Determination of Absolute Stereochemistry and an Alternative Synthesis of Homopumiliotoxin 223G: Identification on Chiral GC Columns with the Natural Alkaloid

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An alternative asymmetric synthesis of (+)-(1*S*,9a*S*)-homopumiliotoxin **223G** (**1**) was accomplished via (1*R*,2*R*,9a*S*)-1-(benzyloxy)-2-hydroxy-1-methyl-3[(*E*)-isobutylidene]quinolizidine **(4),** which was synthesized according to the intramolecular nickel(II)/chromium(II)-mediated cyclization of the *N*-(iodoalkenyl)aldehyde **2**. Compound **4** was converted to the acetate and subjected to reduction with lithium in ammonia, whereupon deprotection of the *O*-benzyl group and removal of the acetoxyl group occurred in a single operation to afford (+)-homopumiliotoxin **223G**. The same sequence using (±)-**4** was applied to the synthesis of racemic **223G**. Gas chromatography of a sample of racemic **223G** showed no separation into enantiomers on four different cyclodextrin-based chiral GC columns. We found, however, that the *O*-acetates of (±)-**223G** gave a nearly baseline separation on either a *β*-cyclodextrin column or a permethylated *β*-cyclodextrin column. The *O*-acetate of synthetic (+)-**223G** was identical on either of these two columns, with the first eluting *O*-acetate from acetylated (±)-**223G** and also with the acetylated **223G** present in a frog skin extract, thus allowing us to confirm unambiguously the 1*S*,9a*S* absolute configurations of natural **223G**.

Neotropical poison-dart frogs of the Dendrobatidae family have been a rich source of a remarkable variety of alkaloids with structurally unique features and biological significance.[1] The pumiliotoxin A class, one of several major groups of the dendrobatid alkaloids, is characterized by the 6-alkylidene-8-hydroxy-8-methylindolizidine ring system and has been divided into two subclasses, the pumiliotoxins and allopumiliotoxins.[2] Another bicyclic alkaloid, in which the indolizidine moiety of the pumiliotoxin subclass of alkaloids is replaced with a quinolizidine ring, namely, homopumiliotoxin **223G** (**1**), has been isolated in small quantities from the Panamanian poison frog *Dendrobates pumilio* and characterized.[3] Examination of skin extracts from nondendrobatid amphibians revealed that homopumiliotoxin **223G** also occurs in the New World genus of bufonid toads *Melanophryniscus*[4] and the Madagascan genus of mantelline frogs *Mantella*.[5] The former genus contains further members of the homopumiliotoxin class, characterized as homopumiliotoxins **319A**, **319B**, and **321B** (Figure 1).[5]

Recent studies concerned with the total synthesis of (+)-homopumiliotoxin **223G** have provided confirmation of the proposed relative stereochemistry of natural homopumiliotoxin **223G**.[6] However, because of the small quantity of material available for study from the natural source, the absolute chemistry and optical rotations have not been determined for all these homopumiliotoxins, although they have tentatively been assigned as 1*S*,9a*S* based on the analogy with the established absolute configurations of the pumiliotoxin class alkaloids, such as **251D**, pumiliotoxin A (**307A**), and pumiliotoxin B (**323A**).[1b]

In the present work, we report an alternative total synthesis of (+)-(1*S*,9a*S*)- homopumiliotoxin **223G** as well as racemic **223G**. Comparison on chiral columns of the 1-*O*-acetates (**6**) of synthetic and natural **223G** allowed us to confirm unambiguously the 1*S*,9a*S* absolute configurations of natural **223G**.

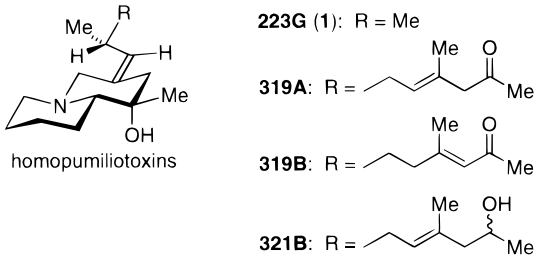

Figure 1. Homopumiliotoxin class alkaloids.

We reported[7] previously that the *N*-(iodoalkenyl) aldehyde **2**, chirally prepared from (*S*)-*N*-Boc-2-acetylpiperidine (**3**), can be subjected to intramolecular chromium(II)-mediated coupling to give the *trans*-quinolizidine **4**. We envisioned that compound **4** could be utilized for an alternative approach to the asymmetric synthesis of (+)-homopumiliotoxin **223G** (**1**) (Scheme 1). An initial attempt at deoxygenation of **4** through the tosylate was unsuccessful, because tosylation of **4** resulted in the formation of a complex mixture. However, upon treatment with acetic anhydride and triethylamine at room temperature, **4** could readily be converted to the acetate **5** in 93% yield. Reduction with lithium in ammonia at −78 °C was carried out to simultaneously remove the *O*-benzyl protecting group and the acetoxyl group to produce (+)-(1*S*,9a*S*)-homopumiliotoxin **223G** (**1**), $[\alpha]^{28}_D = +1.7°$ (*c* 1.00, $CHCl_3$). The hydrochloride salt of **1** had mp 183−184 °C and $[\alpha]^{25}_D = +48.0°$ (*c* 0.48, MeOH). The ^{1}H NMR, vapor-phase FTIR, and mass spectroscopic data of the synthetic substance were identical with those reported for natural **223G**.[3,5]

The same sequence of reactions starting with racemic *N*-Boc 2-acetylpiperidine [(±)-**3**] was also used to synthesize racemic **223G** [(±)-**1**]. A part of the free base was converted

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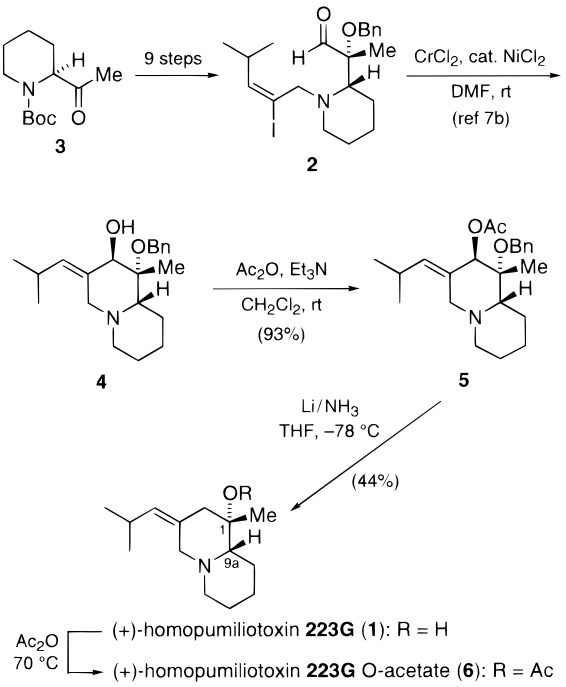
Scheme 1



to the corresponding hydrochloride salt, which was recrystallized from chloroform-EtOAc to give colorless pillars, mp 227−229 °C.

On chiral cyclodextrin-based gas chromatographic columns, the synthetic material of the (+)-enantiomer of **223G** was found to have retention times identical with the natural alkaloid found in frog skin. This strongly suggested that the natural **223G** is also dextrorotatory and that its absolute configuration is 1*S*,9a*S*. However, it remained possible that both enantiomers of **223G** do not separate on those chiral columns. Accordingly, a sample of the racemate of **223G** was subjected to chiral gas chromatography, but unfortunately it showed no separation into enantiomers on four different cyclodextrin-based chiral GC columns (see Experimental Section). We found, however, the *O*-acetates of (±)-**223G** that is (±)-**6**, gave a nearly baseline separation on either a β-cyclodextrin column or a permethylated β-cyclodextrin column (Figure 2). The *O*-acetate of synthetic (+)-**223G** was identical on either of these two columns with the first eluting *O*-acetate from acetylated (±)-**223G** and also with the *O*-acetate of natural **223G**, thus allowing us to unambiguously confirm the 1*S*,9a*S* absolute configurations of natural **223G**.

Experimental Section

Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were taken at 400 MHz. Residual chloroform (7.26 ppm) was used as the internal reference for ^{1}H NMR spectra measured in $CDCl_3$. ^{13}C chemical shifts were reported on the δ scale relative to $CDCl_3$ as an internal reference (77.1 ppm). The EIMS and vapor-phase FTIR spectrum of **223G**−OAc were obtained with a Hewlett-Packard model 5890 gas chromatograph having a 25 m × 0.32 mm i.d. HP-5 fused silica-bonded capillary column programmed from 100 to 280 °C at 10 °C/min interfaced with a Hewlett-Packard model 5971 mass selective detector and a Hewlett-Packard model 5965B IR instrument with a narrow band (4000−750 cm^{-1}) detector and a Hewlett-Packard ChemStation (DOS based). Merck Si gel 60 (230−400 mesh) was used for column chromatography.

(1*R*,2*R*,3*E*,9a*S*)-1-(Benzyloxy)-l-methyl-3-methylpropylidene)octahydro-2*H*-quinolizin-2-yl Acetate (5). To a stirred mixture of **4** (16 mg, 0.049 mmol), which was obtained according to the previously described method,[7b] and triethylamine (15 mg, 0.15 mmol) in CH_2Cl_2 (0.5 mL) was added acetic anhydride (10 mg, 0.098 mmol) at room temperature. After stirring at room temperature for 20 h, the mixture was diluted with CH_2Cl_2 (10 mL), washed with brine and saturated aqueous $NaHCO_3$, and dried over $MgSO_4$. The solvent was removed in vacuo, and the residual oil was purified by chromatography on a Si gel column eluting with 500:90:1 $CHCl_3$−MeOH−concd NH_4OH to give **5** (17 mg, 93%) as a colorless oil: $[\alpha]^{27}_D$ +2.33° (*c* 0.77, $CHCl_3$); IR (neat) 2865, 2836, 2758, 1736, 1669 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.91, (3 H, s), 1.00 (3 H, d, J = 6.5 Hz), 1.15 (3 H, s), 1.21−1.92 (6 H, m), 2.06, (3 H, s), 2.07−2.22 (2 H, m), 2.63 (1 H, dt, J = 6.7, 2.4 Hz), 2.68 (1 H, d, J = 13.8 Hz), 2.95 (1 H, br d, J = 10.3 Hz), 3.48 (1 H, d, J = 12.5 Hz), 4.56 (1 H, d, J = 12.4 Hz), 4.59 (1 H, d, J = 12.4 Hz), 5.40 (1 H, s), 5.48 (1 H, dd, J = 9.1, 1.1 Hz), 7.18−7.34 (5 H); ^{13}C NMR ($CDCl_3$) δ 19.2, 21.4, 6 22.6, 22.8, 23.2,

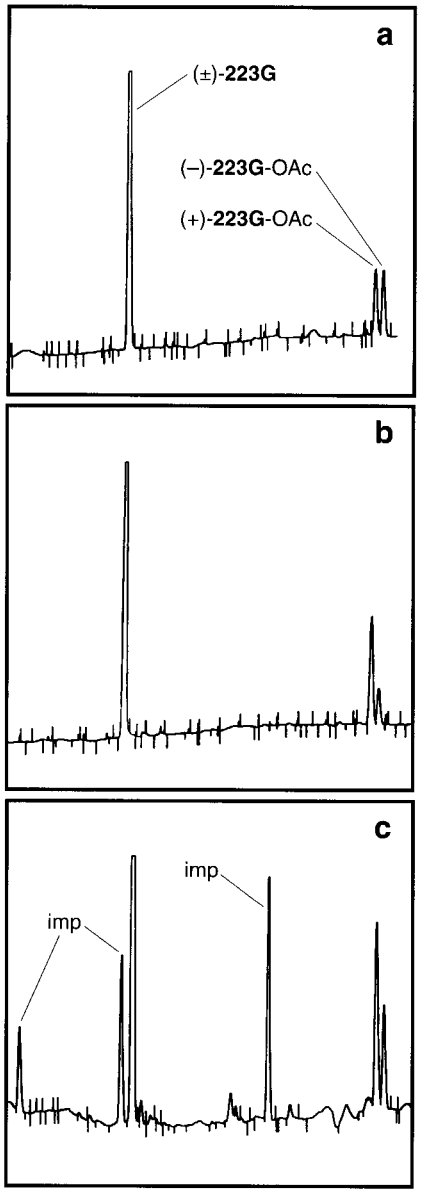


Figure 2. Chiral gas chromatograms of (a) (±)-**223G** heated with Ac_2O for 6 days at 70 °C; leading peak is unacetylated racemate, and the pair of peaks at longer retention time are the (+)- and (−)-*O*-acetate of **223G** as labeled; (b) (±)-**223G** heated with Ac_2O for 6 days at 70 °C, coninjected with synthetic (+)-**223G** treated the same way; and (c) (±)-**223G** heated with Ac_2O for 6 days at 70 °C, coinjected with a sample of the alkaloid fraction from the Madagascan frog *M. viridis*, which had been acetylated for 4 days at 70 °C. In both (b) and (c), the first of the two **223G** *O*-acetate peaks is enhanced. The column used (B) and temperature program are described in the Experimental Section. Imp (impurity) in (c) refers to other alkaloids.

24.1, 24.8, 25.2, 26.5, 52.0, 57.3, 64.3, 66.1, 76.6, 77.6, 127.1, 127.3, 128.1, 134.7, 139.6, 144.6, 172.5; HREIMS *m/z* 371.2488 (calcd for $C_{23}H_{33}NO_3$, 371.2460).

(+)-Homopumiliotoxin 223G (1). A solution of **5** (36 mg, 0.097 mmol) in THF (4 mL) was added to liquid ammonia (10 mL) at −78 °C. To this mixture was added lithium (14 mg, 2.0 mmol) in several portions with stirring at −78 °C and stirring was continued for 1 h. The reaction was quenched by adding saturated aqueous NH_4Cl, and the mixture was extracted with dichloromethane (3 × 20 mL). The combined extracts were washed with saturated aqueous $NaHCO_4$, dried over $MgSO_4$, and concentrated under reduced pressure. The residual oil was chromatographed on Si gel eluting with chloroform−methanol−concd NH_4OH (200:90:1) to give (+)-**1** (9.4 mg, 44%) as a pale yellow oil: $[\alpha]^{28}_D = +1.7°$ (*c* 1.00, $CHCl_3$); IR (neat) 3527, 2954, 2934, 2865, 2802, 2751, 1733, 1674, 1465, 1390, 1323, 1270, 1126, 1062 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.91, (3 H, d, $J = 6.7$ Hz), 0.98 (3 H, d, $J = 6.6$ Hz), 1.10 (3 H, s), 1.22 (1 H, tt, $J = 13.0, 3.8$ Hz), 1.39−1.53 (2 H, m), 1.56−1.63 (1 H, m), 2.05−2.15 (3 H, m), 2.37 (1H, d, $J = 12.2$ Hz), 2.53−2.65 (2 H, m), 2.87 (1 H, d, $J = 11.3$ Hz), 3.49 (1 H, dd, $J = 12.1, 1.0$ Hz), 5.07 (1 H, d, $J = 9.2$ Hz); ^{13}C NMR ($CDCl_3$) δ 23.5 (CH_3), 23.6 (CH_3), 24.2 (CH_2), 24.3 (CH_3), 24.7 (CH_2), 25.6 (CH_2), 26.6 (CH), 49.6 (CH_2), 56.7 (CH_2), 57.0 (CH_2), 69.5 (CH), 69.7 (C), 128.8 (C), 135.2 (CH); EIMS *m/z* (rel int) 223 (M^+, 12), 208 (5), 190 (5), 180 (43), 178 (12), 162 (11), 136 (7), 126 (11), 107 (4), 98 (26), 84 (100); HREIMS *m/z* 223.1939, (calcd for $C_{14}H_{25}NO$, 223.1936).

The free base was treated with methanolic HCl and concentrated in vacuo to give a light brown solid, which was dissolved in MeOH and treated with activated carbon at room temperature. After filtration, the filtrate was concentrated in vacuo, and the residual solid was recrystallized from chloroform−EtOAc to afford the hydrochloride salt of (+)-**1** as colorless prisms: mp 183-184 °C; $[\alpha]^{25}_D = +48.0°$ (*c* 0.48, MeOH); ^1H NMR ($CDCl_3$) δ 0.92, (3 H, d, $J = 6.7$ Hz), 1.16 (3 H, d, $J = 6.5$ Hz), 1.24 (3 H, s), 1.48 (1 H, qt, $J = 13.5, 3.8$ Hz), 1.80 (1 H, br d, $J = 17.1$ Hz), 2.01 (2 H, br t, $J = 15.8$ Hz), 2.26−2.33 (2 H, m), 2.35 (2 H, s), 2.64−2.84 (3 H, m), 3.06 (1 H, dd. $J = 11.8, 9.9$ Hz), 3.50 (1 H, br dd, $J = 11.8, 1.5$ Hz), 4.12 (1 H, d, $J = 12.8$ Hz), 4.15 (1 H, s), 5.44 (1 H, d, $J = 9.8$ Hz), 11.9 (1 H, br s); ^{13}C NMR ($CDCl_3$) δ 15.66 (CH_2), 15.75 (CH_3), 15.86 (CH_2), 15.91 (CH_2), 16.2 (CH_3), 18.8 (CH_3), 20.4 (CH), 41.0 (CH_2), 48.00 (CH_2), 49.3 (CH_2), 63.1 (C), 64.7 (CH), 112.8 (C), 135.9 (CH); FABMS *mlz* 224 $[MH]^+$; *anal.* C 64.35%, H 9.94%, N 5.47%, calcd for $C_{14}H_{25}NO{\cdot}HCl$, C 64.72%, H 10.09%, N 5.39%.

***O*-Acetylation of Synthetic (+)-1 and (+)-1 and Natural Homopumiliotoxin 223G.** Approximately 0.1−0.2 mg of either synthetic material of homopumiliotoxin **223G**, (+)-**1**, or (±)-**1**, was heated for 6 days at 70 °C in small sealed tubes [Wheaton LVI vial (Alltech Assocs., Deerfield, IL)] with teflon-lined caps containing 200 μL of acetic anhydride. The contents were cooled and the reagent removed with a stream of N_2 to near dryness, then MeOH added and the tubes shaken and that solvent removed also. Then 50 μL of MeOH was added, and 1 or 2 μL injections were made on the gas chromatograph as described below, or coinjection mixtures were prepared as indicated below. GC flame ionization detection indicated 30% conversion to the *O*-acetate of homopumiliotoxin **223G** [(+)-**6** or (±)-**6**], under these conditions with 70% recovered starting material: EIMS *m/z* (rel int) 265 $[M]^+$ (3), 222 (43), 205 (49), 190 (100), 176 (19), 162 (31), 148 (9), 134 (13), 131 (14), 121 (26), 107 (11), 97 (14), 84 (92), 69 (81), 55 (32); vapor-phase FTIR 2966, 2945, 2874, 2742, 1749, 1450, 1371, 1238 cm^{-1}.

An aliquot (150 mL) of the alkaloid fraction isolated from five skins (total weight of skins, 0.93 g in 0.93 mL of methanol) of the Madagascan frog *Mantella viridis*, collected 13 km south of Antsirana in January 1994, was evaporated to dryness, dissolved in 50 μL of acetic anhydride, and heated at 70 °C for 4 days. It was then worked up as described above to provide a methanolic solution containing the *O*-acetylated natural **223G**.

Determination of Absolute Stereochemistry of Homopumiliotoxin 223G Using Chiral GC Columns. A Hewlett-Packard model 5890 gas chromatograph with a 3390A integrator-recorder and flame ionization detector was used for all analyses. A temperature program of 100−165 °C at 1.5 °C/min was used. Helium was used as the carrier gas at 20 psi. The following chiral cyclodextrin-based columns (Supelco Inc., Bellefonte, PA) were employed: (α-Dex-120 (column A); β-Dex-120 (B), γ-Dex-120 (C), and, from SGE, Inc. (Phenomenex, Torrance, CA), a permethylated-β-cyclodextrin column (D) (25QC2/CYDEX-B) was obtained. Columns A, B, and C were 30 m × 0.25 mm i.d., 25-μm film thickness, while column D was 25 m × 0.22 mm i.d., 0.25-μm film thickness. Columns A−D failed to resolve (±)-**1**. Columns B and D did, however, provide nearly baseline separation of homopumiliotoxin **223G** *O*-acetates [(+)-**6** and (−)-**6**]. Coinjections were performed by mixing 10- or 20-μL amounts of the acetylated racemic or (+)-reference sample of **223G** (**1**) and 10 or 20 μL of the acetylated frog skin extract, then injecting 1 or 2 μL of the resultant solutions on the GC columns below.

The retention times of either (±)-**1** or (+)-**1** and (+)-**6** and (−)-**6** on columns B and D follows: Chiral column B: (±)-**1** or (+)-**1** = 38.20 min; (+)-**6** = 47.86 min, (−)-**6** = 48.17 min. Chiral column D: (±)-**1** or (+)-**1** = 35.60 min; (+)-**6** = 44.11 min, (−)-**6** = 44.41 min

Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds (+)-**1**, (+)-**1**·HCl, and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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