. ^{13}C NMR: δ 17.2 (*q*), 32.9 (*t*), 48.4 (*q*), 55.5 (*q*), 59.7 (*q*), 73.9 (*d*), 93.6 (*d*), 106.3 (*s*), 106.6 (*s*), 109.8 (*t*), 112.6 (*d*), 117.2 (*s*), 121.9 (*d*), 136.6 (*s*), 147.5 (*s*), 150.2 (*s*), 155.7 (*s*), 159.5 (*s*), 162.8 (*s*), 164.3 (*s*) and 181.9 (*s*). EIMS *m/z* (rel. int.): 385 $[M]^+$ (1), 314 (100), 299 (21), 284 (14).

Acetylation of buntanmine-A (**1**). A soln of **1** (2 mg) in a mixt. of Ac_2O (0.5 ml) and pyridine (0.5 ml) was allowed to stand at room temp. overnight and the reaction mixt. worked-up in the usual manner. Compound **2** (1.5 mg) was obtained as a yellowish oily product. UV λ_{max} nm: 221.4, 256 (sh), 270, 305 (sh), 323 (sh), 331, 398.2. IR ν_{max} cm^{-1}: 1772, 1736, 1629, 1589, 1569, 1201, 900. ^1H NMR: δ 1.72 (6H, *s*, Me and OAc), 2.41 (3H, *s*, OAc), 3.24 (2H, *m*, H-1′), 3.64 (3H, *s*, N-Me), 3.94 (6H, *s*, OMe ×2), 4.77 (2H, *s*, H-4′), 5.53 (1H, *t*, *J* = 7 Hz, H-2′), 6.38 (1H, *s*, H-2), 7.05 (1H, *d*, *J* = 9 Hz, H-7), 8.09 (1H, *d*, *J* = 9 Hz, H-8), 14.09 (1H, *s*, 1-OH). EIMS *m/z*: 469 $[M]^+$, 356 (100%), 326, 314, 299, 298.

Buntansin (**3**). Colourless granules, mp 216–217° (Me_2CO). Found: $[M]^+$, 220.0346, $C_{11}H_8O_5$, requires, 220.0370. UV λ_{max} nm (log ε): 223 (3.95), 231 (3.91), 239 (3.88), 298 (sh, 3.71) and 323.4 (3.88). IR ν_{max} cm^{-1}: 3345, 1742, 1695, 1620, 1500. EIMS *m/z*: 220 $[M]^+$ (100%), 203, 191, 175, 173, 145.

Atalafoline (**9**). Yellow granules, mp 155–158° (Me_2CO), $C_{17}H_{17}NO_6$. UV λ_{max} nm (log ε): 220.6 (4.03), 271.6 (4.61), 300.0 (sh, 3.93), 331.1 (3.96), 394.1 (3.57). IR ν_{max} cm^{-1}: 3400, 1641, 1610, 1595, 1566, 1534. EIMS *m/z* (rel. int.): 331 $[M]^+$ (100), 316 (80), 302 (9), 298 (10), 288 (86), 286 (10), 273 (13), 272 (13), 258 (41), 244 (14), 230 (26). ^1H NMR: δ 3.78, 3.98, 4.02, 4.04 (each 3H, *s*, 3 × OMe and N-Me), 6.44 (1H, *s*, H-4), 6.51 (1H, *s*, 3-OH), 6.99 (1 H, *d*, *J* = 9 Hz, H-7), 8.22 (1 H, *d*, *J* = 9 Hz, H-8), 14.84 (1 H, *s*, 1-OH).

Acetylation of atalafoline (**9**). The same procedures described for acetylation of **1** were carried out to give yellow needles of **9a**, mp 181–182° (*n*-hexane), $C_{19}H_{19}NO_7$. UV λ_{max} nm: 265.3, 273 (sh), 325 (sh), 328, 407.7. IR ν_{max} cm^{-1}: 1761, 1636, 1601, 1560, 1516. EIMS *m/z* (rel. int.): 373 $[M]^+$ (15), 331 (100), 316 (49), 288 (67), 258 (38) and 244 (17). ^1H NMR: δ 2.40 (3H, *s*, OAc), 3.78, 3.94, 4.0, 4.03 (each 3H, *s*, 3 × OMe and N-Me), 6.58 (1H, *s*, H-4), 7.02 (1H, *d*, *J* = 9.2 Hz, H-7), 8.24 (1H, *d*, *J* = 9.2 Hz, H-8), 14.65 (1H, *s*, 1-OH). ^{13}C NMR (62.5 MHz): δ 40.3 (*q*), 56.3 (*q*), 60.9 (*q*), 61.3 (*q*), 90.5 (*d*), 107.5 (*d*), 107.8 (*s*), 122.9 (*d*), 155.4 (*s*), 21.0 (*q*). The signals for the quaternary carbon could not be detected because of the small sample size.

Acknowledgements—We are very grateful to Prof. S. T. Lin (Providence College, Taiwan) for the ^{13}C NMR measurements. We also thank the National Science Council of Republic of China for a grant NSC 75-0201 M 126C-03 to support this work.

REFERENCES

1. Wu, T. S. (1988) *Phytochemistry* **27**, 3717.
2. McPhail, A. T., Ju-Ichi, M., Fujitani, Y., Inoue, M., Wu, T. S. and Furukawa, H. (1985) *Tetrahedron Letters* **26**, 3271.
3. Reish, J., Szendri, K., Minker, E. and Novak, I. (1972) *Pharmazie* **27**, 208.
4. Brown, R. D. and Lahey, F. N. (1950) *Aust. Sci. Res.* **A3**, 593.
5. Fraser, A. W. and Lewis, J. R. (1973) *J. Chem. Soc. Perkin Trans I* 1173.
6. Wu, T. S., Kuoh, C. S. and Furukawa, H. (1983) *Phytochemistry* **22**, 1493.
7. Furukawa, H., Yogo, M. and Wu, T. S. (1983) *Chem. Pharm. Bull.* **31**, 3084.
8. Wu, T. S., Huang, S. C., Jong, T. T., Lai, J. S. and Kuoh, C. S. (1988) *Phytochemistry* **27**, 585.
9. Talapatra, S. K., Mukhopadhyay, S. K. and Talapatra, B. (1975) *Phytochemistry* **14**, 836.
10. Ishii, H., Hosoya, K., Ishikawa, T., Ueda, E. and Haginiwa, J. (1974) *Yakugaku Zasshi* **94**, 322.
11. Burke, B. A. and Parkins, H. (1979) *Phytochemistry* **18**, 1073.
12. Qin, D. K. (1986) *Yaoxuae Xuebao* **21**, 683.
13. Gu, G. M. (1987) *Yaoxue Xuebao* **22**, 886.