23. The Absolute Configuration of 3-Methylpyrrolidine Alkaloids from Poison Glands of Ants Leptothoracini (Myrmicinae)

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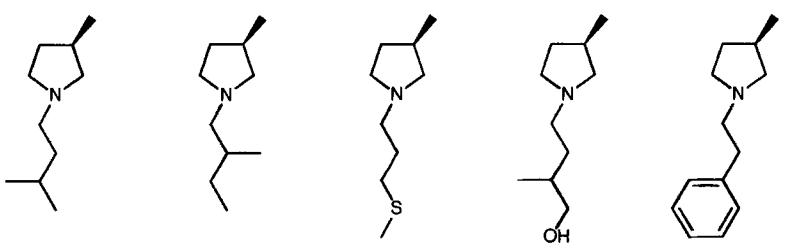
The absolute configuration of 3-methylpyrrolidine alkaloids isolated from the poison gland of ants *Leptothoracini* was determined as (3*R*). The enantiomeric separation by chiral gas chromatography and unambiguous structural assignment of the target compounds are described.

1. Introduction. – Recently, we reported the isolation and identification of the novel 3-methylpyrrolidine alkaloids **1**–**5** as constituents of the poison gland secretion in several species of ants *Leptothoracini* (Myrmicinae) [1]. The content of the poison gland is used for intraspecific signalling, and the basic components of the poison gland secretion of females display a male-attracting behavior [2]. The absolute configuration of natural **1**–**5** remained unknown to date.



1 **2** **3** **4** **5**

The development of modified cyclodextrin columns for gas chromatography (GC) allows separation and determination of enantiomers with unprecedented sensitivity and accuracy [3]. We used this method for determination of the absolute configuration, since purification from the biological extract by column chromatography or crystallization is impossible, due to the low amounts (only some nanograms) available.

2. Results and Discussion. – For the enantiomeric separation of amines, several procedures can be applied [3]. *N*-Trifluoroacetyl (TFA) derivatives of chiral amines are usually separated using chiral phases. Tertiary amines are far more difficult to resolve, only some examples have been reported for the enantiomeric separation of tertiary amines, *e.g.* indolizidines [4]. The enantiomers of the main alkaloid **1**, leptothoracine, are

not separated on chiral columns. However, separation studies on 3-methyl-substituted pyrrolidines revealed that 3-methyl-1-(trifluoroacetyl)pyrrolidine (**8**) was better resolved than 3-methylpyrrolidin-1-ol (**7**) on chiral stationary phases (*Fig. 1*).

To obtain the degradation products of **1**, we applied the *Cope* elimination [5] on the 1-oxide **6** which was synthesized by oxidation of **1** with $MeCOO_2H$ [6] (*Scheme*)[1]). Thermal cleavage of **6** produced pentene and the hydroxylamine **7**, the heterocyclic ring was not cleaved. A separate reduction step became unnecessary, because treatment of **7** with the acetylating reagent methyl-bis(trifluoroacetamide) (MBTFA) directly gave the 1-trifluoroacetyl derivative **8** (*Scheme*). The mechanism of this reductive acetylating reaction is still unknown. Here, it should also be noted that all alkaloids **1**–**5** lead to the same derivative **8**.

Further investigation revealed that thermal cleavage of **6** and reductive acetylation with MBTFA could be performed in the GC injector (260°), starting with small amounts of **6**.

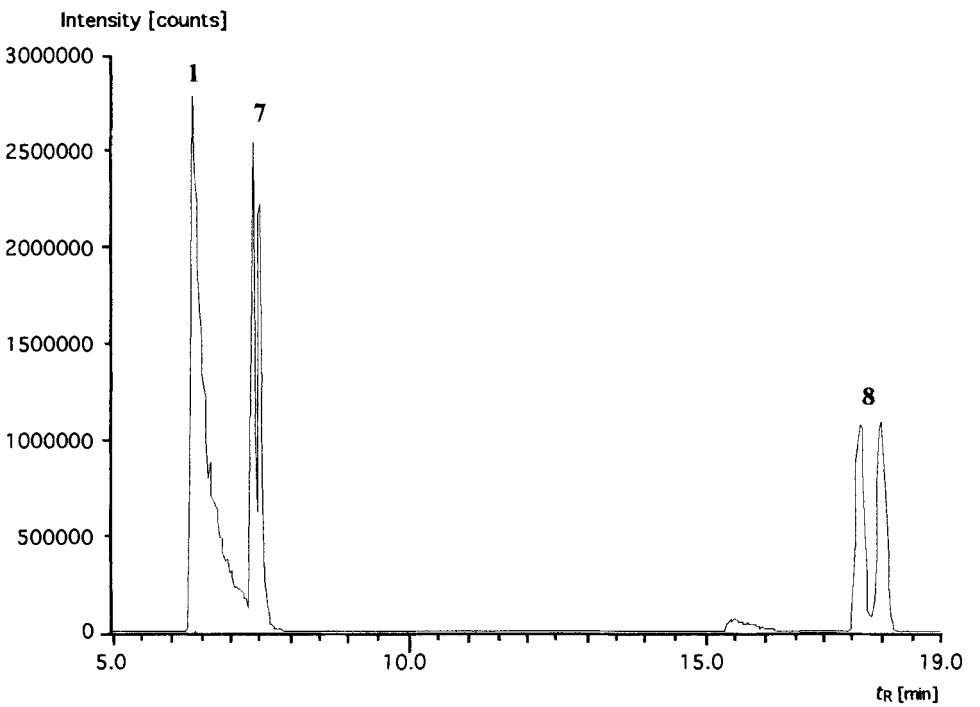
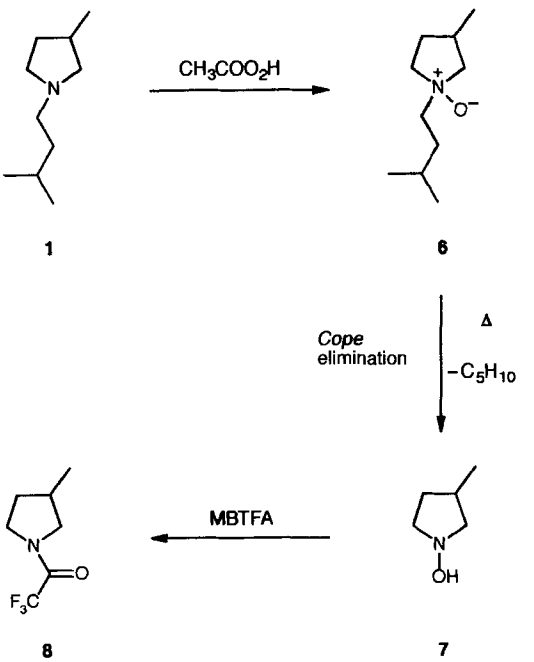


Fig. 1. *Gas-chromatogram of 3-methyl-*N*-(3-methylbutyl)pyrrolidine* (**1**), N*-hydroxy-3-methylpyrrolidine* (**7**), *3-methyl-*N*-trifluoroacetylpyrrolidine* (**8**) *on chiral cyclodexdrine GC column* Lipodex D

[1]) It should be noted that the oxidation created a second chiral center, and the *cis*- and *trans*-diastereoisomers of **6** can be separated by column FC on aluminium oxide. This procedure of diastereoisomeric separation could be a methodology for the determination of the sense of chirality of amines, if a method for stereoselective *N*-oxidation would be available.

Scheme



The determination of the sense of chirality of the natural component **1** was accomplished through comparison of the t_R values with those of the corresponding derivatives (*R*)-**8** and (*S*)-**8** of the synthetic enantiomers (*R*)-**1** and (*S*)-**1** [7] (*Fig. 2*), respectively.

For our investigation of the natural alkaloids **1**–**5**, we used two samples of *Leptothorax acervorum* and one sample of *Harpagoxenus sublaevis*. Since the samples were prepared of 20 poison glands, containing an estimated amount of 200–400 ng of the main component **1**, we developed a method for the transformation of such small amounts into **8**. Synthetic **1** (120 ng) was converted into the 1-oxide **6** with $MeCOO_2H$, followed by extraction of **6** using *Extrelut*® *1* as column material. After evaporating of the solvent, the residue was injected on-line in the GC with MBTFA to obtain **8**. The biological samples and synthetic enantiomers (*R*)-**1** and (*S*)-**1** were treated in the same way. In all GC/MS analysis, the comparison of the t_R values showed that the natural alkaloids **1**–**5** consist of their (3*R*)-enantiomers (*Fig. 2*).

3. Conclusion. – We established the absolute configuration of the novel 3-methylpyrrolidine alkaloids **1**–**5** from poison glands of the ants *Leptothoracini* as the (3*R*). Enantiomeric separation was achieved on chiral stationary phases as its 1-trifluoroacetyl derivatives **8**.

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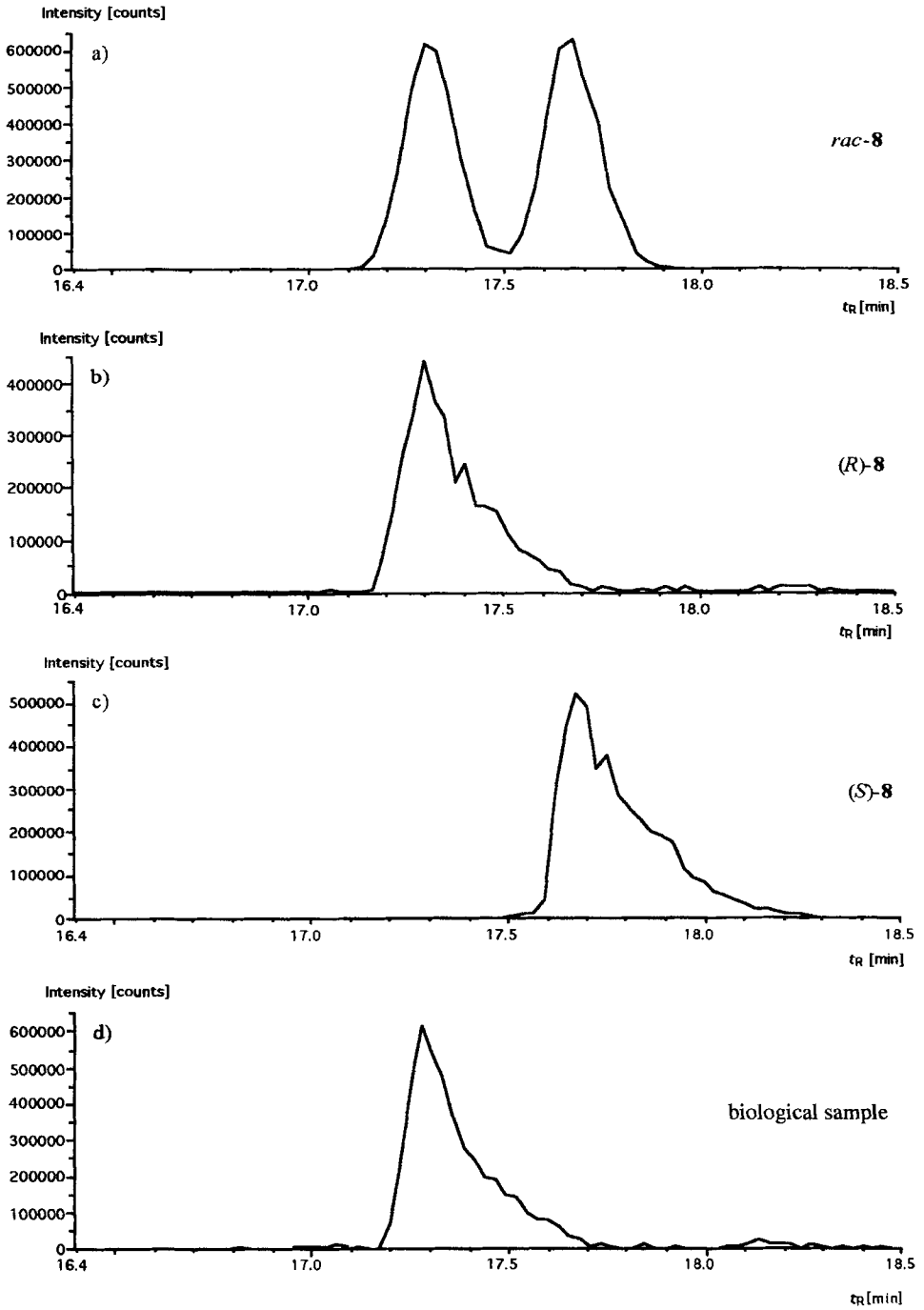

Fig. 2. *Enantiomeric separation of the derivatives* **8** *starting from* a) (rac)-**1**, b) *synthetic* (R)-**1**, c) *synthetic* (S)-**1**, *and* d) *biological sample on a* Lipodex D *GC column*

Experimental Part

1. *General.* B.p. of compounds obtained in small-scale experiments refer to the temp. in a *Büchi Kugelrohr* oven. All reactions were performed in flame-dried reaction vessels under a slight pressure of N_2. Solvents were dried by standard methods. All other commercially available reagents were used without further purification. ^{1}H-NMR: *Bruker ARX-300* (300 MHz), δ in ppm relative to Me_4Si, J in Hz. ^{13}C-NMR: *Bruker ARX-300* (75 MHz), δ in ppm relative to Me_4Si, J in Hz. EI-MS (70 eV): *Finnigan MAT 311 A*; in *m/z* (rel. intensities). GC/MS: *Varian 3700* (GC) coupled directly to a *Finnigan MAT 212* (MS (EI, 70 eV)). For data acquisition, a *Teknivent* data system (*Vector 2*) was used. For identification, the samples were introduced on a fused-silica chiral *Lipodex D*, 25 m × 0.25 mm ID (*Macherey-Nagel*) column; column oven temp.: 1 min at 50°, programmed to 130° at 10°/min; injector temp. 260°; split 60 ml/min; carrier gas He, column pressure 1.2 bar. Retention indices of **1**–**5** on non-chiral columns [1], synthesis of (*R*)-**1** and (*S*)-**1** [7], *rac*-**1** [1], 1,4-dibromo-2-methylbutane [8], and 3-methyl pyrrolidine [1] have already been published.

3-Methylpyrrolidine-1-ol (**7**): Hydroxyammonium chloride (5.4 g, 78.2 mmol) and Na_2CO_3 (8.3 g, 78.2 mmol) were added to a soln. of 1,4-dibromo-2-methylbutane (9.0 g, 39.1 mmol) in EtOH (500 ml). After refluxing for 12 h, the suspension was filtered and the filtrate evaporated. The residue was taken up in CH_2Cl_2 (100 ml), dried ($MgSO_4$), evaporated, and distilled (60°, 30 mbar): **7** (1.5 g, 14.8 mmol; 38%). ^{1}H-NMR (CD_2Cl_2, 80°): 3.21–3.15 (*dd*, J = 7.5, 9, H–C(2)); 3.04–2.99 (*m*, 2 H–C(5)); 2.56–2.49 (*dd*, J = 7.5, 9, H–C(2)); 2.34–2.22 (*m*, H–C(3)); 2.09–1.97 (*m*, H–C(4)); 1.41–1.30 (*m*, H–C(4)); 1.04 (*d*, J = 7, Me–C(3)). ^{13}C-NMR (CD_2Cl_2, 80°): 66.21 (*t*, C(2)); 58.65 (*t*, C(5)); 31.13 (*t*, C(4)); 30.49 (*d*, C(3)); 20.12 (*q*, Me–C(3)). EI-MS: 101 (47, $M^{+\cdot}$), 100 (32), 59 (100), 56 (23), 55 (18), 42 (63). GC/MS: t_R 7.40 and 7.53 min.

3-Methyl-1-(trifluoroacetyl)pyrrolidine (**8**): To a soln. of 3-methylpyrrolidine (100 mg, 1.176 mmol) in CH_2Cl_2 (2 ml), MBTFA (258 mg, 1.176 mmol) was added. After stirring for 2 h at r.t., the mixture was extracted with 2N NaOH (2 ml), the org. phase dried ($MgSO_4$) and evaporated. Bulb-to-bulb distillation (70°, 28 mbar) yielded **8** (140 mg, 0.774 mmol; 66%). Colorless oil. ^{1}H-NMR ($CDCl_3$): 3.68–3.84 (*m*, H–C(2), H–C(5)); 3.45–3.64 (*m*, H–C(5)); 3.03–3.17 (*m*, H–C(2)); 2.22–2.46 (*m*, H–C(3)); 2.02–2.21 (*m*, H–C(4)); 1.46–1.71 (*m*, H–C(4)); 1.11, 1.10 (2*d*, J = 7, Me–C(3)). ^{13}C-NMR ($CDCl_3$): 196.08 (C=O); 118.36, 114.56 (CF_3); 54.17, 53.40 (*t*, C(2)); 47.27, 46.39 (*t*, C(5)); 34.24, 31.42 (*d*, C(3)); 33.96, 31.32 (*t*, C(4)); 17.40, 17.15 (*q*, Me–C(3)). EI-MS: 181 (40, $M^{+\cdot}$), 166 (14), 139 (30), 112 (56), 84 (21), 70 (20), 69 (90), 67 (17), 56 (30), 42 (91), 41 (100). GC/MS: t_R 17.30 and 17.68 min.

Compound (**8**) *in a GC/MS Experiment Starting with Pyrrolidine-1-ol* **7**. A soln. (1 µl) of **7** in CH_2Cl_2 (1 µg/µl) was injected on-line with MBTFA (0.2 µl) in a GC/MS experiment. Only compound **8** was observed. GC/MS: t_R 17.30 and 17.68 min.

3-Methyl-1-(3-methylbutyl)pyrrolidine 1-Oxide (**6**): *3-Methyl-1-(3-methylbutyl)pyrrolidine* (**1**; 2.0 g, 13.0 mmol) was cooled to 0°, and $MeCOO_2H$ (32% in 2N AcOH, 10 ml) was added. After stirring for 12 h at r.t. 10N NaOH was added until pH ≥ 9. The mixture was extracted with CH_2Cl_2 (3 × 100 ml). Drying of the org. layer ($MgSO_4$) and evaporation gave **6** (1.60 g, 9.3 mmol; 72%). White, strongly hygroscopic solid. Multiple signals in the NMR spectra for the same ^{1}H- and ^{13}C-atoms are due to the diastereoisomeric **6** (*cis* and *trans*, *O*–N to Me–C(3)). ^{1}H-NMR ($CDCl_3$): 3.69–3.36 (*m*, H–C(2), *cis*, *trans*; H–C(5), *trans*; H–C(5), *cis*, *trans*); 3.32–3.15 (*m*, H–C(2), *cis*; H–C(5), *cis*; H–C(1′), *cis*, *trans*); 3.13–2.99 (*m*, H–C(3), *trans*); 2.83–2.76 (*m*, H–C(2), *trans*); 2.73–2.60 (*m*, H–C(4), *trans*); 2.51–2.35 (*m*, H–C(3), *cis*); 2.10–2.33 (*m*, H–C(4), *cis*); 1.95–1.81 (*m*, H–C(2′), *cis*, *trans*); 1.72–1.59 (*m*, H–C(3′), *cis*, *trans*); 1.58–1.46 (*m*, H–C(4), *trans*); 1.30 (*d*, J = 7, Me–C(3), *cis*); 1.11 (*d*, J=7, Me–C(3), *trans*); 0.95 (*d*, J = 7, Me_2–C(3′), *cis*, *trans*). ^{13}C-NMR ($CDCl_3$): 75.78, 75.37 (*t*, C(2), *cis*, *trans*); 69.56, 68.65 (*t*, C(5), *cis*, *trans*); 67.91, 67.72 (*t*, C(1′), *cis*, *trans*); 32.89, 32.69 (*t*, C(2′), *cis*, *trans*); 31.60 (*d*, C(3), *cis*); 31.46 (*t*, C(4), *cis*); 30.78 (*t*, C(4), *trans*); 30.56 (*d*, C(3), *trans*); 26.76 (*d*, C(3′), *cis*, *trans*); 22.93 (*q*, Me–C(3′), *cis*, *trans*); 21.07 (*q*, Me–C(3), *cis*); 18.91 (*q*, Me–C(3), *trans*).

Compound **7** *in a GC/MS Experiment.* After injection of 1 µl of a soln. of 3-methyl-1-(3-methylbutyl)pyrrolidine 1-oxide (**6**) in CH_2Cl_2 (1 µg/µl) only **7** was registrated. GC/MS: t_R 7.40 and 7.53 min.

Compound **8** *in a GC/MS Experiment Starting with* **6**. A soln. (1 µl) of **6** was injected on-line with MBTFA (0.2 µl). Only compound **8** was registered. GC/MS: t_R 17.30 and 17.68 min.

Compound **8** *for GC/MS Analysis on a ng Scale.* A soln. (1 µl) of **1** (120 ng/µl) was dropped into a conical vessel. After evaporation of the solvent, $MeCOO_2H$ (32% in 2N AcOH, 2 µl) was added, and the vessel was placed for 30 min, in an ultrasonic bath. For destroying the unreacted $MeCOO_2H$, 20 µl of a soln. of 2N NaOAc, adjusted to pH 8–9 with 2N NaOH, was added. Further 2N NaOH was added dropwise to maintain pH 8–9, until no bubbles were observed. The soln. was placed on a *Extrelut® 1* (30 mg) column. After 10 min, the column was eluted slowly with 200 µl and then fast with 150 µl of CH_2Cl_2. The org. soln. was evaporated, and the 2-µl residue was injected on-line with 0.2 µl of MBTFA. GC/MS: Signals for the enantiomers **8** at t_R 17.30 and 17.68 min.

*Compound (R)-**8** for GC/MS Analysis on a ng Scale.* Compound (*R*)-**1** was treated similary to **8** for GC/MS analysis on a ng scale. GC/MS: Signal for (*R*)-**8** at t_R 17.30 min.

*Compound (S)-**8** for GC/MS Analysis on a ng Scale.* Compound (*S*)-**1** was treated similary to **8** for GC/MS analysis on a ng scale. GC/MS: Signal for (*S*)-**8** at t_R 17.68 min.

Preparation of the Biological Samples for GC/MS Analysis. The biological samples of 20 prepared poison glands were treated similarly to **8** for GC/MS analysis in ng scale. GC/MS: Signal for **8** at t_R 17.28 min.

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