Ouvrardianines A and B, Two New Norditerpenoid Alkaloids from *Aconitum ouvrardianum*

by **Hong Jing**[a]), **Xiao-Dong Yang***[a]), **Jing-Feng Zhao**[a]), **Shu Yang**[b]), **Hong-Bin Zhang**[a]), and **Liang Li***[a])

[a]) Key Laboratory of Medicinal Chemistry for Natural Resources (Yunnan University), Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming 650091, P. R. China (phone: +86-871-5033644-8899; fax: +86-871-5035538; e-mail: liliang5758@hotmail.com or xdyang120@hotmail.com)

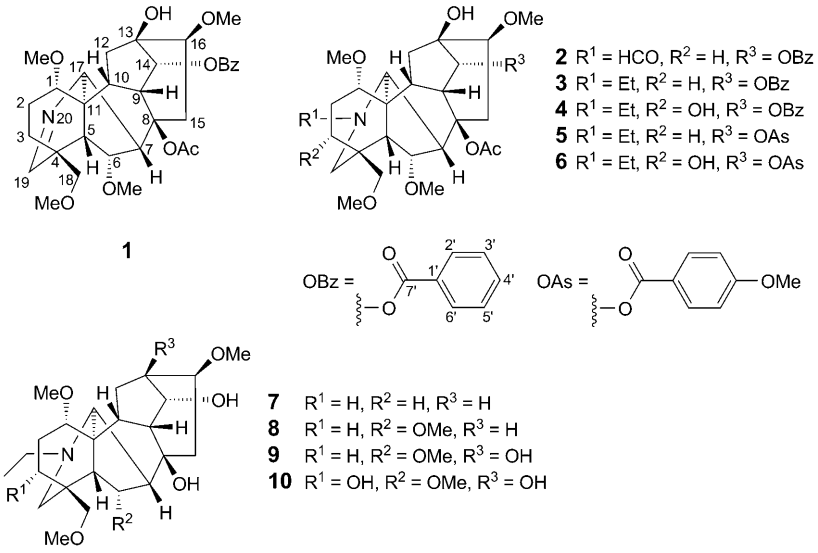
[b]) College of Fundamental and Information Engineering, Yunnan Agricultural University, Kunming 650201, P. R. China

Two new norditerpenoid alkaloids, ouvrardianines A and B (**1** and **2**, resp.), together with eight known compounds, were isolated from *Aconitum ouvrardianum*. The structures of the new compounds were elucidated as (1*α*,6*α*,14*α*,16*β*)-8-(acetyloxy)-13-hydroxy-1,6,16-trimethoxy-4-(methoxymethyl)-aconit-19-en-14-yl benzoate (**1**) and (1*α*,6*α*,14*α*,16*β*)-8-(acetyloxy)-20-formyl-13-hydroxy-1,6,16-trimethoxy-4-(methoxymethyl)aconitan-14-yl benzoate (**2**) on the basis of spectral analyses. The new compound **1** was found to contain the rare C(19)=N imine group.

Introduction. – The genus *Aconitum* (Ranunculaceae) is represented with 208 species in China, mostly growing in the southwestern and northeastern parts of the country on mountains of 1500 meters above sea level or higher [1]. *Aconitum* species produce highly toxic norditerpenoid alkaloids that have attracted considerable interest because of their complex structures, interesting chemistry, and noteworthy physiological effects [2]. *Aconitum ouvrardianum* HAND.-MAZZ. has long been used in Tibetan folk medicine for the treatment of arthralgia, dysmenorrhea and colic [3]. As a continuation of our studies on medicinal plants of *Aconitum* species growing on the Yunnan-Tibet Plateau [4–9], *Aconitum ouvrardianum* was now examined. To the best of our knowledge, no scientific study on this plant has hitherto been reported.

From its roots, two new norditerpenoid alkaloids, named ouvrardianines A and B (**1** and **2**, resp.), as well as eight known norditerpenoid alkaloids, were isolated. The known compounds were identified as chasmaconitine (**3**) [10], indaconitine (**4**) [11], crassicauline A (**5**) [12], yunaconitine (**6**) [13], talatizamine (**7**) [14], chasmaine (**8**) [15], bikhaction (**9**) [16], and pseudaconine (**10**) [17]. Here we report on the isolation and structure elucidation of **1** and **2**.

Results and Discussion. – Ouvrardianine A (**1**) was isolated as an optically active white amorphous solid. Its molecular formula was determined as $C_{32}H_{41}NO_9$ by HR-ESI-MS ($[M+1]^+$ at m/z 584.2825). The IR spectrum showed characteristic absorptions for an OH group (3447 cm^{-1}, br.), an ester group (1717 cm^{-1}), a N=CH moiety (1633 cm^{-1}), and an aromatic ring (1606 and 1512 cm^{-1}). The UV absorption at



259 (4.58) nm is consistent with the presence of a benzoate unit. From the ^{1}H- and ^{13}C-NMR (*Table 1*), HMBC, HMQC, NOESY, and ^{1}H,^{1}H-COSY data (*Fig. 1*), compound **1** was elucidated as (1α,6α,14α,16β)-8-(acetyloxy)-13-hydroxy-1,6,16-trimethoxy-4-(methoxymethyl)aconit-19-en-14-yl benzoate.

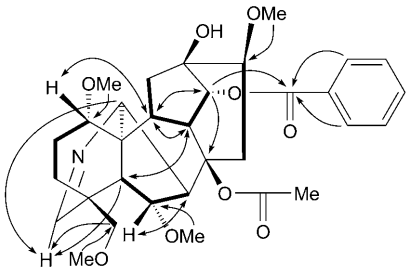


Fig. 1. *Significant ^{1}H,^{1}H-COSY* (—), *HMBC* (→), *and NOESY* (↔) *data for* **1**

The ^{1}H-NMR spectrum of **1** (*Table 1*) showed signals due to five aromatic H-atoms for a monosubstituted benzene (δ(H) 8.05 (*dd*, J = 7.1, 1.4, 2 H), 7.56 (*m*, 1 H), 7.43 (*dd*, J = 7.9, 7.1, 2 H)), four MeO groups (δ(H) 3.55, 3.31, 3.19, and 3.07, each 3 H, *s*), a strongly shielded MeCO group (δ(H) 1.25, *s*), and a methine of an N=CH group (δ(H) 7.33, *s*). The ^{13}C-NMR spectrum (*Table 1*) clearly indicated the presence of a norditerpene moiety (C(1)–C(19)) combined with a benzoyl unit (C(1′) to C(6′), C(=O)–C(1′)), four MeO groups, a MeCO group (δ(C) 169.0 and 20.9), and a N=CH group (δ(C) 165.5). Its spectral characteristics were similar to those of the known compound chasmaconitine (**3**), except for the absence of an N–Et group in **1**. The signals at δ(H) 7.33 (*s*) and δ(C) 165.5 suggested the presence of an N=CH group

Table 1. *^1H- (500 MHz) and ^{13}C-NMR (125 MHz) Data of Ouvrardianine A* (**1**) *in $CDCl_3$.* δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
H–C(1)	3.23 (*t*, *J* = 3.9)	81.7	H–C(16)	3.47 (*t*, *J* = 6.1)	82.6
CH_2(2)	1.64–1.67 (*m*, H_α),	22.2	H–C(17)	3.75 (*s*)	60.8
	1.23–1.27 (*m*, H_β)	27.5	CH_2(18)	3.78 (*d*, *J* = 8.5, H_α),	77.5
CH_2(3)	1.68–1.72 (*m*, H_α),			3.48 (*d*, *J* = 8.5, H_β)	
	1.55–1.61 (*m*, H_β)		H–C(19)	7.33 (*s*)	165.5
C(4)	–	46.1	C(1′)	–	129.6
H–C(5)	2.24 (*d*, *J* = 7.0)	45.4	H–C(2′/6′)	8.05 (*dd*, *J* = 7.1, 1.4, 2 H)	129.2
H–C(6)	3.91 (*d*, *J* = 7.0)	83.2	H–C(3′/5′)	7.43 (*dd*, *J* = 7.9, 7.1, 2 H)	128.1
H–C(7)	3.19 (*s*)	53.6	H–C(4′)	7.56 (*m*)	132.7
C(8)	–	83.9	*C*(=O)–C(1′)	–	165.8
H–C(9)	2.72 (*dd*, *J* = 7.4, 5.2)	42.5	MeO–C(1)	3.07 (*s*)	55.8
H–C(10)	2.21–2.23 (*m*)	39.8	MeO–C(6)	3.55 (*s*)	58.3
C(11)	–	51.2	MeO–C(16)	3.19 (*s*)	56.7
CH_2(12)	2.08–2.11 (*m*, H_α),	35.4	MeO–C(18)	3.31 (*s*)	58.7
	2.16–2.18 (*m*, H_β)		Me*C*OO–C(8)	–	169.0
C(13)	–	74.3	*Me*COO–C(8)	1.25 (*s*)	20.9
H–C(14)	4.94 (*d*, *J* = 5.0)	78.5			
CH_2(15)	2.46 (*dd*, *J* = 15.9, 6.0, H_α),	38.2			
	3.26–3.29 (*m*, H_β)				

instead of the N–Et or N–Me group characteristic of many norditerpenoid alkaloids [18]. The FAB-MS of **1** exhibiting a molecular ion at *m*/*z* 584 ($[M + 1]^+$), compared to *m*/*z* 614 ($[M + 1]^+$) for **3**, is consistent with this contention.

In the HMBC experiment of **1** (*Fig. 1*), the correlation H–C(14) (δ(H) 4.94)/*C*(=O)–C(1′) (δ(C) 165.8) suggested that the benzoyloxy group is positioned at C(14), while the correlations H–C(17) (δ(H) 3.75) and H_β–C(18) (δ(H) 3.48)/C(19) (δ(C) 165.5) suggested that C(19) is involved in the N=CH group. The four MeO groups were assigned as MeO–C(1), MeO–C(6), MeO–C(16), and MeO–C(18), based on the HMQC and HMBC data. The ^{1}H,^{1}H-COSY correlations are shown in *Fig. 1*. The relative configuration of **1** was studied by means of a NOESY experiment (*Fig. 1*). The NOEs H–C(1)/H–C(10), H–C(10)/H–C(14), H–C(14)/H–C(9), and H–C(9)/H–C(10) indicated β-oriented H-atoms at these locations. The coupling constant between H–C(5) and H–C(6) (J = 7.0 Hz) confirmed the β-position of H–C(6), and the NOE between H–C(6) and H–C(7) established the β-orientation of these H-atoms. Further, the NOEs H–C(17)/H_α–C(15) and H_α–C(15)/H–C(16) demonstrated the α-position of H–C(16). The NOEs H–C(16)/H_α–C(15), H–C(17)/H_α–C(12), H–C(5)/H_β–C(2), and H_α–C(2)/H_α–C(3) allowed the steric differentiation of the H-atoms of CH_2(2), CH_2(3), CH_2(12), and CH_2(15).

Ouvrardianine B (**2**) was isolated as an optically active amorphous solid. Its molecular formula was determined as $C_{33}H_{43}NO_{10}$ by HR-ESI-MS ($[M + 1]^+$ at *m*/*z* 614.2911). The IR spectrum showed characteristic absorptions for an OH group (3433 cm^{-1}, br), an ester group (1718 cm^{-1}), an amide moiety (1640 cm^{-1}), and an aromatic ring (1607 and 1513 cm^{-1}). The UV absorption at 260 (4.48) nm is consistent with the presence of a benzoate unit. From the ^{1}H- and ^{13}C-NMR (*Table 2*), HMBC,

HMQC, NOESY, and ^{1}H,^{1}H-COSY data (*Fig. 2*), compound **2** was elucidated as (1α,6α,14α,16β)-8-(acetyloxy)-20-formyl-13-hydroxy-1,6,16-trimethoxy-4-(methoxymethyl)aconitan-14-yl benzoate.

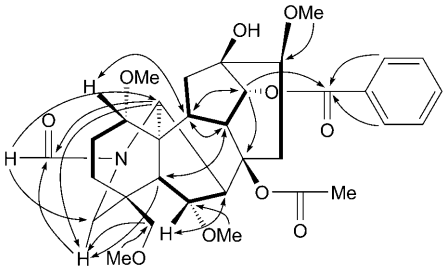


Fig. 2. *Significant ^{1}H,^{1}H-COSY* (—), *HMBC* (→), *and NOESY* (↔) *data for* **2**

Table 2. *^{1}H-* (500 MHz) *and ^{13}C-NMR* (125 MHz) *Data of Ouvrardianine B* (**2**) *in $CDCl_3$*. δ in ppm, *J* in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
H–C(1)	3.10 (*t*, *J* = 3.9)	81.2	H–C(16)	3.44 (*t*, *J* = 7.0)	82.8
CH_2(2)	1.90–1.94 (*m*, H_α),	24.4	H–C(17)	3.67 (*s*)	58.6
	1.44–1.50 (*m*, H_β)		CH_2(18)	3.70 (*d*, *J* = 8.5, H_α),	79.1
CH_2(3)	1.67–1.69 (*m*, H_α),	32.6		3.21 (*d*, *J* = 8.5, H_β)	
	1.57–1.60 (*m*, H_β)		CH_2(19)	3.22 (*d*, *J* = 13.5, H_α),	44.3
C(4)	–	37.4		3.75 (*d*, *J* = 13.5, H_β)	
H–C(5)	2.34 (*d*, *J* = 6.8)	48.4	H–C(21)	8.05 (*s*)	161.9
H–C(6)	4.08 (*d*, *J* = 6.8)	82.1	C(1′)	–	129.5
H–C(7)	2.90 (*s*)	54.4	H–C(2′/6′)	8.06 (*dd*, *J* = 6.3, 1.5, 2 H)	129.2
C(8)	–	83.8	H–C(3′/5′)	7.43 (*dd*, *J* = 7.9, 6.3, 2 H)	128.1
H–C(9)	2.86 (*t*, *J* = 5.9)	43.2	H–C(4′)	7.57 (*m*)	132.8
H–C(10)	2.16–2.20 (*m*)	40.3	*C*(=O)–C(1′)	–	165.8
C(11)	–	49.1	MeO–C(1)	3.14 (*s*)	54.9
CH_2(12)	2.07 (*d*, *J* = 14.7, H_α),	33.6	MeO–C(6)	3.58 (*s*)	58.4
	2.63 (*dd*, *J* = 14.7, 5.4, H_β)		MeO–C(16)	3.20 (*s*)	57.2
C(13)	–	74.3	MeO–C(18)	3.29 (*s*)	58.7
H–C(14)	4.92 (*d*, *J* = 5.2)	78.0	Me*C*OO–C(8)	–	169.3
CH_2(15)	2.52 (*dd*, *J* = 16.3, 5.6, H_α),	38.4	*Me*COO–C(8)	1.27 (*s*)	21.0
	3.05–3.07 (*m*, H_β)				

The ^{1}H-NMR spectrum of **2** (*Table 2*) showed signals due to five aromatic H-atoms for a monosubstituted benzene (δ(H) 8.06 (*dd*, *J* = 6.3, 1.5, 2 H), 7.57 (*m*, 1 H), 7.43 (*dd*, *J* = 7.9, 6.3, 2 H)), four MeO groups (δ(H) 3.58, 3.29, 3.20 and 3.14, each 3 H, *s*), a strongly shielded MeCO group (δ(H) 1.27, *s*), and a H-atom of an HCON group (δ(H) 8.05, *s*). The ^{13}C-NMR spectrum (*Table 2*) clearly indicated the presence of a norditerpene moiety (C(1)–C(19)) combined with a benzoyl unit (C(1′) to C (6′), C(=O)–C(1′)), four MeO groups, a MeCO group (δ(C) 169.3 and 21.0), and an HCON group (δ(C) 161.9). Its spectral characteristics were similar to those of the known compound chasmaconitine (**3**), except that a formyl group (HCO, δ(C) 161.9,

C(21)) in compound **1** replaced the ethyl group (δ(C) 49.9 and 13.8, C(21) and C(22)) in **3**. The signals at δ(H) 8.05 (*s*) and δ(C) 161.9 suggested the presence of an HCON instead of the N–Et or N–Me group characteristic of norditerpenoid alkaloids. The FAB-MS of **2** exhibiting a molecular ion at *m*/*z* 614 ($[M + 1]^+$) compared to *m*/*z* 614 ($[M + 1]^+$) for **3** is consistent with this contention.

In the HMBC plot of **2** (*Fig. 2*) the correlation H–C(14) (δ(H) 4.92)/*C*(=O)–C(1′) (δ(C) 165.8) suggested that the *O*-benzoyl group is at C(14), while the correlations H–C(17) (δ(H) 3.67) and H–C(19) ($\delta(H_\beta)$ 3.75)/C(21) (δ(C) 161.9) as well as H–C(21) (δ(H) 8.05)/C(17) (δ(C) 58.6) and C(19) (δ(C) 44.3) suggested that C(21) is involved in the position of the HCON group. The four MeO groups were assigned as MeO–C(1), MeO–C(6), MeO–C(16), and MeO–C(18), based on the HMQC and HMBC data. ^{1}H,^{1}H-COSY correlations of **2** are shown in *Fig. 2*. The relative configuration of **2** was identical with that of **1**, as can be seen from the NOESY data (*Figs. 1* and *2*).

This work was supported by the *Natural Science Foundation of Yunnan Province* (2005B0001Q and 2007B0006Z), which is gratefully acknowledged.

Experimental Part

General. M.p.: *XT-4* melting-point apparatus, uncorrected. $[\alpha]_D$: *Jasco-20C* digital polarimeter. UV Spectra: *UV 210A* spectrometer; λ_{max}(log ε) in nm. IR Spectra: *Bio-Rad-FTS-135* spectrometer; in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker Avance-DRX-500* instrument; Me_4Si as internal reference, δ in ppm, *J* in Hz. EI-MS: *VG Autospec-3000* mass spectrometer; in *m*/*z* (rel. %).

Plant Material. The roots of *A. ouvrardianum* HAND.-MAZZ. were collected in Deqin County, Yunnan Province, P. R. China, in September 2001. The identity of the plant material was verified by Prof. *Zhi-Hao Hu*, Department of Biology, School of Life Science, Yunnan University, P. R. China. A voucher specimen (No. 01-006) was deposited in the Key Laboratory of Medicinal Chemistry for Natural Resources, Yunnan University, Kunming, P. R. China.

Extraction and Isolation. The ground roots (4.2 kg) of *Aconitum ouvrardianum* were extracted with 95% EtOH (5 × 20 l) at r.t. The EtOH extract was evaporated to yield a residue, which was suspended in H_2O and then extracted with petroleum ether (PE), AcOEt, and BuOH, in this order. The AcOEt extract (48 g) was subjected to column chromatography (CC; SiO_2; PE/AcOEt/Et_3N 50 : 1 : 0.1 → 0 : 1 : 0.1): *Fractions 1–5*. *Fr. 2* was further purified by CC (1. SiO_2; PE/AcOEt/Et_3N 8 : 1 : 0.1 → 0 : 1 : 0; 2. *Sephadex LH-20*, MeOH): **3** (25 mg), **5** (58 mg), and **7** (40 mg). *Fr. 4* was further purified by CC (1. SiO_2; PE/AcOEt/Et_3N 2 : 1 : 0.1 → 1 : 10 : 0.1; 2. *Sephadex LH-20*, MeOH): **2** (8 mg), **4** (22 mg), and **8** (18 mg). The BuOH extract (42 g) was subjected to CC (SiO_2; PE/AcOEt/Et_3N 20 : 1 : 0.1 → 0 : 1 : 0.1): *Fractions 1–8*). *Fr. 4* was further purified by CC (SiO_2; PE/AcOEt/Et_3N 8 : 1 : 0.1 → 0 : 1 : 0): **1** (5 mg), **6** (92 mg), and **9** (9 mg). *Fr. 7* was further purified by CC (1. SiO_2; PE/AcOEt/Et_3N 0 : 1 : 0.1 → 1 : 10 : 0.1; 2. *Sephadex LH-20*, MeOH): **10** (12 mg).

Ouvrardianine A (=*(1α,6α,14α,16β)-8-(Acetyloxy)-13-hydroxy-1,6,16-trimethoxy-4-(methoxymethyl)aconit-19-en-14-yl Benzoate*; **1**). White amorphous solid. $[\alpha]_D^{25} = +50.70$ ($c = 0.377$, MeOH). UV (MeOH): 234 (4.48), 259 (4.58). IR (KBr): 3447, 2963, 2820, 1717, 1633, 1606, 1512, 1461, 1370, 1281, 1261, 1230, 1169, 1097, 1021, 945, 912, 850, 802, 772, 761. ^{1}H- and ^{13}C-NMR: *Table 1*. FAB-MS (pos.): 584 (100, $[M + 1]^+$), 568 (3), 538 (2), 524 (5), 490 (1), 461 (1), 434 (1), 402 (2), 356 (1), 326 (1), 282 (1), 223 (1), 186 (1), 149 (1), 106 (9), 71 (2). HR-ESI-MS: 584.2825 ($[M + 1]^+$, $C_{32}H_{42}NO_9^+$; calc. 584.2860).

Ouvrardianine B (=*(1α,6α,14α,16β)-8-(Acetyloxy)-20-formyl-13-hydroxy-1,6,16-trimethoxy-4-(methoxymethyl)aconitan-14-yl Benzoate*; **2**): White amorphous solid. $[\alpha]_D^{25} = -28.17$ ($c = 0.142$, MeOH). UV (MeOH): 260 (4.48), 313 (3.71), 397 (2.97). IR (KBr): 3433, 2928, 2854, 1718, 1640, 1607, 1513, 1462, 1371, 1345, 1278, 1258, 1169, 1107, 1091, 1021, 991, 850, 772. ^{1}H- and ^{13}C-NMR: *Table 2*. FAB-MS (pos.):

614 (100, $[M + 1]^+$), 584 (30), 553 (16), 536 (5), 494 (6), 462 (4), 386 (4), 368 (13), 279 (4), 233 (5), 186 (51), 149 (20), 106 (20), 91 (19). HR-ESI-MS: 614.2911 ($[M + 1]^+$, $C_{33}H_{44}NO_{10}^+$; calc. 614.2965).

REFERENCES

[1] Institute of Botany, Chinese Academy of Science, 'Flora Reipublicae Sinicae', Science Press, Beijing, 1979, Vol. 27, p. 113 – 300.
[2] S. W. Pelletier, N. V. Mody, B. S. Joshi, L. C. Schramm, in 'Alkaloids: Chemical and Biological Perspectives', Ed. S. W. Pelletier, J. Wiley & Sons, New York, 1984, Vol. 2, pp. 205 – 462.
[3] Yunnan Medicinal Material Company, 'Index Chinese Medicines Resources Yunnanensis', Science Press, Beijing, 1993, p. 352.
[4] J.-H. Yang, Z.-Y. Li, L. Li, Y.-S. Wang, *Phytochemistry* **1999**, *50*, 345.
[5] L. Li, J. Zhao, Y. B. Wang, H. B. Zhang, *Helv. Chim. Acta* **2004**, *87*, 866.
[6] Z. Y. Li, J. Zhao, J.-H. Yang, H. B. Zhang, L. Li, *Helv. Chim. Acta* **2004**, *87*, 2085.
[7] Y.-B. Wang, R. Huang, H.-B. Zhang, L. Li, *Helv. Chim. Acta* **2005**, *88*, 1081.
[8] S. Yang, X.-D. Yang, J.-F. Zhao, H.-B. Zhang, L. Li, *Helv. Chim. Acta* **2007**, *90*, 1160.
[9] X.-D. Yang, S. Yang, J. Yang, J.-F. Zhao, H.-B. Zhang, L. Li, *Helv. Chim. Acta* **2008**, *91*, 569.
[10] S. W. Pelletier, S. A. Ross, J. T. Etse, *Heterocycles* **1988**, *27*, 2467.
[11] D. L. Chen, X. X. Jian, F. P. Wang, *West China J. Pharm. Sci.* **2002**, *17*, 326.
[12] F. Wang, Q. Fang, *Planta Med.* **1981**, *42*, 375.
[13] S. Y. Chen, *Acta Chim. Sin.* **1979**, *37*, 15.
[14] C. Konno, M. Shirasaka, H. Hikino, *J. Nat. Prod.* **1982**, *45*, 128.
[15] S. W. Pelletier, Z. Djarmati, *J. Am. Chem. Soc.* **1976**, *98*, 2626.
[16] J. B. Hanuman, A. Katz, *Phytochemistry* **1994**, *36*, 1527.
[17] S. W. Pelletier, N. V. Mody, A. P. Venkov, S. B. Jones Jr., *Heterocycles* **1979**, *12*, 779.
[18] D. Csupor, P. Forgo, I. Máthé, J. Hohmann, *Helv. Chim. Acta* **2004**, *87*, 2125.

Received August 7, 2008