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ABSOLUTE CONFIGURATION OF ANABASINE FROM *Messor* AND *Aphaenogaster* ANTS

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Abstract—A method has been developed to assign the absolute configuration and enantiomeric excess of anabasine based on small amounts of material (in the microgram range), by derivatization with (+)-menthylchloroformate followed by capillary GC analysis of the resulting carbamate(s). This method was applied to three samples of anabasine isolated from *Messor* and *Aphaenogaster* ants. In *Messor sanctus*, only (2′*S*)-anabasine was present, whereas in *Aphaenogaster subterranea* and *A. miamiana* (2′*S*)-anabasine was determined to have an ee of 78 and 24%, respectively.

Key Words—Alkaloids, anabasine, ants, *Messor*, *Aphaenogaster*, absolute configuration.

INTRODUCTION

The alkaloid anabasine (**1**) has been isolated from several plant genera, such as *Anabasis*, *Nicotiana*, *Duboisia*, *Malacocarpus*, and *Marsdenia* (Leete, 1983). (−)-Anabasine ($[\alpha]_D^{20}$ −82.2, neat) from *Anabasis aphylla* L. was shown to have the (2′*S*) absolute configuration (Lukes et al., 1962) (Figure 1), but values of the optical rotation of anabasine from different sources vary considerably (from −82.2 to 0) (Leete, 1983). More recently, anabasine has also been reported from animal sources, e.g., in two of the 19 species of hoplonemertine worms surveyed

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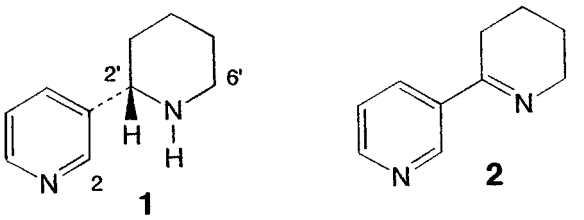


FIG. 1. Structures of (2′*S*)-(−)-anabasine [(2′*S*)-**1**] and anabaseine (**2**).

(Kem, 1988), in the poison gland of the ants *Messor ebeninus* (Coll et al., 1987) and *M. bouvieri* (Jackson et al., 1989), and as a minor component in *M. capensis* (Brand and Mpuru, 1993) and *Aphaenogaster rudis*, where it coexists with anabaseine (**2**) (Figure 1) (Attygalle et al., 1998).

From a biosynthetic point of view, it would be interesting to compare the absolute configuration of anabasine (**1**) isolated from plants and from animals (worms and ants). To this end, an effective and practical procedure to assign the absolute configuration of this alkaloid and applicable to quantities in the microgram range was needed. Although the separation of the enantiomers of anabasine as their trifluoroacetyl derivatives on a dipentyl β-cyclodextrin GC column has been reported (Armstrong et al., 1990), this method is not very convenient since this column is not commercially available. Our attempts to separate the anabasine enantiomers on two commercial chiral columns (CP-Chirasil Val and CP-Chirasil-Dex CB) were unsuccessful as the alkaloids did not elute from these columns at the temperature limit. In this paper, we have developed a method based on the reaction of (±)-anabasine with (+)-menthylchloroformate, affording the corresponding menthoxycarbonyl derivatives, followed by comparison of their retention time in capillary gas chromatography with that of a standard of known absolute configuration. This procedure allowed us to assign the absolute configuration and to measure the ee of anabasine (**1**), which was detected in three ant species: *Messor sanctus*, *Aphaenogaster subterranea*, and *A. miamiana* (Myrmicinae: Myrmicini).

METHODS AND MATERIALS

Sample Collection. Colonies of *Messor barbarus* and *M. sanctus* were collected at Port Leucate (southern France). Colonies of *Messor structor* were collected in the area of Toulouse (southern France). Colonies of *Aphaenogaster subterranea* were collected in Bouconne forest (Midi-Pyrénées), whereas *Aphaenogaster senilis* were collected at Consprons (Banyuls-sur-Mer, southern France). Colonies of *Aphaenogaster miamiana* were collected near Gainesville, Florida.

Analyses of Ants for Alkaloids. Ants were separated manually from the soil and dipped into MeOH. Five hundred ants of each species were then exhaustively

extracted with $CHCl_3$–MeOH (1 : 1). The extract was evaporated under vacuum and submitted to filtration on an alumina column, first eluting with CH_2Cl_2 (10 ml) to remove nonpolar compounds, then with CH_2Cl_2–MeOH (1 : 1; 20 ml) to yield a polar fraction which was analyzed by TLC and GC-MS for the presence of alkaloids.

Chemical Analyses. UV spectra were taken on a Philips PU 8700 UV-VIS spectrophotometer in MeOH. IR spectra were recorded on a Bruker IFS 25 instrument as a film on a NaCl disk. NMR spectra were recorded in $CDCl_3$ at 600 and 150.87 MHz (Varian Unity 600 instrument) or at 250 MHz (Bruker WM 250). Chemical shifts (δ) are reported in parts per million from internal TMS, and the coupling constants are given in hertz. Mass spectra were performed on a Fisons VG Autospec mass spectrometer in the electron impact (70 eV) mode. Optical rotations were measured on a Perkin Elmer 141 polarimeter (Na vapor lamp) in a 10-cm cell at room temperature. Flash liquid chromatography was performed over Macherey-Nagel Si gel (0.04–0.063 mm) and thin-layer chromatography analyses (TLC) on Polygram SilG/UV_{254} precoated plates (0.25 mm). The compounds were visualized under UV_{254} light, and/or by spraying with Dragendorff reagent. GC-MS analyses were carried out on a Fisons VG Autospec mass spectrometer in the electron impact (70 eV) mode, coupled to a Carlo Erba GC 8065 gas chromatograph equipped with a split injector, and with a 25-m × 0.25-mm CP-Sil 5CB fused silica column (Chrompack). The conditions were: 1 min at 150°C, then increased 10°C/min to 320°C, then maintained 2 min at 320°C. Carrier gas was helium, and injector and detector temperatures were 250°C. GC analyses were performed on a Varian gas chromatograph equipped with a 25-m × 0.53-mm CP-Sil 19CB fused silica column (Chrompack). The carrier gas was nitrogen, and oven and injector temperatures were maintained at 250°C and the detector temperature at 270°C.

*Derivatization of (±)-Anabasine with (+)-(1S,2R,5S)-Menthylchloroformate [(+)−**3**]*. To (±)-anabasine (1.0 mg, 0.0062 mmol) in a 300-μl Reacti-vial was added anhydrous THF (150 μl), NEt_3 (5 μl, 0.036 mmol) and finally (+)-**3** (4.0 μl, 0.018 mmol). After stirring at 60°C under argon for 2 hr, the reaction mixture was poured into 10% aqueous NaOH (2 ml), and the aqueous phase extracted with diethyl ether (3 × 1 ml). Drying and concentration of the combined organic extracts *in vacuo* afforded an oily residue, which was purified by flash chromatography on silica gel (CH_2Cl_2, then CH_2Cl_2–MeOH 9 : 1) to quantitatively afford a mixture of (2′*R*)-**4** and (2′*S*)-**4** (2.1 mg, 0.0061 mmol). They were separated by reverse-phase HPLC (Chromspher C_{18}, 5 μm, 10 × 250 mm, CH_3CN–H_2O, 65 : 35, flow: 5 ml/min).

(2′R)- [(1S,2R,5S)-Menthoxycarbonyl]anabasine [(2′*R*)−***4***]. $[\alpha]_D^{20}$ +110 (c 0.135, CH_2Cl_2); EI-MS (70 eV) m/z 344 ($M^{+\cdot}$, $C_{21}H_{32}N_2O_2$, 6), 216 (10), 207 (100), 206 (66), 189 (22), 162 (38), 138 (7), 133 (14), 128 (7), 92 (21), 83 (53), 69 (26), 55 (38); IR (film) 2928–2866, 1694, 1422, 1265, 1169, 1037, 810-714 cm^{-1}; UV (MeOH) λ_{max} (ε) 203 (6800), 262 (1900); GC: t_r = 13.0 min; ^{1}H NMR

(600 MHz, $CDCl_3$) δ 8.52 (m, 2 H, H-2, H-6), 7.57 (d, 8.0 Hz, 1 H, H-4), 7.32 (m, 1 H, H-5), 5.51 (bs, 1H, H-2′), 4.65 (td, $J = 11.0, 4.0$, 1 H, H-8′), 4.12 (bd, $J = 13.0$, 1 H, H-6′eq), 2.79 (td, $J = 14.0, 5.0$, 1 H, H-6′ax), 2.28 (bd, $J = 14.0$, 1 H, H-3′eq), 2.12 (bd, $J = 11.0$, 1 H, H-13′eq), 1.96 (tt, $J = 13.8, 4.8$, 1 H, H-3′ax), 1.67 (m, 4 H, H-4′eq, H-10′eq, H-11′eq, H15′), 1.62 (m, 1 H, H-5′eq), 1.52 (m, 2 H, H-5′ax and H-12′ax), 1.43 (bq, $J = 13.0$, 1 H, H-4′ax), 1.30 (m, 1 H, H-9′ax), 1.04 (m, 1 H, H-10′ax), 0.98 (q, $J = 11.8$, 1 H, H-13′ax), 0.91 (d, $J = 6.7$, 3 H, CH_3-14′), 0.86 (m, 1H, H-11′ax), 0.78 (d, $J = 5.0$, 3 H, CH_3-16′ or 17′), 0.73 (d, $J = 6.3$, 3 H, CH_3-16′ or 17′).

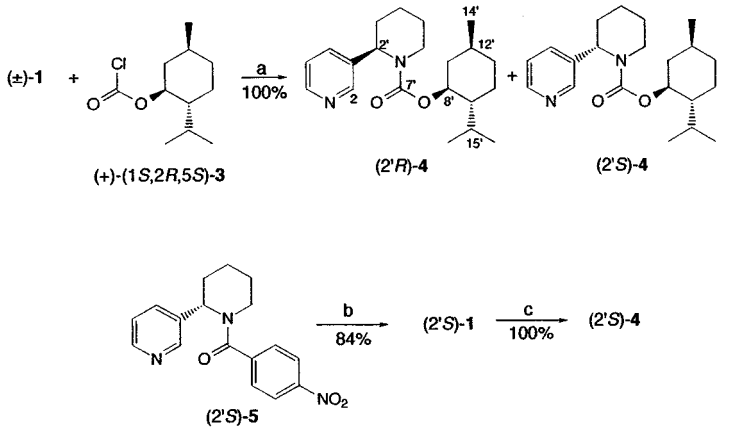
*(2′S)-[(1S,2R,5S)-Menthoxycarbonyl]anabasine [(2′S)-**4**].* $[\alpha]_D^{20}$ −100 (c 0.485, CH_2Cl_2); EI-MS (70 eV) m/z 344 ($M^{+\cdot}$, $C_{21}H_{32}N_2O_2$, 6), 216 (10), 207 (100), 206 (66), 189 (22), 162 (38), 138 (7), 133 (14), 128 (7), 92 (21), 83 (53), 69 (26), 55 (38); IR (film) 2956–2872, 1699, 1410, 1261, 1038, 963, 815-712 cm^{-1}; UV (MeOH) λ_{max} (ε) 203 (4600), 262 (1800); GC: $t_r = 13.5$ min; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.54 (m, 2 H, H-2, H-6), 7.62 (d, $J = 8.0$, 1 H, H-4), 7.36 (dd, $J = 8.0, 4.0$, H-5), 5.53 (bs, $J = bd$, 1 H, H-2′), 4.61 (td, $J = 11.0, 4.0$, 1 H, H-8′), 4.13 (bd, $J = 14.0$, 1 H, H-6′eq), 2.79 (ddd, $J = 14.0, 11.0, 4.0$, 1 H, H-6′ax), 0.92 (d, $J = 6.7$, 3 H, CH_3-14′), 0.79 (d, $J = 6.9$, 3 H, CH_3-16′ or -17′), 0.73 (d, $J = 6.9$, 3 H, CH_3-16′ or 17′). The derivatization procedure described above was applied to the alkaloid fractions coming from *M. sanctus*, *A. subterranea*, and *A. miamiana*. The menthoxycarbamates thus obtained were analyzed by capillary GC and by GC-MS.

*Hydrolysis of (2′S)-Anabasine-p-NO_2-Benzamide [(2′S)-**5**] into (2′S)-Anabasine.* (2′*S*)-**5** (Pfrengle and Kunz, 1989) was kindly provided by Prof. H. Kunz (University of Mainz). (2′*S*)-**5**, $[\alpha]_D^{20}$ −130 (c 3, MeOH) (19.6 mg, 0.063 mmol) was dissolved in a mixture of 33% HBr–AcOH (0.6 ml) in a 1 ml Reacti-vial. The mixture was stirred at 80°C for 45 hr and was then poured into NH_4OH (8 M) and the aqueous phase quickly extracted with CH_2Cl_2. The combined organic extracts were concentrated *in vacuo* to afford an oily residue that was purified by flash chromatography on a silica gel column (CH_2Cl_2–MeOH 9 : 1) to afford (8.5 mg) (2′*S*)-anabasine in an 84% yield.

*(2′S)-Anabasine [(2′S)-**1**].* $[\alpha]_D^{20}$ −21 (c 0.5, MeOH); ^{1}H NMR data of *(2′S)*-**1** were identical to those of our synthetic material and to literature data (Yang and Tanner, 1997). Treatment of this sample with (+)-**3** as described above furnished (2′ S)-[(1S,2*R*,5*S*)-menthoxycarbonyl]anabasine [(2′S)-**4**], which gave a single peak at $t_r = 13.5$ min in capillary GC.

RESULTS

Development of a Procedure to Assign the Absolute Configuration of Anabasine. We first applied to a sample of (±)-anabasine [(±)-**1**], synthesized according



SCHEME 1. Derivatization of (±)-**1** with (+)-(1*S*,2*R*,5*S*)-menthylchloroformate, and synthesis of (2′*S*)-**4**. Reagents and conditions: (a) NEt_3, THF, 60°C, 2 hr; (b) 33% HBr–AcOH, 80°C, 45 hr; (c) (+)-(1*S*,2*R*,5*S*)-**3**, NEt_3, THF, 60°C, 2 hr.

to Deo and Crooks (1996), the procedure that was developed for the solenopsins (Leclercq et al., 1994), but we could not separate the resulting diastereomeric MTPA amides by capillary GC or by HPLC. In contrast, treatment of (±)-**1** with an excess of (+)-menthylchloroformate [(+)-**3**] quantitatively furnished the diastereomeric carbamates (2′*R*)-**4** and (2′*S*)-**4** (Scheme 1), which could be separated by reverse-phase HPLC on a C_{18} column. Moreover, their retention times in capillary GC on a CP-Sil 19CB column were also different (Figure 2). The two diastereomers were identified by GC comparison with a reference sample of (2′*S*)-**4**, which was prepared from (2′*S*)-anabasine-*p*-nitrobenzamide [(2′*S*)-**5**] (Pfrengle and Kunz, 1989) (Scheme 1). Under our GC conditions, the retention time of (2′*R*)-**4** was 13.0 min and that of (2′*S*)-**4**, 13.5 min (Figure 2). We have also assigned all the proton signals of (2′*R*)-**4** by a 2D NMR study at 600 MHz.

Detection of Anabasine and Anabaseine in Messor *and* Aphaenogaster *Ants*. Five hundred ants of each species were exhaustively extracted, and each extract was checked for the presence of anabasine (**1**) by TLC and GC-EI-MS. Compound **1** was present as the sole alkaloid in two of the six species, *Messor sanctus* and *Aphaenogaster miamiana*. In TLC, the alkaloidic fraction of *A. senilis* exhibited one major spot less polar than anabasine. GC-EI-MS analysis revealed the presence of one major peak identified as anabaseine (**2**) (Wheeler et al., 1981). No trace of anabasine was detected in this species. In contrast, the two compounds were present in the venom of *A. subterranea*, in an approximately 60 : 40 ratio. Finally, neither anabasine nor anabaseine could be detected in the venom of *M. barbarus* and *M. structor*. The relative amounts of anabasine (**1**) and anabaseine (**2**) identified in the venom of *Messor* and *Aphaenogaster* ants are reported in Table 1.

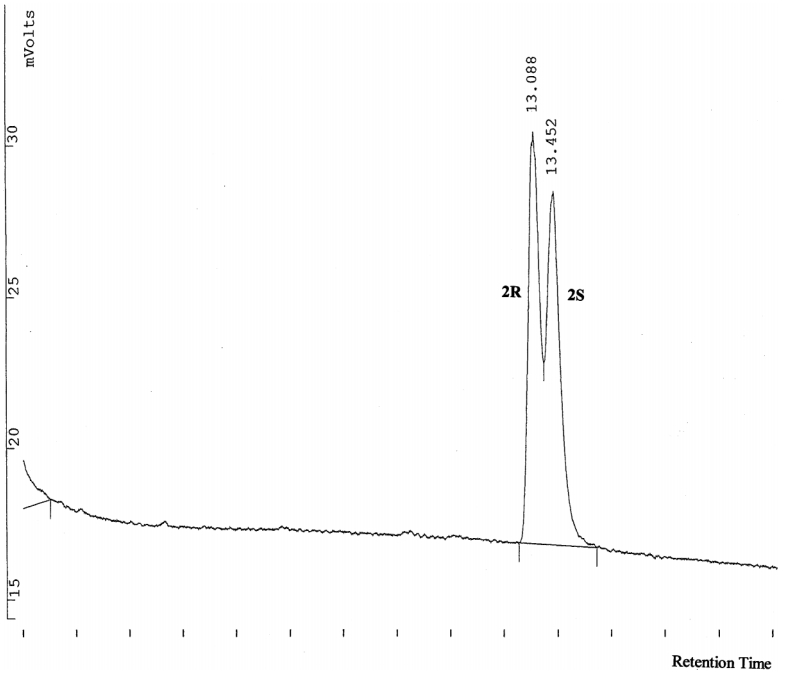


FIG. 2. GC (FID) separation of (2′*S*)-**4** and (2′*R*)-**4**.

Absolute Configuration and Enantiomeric Excess of Anabasine from Messor *and* Aphaenogaster *Ants*. The procedure described above was applied to determine the absolute configuration of anabasine isolated from the venom of *M. sanctus*, *A. subterranea*, and *A. miamiana*. In *M. sanctus*, only one diastereoisomer was present after derivatization, whose retention time corresponded to that of synthetic

TABLE 1. RELATIVE AMOUNTS OF **1** AND **2** IN VENOMS OF *Messor* AND *Aphaenogaster* ANTS

	Anabasine (**1**)	Anabaseine (**2**)
M. sanctus	100	0
M. barbarus	0	0
M. structor	0	0
A. subterranea	60[a]	40[a]
A. senilis	0	100
A. miamiana	100	0

[a] Approximate figures, due to peak superimposition.

(2′*S*)-**4**. Thus, anabasine is present in the venom of *M. sanctus* as a single enantiomer having the 2′*S* configuration.

In *A. subterranea* and *A. miamiana*, anabasine was present as a mixture of *R* and *S* enantiomers, with the *S* enantiomer predominating. Since we have checked that no kinetic resolution occurs during conversion of anabasine into diastereoisomers **4**, the enantiomeric excess of (*S*)-anabasine from *A. subterranea* and *A. miamiana* could be determined to be 78% and 24%, respectively, from the (2′*S*)-**4**/(2′*R*)-**4** ratio.

DISCUSSION

The data reported here on the ee of anabasine from ants are too limited to make any generalization. However, it should be pointed out that our results are similar to those obtained with plants, where anabasine either exists as a single enantiomer with the *S* configuration or as *S*/*R* mixtures, with the *S* enantiomer being generally predominant (Leete, 1983). Several hypotheses can be put forward to explain the wide range of enantiomeric purity observed for anabasine samples isolated from both plants and ants: (1) poor stereochemical control of the enzymatic reaction leading to the formation of the (2′*S*)-stereogenic carbon, (2) co-occurrence of two different biosynthetic routes for the (+) and (−) enantiomers, and (3) chemical or enzymatic isomerization of enantiomerically pure anabasine. The relative contribution of these different possibilities is not known yet. In any case, the detection of a single anabasine enantiomer in *M. sanctus* shows that our method of analysis does not lead to any racemization.

The presence of anabasine and anabaseine in the genus *Aphaenogaster* could be of taxonomic significance, since it supports the current classification of these ants with both genera being placed within the same tribe Pheidolini (Bolton, 1995); the genus *Messor* was even once considered as a subgenus of *Aphaenogaster* (Forel, 1890). However, it is striking that two of the three *Messor* species analyzed in this study do not contain alkaloids, at least in amounts sufficient to be detected by TLC and CG-MS.

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