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Bishordeninyl terpene alkaloids from *Zanthoxylum avicennae*

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Abstract

In addition to (−)-culantraramine and (−)-culantraraminol the bishordeninyl terpene alkaloids, (−)-culantraramine *N*-oxide, (−)-culantraraminol *N*-oxide and avicennamine, have been isolated from the leaves of *Zanthoxylum avicennae*. Their structures have been assigned by MS and especially by NMR investigations.

Keywords: Zanthoxylum avicennae; Rutaceae; Bishordeninyl terpene alkaloids; (−)-Culantraramine; (−)-Culantraraminol; (−)-Culantraramine *N*-oxide; (−)-Culantraraminol *N*-oxide; Avicennamine

1. Introduction

Zanthoxylum avicennae DC (Rutaceae) is an 8 m high shrub (Pham-hoang Ho, 1992) growing in North and South Vietnam, Cambodia and Laos (Do Tat Loi, 1991). A decoction of its stems is used as a stomach tonic and as a counter-poison to snake bite (Perry, 1980). Previous investigations have shown that the plant contains alkaloids (Fish, Gray, & Waterman, 1975; Wenzhu Wu & Zonghong Zhu, 1992; Miao Zhenchun, Wu Wenzhu, & Feng Rui, 1993), terpenoids (Shifa Cheng, Liangfeng Zhu, Biyao Lu, Yuexin Yu, & Zhujin Liu, 1990), flavonoids (Fish et al., 1975) and coumarins (Fish et al., 1975; Gray, Waigh, & Waterman, 1975). In continuation of our phytochemical studies on Vietnamese plants (Nguyen Hoang Anh, Ripperger, Porzel, Tran Van Sung, & Adam, 1997) we have isolated from the leaves of this species the optically active bishordeninyl terpene alkaloids (−)-culantraramine (**1**) and (−)-culantraraminol (**4**), hitherto only known as the racemates (Schroeder & Stermitz, 1985), and the new alkaloids (−)-culantraramine *N*-oxide (mixture of **2**+**3**), (−)-culantraraminol *N*-oxide (mixture of **5**+**6**) and avicennamine (**7**), the structures of which have been elucidated as outlined in Section 2.

2. Results and discussion

The alkaloids were obtained from the methanol extract of the leaves via partition between chloroform and water and subsequent chromatographic procedures. Column chromatography on silica gel afforded firstly (−)-culantraramine (**1**) and (−)-culantraraminol (**4**), identified by comparison of the NMR data with those of their known racemates (Schroeder & Stermitz, 1985). As expected for *N*-oxides the electron-impact mass spectra of the further constituents (−)-culantraramine *N*-oxide (mixture of **2**+**3**) and (−)-culantraraminol *N*-oxide (mixture of **5**+**6**) displayed $[M–O]^+$ ions ($C_{32}H_{46}N_2O_2$ and $C_{32}H_{48}N_2O_3$, respectively). The electrospray ionization mass spectra showed quasi-molecular ions $[M+H]^+$. The NMR signals of the compounds (Tables 1 and 2) as well as their structures were assigned on the basis of their gradient-selected HSQC, ^1H–^1H COSY (in the case of **2**+**3** long-range), gradient-selected HMBC and NOESY spectra. The data corresponded to those of culantraramine and culantraraminol (Schroeder & Stermitz, 1985). C-3 to C-5 were not correctly assigned by Schroeder & Stermitz (1985). The signals for H-8′ and H-9′ Table 1 of the alkaloids **2**, **3**, **5** and **6** showed low-field shifts in comparison with those of compound **7** indicating protonation (Schroeder & Stermitz, 1985) due to acidity in the $CDCl_3$. Some of the ^{13}C signals appeared twice (especially for C-1, C-2, C-2′ and C-2″) indicating a mixture of two regioisomeric compounds.

The elemental composition of avicennamine (**7**) was shown to be $C_{32}H_{48}N_2O_3$ by high-resolution mass spectrometry. The constitution followed from the gradient-selected HSQC, ^1H–^1H COSY and gradient-selected HMBC spectra. Tables 1–2 contain the ^1H and ^{13}C NMR data. The relative configuration was elucidated by NOESY spectroscopy (Fig. 1). A *cis*-relationship of the

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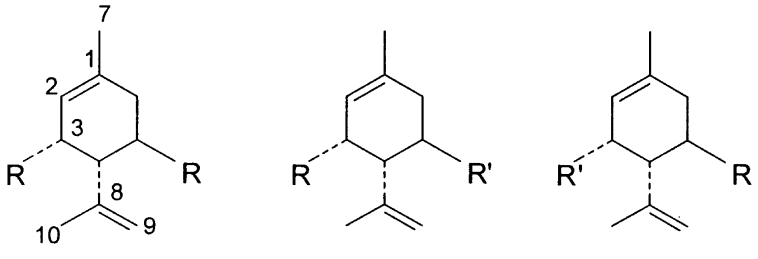

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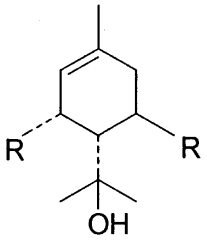

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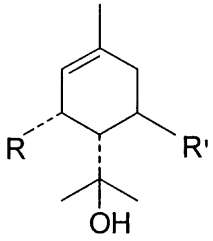

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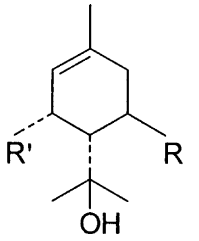

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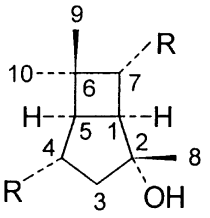

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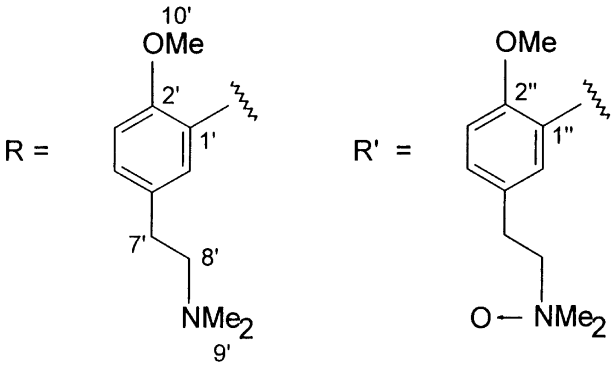

Structure 1

two rings could be assumed because of steric reasons. A NOE between H-1/H-5 (Dreiding model: 2.8 Å) was observed. A NOE between H-5/Me-10 (2.5 Å) indicated their *cis*-position and, therefore, *trans*-arrangement of Me-9. Further NOEs between H-4/Me-9 (1.4 Å), H-7/Me-9 (2.8 Å), H-3β/H-7 (3.0 Å) and H-7/Me-8 (2.4 Å) revealed corresponding *cis* relationships. NOEs between H-3β/H-4 (2.4 Å) and H-3β/Me-8 (2.6 Å) corroborated these assignments. Strucure **7** followed from these data for avicennamine, representing a new type of bishordeninyl alkaloids with a bicyclo[3,2,0]heptane monoterpene unit.

The absolute configurations of the alkaloids **1**–**7** have not been established.

Table 1
^{1}H NMR data of compounds **2**, **3**, **5**, **6** and **7** ($CDCl_3$, δ values, J (Hz) in parentheses)

Position	**2**+**3**[a]	**5**+**6**[a]	**7**[b]
1	–	–	3.15 m
2	5.43 m	5.50 br s	–
3	4.23 d (4.7)	ca 4.15 m	2.07 (α) br d (11.6), 2.61 (β) m
4	3.07 m	2.65 m	3.81 m
5	2.56 m	2.00 m	2.52 m
6	2.32 m	2.39 m	–
7	1.79 s	1.79 s	3.20 m
8	–	–	1.18 s
9	4.23 m	0.67 s	1.24 s
10	1.36 s, 1.40 s	0.67 s	0.73 s
3′, 3″	ca 6.7 m	6.76 d (8.5), 6.77 d (8.8)	6.75 d (8.2)[c], 6.79 d (8.2)[d]
4′, 4″	6.99 dd (8.3, 2.2), 7.04 dd (8.3, 2.2)	ca 7.02 m	7.01 dd (8.1, 2.1), –
6′, 6″	7.10 d (2.2), 7.11 d (2.2)	7.22 d (1.8)	7.11 d (1.9)[c], 7.32 d (1.9)[d]
7′	2.85 m, 2.92 m	ca 2.95 m	2.73 m
7″	3.07 t (8.0), 3.18 t (8.0)	3.12 t (8.2), 3.21 t (8.1)	–
8′	2.85 m, 2.92 m	ca 2.95 m	2.55 m
8″	3.60 m, 3.68 m	ca 3.70 m	–
9′	2.56 s, 2.61 s	2.65 s	2.35 s, 2.36 s
9″	3.34 s, 3.37 s, 3.44 s, 3.47 s	3.38 s, 3.43 s, 3.46 s, 3.49 s	–
10′, 10″	3.65 s, 3.73 s	3.80 s	3.78 s[c], 3.84 s[d]

[a] 500 MHz.
[b] 300 MHz.
[c] Signals for 7-substituent.
[d] Signals for 4-substituent.

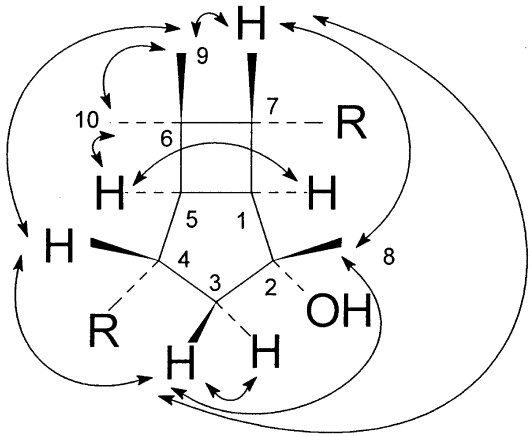

Fig. 1. NOEs of avicennamine (**7**).

3. Experimental

Leaves of *Zanthoxylum avicennae* DC. were collected in the National Park Cuc Phuong, Ninh Binh, Vietnam in December 1996. The species was identified by Dr. Tran Dinh Dai, Hanoi. A voucher specimen was deposited in the Herbarium of the Institute of Ecology and Natural Resources, National Centre for Natural Science and Technology of Vietnam, Hanoi. The plant material was dried at 45°C, ground (1.3 kg) and extracted with 95% MeOH at room temp. MeOH was removed by distillation in vacuo and the aq. soln was extracted with *n*-hexane followed by EtOAc and *n*-BuOH. The *n*-BuOH was evapd in vacuo, the residue was partitioned between $CHCl_3$ and H_2O. Evapn of $CHCl_3$ in vacuo gave a mixture of alkaloids. This was chromatographed over silica gel with $CHCl_3$, shaken with an equal quantity of conc. NH_3–EtOAc (4:1). Increasing amounts (0–16%) of MeOH were added to this mixture leading to the alkaloids in the following sequence.

3.1. (−)-Culantraramine (*1*)

Oil, yield 0.01%. $[\alpha]^{22}_D$ −196.7° ($CHCl_3$, *c* 1.0), lit. Schroeder and Stermitz (1985): 0°. CD (MeOH): $\Delta\varepsilon_{280}$ +3.62, $\Delta\varepsilon_{235}$ +9.14, $\Delta\varepsilon_{207}$ −84.5. R_f 0.68 (silica gel, EtOAc–MeOH–conc. NH_3 (12:2:1)). ^{13}C NMR (75.5 MHz, C_6D_6): identical with the data of lit. Miao Zhenchun et al. (1993), exception δ 111.7 (C-9) instead of 110.7. EI-MS (70 eV) *m*/*z* (rel. int.): 490.3542 $[M]^+$

Table 2
^{13}C NMR data of compounds **2**, **3**, **5**, **6** and **7** ($CDCl_3$, δ values)

Position	**2**+**3**[a]	**5**+**6**[b]	**7**[a]
1	134.8, 135.3	134.6	50.4
2	123.6, 124.0	124.9	82.7
3	36.8, 36.9	34.0	48.3
4	48.9	50.7	39.2
5	45.2	22.4	53.1
6	39.6	ca. 37.0	38.6
7	23.1	23.0	45.7
8	145.0, 145.2	73.7	22.8
9	110.7, 110.9	ca. 29.8	25.4
10	23.2, 23.7	ca. 29.8	26.5
1′, 1″	134.2, 134.3	134.6	129.5[c], 136.0[d]
2′, 2″	155.2, 155.9, 156.6, 157.3	155.3	155.1[d], 156.2[c]
3′, 3″	109.8, 110.1, 110.3, 110.6	110.8, 111.0, 111.3	109.4[c], 110.5[d]
4′, 4″	123.3, 126.5, 126.9	127.3, 127.4, 127.7	126.5[c], 126.7[d]
5′, 5″	130.8, 130.9	132.5, 132.7	131.3[c], 131.7[d]
6′, 6″	131.1	132.0	128.4, 128.5
7′	33.4, 33.6	31.1	33.3[d], 33.5[c]
7″	29.6, 29.7	29.0, 29.3, 29.7	–
8′	61.6, 62.0	59.9	61.4, 61.7
8″	72.4, 72.7	71.2	–
9′	45.2, 45.3	43.6, 43.7	45.0, 45.2
9″	58.5	57.2, 57.3, 57.6	–
10′, 10″	54.9, 55.4, 55.5	55.6, 55.7	54.8[c], 55.3[d]

[a] 75.5 MHz.
[b] 125.7 MHz.
[c] Signals for 7-substituent.
[d] Signals for 4-substituent.

($C_{32}H_{46}N_2O_2$, calcd 490.3559) (4.5), 58 $[CH_2NMe_2]^+$ (100).

3.2. (−)-Culantraraminol (*4*)

The alkaloid was purified by CC (silica gel, EtOAc–MeOH–conc. NH_3 (12:1:1)) and prep. TLC (silica gel, EtOAc–MeOH–$NHEt_2$ (40:5:3)). Oil, yield 0.004%. $[\alpha]^{24}_D$ −112.2° ($CHCl_3$, *c* 0.25), lit. Schroeder and Stermitz (1985): 0°. CD (MeOH): $\Delta\varepsilon_{277}$ +1.24, $\Delta\varepsilon_{241}$ −1.05, $\Delta\varepsilon_{236}$ +0.92, $\Delta\varepsilon_{207}$ −39.7. R_f 0.48 (silica gel, EtOAc–MeOH–conc. NH_3 (12:2:1)). ^{1}H NMR (300 MHz, $CDCl_3$): identical with the data of lit. Nguyen Hoang Anh et al. (1997). EI-MS (70 eV) *m*/*z* (rel. int.): 508.3663 $[M]^+$ ($C_{32}H_{48}N_2O_3$, calcd 508.3669) (4), 58 $[CH_2NMe_2]^+$ (100). Hydrobromide: m.p. 296–298° (from EtOH–EtOAc).

3.3. (−)-Culantraramine N-oxide (*2*+*3*)

The mixture of both alkaloids was further purified by prep. TLC (EtOAc–MeOH–conc. NH_3 (6:1:1)). Oil, yield 0.0012%. $[\alpha]^{25}_D$ −200.8° ($CHCl_3$, *c* 0.50). CD (MeOH): $\Delta\varepsilon_{281}$ +3.32, $\Delta\varepsilon_{236}$ +10.38, $\Delta\varepsilon_{207}$ −70.2. R_f 0.21 (silica gel, EtOAc–MeOH–conc. NH_3 (12:2:1)). EI-MS (70 eV) *m*/*z* (rel. int.): 490.3559 $[M–O]^+$ ($C_{32}H_{46}N_2O_2$, calcd 490.3559) (10), 58 $[CH_2NMe_2]^+$ (100). ESI-MS (positive ions): 507 $[M+H]^+$, 446 $[M+H–NMe_2OH]^+$.

3.4. (−)-Culantraraminol N-oxide (*5*+*6*)

The mixture of both alkaloids was further purified by CC (silica gel, EtOAc–MeOH–conc. NH_3 (6:1:1)) and prep. TLC (EtOAc–MeOH–conc. NH_3 (6:1:1)). Oil, yield 0.0006%. $[\alpha]^{25}_D$ −256.1° ($CHCl_3$, *c* 0.20). CD (MeOH): $\Delta\varepsilon_{280}$ +0.87, $\Delta\varepsilon_{236}$ +3.02, $\Delta\varepsilon_{206}$ −37.4, $\Delta\varepsilon_{194}$ +26.1. R_f 0.18 (silica gel, EtOAc–MeOH–conc. NH_3 (12:2:1)). EI-MS (70 eV) *m*/*z* (rel. int.): 508.3651 $[M–O]^+$ ($C_{32}H_{48}N_2O_3$, calcd 508.3665) (3), 58 $[CH_2NMe_2]^+$ (100). ESI-MS (positive ions): 525 $[M+H]^+$, 507 $[M+H–H_2O]^+$, 446 $[M+H–H_2O–NMe_2OH]^+$.

3.5. Avicennamine (*7*)

The alkaloid was purified by CC (silica gel, EtOAc–MeOH–conc. NH_3 (12:1:1)) and prep. TLC (silica gel, EtOAc–MeOH–$NHEt_2$ (40:5:3), 3×). Amorphous, yield 0.0018%. $[\alpha]^{28}_D$ −52.9° ($CHCl_3$, *c* 1.00). CD (MeOH): $\Delta\varepsilon_{287}$ −0.72, $\Delta\varepsilon_{235}$ −4.50. R_f 0.38 (silica gel, EtOAc–MeOH–conc. NH_3 (12:2:1)). EI-MS (70 eV) *m*/*z* (rel. int.): 508.3644 $[M]^+$ ($C_{32}H_{48}N_2O_3$, calcd 508.3665) (10), 58 $[CH_2NMe_2]^+$ (100).

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