Novel Sponge-Derived Amino Acids. 11. The Entire Absolute Stereochemistry of the Bengamides

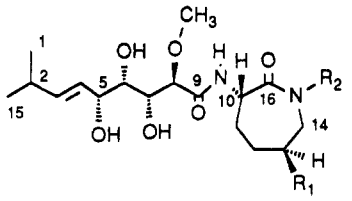
Madeline Adamczeski, Emilio Quiñoà, and Phillip Crews*

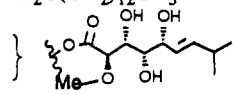
Department of Chemistry and Institute of Marine Sciences, University of California, Santa Cruz, California 95064

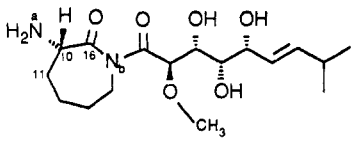
Received July 11, 1989

The complete stereochemistry of bengamides A (1) and B (2) can be assigned as 5*R*,6*S*,7*R*,8*R*,10*S*,13*S*, and this absolute stereochemistry can also be extended to other bengamide derivatives C, D, E, and F (3–6) and isobengamide E (7). The absolute stereochemical results were obtained by isolating a monohydroxy lactone 12, which was then converted to dihydroxy lactone 11. The relative stereochemistry of 11 had been previously shown to be the same as that of the 2-methoxy-3,4,5-trihydroxy-8-methylnon-6(*E*)-enoyl side chain (A) in the bengamides. The absolute stereochemistry of lactone 12, deduced by esterifying with *O*-methylmandelic acids followed by an analysis of their different ^{1}H NMR chemical shifts, gave the absolute configuration of the stereocenters in A. Combining the new assignments with those obtained previously for the δ-hydroxycaprolactam moiety of the bengamides completed assignment of the absolute configuration at all chiral sites.

Our reports of the bengamides A–F (1–6) and isobengamide E (7), a new category of amino acid derivatives from a Choristid sponge, included absolute stereochemistry for one or two out of five, six, or 10 chiral centers.[1] These



Bengamide		R_1	R_2
A	(1)	$-O_2C(CH_2)_{12}CH_3$	H
B	(2)	$-O_2C(CH_2)_{12}CH_3$	CH_3
C	(3)		H
D	(4)		CH_3
E	(5)	H	H
F	(6)	H	CH_3

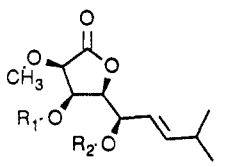


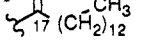
*iso*bengamide E (7)

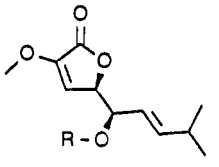
anti infectious disease active compounds[2] combine the elements of a caprolactam, originating from a δ-(*S*)-hydroxy-(*S*)-lysine or an (*S*)-lysine, and one or two complex side chains, likely derived by the union of a C_4-diketide and leucine.[1a] The absolute stereochemistry deduced for the lactam ring could not be interrelated to the relative stereochemistry proposed for the four contiguous stereocenters in the 2(*R**)-methoxy-3(*R**),4(*S**),5(*R**)-trihydroxy-8-methylnon-6(*E*)-enoyl side chain (A) due to the noncrystalline nature of the bengamides and because only one of their degradation products was of known absolute chirality.[1a] We now describe the comprehensive absolute stereochemistry of the bengamides, which will facilitate their future total synthesis or biosynthetic studies.

Results and Discussion

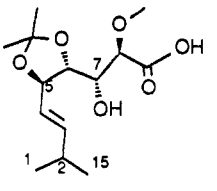
We envisioned that the *O*-methylmandelate ester method would be applicable since it has been widely used to establish the absolute configuration of secondary alcohols.[3] Unfortunately, there were no secondary monoalcohol derivatives at hand containing the side chain A because acetolysis of the bengamides or isobengamide E yielded secondary hydroxy derivatives such as 8 or 9. Even though



8 $R_1 = R_2 = Ac$
11 $R_1 = R_2 = H$
12 $R_1 = H$, $R_2 =$ 



9 R = Ac



10

hydrolysis of isobengamide E yielded the requisite monoalcohol derivative **10**, the paucity of it or of additional isobengamide E did not allow us to proceed. Although the dihydroxy lactone 11 was isolated from the crude sponge extracts, the prospects of isolating a monoalcohol analogue

(1) (a) Adamczeski, M.; Quiñoà, E.; Crews, P. *J. Am. Chem. Soc.* **1989**, *111*, 647. (b) Quiñoà, E.; Adamczeski, M.; Crews, P.; Bakus, G. J. *J. Org. Chem.* **1986**, *51*, 4494.

(2) Antiparasitic activity against the parasite *Nippostrongylus brasiliensis* was as follows. **Levamisole** (at 0.5 μg/mL, a standard), % reduction vs control: casts, 100; viability, 99; motility, 90. **Bengamide A** (at 50 μg/mL): casts, 95; viability, 100; motility, 100. **Bengamide B** (at 3 μg/mL): casts, 95; viability, 100; motility, 100. Antimicrobial properties for bengamides A and B are respectively as follows: MIC (μg/mL) against *Streptococcus pyrogenes* = 4 and 2; neither is active against *Candida albicans* or *Trichophyton mentagrophytes*. Additional discussions can be found in U.S. Patent No. 4,831,135 issued on May 16, 1989.

(3) (a) Dale, J. A.; Mosher, H. S.; *J. Am. Chem. Soc.* **1973**, *95*, 512. (b) Trost, B. M.; Curran, D. P. *Tetrahedron Lett.* **1981**, 4929. (c) Trost, B. M.; Belletire, J. L.; Godleski, S.; McDougal, P. G.; Balkovec, J. M. *J. Org. Chem.* **1986**, *51*, 2370.

0022-3263/90/1955-0240$02.50/0 © 1990 American Chemical Society

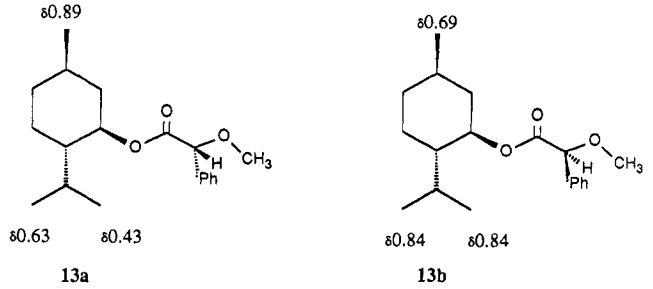


Figure 1. Selected ^1H NMR shifts of the *R*/*S* pairs of *O*-methylmandelates of (−)-menthol.

seemed bleak.[1a] Serendipitously, recent efforts to reisolate several of the bengamides also provided a new monohydroxy lactone, **12** (104 mg, waxy solid), in sufficient quantity to be used for the derivatization experiments. Lactone **12** differs from **11** by the presence of a myristate group esterified at the 5-position,[4] and its structure was elucidated from LREIMS (M^+ = 440, $C_{25}H_{44}O_6$), IR (3600–3200, 1790, 1738 cm^{-1}), and exhaustive NMR analysis: ^1H, ^{13}C, APT, and ^1H–^1H COSY. A study of the ^1H NMR *J*'s and ^{13}C δ's indicated that the relative stereochemistry of **12** was the same as that of **11**, which was further confirmed when hydrolysis of **12** followed by acetylation afforded the identical diacetylated lactone **8**, which had been previously obtained from **11** [**8** from respectively **11** ($[\alpha]^{20}_D$ = −15.0°, *c* = 0.026, MeOH) and **12** ($[\alpha]^{20}_D$ = −13.8°, *c* = 0.019, MeOH)].

Desiring to check both the validity of the method and the reagents [(*R*)- and (*S*)-methoxyphenylacetic acids], we synthesized the (*R*)- and the (*S*)-*O*-methylmandelate esters of a chiral standard, (1*R*,2*S*,5*R*)-(−)-menthol. The ^1H NMR spectra of both diastereomers showed the expected chemical shift patterns (Figure 1) according to projections drawn first by Mosher[3a] and subsequently by Trost[3b,c] as substituents in the vicinity of the *O*-methylmandelate aryl group are shielded. Alternatively, such conformational models cannot be easily verified by NMR because *J* values do not provide data about the proximity of the mandelate aryl to the alcohol substituents. Additional insights about the differential aryl anisotropy were sought from an NMR NOESY study. Polarization transfer was measured from the aromatic to the methyl protons of another simple model compound, **14**, the (*R*)-*O*-methylmandelate ester of 2-propanol. The largest NOESY effect was observed between the phenyl (δ = 7.25) and the more shielded (δ = 1.11) of the two diastereotopic methyls. Furthermore, the methine hydrogen of the mandelate (δ = 4.71) exhibited the most intense NOESY correlation to the downfield methyl (δ = 1.23). These observations are consistent with the preferred conformation, represented in Figure 2.[3,5]

The (*R*)- and (*S*)-mandelate esters of lactone **12** (**15a** and **15b**, respectively) were prepared, and their ^1H NMR shifts were examined (Figure 3). In comparison to the *R* derivative **15a**, the *S* derivative **15b** showed substantial upfield shifts (absolute difference in parts per million) of H-5 (0.33), H-4 (0.36), H-3 (0.76), H-2 (0.22), Me-1 (0.14), and

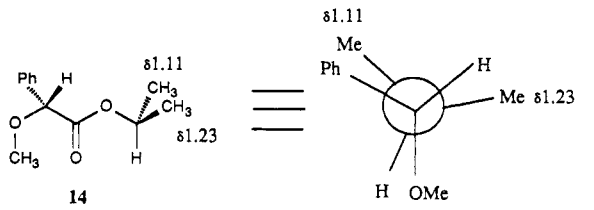


Figure 2. Selected ^1H NMR shifts of the (*R*)-*O*-methylmandelate of 2-propanol.

Me-15 (0.14). Conversely, the *R* ester **15a** showed upfield shifts of H-8 (0.07) and the OMe (0.29) when compared to the *S* ester **15b**. In view of the discussion above, the different shielding effects by the phenyl group of the (*S*)- or (*R*)-*O*-methylmandelate esters on separate regions of **15** can be rationalized according to the diagrams in Figure 3. Accordingly, the assignment of *R* absolute configuration can be made at C-7 in **12**. Since the relative stereochemistry of **11** and **12** had already been established and the caprolactam absolute stereochemistry was known,[1a] the bengamides A and B can now be specified as 5*R*,6*S*,7*R*,8*R*,10*S*,13*S*. These absolute stereochemical details can further be extended to the other five bengamide derivatives (**3**–**7**), including their additional ester or amide side chains, which have the same absolute chirality as those of bengamide A (**1**). These results will facilitate work that is in progress to unravel the biosynthetic origin of these unusual ketide amino acids.

Experimental Section

General experimental procedures are as described in ref 1a. Abbreviations for terms pertaining to spectroscopic experiments include LREIMS (low-resolution electron-impact mass spectrometry), LRCIMS (low-resolution chemical-ionization mass spectrometry), APT (attached-proton test), COSY (correlation spectroscopy), and NOESY (nuclear Overhauser enhancement spectroscopy). The standard procedure for the preparation of all *O*-methylmandelate derivatives is as follows. A catalytic amount of DMAP [4-(dimethylamino)pyridine] was added to a solution of the title alcohol (≈0.01 mmol) with *O*-methylmandelic acid (≈ 0.01 mmol) and DCC (dicyclohexylcarbodiimide, ≈0.01 mmol) in methylene chloride (2 mL). After 24 h, the dicyclohexylurea was removed by filtration and the solvent removed in vacuo. The filter cake was washed with hexane (3 × 1 mL), and the combined filtrates were washed with cold 1 N HCl (2 × 0.5 mL), saturated $NaHCO_3$ (2 × 0.5 mL), and saturated NaCl (2 × 0.5 mL). The organic phase was filtered and the solvent removed to afford a pure adduct.

Collection and Isolation Procedures. Preserved sponge (collection no. 87036a = 3.4 kg wet wt) was soaked with MeOH followed by CH_2Cl_2, and the combined organics were concentrated and yielded 17.46 g of crude viscous oil. The extract was then successively partitioned as described in ref 1a. Compound **12** (104 mg) was obtained from flash chromatography of the CCl_4 partition fraction (3.31 g) over silica gel (835 g) using a solvent gradient 98:2 CH_2Cl_2/MeOH to 100% MeOH.

(2*R*,3*R*,4*S*,5*R*)-3-Hydroxy-2-methoxy-8-methyl-5-tetradecanoylnon-6-ene 1,4-lactone (12): waxy solid; $[\alpha]^{20}_D$ = −3.3° (*c* = 0.045, MeOH); IR (neat) 3600–3200, 1790, 1738, 1558 cm^{-1}. NMR ($CDCl_3$) shifts in ppm from Me_4Si [atom number], ^{13}C δ values at 75 MHz, ^1H δ and *J* values at 300 MHz: [1] 22.0, 0.98 (d = 6.9, Me); [2] 30.9, 2.32 (m); [3] 144.2, 5.92 (ddd = 16.5, 6.6, 0.9); [4] 119.6, 5.43 (ddd = 15.8, 6.6, 1.1); [5] 71.8, 5.67 (dd = 8.9, 2.1); 80.6, 4.23 (dd = 9.0, 3.0); [7] 67.6, 4.41 (dd = 4.2, 3.0); [8] 78.4, 4.07 (d = 4.5); [9] 172.6 (*); [15] 22.0, 0.98 (d = 6.9, Me); [17] 172.5 (*); [18] 34.5, 2.32 (br t = 7.5); [19] 25.0, 1.61 (m, 2 H); [20–27] 29.1–29.9, 1.4–1.2 (br s); [28] 32.0, 1.4–1.2 (br s); [29] 22.7, 1.4–1.2; [30] 14.2, 0.85 (dd = 6.8, 2.0); [OCH_3] 59.3, 3.66 (s, 3 H). LREIMS *m*/*z* (relative intensity): 440 [M^+ (2)], 297 [$C_{19}H_{36}O_2$ + H (9)], 225 [$C_{14}H_{27}O_2$ − 2 H (18)], 213 [M^+ − $C_{14}H_{27}O_2$ (100)], 57 [C_4H_8 + H (45)]. LRCIMS (isobutane) *m*/*z* (relative intensity): 440 [M^+ (8)], 423 [M^+ − OH (13)], 409 [M^+ − MeO (5)], 397 [M^+

(4) Numbering was assigned to be consistent with the system used in ref 1a.

(5) Still unexplained is the inference (see ref 3) that the mandelate esters and *O*-methylmandelate esters of the same alcohol ought to adopt analogous conformations in spite of the inability of the latter to engage in intramolecular hydrogen bonding. Interestingly, a recent molecular mechanics calculation (Ivanov, P. M. *J. Mol. Struct.* **1986**, *140*, 359) concluded, without experimental verification, that one lowest energy conformer predominated for the adduct of (−)-menthol and (*S*)-mandelic acid (see **13b**) whereas five nearly equal low-energy conformers were calculated for the adduct of (−)-menthol and (*R*)-mandelic acid (see **13a**).

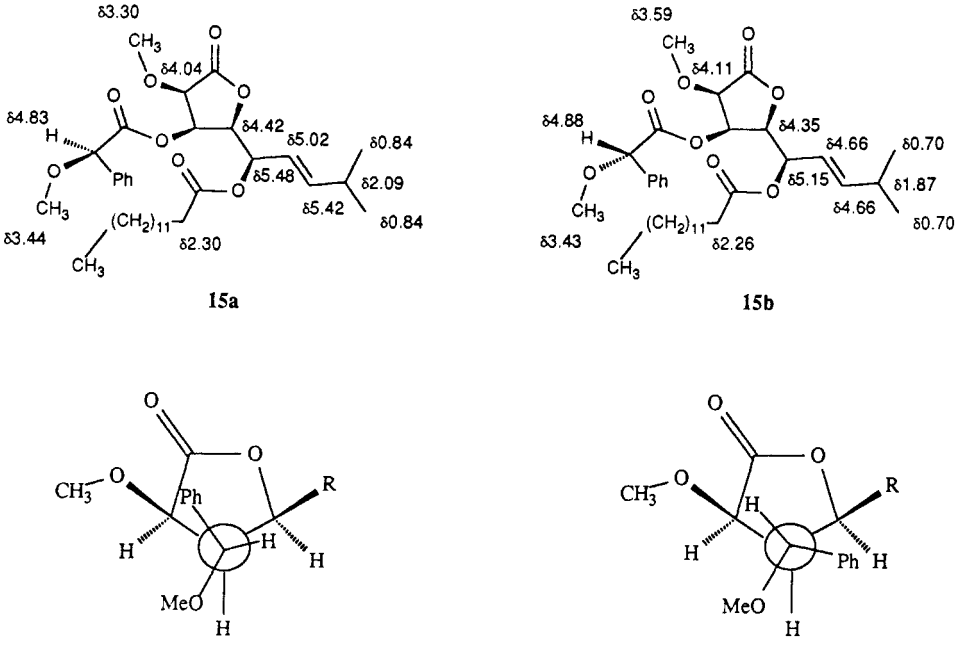


Figure 3. ^1H NMR shifts of the *R*/*S* pairs of *O*-methylmandelates of (−)-**12**.

$- C_3H_7$ (10)], 229 [$M^+ - C_{14}H_{27}O$ (12)], 227 [$C_{14}H_{27}O_2$ (18)], 213 [$M^+ - C_{14}H_{27}O_2$ (100)].

(*R*)-*O*-Methylmandelic acid derivative of (−)-menthol (13a): IR (neat) 2957, 2871, 1729, 1600 cm^{-1}. NMR ($CDCl_3$) shifts in ppm from Me_4Si [atom number], ^1H δ and *J* values at 300 MHz: [1] 4.65 (ddd = 11.1, 11.1, 4.5); [2–7] 1.7–1.0 (m, 8 H); [8] 0.63 (d = 6.9); [9] 0.43 (d = 6.9); [10] 0.89 (d = 6.3); [12] 4.71 (s); [OCH_3] 3.41 (s, 3 H); [Ph] 7.3–7.7 (m, 5 H).

(*S*)-*O*-Methylmandelic acid derivative of (−)-menthol (13b): IR (neat) 2960, 2870, 1730, 1600 cm^{-1}. NMR ($CDCl_3$) shifts in ppm from Me_4Si [atom number], ^1H δ and *J* values at 300 MHz: [1] 4.74 (ddd = 11.1, 11.1, 4.5); [2–7] 1.8–1.2 (m, 8 H); [8–9] 0.84 (d = 6.3, 2 H); [10] 0.69 (d = 6.6); [12] 4.74 (s); [OCH_3] 3.41 (s, 3 H); [Ph] 7.3–7.7 (m, 5 H).

(*R*)-*O*-Methylmandelic acid derivative of 2-propanol (14): IR (neat) 3063, 1745, 1705, 1667, 1496, 1315 cm^{-1}. NMR ($CDCl_3$) shifts in ppm from Me_4Si [atom number], ^1H δ and *J* values at 300 MHz: [1] 1.23 (d = 6.3, Me); [2] 1.11 (d = 6.3, Me); [3]; 5.04 (sept = 6.3); [5] 4.71 (s); [OCH_3] 3.40 (s, 3 H); [Ph] 7.6–7.2 (m, 5 H).

(*R*)-*O*-Methylmandelic acid derivative of 12 (15a): IR neat 2925, 2855, 1800, 1750, 1650, 1560, 1540, 1460, 1360, 1170, 1010, 970 cm^{-1}. NMR ($CDCl_3$) shifts in ppm from Me_4Si [atom number], ^1H δ and *J* values at 300 MHz: [1] 0.84 (m); [2] 2.09 (oct = 6.9); [3] 5.42 (dd = 15.9, 6.9); [4] 5.02 (ddd = 16.2, 8.1, 0.9); [5] 5.48 (t = 8.4); [6] 4.42 (dd = 9.3, 2.7); [7] 5.63 (dd = 4.5, 3.3); [8] 4.04 (d = 4.5); [15] 0.84 (m); [18] 2.30 (dt = 7.5, 1.2); [19–29] 1.2–1.4 (br s); [30] 0.84 (t = 6.5); [32] 4.83 (s); [OCH_3 at C-8] 3.30 (s, 3 H); [OCH_3 of mandelate] 3.44 (s, 3 H); [Ph] 7.6–7.2 (m, 5 H). LREIMS *m*/*z* (relative intensity): 468 [$M^+ - C_8H_9O$ + H (1)], 379 [$M^+ - C_{14}H_{27}O$ + 2H (34)], 361 [$M^+ - C_{14}H_{27}O_2$ (30)], 309 [$C_{20}H_{37}O_2$ (40)], 279 [$C_{14}H_{15}O_6$ (1)], 225 [$C_{14}H_{27}O_2$ − 2H (37)], 212 [$C_{14}H_{27}O$ + H (42)], 163 [$C_9H_9O_3$ − 2H (39)], 151 [$C_9H_9O_2$ + 2H (37)], 121 [C_8H_9O (65)], 70 [C_5H_9 + H (67)], 58 [C_4H_8 + 2H (80)].

(*S*)-*O*-Methylmandelic acid derivative of 12 (15b): IR (neat) 2920, 2850, 1800, 1750, 1660, 1550, 1450, 1370, 1160, 1020, 980 cm^{-1}. NMR ($CDCl_3$) shifts in ppm from Me_4Si [atom number], ^1H δ and *J* values at 300 MHz: [1] 0.70 (d = 6.6); [2] 1.87 (oct = 6.9); [3] 4.66 (m); [4] 4.66 (m); [5] 5.15 (dd = 9.6, 6.9); [6] 4.35 (dd = 9.6, 3.0); [7] 5.57 (dd = 4.2, 2.7); [8] 4.11 (d = 4.2); [15] 0.70 (d = 6.6); [18] 2.26 (dt = 7.5, 1.7); [19–29] 1.2–1.4 (br s); [30] 0.86 (t = 6.5); [32] 4.88 (s); [OCH_3 at C-8] 3.59 (s); [OCH_3 of mandelate] 3.43 (s); [Ph] 7.6–7.2 (m, 5 H). LREIMS *m*/*z* (relative intensity): 361 [$M^+ - C_{14}H_{27}O_2$ (22)], 309 [$C_{20}H_{37}O_2$ (20)], 281 [$C_{14}H_{15}O_6$ + 2H (12)], 225 [$C_{14}H_{27}O_2$ − 2H (19)], 211 [$C_{14}H_{27}O$ (13)], 167 [$C_9H_9O_3$ + 2H (2)], 151 [$C_9H_9O_2$ + 2H (21)], 121 [C_8H_9O (100)], 71 [C_5H_9 + 2H (60)], 57 [C_4H_8 + H (90)].

Hydrolysis of Lactone 12 to Lactone 11. A solution of **12** (5.4 mg) in 1% MeOH/KOH (1 mL) was placed, in the dark, under a nitrogen atmosphere, and allowed to stand at room temperature for 24 h. Next, after being neutralized with a 1% solution of HCl, the reaction mixture was partitioned between H_2O (2 mL) and CH_2Cl_2 (3 × 2 mL). The combined organic extracts were concentrated to dryness in vacuo (less then 40 °C) to obtain myristic acid and **11** after HPLC (ODS, MeOH/H_2O, 85:15).

Acetylation of Lactone 11 to Lactone 8. A solution of **11** (2.9 mg), obtained from **12**, in dry pyridine (0.5 mL) and acetic anhydride (0.5 mL) at room temperature was placed in the dark under nitrogen for 24 h. The reaction mixture was concentrated to dryness to afford diacetate **8** in quantitative yield.

Acknowledgment. Partial research support was from NOAA, National Sea Grant College Program, Department of Commerce, under Grant No. NA85AA-D-SG140, project number R/MP-41, through the California Sea Grant College Program. M.A. was a Sea Grant trainee 1989–90.

Registry No. **1**, 104947-68-4; **2**, 104947-69-5; **3**, 104975-72-6; **4**, 118477-02-4; **5**, 118477-03-5; **6**, 118477-04-6; **7**, 118477-05-7; **8**, 123805-15-2; **11**, 123805-16-3; **12**, 123642-97-7; **13a**, 113581-35-4; **13b**, 123642-96-6; **14**, 123642-98-8; **15a**, 123642-99-9; **15b**, 123643-00-5; (*R*)-MeOCH(Ph)COOH, 3966-32-3; (*S*)-MeOCH(Ph)COOH, 26164-26-1; (−)-menthol, 2216-51-5.