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Gentrymine B, an *N*-Quaternary *Ancistrocladus* Alkaloid: Stereoanalysis, Synthesis, and Biomimetic Formation from Gentrymine A[1]

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Abstract—The total synthesis of the *N*-quaternary isoquinoline alkaloid gentrymine B (**1**) and of its unnatural enantiomer as well as its oxidative degradation is described. A further proof of stereostructure and hints at the biosynthetic origin of the unusual *S*,*S*-configuration in gentrymine B (**1**) were obtained by an additional biomimetic synthesis of **1** from the related—but 1*R*-configured—natural product gentrymine A (**2**), by *N*-methylation and subsequent spontaneous epimerization at C-1.

Introduction

Gentrymine B (**1**) from *Ancistrocladus korupensis*[2] is the only *N*-quaternary isoquinolinium alkaloid found in any of the Ancistrocladaceae (or the related Dioncophyllaceae) plants as yet investigated.[3] Moreover, none of the other 1,3-*trans*-configured mono- or dimeric naphthylisoquinoline alkaloids from that Cameroonian plant has 1*S*,3*S*-configuration, but they are all 1*R*,3*R*-configured, like, e.g. the antimalarial naphthylisoquinoline alkaloid korupensamine A (**3**)[4] or the heterodimeric, anti-HIV active michellamine B (**4**).[5] The absolute configuration of **1** was assigned by quantum chemical CD calculations and has been rationalized by a presumable biosynthetic origin of gentrymine B (**1**) from the related, but *cis*-configured tetrahydroisoquinoline gentrymine A (**2**) by *N*-methylation, followed by a postulated C-1 epimerization, thus changing its 1*R*,3*S*-configuration into 1*S*,3*S*.[2] In this paper, we describe the confirmation of the absolute stereostructure of **1** by total synthesis and by ruthenium-mediated oxidative degradation to give simple, easy-to-analyze amino acids. The biomimetic transformation of **2** into **1**, indeed with the anticipated spontaneous configurative inversion at C-1, makes the biosynthetic origin of **1** from **2** plausible, thus explaining the unusual 1*S*,3*S* stereochemical array (Fig. 1).

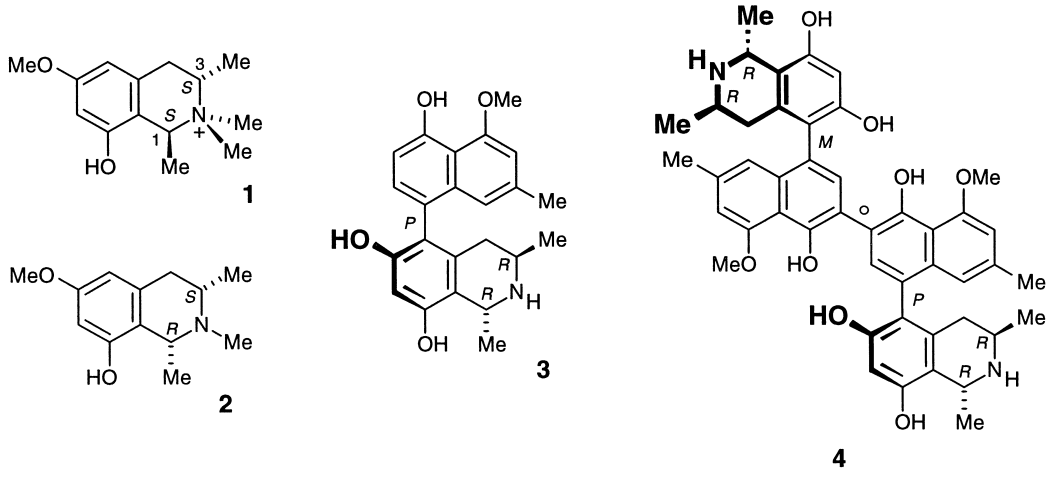

Figure 1. Isoquinoline alkaloids of ***Ancistrocladus korupensis.***

Keywords: gentrymine B; *Ancistrocladus korupensis*; biomimetic synthesis; isoquinoline alkaloids.

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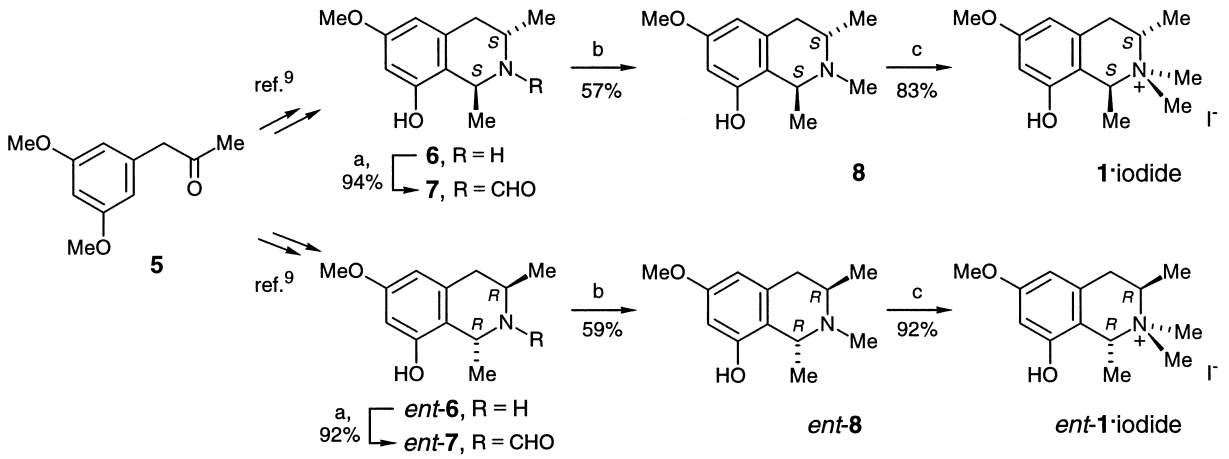


Scheme 1. Enantio-divergent synthesis of gentrymine B (**1**) and its enantiomer. Reagents and conditions: (a) pivalic–formic anhydride, CH_2Cl_2; (b) LAH, THF; (c) MeI, CH_2Cl_2.

Results and Discussion

Synthesis of gentrymine B and its enantiomer

As the starting material for the synthesis of gentrymine B (**1**), we chose the tetrahydroisoquinoline **6** previously prepared from the arylpropanone **5** in either enantiomeric form.[6] The close structural similarity of this synthetic building block with the target molecule **1** only required the introduction of two *N*-methyl groups, which, according to earlier experience,[7] should be performed *consecutively*, rather than by a direct, double *N*-methylation. Thus **6** was *N*-formylated with pivalic–formic anhydride to give **7** (Scheme 1), followed by LAH-reduction in THF to yield **8**. This *N*-tertiary amine was then further *N*-methylated, now with methyl iodide, to give gentrymine B (**1**), which crystallized as its iodide.[8] By the same synthetic procedure, the unnatural enantiomer of gentrymine B, *ent*-**1** was synthesized, now via the 1*R*,3*R* building block, *ent*-**6**, which is available from the same keto precursor **5**. All of the spectroscopic data of **1**·iodide and *ent*-**1**·iodide were found to be identical to those of the natural product, gentrymine B (**1**),[2] the optical rotations and the CD spectra of the synthetic products confirming the natural product to possess structure **1**, i.e. with 1*S*,3*S*-configuration.

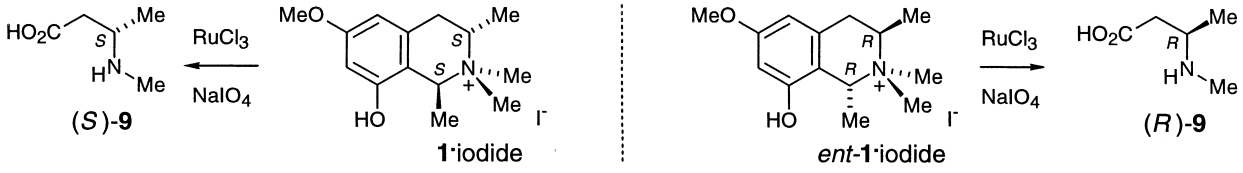
Degradative stereoanalysis of 1

Further support of the correct absolute configuration of gentrymine B was obtained from a ruthenium-mediated oxidative degradation according to a procedure originally developed for the stereoanalysis of *N*-secondary and *N*-tertiary tetrahydroisoquinolines,[9] which has recently been extended to an *N*-quaternary salt.[7] By application of the method now to **1** and *ent*-**1**, significant peaks for the Mosher derivatives of the corresponding stereoisomers of *N*-methyl-3-aminobutyric acid, (*S*)-**9** and (*R*)-**9**, respectively, were identified (see Scheme 2), thus confirming the stereochemical attributions deduced previously. In agreement with earlier observations,[9] the amino acid derivatives of alanine and *N*-methyl alanine were less qualified to prove the stereostructure.

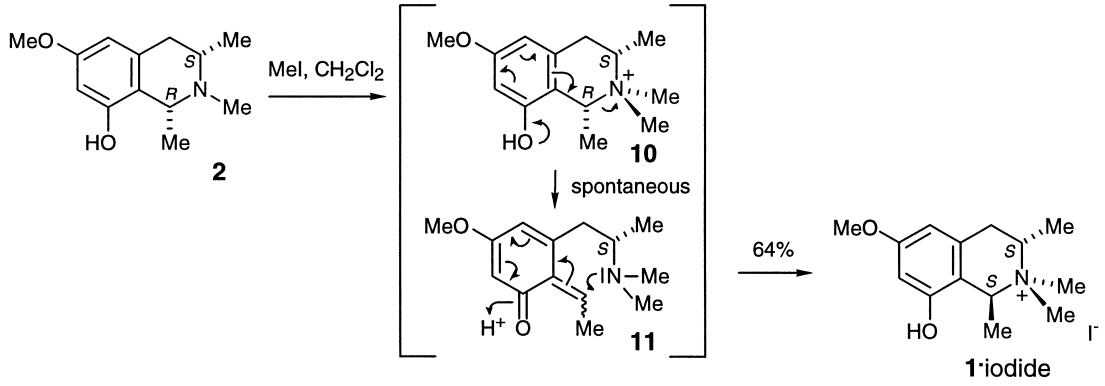
Biomimetic synthesis of gentrymine B (1) from gentrymine A (2)—with stereoinversion at C-1!

In an attempt to rationalize the unusual 1*S*,3*S*-configuration of gentrymine B (**1**), which is different from all the other *trans*-1,3-configured naphthylisoquinolines isolated from the same plant, a biomimetic synthesis of gentrymine B (**1**) from the likewise 3*S*-, but 1*R*- and thus *cis*-configured *N*-monomethylated analog of **1**, gentrymine A (**2**),[4] was desirable. Our biosynthetic concept, as formulated previously,[2] implies an enzymic second *N*-methylation of **2** to give the still *cis*-configured *N*-quaternary tetrahydroisoquinolinium salt **10**, which should then isomerize via the open-chain intermediate **11** to give the thermodynamically more stable[6] *trans*-diastereomer, with inversion of configuration at C-1. Indeed, treatment of natural gentrymine A (**2**)[4] with methyl iodide as for the second *N*-methylation of **8** described above, again gave authentic gentrymine B (**1**), in a smooth reaction, without any hints at the corresponding intermediate *cis*-configured primary product **10** (Scheme 3).[10] This efficient biomimetic partial synthesis convincingly illustrates that in *A. korupensis*, gentrymine B (**1**) might likewise originate from **2**, now, e.g. with *S*-adenosylmethionine as a biological *N*-methylating agent.

Similar C-1 isomerizations have been found to occur spontaneously, e.g. for the isoquinoline alkaloid lophocerine,



Scheme 2. Ruthenium-mediated oxidative degradation of **1**.



Scheme 3. Presumable course of the biomimetic transformation of gentrymine A (**2**) to gentrymine B iodide (**1**·iodide).

which probably racemizes during isolation workup.[11] In a more directed, enzymic way, the β-carboline vincoside is transformed into strictosidine, its C-1 epimer,[12] and the morphine precursor (*S*)-reticuline is biosynthetically isomerized into (*R*)-reticuline, here via the corresponding dihydroisoquinolinium salt.[13] The smooth spontaneous isomerization reaction at C-1, without leaving even a trace of the original *cis* array, might be one of possibly numerous examples of such direct configurational transformations whose occurrence in nature may not yet have been recognized.

Experimental

Melting points: Reichert-Jung Thermovar hot-plate, uncorrected. IR: Perkin–Elmer 1420 infrared spectrophotometer, reported in wave numbers (cm^{-1}). CD spectra: Jobin Yvon Dichrograph CD 6, Δ_ϵ in cm^2/mol at the given wavelength λ (nm). NMR: Bruker AC 200, Bruker WM 400, and Bruker DMX 600 spectrometers. Chemical shifts δ in parts per million (ppm) with the proton signals in the deuterated solvent as the internal reference for ^1H and ^{13}C NMR. Coupling constants, *J*, in Hertz. Mass spectra: Finnigan MAT 8200 and Finnigan MAT 90, 70 eV in the EI mode. Elemental analyses: Microanalytical Laboratory of the University of Würzburg, LECO CHNS-932. TLC: precoated silica gel 60 F_{254} plates (Merck). GC-MSD: Hewlett Packard 5890 Series II GC with a Hewlett Packard 5971A MSD. Transferline temperature maintained at 280°C, resulting in a source temperature of 180°C. GC-EIMS: ionizing energy of 70 eV. Selected ion monotoring (SIM) at mass numbers 102 and 116. Chromatographic separation of amino acid derivatives: nonpolar fused silica capillary column Hewlett Packard Ultra 2.25 m×0.32 mm (i.d.)×0.52 μm (film thickness), crosslinked 5% diphenyl and 95% dimethylpolysiloxane with an on-column injector maintained at 210°C. Carrier gas: He with a column head pressure of 40 kPa. Column temperature: from 100 to 160°C by 30°C min^{-1}, then raised to 190°C by 1°C min^{-1}, and finally increased to 270°C by 40°C min^{-1}. Retention times of the standards (*R*)-**9** and (*S*)-**9**: 18.73 and 18.94 min, respectively.

(1*S*,3*S*)-*N*-Formyl-8-hydroxy-6-methoxy-1,3-dimethyl-1,2,3,4-tetrahydroisoquinoline (7). A solution of 39.9 mg (193 μmol) **6**[6] and 28.0 μl (386 μmol) pivalic–formic anhydride[14] in 2 ml of CH_2Cl_2 was stirred at room temperature for 2 h. The solvent was removed in vacuo and the residue was crystallized from petroleum ether/CH_2Cl_2 to give **6** (42.9 mg, 94%) as colorless needles; mp 182°C; $[\alpha]_D^{25}=-75.6$ (*c*=0.30 in methanol); IR (KBr): $\tilde{\nu}$ 3300–2500 (OH), 2940 (C–H), 1620 (C=O), 1600 (C=C), 1370, 1340, 1180 (C–O), 1140 (C–O), 1051 (C–O), 830; ^1H NMR (200 MHz, CD_3OD), 2:1 mixture of interconverting rotational isomers with respect to the *N*-formyl bond, major isomer: δ=1.19 (d, *J*=6.6 Hz, 3H, 3-CH_3), 1.33 (d, *J*=6.5 Hz, 3H, 1-CH_3), 2.52–2.64 (m, 1H, 4-H_{ax}, overlapping), 3.02–3.17 (m, 1H, 4-H_{eq}, overlapping), 3.71 (s, 3H, OCH_3), 4.14 (m_c, 1H, 3-H), 5.42 (q, *J*=6.4 Hz, 1H, 1-H), 6.24–6.30 (m, 2H, 5-H, 7-H, overlapping), 8.24 (s, 1H, CHO); minor isomer: δ=0.93 (d, *J*=6.5 Hz, 3H, 3-CH_3), 1.39 (d, *J*=6.8 Hz, 3H, 1-CH_3), 2.52–2.64 (m, 1H, 4-H_{ax}, overlapping), 3.02–3.17 (m, 1H, 4-H_{eq}, overlapping), 3.73 (s, 3H, OCH_3), 4.50 (m_c, 1H, 3-H), 5.13 (q, *J*=6.7 Hz, 1H, 1-H), 6.24–6.30 (m, 2H, 5-H, 7-H, overlapping), 8.34 (s, 1H, CHO); ^{13}C NMR (50 MHz, CD_3OD), major isomer: δ=20.53 (1-CH_3 or 3-CH_3), 21.60 (1-CH_3 or 3-CH_3), 37.22 (C-4), 45.44 (C-3), 49.70 (C-1), 55.60 (OCH_3), 100.75 (C-7), 106.13 (C-5), 117.98 (C-9), 136.28 (C-10), 155.60 (C-8), 160.82 (C-6), 163.45 (CHO); minor isomer: δ=19.23 (1-CH_3 or 3-CH_3), 25.46 (1-CH_3 or 3-CH_3), 35.19 (C-4), 47.08 (C-3), 48.43 (C-1), 55.64 (OCH_3), 100.66 (C-7), 106.72 (C-5), 117.22 (C-9), 136.84 (C-10), 155.06 (C-8), 161.14 (C-6), 165.27 (CHO); MS: *m/z* (%)=235 (15) [M^+], 220 (100) [M^+-CH_3], 192 (32) [M^+-H_2O]; Anal. calcd for $C_{13}H_{17}NO_3$ (235.3): C, 66.36; H, 7.28; N, 5.95. Found: C, 65.99; H, 7.42; N, 5.68.

(1*R*,3*R*)-*N*-Formyl-8-hydroxy-6-methoxy-1,3-dimethyl-1,2,3,4-tetrahydroisoquinoline (*ent*-7). The preparation was performed from *ent*-**6**[6] in analogy to the synthesis of **7** (92%); mp 186°C; $[\alpha]_D^{28}=+67.0$ (*c*=0.92 in methanol); spectroscopic data (^1H, ^{13}C NMR, MS) identical to those of **7**.

(1*S*,3*S*)-8-Hydroxy-6-methoxy-*N*-methyl-1,3-dimethyl-1,2,3,4-tetrahydroisoquinoline (8). To a solution of 45.4 mg (193 μmol) of **7** in 2 ml of dry THF, LAH (29.3 mg, 773 μmol) was added at 0°C under argon and the reaction mixture was stirred for 1 h at room temperature. The suspension was hydrolyzed with 100 μl of water, adjusted to pH 10–11 with 2 N HCl, and extracted several times with CH_2Cl_2/ethyl acetate (9:1). After evaporation of the organic solvent, filtration on silica gel (7.5% NH_3) with CH_2Cl_2/

methanol (9:1) as the eluent yielded **8** as an amorphous yellow powder (27.0 mg, 57%); $[\alpha]_D^{25}=-5.6$ (c=0.30 in methanol); IR (KBr): $\tilde{\nu}$ 3000–2800 (OH), 2950 (C–H), 1600 (C=C), 1570 (C=C), 1420, 1320, 1300, 1200 (C–O), 1150 (C–O), 1130 (C–O); ^{1}H NMR (200 MHz, $CDCl_3$): δ=1.23 (d, J=6.6 Hz, 3H, 3-CH_3), 1.37 (d, J=6.7 Hz, 3H, 1-CH_3), 2.37 (s, 1H, NCH_3), 2.59 (s, 1H, 4-H), 2.63 (s, 1H, 4-H), 3.71 (s, 3H, OCH_3), 3.30 (mc, 1H, 3-H), 4.06 (q, J=6.3 Hz, 1H, 1-H), 6.16 (d, J=2.4 Hz, 1H, 5-H or 7-H), 6.18 (d, J=2.3 Hz, 1H, 5-H or 7-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=17.30 (1-CH_3 or 3-CH_3), 19.11 (1-CH_3 or 3-CH_3), 33.49 (C-4), 36.19 (C-3), 46.62 (C-1), 55.22 (OCH_3), 55.31 (NCH_3), 100.17 (C-7), 105.40 (C-5), 118.41 (C-9), 135.98 (C-10), 153.77 (C-8), 158.68 (C-6); MS: m/z (%)=221 (1) [M^+], 206 (100) [M^+-CH3], 190 (9) [M^+-OCH_3], 177 (9); Anal. calcd for $C_{13}H_{19}NO_2$ (221.1): C, 70.61; H, 8.66; N, 6.33. Found: C, 70.83; H, 8.72; N, 6.03.

(1*R*,3*R*)-8-Hydroxy-6-methoxy-*N*-methyl-1,3-dimethyl-1,2,3,4-tetrahydroisoquinoline (*ent*-8). The synthesis was achieved starting from *ent*-**7** following the protocol for the preparation of **8** (59%); $[\alpha]_D^{25}=+0.60$ (c=0.53 in methanol); spectroscopic data (^{1}H, ^{13}C NMR, MS) in accordance with **8**.

Gentrymine B iodide (1·iodide) by methylation of 8. To a solution of **8** (6.23 mg, 28.9 μmol) in 1 ml of CH_2Cl_2, 7.25 μl (115 μmol) methyl iodide were added. After stirring at room temperature for 96 h, the solvent was evaporated under reduced pressure and the residue was crystallized from acetone to give **1**·iodide as colorless cubes (7.71 mg, 83%); mp 126°C; $[\alpha]_D^{25}=-29.8$ (c=0.30 in methanol) [Refs. 2,8 −36 (c=0.11 in methanol)]; CD: $\Delta\epsilon_{209}$ −2.7, $\Delta\epsilon_{214}$ −3.1, $\Delta\epsilon_{223}$ −2.7, $\Delta\epsilon_{234}$ −3.2, $\Delta\epsilon_{251}$ −0.5, $\Delta\epsilon_{283}$ −2.1; IR (KBr): $\tilde{\nu}$ 3500–3000 (OH), 1600 (C=C), 1580 (C=C), 1420, 1140 (C–O); ^{1}H NMR (600 MHz, CD_3OD): δ=1.49 (d, J=6.5 Hz, 3H, 3-CH_3), 1.65 (d, J=6.6 Hz, 3H, 1-CH_3), 2.92 (dd, J=18.6 Hz, J=12.2 Hz, 1H, 4-H_{ax}), 2.94 (s, 1H, NCH_{3ax}), 3.16 (dd, J=18.6, 4.8 Hz, 1H, 4-H_{eq}), 3.21 (s, 1H, NCH_{3eq}), 3.73 (s, 3H, OCH_3), 4.12 (m_c, 1H, 3-H), 4.76 (q, J=6.7 Hz, 1H, 1-H), 6.30 (d, J=2.2 Hz, 1H, 5-H), 6.32 (d, J=2.3 Hz, 1H, 7-H). ^{13}C NMR (151 MHz, $CDCl_3$): δ=14.73 (3-CH_3), 16.80 (1-CH_3), 33.66 (C-4), 44.90 (NCH_{3ax}), 50.46 (NCH_{3eq}), 55.75 (OCH_3), 59.37 (C-3), 69.07 (C-1), 101.30 (C-7), 104.72 (C-5), 114.09 (C-9), 132.66 (C-10), 156.17 (C-8), 162.00 (C-6); MS: m/z (%)=221 (1) [M^+-CH_3I], 220 (4) [M^+-CH_4I], 206 (100) [221−CH_3], 190 (10) [M^+-OCH_3], 177 (10), 142 (22) [CH_3I^+], 72 (72) [$C_4H_{10}N^+$]; Anal. calcd for $C_{14}H_{22}NO_2I$ (363.2): C, 46.29; H, 6.10; N, 3.86. Found: C, 45.92; H, 6.37; N, 3.83. The compound was found to be identical with the material isolated from *A. korupensis*.[2]

***ent*-Gentrymine B·iodide (*ent*-1·iodide) by methylation of ent-8.** The same procedure was used as for the preparation of **1** (92%); mp 124°C; $[\alpha]_D^{27}=+27.0$ (c=0.89 in methanol); CD: $\Delta\epsilon_{209}$ +0.3, $\Delta\epsilon_{218}$ +0.6, $\Delta\epsilon_{225}$ +0.4, $\Delta\epsilon_{232}$ +0.6, $\Delta\epsilon_{251}$ +0.1, $\Delta\epsilon_{281}$ +0.3; spectroscopic data (^{1}H, ^{13}C NMR, MS) in accordance with synthetic material of **1**·iodide.

Oxidative degradation. Gentrymine B iodide (**1**·iodide) or *ent*-gentrymine B iodide (*ent*-**1**·iodide) (1.50 mg, 4.13 μmol), and $RuCl_3\cdot 6H_2O$ (0.1 mg, 0.4 μmol) were added with stirring to a two-phase mixture of MeCN (100 μl), CCl_4 (100μl), and 0.1 M aqueous sodium phosphate buffer (200μl, pH 6) at room temperature. Over 60 min, $NaIO_4$ (25.7 mg, 120 μmol) was added in several portions and the mixture was stirred at room temperature for another 1.5 h. For extraction of the resulting amino acids, water (700 μl) was added and the aqueous phase was washed twice with portions of $CHCl_3$ (300 μl), and lyophilized. The residue was extracted with 1.5 ml of dry methanol followed by separation of the insoluble inorganic salts by centrifugation. The resulting methanolic solution of amino acids was used for Mosher derivatization with (*R*)-MTPA-Cl [(*R*)-α-methoxy-α-trifluoromethylphenylacetic acid chloride].[9] The GC analysis in SIM-mode (m/z=102, 116) of the derivatized degradation products, which was carried out as described earlier,[9] showed significant peaks for Mosher esters of *N*-methyl-3-aminobutyric acid. Retention times [(*R*)-**9**: 18.72 min; (*S*)-**9**: 18.95 min] matched with those of authentic samples.

Gentrymine B iodide (1·iodide) by biomimetic methylation of gentrymine A (2). A mixture of gentrymine A[4] (**2**, 10.6 mg, 47.7 μmol) and MeI (15.0 mg, 238 μmol) in 1 ml of CH_2Cl_2 was stirred at room temperature for 96 h. After removal of the solvent in vacuo, the residue was crystallized from acetone to give gentrymine B iodide (**1**·iodide, 11.1 mg, 64%) as colorless cubes; mp 128°C, $[\alpha]_D^{25}=-28.3$ (c=0.30 in methanol) [Refs. 2,8 −36 (c=0.11 in methanol)]; physical and spectroscopic (^{1}H, ^{13}C NMR, MS) data of **1** identical with those of gentrymine B (**1**) gained by total synthesis (see above) and by isolation.[2]

Acknowledgements

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