Pergamon

0031-9422(95)00856-X

Phytochemistry, Vol. 42, No. 1, pp. 217–219, 1996

0031-9422/96 $15.00 + 0.00

ALKALOIDS FROM ROOT BARK OF *ZANTHOXYLUM SIMULANS*

IH-SHENG CHEN, SHWU-JEN WU,* YANN-LII LEU,† INN-WHA TSAI and TIAN-SHUNG WU†‡

Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical College, Kaohsiung, Taiwan, R.O.C.; *China Junior College of Medical Technology, Tainan Hsien, Taiwan R.O.C.; †Department of Chemistry, National Cheng Kung University, Tainan, Taiwan, R.O.C.

(*Received* 31 *August* 1995)

Key Word Index—*Zanthoxylum simulans*; Rutaceae; root bark; alkaloids; aporphines; benzo[*c*]-phenanthridines; 2-quinolones.

Abstract—A new 6*a*,7-dehydroaporphine alkaloid, *N*-acetyldehydroanonaine and a new 2-quinolone alkaloid, simulansine (**2**), together with 21 known compounds were isolated from the root bark of *Zanthoxylum simulans*. Their structures were established on the basis of spectral and chemical evidence.

INTRODUCTION

In continuation of our phytochemical survey of *Zanthoxylum simulans*, we have reported the isolation of six new alkaloids from the root bark and root wood of this plant [1–3]. Further examination of the $CHCl_3$ and MeOH extracts and the acid-soluble part of the root bark led to the isolation of two further new alkaloids, *N*-acetyldehydroanonaine (**1**) and simulansine (**2**), together with 21 known compounds and an unknown alkaloid. In this paper, we describe the structural determination of these compounds.

RESULTS AND DISCUSSION

N-Acetyldehydroanonaine (**1**) was isolated as prisms. It exhibited a $[M]^+$ at *m/z* 305 which, by high-resolution mass spectrometry, was found to correspond to the molecular formula, $C_{19}H_{15}NO_3$. UV absorption bands at 212, 230, 254, 289, 330, 375 nm indicated a highly conjugated system similar to that of dehydroaporphine [4]. The presence of an *N*-acetyl group was confirmed by the IR band at 1670 cm^{-1} and the mass spectral fragment ions at *m/z* 262 $[M - COMe]^+$ and *m/z* 248 $[M-NCOMe]^+$, and also by an acetyl signal at δ 2.33 (3H, *s*) in the ^1H NMR spectrum. The ^1H NMR spectrum of **1** revealed one methylenedioxy group at δ 6.27 (*s*, 2H) and an ethylene group at δ 3.18 and 4.19 (each 2H, *t*, $J = 5.7$ Hz). Four mutually coupling aromatic protons at δ 7.56–7.63 (2H, *m*), 7.78 (1*H*, *m*) and 9.04 (1H, *m*) were attributed to H-9,-10, H-8 and H-11, respectively. Two singlet signals at δ 7.19 and 7.62 were assigned to H-3 and H-7, respectively. The substituents at C-1, C-2 and N were deduced from a to be within NOE distance of the 4-CH_2 (δ 3.18), and 7-H (δ 7.27) was similarly placed for 8-H (δ 7.78) and *N*-Ac (δ 2.33). On the basis of these results, the structure of *N*-acetyldehydroanonaine can be represented as **1**.

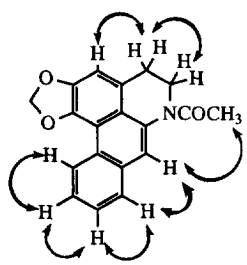
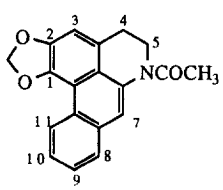
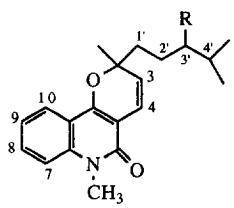


Fig. 1. NOESY correlation of compound **1**.

Simulansine (**2**) was obtained as an oil which possessed the molecular formula $C_{20}H_{25}NO_3$ by high-resolution mass spectrometry. The ^1H NMR, IR and UV spectra were reminiscent of the 2-quinolone alkaloids, zanthosimuline (**3**) and huajiaosimuline (**4**), which have previously been isolated from *Z. simulans* [1, 3]. The ^1H NMR spectrum differed from that of **3** and **4** only by the presence of signals at δ 1.18, 1.15 (each 3H, *d*, $J = 6.0$ Hz, $2 \times$ Me), 1.68–2.22 (5H, *m*), 3.37 (1H, *br d*, $J = 9.0$ Hz, H-3′) for the 3-hydroxy-2-methylpentyl group instead of a 2-methyl-2-pentenyl group in **3** and a 3-oxo-2-methylpentyl group in **4**, respectively. This proposition was further supported by the IR band at 3425 cm^{-1} for a hydroxyl group and the fragments at *m/z* 240 $[M - CH_2CH(OH)CH(Me)]^+$



1



2 R=OH
4 R=O

spectral data and TLC [1]. Consequently, structure **2** was suggested for simulansine.

The known benzo[*c*]phenanthridine alkaloids (norchelerythrine (**5**) [5], 6-acetonyldihydrochelerythrine (**6**) [6], bocconoline (**7**) [7], decarine (**8**) [8], oxychelerythrine (**9**) [7], arnottianamide (**10**) [8], dihydrochelerythrine (**11**) [9], chelerythrine (**12**) [9]), quinoline alkaloids (zanthobisquinolone (**13**) [2], γ-fagarine (**14**) [10], simulanoquinoline (**15**) [1], toddaquinoline (**16**) [5], 8-methoxy-flindersine (**17**) [11], 8-methoxy-*N*-methylflindersine (**18**) [11], dictamnine (**19**) [10], skimmianine (**20**) [5], elutine (**21**) [12], 4-methoxy-2-quinolone (**22**) [13]), aporphine alkaloids, (−)-*N*-acetylanonaine (**23**) [14], (−)-*N*-acetylasimilobine (**24**) [14], *N*-acetylnormuciferine (**25**) [14]) were also isolated from the root bank of *Z. simulans*. These compounds were identified by the comparison of their spectral data (UV, IR, ^{1}H NMR and mass spectra) and/or mmp with corresponding authentic samples.

EXPERIMENTAL

Mps, uncorr. IR: KBr or $CHCl_3$. UV: MeOH. ^{1}H NMR (200 and 270 MHz): $CDCl_3$, chemical shifts in ppm (δ) with TMS as int. standard. MS: direct inlet.

Plant material. Root bark of *Z. simulans* Hance was collected from Taichung Hsien, Taiwan, in October 1985.

Extraction and separation. The extraction procedure is described in our previous paper [1]. The $CHCl_3$-soluble part (fr. A3) was concd to give a brown syrup (11.43 g) which directly chromatographed on silica gel eluting with $CHCl_3$, $CHCl_3$–EtOAc (100:1, 20:1 and 10:1), $CHCl_3$–MeOH (20:1 and 10:1) to yield 11 frs. Fr. 1 was filtered and washed with MeOH to give **13** (3 mg). The filtrate was evapd to give a brown syrup which was purified by prep. TLC on silica gel with benzene–EtOAc (10:1) to yield **1** (3 mg). Fr. 2 was chromatographed on silica gel using hexane–EtOAc (10:1) to give **5** (5 mg) and **6** (3 mg). Fr. 3 was separated by silica gel CC eluting with benzene–EtOAc (10:1) to obtain **7** (7 mg) and **14** (2 mg). Fr. 4 was washed with MeOH to give solid **23** (152 mg). Fr. 5 was washed with Me_2CO to afford **8** (121 mg) as an orange-brown solid. After removal of **8**, the filtrate was benzene EtOAc (5:1) to yield **24** (8 mg) and **16** (3 mg). Fr. 7 was washed with MeOH to give **10** (20 mg). After removal of **10**, the filtrate was evapd and subjected to prep. TLC over silica gel in $CHCl_3$–EtOAc (10:1) to obtain **17** (2 mg), **2** (5 mg) and **10** (3 mg). The MeOH-soluble part (fr. A4) was concd to give a brown residue (10.45 g) which was treated as $CHCl_3$ described for the soluble part (fr. A3) to give **11** (2 mg), **5** (1.5 mg), **6** (2 mg), **23** (5 mg), **8** (3 mg), **9** (2 mg) and **10** (4 mg), successively. The acid-soluble part was neutralized with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ soln was shaken with 5% aq. NaOH soln, then dried (K_2CO_3) and concd to small vol; 10% HCl was added to yield a yellow ppt. This ppt. was crystallized repeatedly from MeOH–Et_2O to obtain **12** (0.34 g). The $CHCl_3$-HCl aq. soln was treated with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ soln was evapd under red. pres. to give *tert.* nonphenolic bases (23.18 g). The aq. NaOH soln was treated with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ soln was evapd under red. pres. to yield tert. phenolic bases (0.35 g). These were chromatographed on a silica gel column and eluted with gradient of $CHCl_3$–MeOH to give three frs. Fr. 1 (2.25 g) was washed with Et_2O, then purified by recrystallization to give **23** (462 mg). The filtrate was subjected to silica gel cc and eluted with a gradient of benzene–EtOAc to yield **19** (33 mg), **6** (1.5 mg), **18** (11 mg), **7** (3 mg), **23** (152 mg) and **14** (1.7 mg), successively. Compound **20** (1.04 g) was separated from fr. 2 (5.32 g) by filtration. The filtrate was chromatographed on silica gel and eluted with benzene–EtOAc (10:1) to give **5** (1.7 mg), **23** (56 mg), **20** (35 mg), **9** (45 mg), **25** (4.2 mg) and **24** (3.1 mg). Fr. 3 (11.1 g) was rechromatographed on silica gel and using benzene–EtOAc (5:1) to yield (4 mg) and unknown **a** (3.1 mg). The tert. phenolic bases were chromatographed on a silica gel column using benzene–EtOAc (10:1 and 2:1) to give **10** (2 mg), **24** (1.5 mg) and **22** (0.7 mg).

N-Acetyldehydroanonaine (**1**). Prisms, mp. 151–153° (MeOH). HRMS: found, $[M]^+$ 305.1054; $C_{19}H_{15}NO_3$, requires 305.1051. UV λ_{max} nm (log ε): 212 (4.16), 230 (3.91), 254 (4.45), 289 (3.79), 330 (3.71), 375 (3.23). IR ν_{max}^{KBr} cm^{-1}: 1730, 1670, 1580, 1500, 860. ^{1}H NMR (Me_2CO-d_6): δ 2.28 (3H, *s*, NCOMe), 3.18 (2H, *t*, J = 6.1 Hz, H-4), 4.11 (2H, *t*, J = 6.1 Hz, H-5), 6.34 (2H, *s*, $-OCH_2O-$), 7.19 (1H, *s*, H-3), 7.60 (2H, *m*, H-9, 10), 7.62 (1H, *s*, H-7), 7.87 (1H, *m*, H-8), 9.03 (1H, *m*, H-11). ^{1}H NMR ($CDCl_3$): δ 2.33 (3H, *s*, NCOMe), 3.18 (2H, *t*, J = 5.7 Hz, H-4), 4.19 (2H, *t*, J = 5.7 Hz, H-5), 6.27 (2H, *s*, $-OCH_2O-$), 7.07 (1H, *s*, H-3), 7.27 (1H, *br s*, H-7), 7.56–7.63 (2H, *m*, H-9, 10), 7.78 (1H, *m*, H-8), 9.04 (1H, *m*, H-11). EIMS *m/z* (rel. int.): 306 (30), 305 ($[M]^+$, 96), 264 (29), 263 (100), 262 (31), 261 (25), 248 (13), 232 (18), 204 (31), 203 (21), 177 (11), 176 (22).

Simulansine (**2**). Oil. HRMS: found $[M]^+$ 327.1834;

NMR: δ 1.15, 1.18 (each 3H, *d*, $J = 6.0$ Hz, 2 × Me), 1.49 (3H, *s*, Me), 1.68–2.22 (5H, *m*, H-1′, H-2′, H-4′), 3.37 (1H, *d*, $J = 9.0$ Hz, H-3′), 3.69 (3H, *s*, *N*-Me), 5.50 (1H, *d*, $J = 10.0$ Hz, H-3), 6.81 (1H, *d*, $J = 10.0$ Hz, H-4), 7.23 (1H, *t*, $J = 7.8$ Hz, H-9), 7.32 (1H, *d*, $J = 7.8$ Hz, H-7), 7.55 (1H, *t*, $J = 7.8$ Hz, H-8), 7.94 (1H, *br d*, $J = 7.8$ Hz, H-10). EIMS *m/z* (rel. Int.): 327 ($[M]^+$, 30), 319 (3), 286 (11), 242 (7), 226 (100), 204 (10), 188 (8).

Oxidation of **2**. A CH_2Cl_2 soln of **2** (1 mg) was treated with CrO_3 (3 mg) at room temp. for 30 min. The mixt. was treated in the usual way to give an oily product which was identified as **4** by comparison of spectral data and TLC.

Acknowledgement—We thank Miss L. N. Lai, National Cheng Kung University, for measuring mass spectra.

REFERENCES

1. Wu, S. J. and Chen, I. S. (1993) *Phytochemistry* **34**, 1659.
2. Chen, I. S., Wu, S. J., Lin, Y. C., Tsai, I. L., Seki, H., Ko, F. N. and Teng, C. M. (1994) *Phytochemistry* **36**, 237.
3. Chen, I. S., Wu, S. J., Tsai, I. L., Wu, T. S., Pezzuto, J. M., Lu, M. C., Chai, H. and Suh, N. and Teng, C. M. (1994) *J. Nat. Prod.* **57**, 1206.
4. Shamma, M. (1972) in *Organic Chemistry*, vol. 5 (A. T. Blomquist and H. Wasserman, eds), Academic Press, New York.
5. Chen, I. S., Tsai, I. L., Wu, S. J., Sheen, W. S., Ishikawa, T. and Ishii, H. (1993) *Phytochemistry* **32**, 1065.
6. Ishii, H., Ishikawa, T., Mihara, M. and Akaike, M. (1983) *Yakugaku Zasshi* **103**, 279.
7. Ishii, H., Ishikawa, T., Akaike, M., Tohjoh, T., Toyoki, M., Ishikawa, M. and Chen, I. S. (1984) *Yakugaku Zasshi* **104**, 1030.
8. Ishii, H., Ishikawa, T., Lu, S. T. and Chen, I. S. (1976) *Yakugaku Zasshi* **96**, 1458.
9. Ishii, H., Ishikawa, T. and Haginiwa, J. (1977) *Yakugaku Zasshi* **97**, 890.
10. Ishii, H., Hosoya, K., Ishikawa, T., Veda, E. and Haginiwa, J. (1974) *Yakugaku Zasshi* **94**, 322.
11. Hifnawy, M. S., Vaguette, J., S'evenet, T., Pousset, J. L. and Cav'e, A. (1977) *Phytochemistry* **16**, 1035.
12. Mester, I. (1973) *Fitoterapia* **44**, 123. [C. A. **81**, 23041a (1974)].
13. Correa, D. B., Gottlieb, O. R., Padua, A. P. and Rocha, A. I. (1976) *Rev. Latinoam. Quim.* **7**, 43 [CA **84**, 161790g (1976)].
14. Hufford, C. D. (1976) *Phytochemistry* **15**, 1169.