(−)-Echinobetaine A: Isolation, Structure Elucidation, Synthesis, and SAR Studies on a New Nematocide from a Southern Australian Marine Sponge, *Echinodictyum* sp.

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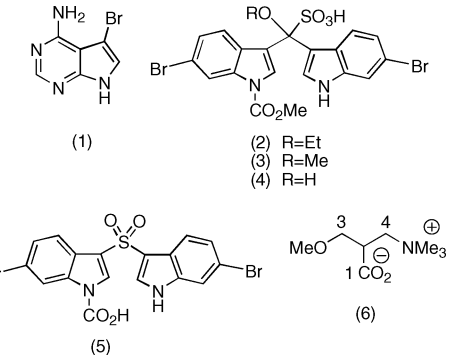
A nematocidal agent present in a southern Australian marine sponge of the genus *Echinodictyum* has been isolated and identified by detailed spectroscopic analysis and total synthesis as the novel betaine (−)-echinobetaine A (**6**). Preliminary SAR investigations have been undertaken.

During our investigations into new agrochemical agents from Australian marine organisms we examined a sponge specimen, *Echinodictyum* sp., collected by beam trawl at a depth of 85 m during a 1995 scientific expedition to the Great Australian Bight, Australia. The EtOH extract of this *Echinodictyum* sp. displayed growth inhibitory properties against the bacteria *Serratia marcescens*, *Micrococcus luteus*, and *Staphylococcus aureus,* but more importantly it displayed excellent in vitro antiparasitic activity against the endo parasite *Haemonchus contortus*. The latter parasite inflicts serious economic damage to the agricultural sector worldwide through its impact on the health and productivity of livestock such as sheep. While agrochemicals exist to combat such parasites, the increasing incidence of resistance requires that the search for new and improved antiparasitics be both vigorous and ongoing. Experience has shown us that new antiparasitic lead compounds can be discovered through the bioassay-directed exploration of marine biodiversity,[1−9] with *Echinodictyum* being a promising target for just such an investigation.

Before embarking on this study we reviewed the existing chemical literature on the genus *Echinodictyum*. The first published account (in 1983)[10] reported the bioassay-directed isolation and subsequent identification of the previously known synthetic compound 4-amino-5-bromopyrrolo[2,3-*d*]pyrimidine (**1**), as a potential bronchodilator. To the best of our knowledge the only other report of novel metabolites from an *Echinodictyum* sp. was an early account by us in 1999.[7] In that report we described a series of novel antibacterial agents, echinosulfonic acids A−C (**2**−**4**) and echinosulfone (**5**). At that time our primary interest in *Echinodictyum* focused on the in vitro antiparasitic activity displayed by the EtOH extracts of at least two specimens in our collection, both from the Great Australian Bight. Although the echinosulfonic acids **2**−**4** and echinosulfone (**5**) were identified as natural antibacterial agents, these compounds were not responsible for the antiparasitic properties displayed by the *Echinodictyum* extract. After our earlier report, we persisted in our investigations to the point where we now report the isolation, characterization, structure elucidation, and synthesis of a nematocidal agent, (−)-echinobetaine A (**6**), from this same *Echinodictyum* sp.



Results and Discussion

The EtOH extract of the *Echinodictyum* specimen was decanted, concentrated in vacuo, and triturated with CH_2Cl_2, after which the residue was partitioned between n-BuOH and H_2O. Whereas the n-BuOH solubles displayed antibacterial activity and ultimately led to the isolation and identification of the echinosulfonic acids A−C (**2**−**4**) and echinosulfone (**5**),[7] the crude H_2O solubles displayed significant antiparasitic activity against *H. contortus*.

The H_2O solubles were further fractionated by elution through Sephadex G-10 (H_2O) followed by C_{18} HPLC (0.1% TFA in H_2O) to yield the nematocide (−)-echinobetaine A (**6**) (*H. contortus*, LD_{99} 83 μg/mL).

High-resolution ESI(+)MS analysis of **6** revealed a highest mass ion at *m/z* 176 measuring for $C_8H_{18}NO_3$ (Δ −0.4 mmu). Examination of the NMR (D_2O, 400 MHz) data for **6** revealed resonances consistent with an OMe (^{1}H: δ 3.19 (s); ^{13}C: 58.6 ppm), an NMe_3 (^{1}H: δ 2.97 (s); ^{13}C: 53.3 ppm), and two deshielded diastereotopic methylenes (^{1}H: δ 3.31 (dd, J = 13.6, 1.6 Hz) and 3.78 (dd, J = 13.6, 8.0 Hz); ^{13}C: 65.1 ppm; as well as ^{1}H: δ 3.56 (m); ^{13}C: 71.8 ppm) flanking a deshielded methine (^{1}H: δ 3.15 (m); ^{13}C: 41.6 ppm), accounting for all but CO_2H of the proposed molecular formula. Furthermore, the appearance of a ^{13}C NMR resonance at 175.1 ppm was consistent with the remaining functional unit in **6** being a carboxylic acid. Given the deshielded character of the methylene carbons, and the co-occurrence of quaternary ammonium and carboxylic acid functionalities, the most probable structure for (−)-echinobetaine A (**6**) was that shown. Analysis of the

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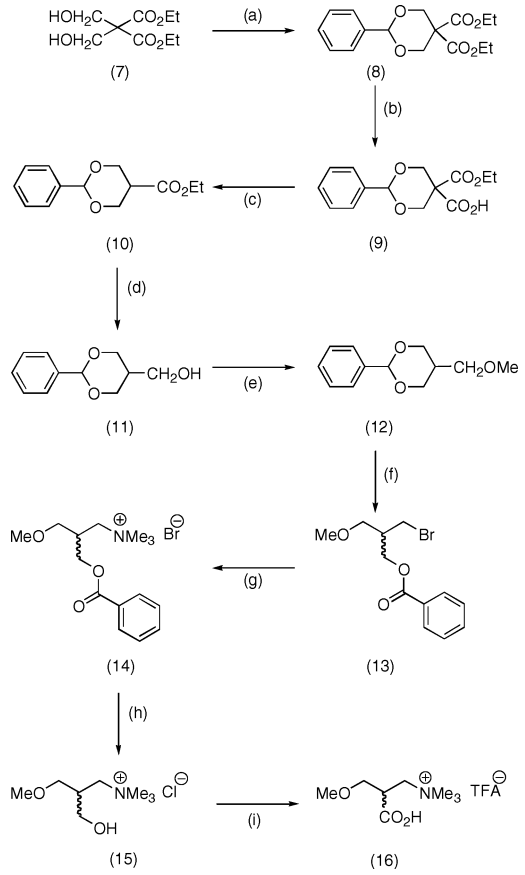
Table 1. NMR (D_2O, 400 MHz) Data for (−)-Echinobetaine A (**6**)

	^{13}C δ	^{1}H δ (m, J Hz)	COSY	gHMBC ($^{1}H-^{13}C$)
1	175.1			
2	41.6	3.15 (m)	3-H_a, 3-H_b, 4-H_2	
3-H_a	65.1	3.78 (dd. J = 13.6, 8.0 Hz)	H-2, 3-H_b	NMe_3, C-1
3-H_b		3.31 (dd, J = 13.6, 1.6 Hz)	2-H, 3-H_a	NMe_3, C-1
NMe_3	53.3	2.97 (s)		C-3, NMe_3
4	71.8	3.56 (m)	2-H	C-3, OMe
OMe	58.6	3.19 (s)		C-4

Scheme 1. Synthesis of (±)-Echinobetaine A (**16**)[a]

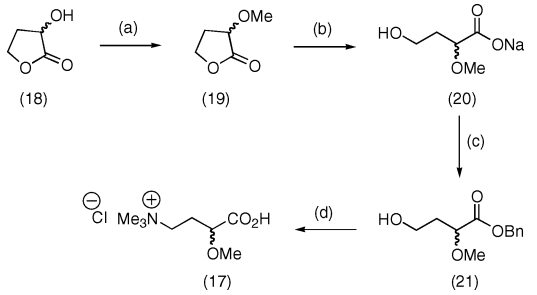


[a] (a) PhCHO, 100%; (b) i KOH, ii HCl, 100%; (c) piperidine, 100%; (d) $LiAlH_4$, 96%; (e) MeI, NaH, 91%; (f) NBS, $BaCO_3$, 95%; (g) NMe_3, benzene, 98%; (h) 1 M HCl; (i) $AcOH_{aq}$, 25%.

2D NMR COSY and gHMBC data for **6** (see Table 1) supported this assignment; however, to provide unambiguous evidence, we undertook the total synthesis of (±)-echinobetaine A (**16**) as outlined in Scheme 1.

Preparation of the benzoate intermediate **13** proceeded in six steps following the method of Bosies et al. (see Scheme 1).[11] Quantitative conversion of the readily available diester **7** to the acetal **8** was followed by quantitative conversion to the ester **9**. Decarboxylation of **9** yielded **10** as a mixture of *cis* and *trans* isomers (3:4), while subsequent reduction and methylation returned the alcohol **11** (96%) and methyl ether **12** (91%), respectively. Opening of the acetal in **12** with NBS yielded the key intermediate **13** (95%), which was smoothly converted to the corresponding trimethylammonium **14** (98%). Acid hydrolysis of the benzoate ester moiety in **14** returned the alcohol **15**, while unoptimized oxidation of **15** returned (±)-echinobetaine A (**16**) in 25% yield after purification by HPLC. Synthetic (±)-echinobetaine A (**16**) was identical with natural (−)-echinobetaine A (**6**) by NMR, ESI(+)MS, and IR and by coelution on HPLC.

Scheme 2. Synthesis of the (±)-2-Methoxy-γ-aminobutyric Acid Betaine Methyl Ether (**17**)[a]



[a] (a) Ag_2O, MeI, 99%; (b) NaOH, 100%; (c) $PhCH_2Br$, DMF, 30%; (d) i PTsOH, ii NMe_3, 56%.

Of particular note, the nematocidal properties for (±)-echinobetaine A (**16**) were an order of magnitude weaker than those for the natural product (−)-echinobetaine A (**6**) (LD_{99} 83 μg/mL), highlighting the significance of stereochemistry in the echinobetaine A pharmacophore. Ongoing studies are directed at assignment of absolute stereochemistry to (−)-echinobetaine A (**6**), as well as chiral resolution of (±)-echinobetaine A (**16**), to yield authentic synthetic samples of both enantiomers to support future SAR investigations.

In an attempt to further define the nematocidal pharmacophore revealed by echinobetaine A (**6**) we prepared the isomer **17** in four steps from (±)-α-hydroxybutyrolactone (**18**) (see Scheme 2). In this sequence commercially available **18** was converted to the methyl ether **19**, which was subsequently ring opened to the hydroxycarboxylate salt **20**. The salt **20** was then converted to the benzyl ester **21**, which was in turn transformed into the target product, (±)-2-methoxy-γ-aminobutyric acid betaine methyl ether (**17**). The isomeric synthetic analogue **17** displayed only very modest nematocidal activity (LD_{99} 438 μg/mL), whereas all intermediates prepared during the synthesis of **16** and **17** were inactive. From this bioassay data it would appear that the (−)-echinobetaine A (**6**) nematocidal pharamacophore does not extend to isomeric methoxy betaines in general and does appear to be stereo-dependent.

(−)-Echinobetaine A (**6**) represents the first example of a new class of antiparasitic natural products and illustrates the all too often unrealized and untapped potential of small molecular weight water-soluble marine metabolites. Given the promising antiparasitic activity displayed by the original *Echinodictyum* extract, we are confident that as yet unidentified water-soluble metabolites with even greater antiparasitic activity await discovery. Our future efforts will extend synthetic studies to further explore and define the (−)-echinobetaine A (**6**) pharmacophore, as well as support ongoing exploration of *Echinodictyum* extracts to detect, isolate, and identify new and hopefully more potent antiparasitics.

Experimental Section

General Experimental Procedures. As previously described.[12]

Animal Material. An *Echinodictyum* sp. (Museum of Victoria Registry Number MVF88741) of marine sponge was collected during a scientific expedition to the Great Australian Bight aboard the *RV Franklin* in July 1995, as previously described.[7]

Extraction and Isolation. The EtOH extract of the *Echinodictyum* specimen was decanted and concentrated in vacuo to yield a bright yellow-orange solid (4.08 g), which was

triturated with CH_2Cl_2. The CH_2Cl_2–insoluble materials were partitioned between n-BuOH and H_2O. The H_2O solubles (2.96 g) were concentrated in vacuo, and the residue was fractionated by Sephadex G-10 (H_2O) chromatography (2.5 × 30 cm column), followed by isocratic C_{18} HPLC (2.0 mL/min H_2O + 0.1% TFA through a Zorbax 10 μm 250 × 10 mm column) to yield (−)-echinobetaine A (**6**) (25 mg, 0.044% specimen dry weight) as the TFA salt. All fractionation studies were supported by bioassay.

(−)-Echinobetaine A (6): $[\alpha]^{22}_D$ −49° (*c* 0.59, MeOH); IR (neat) ν_{max} 1683 cm^{-1}; ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100 MHz, D_2O), see Table 1; HRESI(+) MS *m/z* 176.1283 (calcd for $C_8H_{18}NO_3$, 176.1287).

Diethyl 6-Phenyl-[1,5]-dioxane-3,3-dicarboxylic Acid Diester (8). A solution of diethyl bis-hydroxymethylmalonate (**7**) (22.0 g, 0.1 mol), PhCHO (10.6 g, 0.1 mol), and *p*-TsOH (0.2 g) was refluxed in toluene (200 mL) for 24 h using a Dean Stark trap, after which the toluene was removed in vacuo and the residue purified by distillation to afford the acetal **8** (7.64 g, 25%): bp_{1mmHg} 160−170 °C; IR (Nujol) ν_{max} 1741 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43−7.31 (m, Ph), 5.46 (s, 6-H), 4.83 (d, J = 11.5 Hz, 2- and 4-H_{2a}), 4.31 (q, J = 7.1 Hz, CO_2CH_2-CH_3), 4.17 (q, J = 7.1 Hz, $CO_2CH_2CH_3$), 4.14 (d, J = 11.5 Hz, 2- and 4-H_{2b}), 1.29 (t, J = 7.1 Hz, $CO_2CH_2CH_3$), 1.23 (t, J = 7.1 Hz, $CO_2CH_2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.6 and 166.7 (2 × $CO_2CH_2CH_3$), 137.3, 129.0, 128.1, and 126.0 (Ph), 101.6 (C-6), 69.4 (C-4 and C-2), 61.9 ($CO_2CH_2CH_3$), 53.1 (C-3), 13.9 ($CO_2CH_2CH_3$); ESI(+)MS *m/z* 331 (M + Na).

Ethyl 6-Phenyl-[1,5]-dioxane-3,3-dicarboxylic Acid Monoester (9). An EtOH solution of the acetal **8** (6.58 g, 0.021 mol) and KOH (1.71 g, 0.03 mol) was stirred at RT for 4 h, after which the EtOH was evaporated in vacuo and the residue treated with 1 M HCl (40 mL) to return the monoester **9** as a white solid (5.50 g, 95%): IR (KBr) ν_{max} 3280−2544 (br), 1741, 1716 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45−7.35 (m, Ph), 6.03 (s, CO_2H), 5.50 (s, 6-H), 4.88 (d, J = 11.6 Hz, 2- and 4-H_{2a}), 4.35 (q, J = 7.2 Hz, $CO_2CH_2CH_3$), 4.18 (d, J = 11.6 Hz, 2- and 4-H_{2b}), 1.34 (t, J = 7.2 Hz, $CO_2CH_2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9 (CO_2H), 167.5 ($CO_2CH_2CH_3$), 137.1, 129.3, 128.3, and 126.1 (Ph),101.8 (C-6), 69.3 (C-4 and C-2), 62.5 ($CO_2CH_2CH_3$), 53.2 (C-3), 14.0 ($CO_2CH_2CH_3$); ESI(+)MS *m/z* 303 (M + Na).

Ethyl 6-Phenyl-[1,5]-dioxane-3-carboxylic Acid Ester (10). An anhydrous pyridine solution (10 mL) of the ester **9** (5.0 g, 0.018 mol) and piperidine (30 μL) was refluxed for 5 h, after which the solution was added dropwise to ice cold HCl_{aq} (37%, 13 mL). The resulting solution was extracted with CH_2Cl_2 (3 × 50 mL) and the combined organic layer dried (anhydrous $MgSO_4$) and concentrated in vacuo to afford the decarboxylated product **10** as a mixture of minor (43%) and major (57%) isomers (3.0 g, 72%): IR (Nujol) ν_{max} 1736 cm^{-1}; *minor isomer* ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49−7.35 (m, Ph), 5.52 (s, 6-H), 4.73 (d, J = 11.0 Hz, 2- and 4-H_{2a}), 4.28 (q, J = 7.0 Hz, $CO_2CH_2CH_3$), 4.11 (dd, J = 11.0, 3.3 Hz, 2- and 4-H_{2b}), 2.41 (br m, 3-H), 1.32 (t, J = 7.0 Hz, $CO_2CH_2CH_3$); *major isomer* ^{1}H NMR δ7.49−7.35 (m, Ph), 5.44 (s, 6-H), 4.47 (dd, J = 11.9, 4.8 Hz, 2- and 4-H_{2a}), 4.16 (q, J = 7.0 Hz, CO_2CH_2-CH_3), 4.00 (dd, J = 11.9, 11.9 Hz, 2- and 4-H_{2b}), 3.42 (m, 3-H) 1.23 (t, J = 7.0 Hz, $CO_2CH_2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1 and 169.9 (2 × $CO_2CH_2CH_3$), 138.0, 137.7, 129.0, 128.9, 128.2, 128.1, 126.1, and 126.0 (Ph), 101.8 and 101.3 (2 × C-6), 68.0 and 67.1 (2 × C-4 and C-2), 61.1 and 60.8 (2 × CO_2CH_2-CH_3), 39.9 (C-3), 14.1 (2 × $CO_2CH_2CH_3$); ESI(+)MS *m/z* 259 (M + Na).

(6-Phenyl-[1,5]-dioxan-3-yl)methanol (11). The monoester **10** (3.3 g, 0.014 mol) was treated with $LiAlH_4$ (2.3 g, 0.061 mol) in refluxing dry Et_2O (20 mL) under a N_2 atmosphere for 3 h. The reaction was then quenched with ice and following the dropwise addition of EtOAc (18 mL), water (35 mL), and 15% NaOH (9 mL) was extracted with Et_2O (3 × 50 mL). After drying (anhydrous $MgSO_4$) the organic phase was concentrated in vacuo to yield the alcohol **11** (2.5 g, 94%) as minor (42%) and major (58%) isomers: IR (Nujol) ν_{max} 3390−3308 (br) cm^{-1}; *minor isomer* ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50−7.32 (m, Ph), 5.52 (s, 6-H), 4.25 (d, J = 11.2 Hz, 2- and 4-H_{2a}), 4.15−4.11 (m, 2- and 4-H_{2b}), 4.06 (d, J = 7.6 Hz, CH_2-OH), 1.69 (br s, 3-H); *major isomer* ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50−7.32 (m, Ph), 5.43 (s, 6-H), 4.32 (dd, J = 11.6, 4.8 Hz, 2- and 4-H_{2a}), 3.74 (dd, J = 11.6, 11.6 Hz, 2- and 4-H_{2b}), 3.52 (d, J = 6.0 Hz, CH_2OH), 2.39 (m, 3-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.3, 138.1, 128.9, 128.8, 128.2, 126.0, and 125.9 (Ph), 101.8 and 101.4 (2 × C-6), 69.6 and 67.7 (2 × C-4 and C-2), 61.5 and 60.9 (2 × CH_2OH), 36.8 and 36.4 (2 × C-3); ESI-(+)MS *m/z* 217 (M + Na).

3-Methoxymethyl-6-phenyl-[1,5]-dioxane (12). A dry THF solution of the alcohol **11** (2.48 g, 0.013 mol) and NaH (1.53 g, 0.064 mol) was refluxed under a N_2 atmosphere for 1 h, after which MeI (9 mL, 0.11 mol) was added dropwise and the reaction refluxed for a further 2 h. On cooling the reaction mixture was extracted with Et_2O (3 × 50 mL), and the organic phase washed with brine (3 × 20 mL), dried (anhydrous $MgSO_4$), and concentrated in vacuo to yield the methyl ether **12** (2.46 g, 91%) as minor (36%) and major (64%) isomers: *minor isomer* ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50−7.31 (m, Ph), 5.50 (s, 6-H), 4.21 (d, J = 10.8 Hz, 2- and 4-H_{2a}), 4.11−4.08 (m, 2- and 4-H_{2b}), 3.76 (d, J = 7.2 Hz, CH_2OMe), 3.41 (s, CH_2OMe), 1.74 (br m, 3-H); *major isomer* ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50−7.32 (m, Ph), 5.42 (s, 6-H), 4.28 (dd, J = 11.8, 4.4 Hz, 2- and 4-H_{2a}), 3.73 (dd, J = 11.8, 11.8 Hz, 2- and 4-H_{2b}), 3.32 (s, CH_2OMe), 3.24 (d, J = 5.6 Hz, CH_2OH), 2.47 (m, 3-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.4, 138.2,128.5, 127.9, 125.8, and 125.7 (Ph), 101.5 and 101.1 (2 × C-6), 71.3 and 70.6 (2 × CH_2OMe), 69.5 and 67.6 (2 × C-4 and C-2), 58.7 and 58.6 (2 × CH_2OMe), 34.6 and 34.4 (2 × C-3); ESI(+)MS *m/z* 231 (M + Na).

3-Bromo-2-(methoxymethyl)-1-propyl Benzoate (13). A mixture of the methyl ether **12** (2.46 g, 0.012 mol), freshly recrystallized *N*-bromosuccimide (2.2 g, 0.006 mol), and $BaCO_3$ (0.359 g, 0.002 mol) was refluxed in dry CH_2Cl_2 (30 mL) for 24 h under a N_2 atmosphere. After cooling, the reaction was concentrated in vacuo and the residue triturated with hexane, filtered, and dried in vacuo to yield the bromobenzoate **13** (3.3 g, 95%): IR (neat) ν_{max} 1724 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (d, J = 7.4 Hz, 2′-H and 6′-H), 7.58 (t, J = 7.4 Hz, 4′-H), 7.46 (t, J = 7.4 Hz, 3′-H and 5′-H), 4.45 (dd, J = 11.2, 6.4 Hz, 1-H_{2a}), 4.38 (dd, J = 11.2, 6.4 Hz, 1-H_{2b}), 3.61 (d, J = 5.6 Hz, 4-H_2), 3.56 (dd, J = 7.8, 5.0 Hz, 3-H_{2a}), 3.51 (dd, J = 7.8, 5.0 Hz, 3-H_{2b}), 3.37 (s, OMe), 2.47 (m, 2-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0 (CO_2Ph), 132.9, 129.8, 129.4, and 128.3 (CO_2Ph), 70.9 (C-4), 63.6 (C-1), 58.9 (OMe), 40.5 (C-2), 31.9 (C-3); ESI(+)MS *m/z* 309/311 (M + Na).

(±)-3-Trimethylammonium-2-(methoxymethyl)-1-propyl) Benzoate (14). A solution of the bromobenzoate **13** (3.3 g, 0.013 mol) in 23% trimethylamine in dry benzene (200 mL) was stirred for 48 h, after which the resulting precipitate was filtered and washed with Et_2O (50 mL) to yield the insoluble trimethylammonium salt **14** (3.40 g, 98%). Recrystallization from methanol/EtOAc returned a white solid with a melting point of 125−127 °C: IR (Nujol) ν_{max} 1720 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 229 (7400); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 7.8 Hz, 2′-H and 6′-H), 7.56 (t, J = 7.8 Hz, 4′-H), 7.43 (t, J = 7.8 Hz, 3′-H and 5′-H), 4.55 (dd, J = 11.8, 5.2 Hz, 1-H_{2a}), 4.34 (dd, J = 11.8, 7.2 Hz, 1-H_{2b}), 3.86 (dd, J = 13.4, 4.4 Hz, 3-H_{2a}), 3.70 (dd, J = 13.4, 3.2 Hz, 3-H_{2b}), 3.61 (dd, J = 9.2, 4.4 Hz, 4-H_{2a}), 3.51 (s, NMe_3), 3.46 (dd, J = 9.2, 7.2 Hz, 4-H_{2b}), 3.34 (s, OMe), 2.71 (br m, 2-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3 (CO_2Ph), 133.3, 129.5, 129.1, and 128.4 (CO_2Ph), 71.4 (C-4), 65.8 (C-3), 64.3 (C-1), 59.1 (OMe), 53.6 (NMe_3), 34.7 (C-2); HRESI(+)MS *m/z* 266.1764 (calcd for $C_{15}H_{24}NO_3$, 266.1756).

(±)-3-Trimethylammonium-2-(methoxymethyl)-1-propanol (15). The trimethylammonium salt **14** (1.65 g, 0.006 mol) was refluxed for 24 h in 1 M HCl (80 mL), then washed with EtOAc, and the aqueous layer was concentrated in vacuo to yield the alcohol **15** (840 mg, 87%). Recrystallization from MeOH/EtOAc returned flake-like crystals with a melting point of 123−124 °C: IR (Nujol) ν_{max} 3275 cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ 3.49 (dd, J = 11.6, 5.7 Hz, 3-H_{2a}), 3.42 (dd, J = 11.6, 5.7 Hz, 3-H_{2b}), 3.36 (dd, J = 10.4, 5.2 Hz, 1-H_{2a}), 3.30 (dd, J = 10.4, 6.4 Hz, 1-H_{2b}), 3.19 (d, J = 4.0, Hz, 4-H_2), 3.23 (s, OMe), 3.00 (s, NMe_3), 2.19 (m, 2-H); ^{13}C NMR (100 MHz, D_2O) δ 71.5

(C-4), 65.5 (C-3), 60.9 (C-1), 58.4 (OMe), 53.2 (NMe_3), 36.0 (C-2); HRESI(+)MS *m/z* 162.1489 (calcd for $C_8H_{20}NO_2$, 162.1494).

(±)-Echinobetaine A (16). A 54% $AcOH_{aq}$ solution of the alcohol **15** (0.142 g, 0.97 mmol) and $KMnO_4$ (0.278 g, 1.8 mmol) was stirred for 3 h at 50 °C, after which the reaction was filtered and concentrated in vacuo. The resultant solid was eluted through a C_{18} solid-phase extraction (SPE) cartridge with H_2O. The solvent was removed in vacuo and the residue subjected to isocratic C_{18} HPLC (2.0 mL/min H_2O + 0.1% TFA, Zorbax 10 μm 250 × 10 mm column) to yield (±)-echinobetaine A (**16**) (43 mg, 25%): IR, ^{1}H NMR (400 MHz, D_2O), and ^{13}C NMR (100 MHz, D_2O) identical with (−)-echinobetaine A (**6**); HRESI(+)MS *m/z* 176.1282 (calcd for $C_8H_{18}NO_3$, 176.1287).

(±)-2-Methoxy-γ-butyrolactone (19). A solution of the (±)-2-hydroxy-γ-butyrolactone (**18**) (1.87 g, 18.3 mmol), MeI (11 mL, 176 mmol), and Ag_2O (17.8 g, 77 mmol) was refluxed under a N_2 atmosphere in $CHCl_3$ (50 mL) for 1 h. The reaction was cooled and filtered, and the clear solution was concentrated in vacuo to yield the methoxy butyrolactone **19** (2.10 g, 99%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.40 (m, 4-H_{2a}), 4.23 (m, 4-H_{2b}), 4.02 (t, J = 7.6 Hz, 2-H), 3.56 (s, OMe), 2.50 (m, 3-H_{2a}), 2.23 (m, 3-H_{2b}); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.6 (C-1), 74.6 (C-2), 65.0 (C-4), 57.6 (OMe), 28.9 (C-3).

Sodium (±)-4-Hydroxy-2-methoxybutyrate (20). A solution of the methoxy butyrolactone **19** (2.10 g, 18.1 mmol) in 2 M NaOH (6 mL) and MeOH (6 mL) was stirred for 16 h at RT and the reaction mixture concentrated in vacuo to return the crude sodium salt **20** (2.80 g, 100%), which was used without further purification: ^{1}H NMR (D_2O, 300 MHz) δ 3.56 (dd, J = 8.1, 4.8 Hz, 2-H), 3.48 (t, J = 7.2 Hz, 4-H_2), 3.12 (s, OMe), 1.70 (m, 3-H_2); ^{13}C NMR (D_2O, 75 MHz) δ 180.3 (C-1), 79.9 (C-2), 58.2 (C-4), 57.0 (OMe), 35.0 (C-3).

Benzyl (±)-4-Hydroxy-2-methoxybutanoate (21). The sodium salt **20** (2.80 g, 18.00 mmol), benzyl bromide (2.2 mL, 18.7 mmol), and 18-crown-6-(1,4,7,10,13,16-hexaoxacyclooctadecane) (1.60 g, 6.2 mmol) were stirred at 40 °C in DMF (30 mL) under a N_2 atmosphere for 4 days, after which the reaction mixture was quenched with H_2O (30 mL), extracted with Et_2O, washed with brine, dried over anhydrous Mg_2SO_4, and concentrated in vacuo to yield the benzyl ester **21** (1.20 g, 30%), which was purified by rapid silica filtration eluting with 30% ethyl acetate/petroleum spirit: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.37 (m, OCH_2Ph), 5.23 (d, J = 12.4 Hz, $OCH_{2a}Ph$), 5.18 (d, J = 12.4 Hz, $OCH_{2b}Ph$), 4.03 (dd, J = 7.6, 4.8 Hz, 2-H), 3.76 (t, J = 5.2 Hz, 4-H_2), 3.43 (s, OMe), 2.01 (m, 3-H_2); ^{13}C NMR (D_2O, 100 MHz) δ 172.2 (C-1), 128.6, 128.5, 128.4, and 128.3 (OCH_2Ph), (79.1 (C-2), 66.7 (OCH_2Ph), 59.7 (C-4), 58.4 (OCH_3), 35.1 (C-3).

(±)-2-Methoxy-γ-aminobutyric Acid Betaine Methyl Ether (17). A solution of the benzyl ester **21** (1.20 g, 5.35 mmol) and *p*-toluenesulfonyl chloride (2.04 g, 10.7 mmol) was stirred in dry pyridine at 5 °C for 3 h. Diethyl ether was added and the organic phase washed sequentially with water, 5% copper sulfate, and water, after which it was dried with anhydrous $MgSO_4$ and concentrated in vacuo to yield a 4-tosyl intermediate (1.39 g, 68%). The tosyl intermediate was heated to 95 °C with a solution of trimethylamine in benzene in a sealed tube for 16 h, after which the reaction mixture was concentrated in vacuo to a clear, viscous oil (1.60 g, 91%), which was dissolved in 4 M HCl (15 mL) and dioxane (5 mL) and stirred at RT for 16 h. The reaction mixture was concentrated in vacuo to yield a viscous oil, which was purified by C_{18} SPE (10% stepwise elution from 100% H_2O to 100% MeOH). The 100% H_2O fractions were combined, concentrated in vacuo, and further purified by anion exchange SPE (preconditioned and sample loaded from 10 mL of 0.1 M $NaCl_{aq}$, elution with H_2O) to yield the trimethylammonium **17** (350 mg, 56%): IR (neat) ν_{max}1736 cm^{-1}; ^{1}H NMR (D_2O, 400 MHz) δ 3.89 (dd, J = 8.0, 3.6 Hz, 2-H), 3.30 (dt, J = 12.4, 4.8 Hz, 4-H_2), 3.23 (s, OMe), 2.96 (s, NMe_3), 2.20 (m, 3-H_{2a}), 2.00 (m, 3-H_{2b}); ^{13}C NMR (D_2O, 75 MHz) δ 174.8 (C-1), 76.8 (C-2), 62.8 (C-4), 58.1 (OMe), 53.1 (NMe_3), 25.5 (C-3); HRESI(+)MS *m/z* 176.1282 (calcd for $C_8H_{18}NO_3$, 176.1287).

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