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STRUCTURE AND SYNTHESIS OF A NEW BUTANOLIDE FROM A MARINE ACTINOMYCETE

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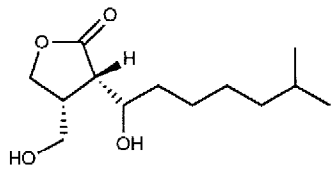
Summary: *The novel butanolide* **2**, (1'*R*, 2*S*, 4*S*)-2-(1-hydroxy-6-methylheptyl)-4-hydroxymethyl-butanolide, *was isolated from the culture broth of a marine actinomycete. The structure of* **2** *was assigned by analysis of spectral data and the absolute configuration was determined by synthesis.*

Actinomycetes are a rich source of secondary metabolites with diverse biological activities.[1] A class of butanolides known as signal compounds, which initiate and regulate the production of secondary metabolites, has been isolated from some actinomycete species, e.g. virginiae butanolide A (**1**) isolated from *S. virginiae* .[2] As a part of an ongoing project to study the secondary metabolites of marine bacteria, we investigated the fermentation products of an actinomycete species, CNB-228, isolated from sediments in the Bahamas Islands. From the fermentation broth we isolated a butanolide (**2**), which is similar to those of the previously known virginiae butanolides, but possesses a markedly different substitution pattern on the γ-lactone ring. Herein we report the isolation, structure elucidation and total synthesis of this compound.

The actinomycete was grown in liquid culture and the whole culture broth was extracted with ethyl acetate.[3] The organic extract was separated by flash chromatography on silica using a solvent gradient (isooctane/ethyl acetate), followed by normal phase HPLC using 1:1 ethyl acetate/isooctane, to obtain **2** as a pale yellow oil.



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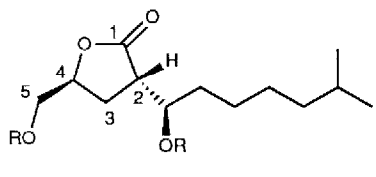


2, R = H
3, R = Ac

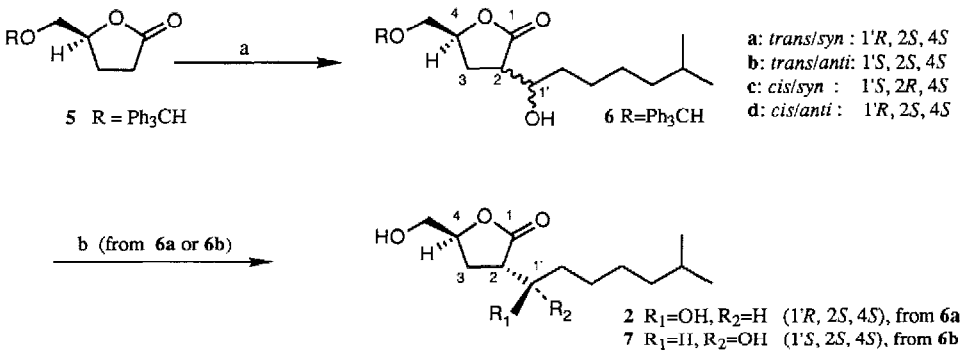
A molecular formula of $C_{13}H_{24}O_4$ was determined by HREIMS and agreed with ^{13}C and ^{1}H NMR data.[4] The absorption bands observed in the IR spectrum of **2** at 3350 and 1750 cm^{-1} indicated OH and ester functionalities. Proton NMR COSY experiments readily defined the C2-

C5 fragment as well as an isopropyl group. To satisfy the molecular formula, which requires one degree of unsaturation, **2** had to be monocyclic. This required that two of the oxygen bearing carbons be carbinols and the other the linked to the ester moiety to form a ring. Upon treatment with acetic anhydride in pyridine, **2** yielded a diacetate **3**. When the ^{1}H NMR spectra of **2** and **3** were compared,[4] the chemical shifts of the resonances assigned to protons on C2 and C5 showed significant differences whereas the chemical shift of the resonance assigned to the proton at C4 was unchanged. Thus **2** contained a γ-lactone ring with the ester linkage at C4.

An nOe effect between H2 and H3β and also between H4 and H3α established the relative stereochemistry of the lactone ring shown in **2**. In order to establish the configuration of **2** at C1', both epimers at C1' were synthesized (Scheme 1). Synthesis of target **2** relied on the stereocontrolled aldol condensation between 6-methylheptanal[5] and lactone **5**[6] in which the bulky substituent at C-4 was expected to play a decisive role in the formation of isomers **6a-d**. The stereochemical assignment of **6a-d** was carried out by a combination of techniques: The *trans* or *cis* relation was unambiguously established by differential nOe experiments between the lactone ring protons and C1'H. The *syn* or *anti* relation was determined on the basis of the H-H coupling constants of the C2-C1' fragment.[9] The epimeric relationship between **6a** and **6b** was confirmed by chemical transformations, since treatment of **6b** under Mitsunobu conditions,[10] followed by alkaline hydrolysis of the intermediate formate, cleanly afforded **6a**. The relative stereochemistry of **6b** was determined by X-ray diffraction analysis[11] to directly confirm the above stereochemical assignments.

Condensation of **5** with 6-methylheptanal under kinetic conditions[7] proceeded with reasonable stereocontrol (*trans/cis* ratio 85/15, based on isolated compounds). The stereoselection observed for the new chiral center at C1' was in agreement with the postulated operating model for these condensations,[8] *anti* isomers being the major products. Thus, *trans/anti* (1'*S*,2*S*) vs. *trans/syn* (1'*R*,2*S*) ratios of around 70/30 were observed.[9] Chromatographic separation of the mixture of diastereomers **6** followed by removal of the protecting group afforded target **2**. Both natural and synthetic **2** had identical ^{1}H NMR spectra, with a 2.6 Hz coupling between H1' and H2. The analogous coupling observed for synthetic epimer **7** was 8 Hz. This established the relative stereochemistry of **2** at C1'. The assignment of the absolute configuration of **2** as shown follows from the identical specific rotations observed for both natural and synthetic **2** (+19.1° and +22.3° respectively). Conformational analysis using molecular mechanics[12] predicted hydrogen bonding between the C1'OH hydrogen and the ester carbonyl in **2**. This H-bonding causes the infrared carbonyl absorption of **2** to appear at 1750 cm^{-1}—an unusually low value for a γ-lactone.

Signal butanolides play significant, and only partially undestood, roles in the metabolic activities of bacteria of the order Actinomycetales. Although we have not been able to demonstrate a physiological function for butanolide **2**, it is clear that this molecule is closely related to the hydroxymethyl, α-hydroxyalkyl-butanolides of demonstrated importance in the metabolism of terrestrial actinomycetes.



a) (1) LDA, 1.2 equiv., THF, -78°C, 20 min. (2) 6-methylheptanal, THF, -78°C, 1 min. (71%);
b) H_2 Pearlman's catalyst, MeOH (98%)

SCHEME 1

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References and Notes

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2. Sakuda, S.; Yamada, Y., *Tetrahedron Lett.*, **1991**, 32, 1817 and references therein.
3. The unidentified actinomycete (CNB-228) was isolated from a sediment sample collected at -80 ft. depth in the Bahamas Islands. The bacterium was cryo-preserved until used. An inoculum, prepared from a thawed sample, was inoculated into 10 Fernbach flasks (2.5 L vol.) each containing 1L of a medium prepared with starch (10 g), yeast extract (4g), peptone (2g), sea water 750 mL, deionized water 250 mL and tris buffer (pH 8.0) 10 mL and the flasks were shaken at 230 rpm at room temperature for 7 days. The whole culture broth was extracted 2x with equal volumes of EtOAc (20 L total). The EtOAc extract was dried (Na_2SO_4) and subjected to standard vacuum flash silica chromatography. Fractions containing **2** were combined and purified by HPLC (1:1 EtOAc/isooctane) to obtain **2** as a pale yellow oil (12 mg from 10 L of culture).
4. Compound **2**: $[\alpha]_D$=+22.3 (c=0.61, CH_2Cl_2), ^{1}HNMR (400 MHz, $CDCl_3$), 0.81 (d, J=7.2 Hz, 6H, CH_3 isopropyl), 1.05-1.55 (m, 9H, aliphatic side chain), 2.05 (ddd, J=4Hz, J'=10 Hz, J"=12.8 Hz, 1H, C3Hβ), 2.35 (ddd, J=J'=8 Hz, J"=12.8 Hz, 1H, C3Hα), 2.78 (ddd, J=2.6 Hz, J'=8Hz, J"=10.5 Hz, 1H, C2H), 3.55 (dd, J=7Hz, J'=2.5Hz, 1H, C5H), 3.83 (dd, 1H, J=7Hz, J'=1.5 Hz, C5'H), 4.08 (m, 1H, C1'H), 4.62 (m, 1H, C4H); ^{13}CNMR ($CDCl_3$): 179.9(C1), 80.0(C4), 69.9(C1'), 64.4(C5), 46.3(C2), 38.8(C2'), 34.9(C3'), 27.8(C6'), 27.1(C4'), 26.0(C5'), 23.0(C3), 22.5(2 x C7').

   Compound **3**: ^{1}H NMR (400 MHz, $CDCl_3$), 0.86 (d, J=7 Hz, 6H, CH_3 isopropyl), 1.30-1.51 (m, 9H, aliphatic side chain), 2.04 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.14 (ddd, J=4Hz, J'=10Hz,

J"=13Hz, 1H, C3Hβ), 2.43 (ddd, J=J'=8Hz, J"=13Hz, 1H, C3Hα), 2.92 (ddd, J=4Hz, J'=8Hz, J"=10Hz, 1H, C2H), 4.14 (dd, J=4Hz, J'=12Hz, 1H, C5H), 4.27 (dd, J=3Hz, J'=12Hz, 1H, C5H'), 4.75 (m, 1H, C4H), 5.30 (ddd, J=4Hz, J'=J"=7Hz, 1H, C1'H); ^{13}C NMR ($CDCl_3$), 75.5 (C4), 72.1(C6), 65.5 (C5), 43.1 (C2), 38.7 (C2'), 32.5 (C3'), 27.9 (C6'), 27.1 (C4'), 25.6 (C5'), 25.1 (C3), 22.6 (2 x C7'), 21.0, and 20.8 (2 x CH_3) (no ester carbonyl carbons were observed due to the small sample size).

Compound **7**: $[\alpha]_D$=+30.1 (c=0.72, CH_2Cl_2), ^1H NMR (400 MHz, $CDCl_3$), 0.79 (d, J=7.2 Hz, 6H, CH_3 isopropyl), 1.08-1.52 (m, 9H, aliphatic side chain), 2.08 (m, 1H, C3Hα), 2.23 (m, 1H, C3Hβ), 2.75 (m, 1H, C2H), 3.60 (dd, 1H, J=6.2 Hz, J'=2.0 Hz, C5H), 3.67 (m, 1H, C1'H), 3.82 (dd, 1H, J=6.2 Hz, J'=1.8 Hz, C5'H), 4.60 (m, 1H, C4H); ^{13}C NMR ($CDCl_3$): 179.2, 79.1, 72.5, 64.3, 45.0, 38.9, 34.7, 27.8, 27.3, 27.2, 25.5, 22.5.

5. Stowell, J.C.; King, B.T. , *Synthesis*, **1984**, 278.
6. Tomioka, K.; Cho, Y.; Sato, F.; Koga,K. , *J.Org.Chem.* **1988**, *53*, 4094, and references cited therein.
7. In a typical experiment, a 0.3 M solution of the starting lactone in THF was added dropwise to 1.2 equiv. of a 1.0 N solution of LDA in THF at -78 °C . After stirring at that temperature for 20 min., 1.2 equiv. of a cooled (-78º C) 0.5 M solution of 6-methylheptanal was added and stirring was maintained for 1 minute at -78ºC. Quenching of the reaction mixture with sat. aq. NH_4Cl followed by ether extraction afforded a crude mixture which was purified by Si flash chromatography with hexanes-EtOAc (70:30) to give pure **6b** and **6c**. Mixtures of the remaining stereoisomers **6a** and **6d**, were purified by flash chromatography using $CHCl_3$-acetone (99:1).
8. Heathcock, C.H. *Asymmetric Synthesis* ; J.D.Morrison, J.W.Scott, Eds.; Academic Press, New York, 1984; Vol. 3, Chapter 2
9. Ratios were determined by HPLC analysis (Whatman analytical Partisil 5 ODS-3 column, 25 cm length; solvent system: MeOH-H_2O 80/20; flow rate 1.5 ml/ml).
10. Mitsunobu, O. *Synthesis*, **1981**, 1.
11. A flat plate of **6b** grown from n-pentane was used for X-ray diffraction analysis and was found to belong to the Monoclinic space group $P2_1$with cell constants a = 10.679(4), b = 18.881(5) and c = 13.937(3) Å and β = 98.7610°. A total of 3907 independent diffraction maxima with 2θ ≤ 115° were collected with θ - 2θ scans and graphite monochromated Cu Kα radiation. The structure was solved with PATSEE and refined with full matrix least-squares refinements with anisotropic heavy atoms and fixed riding hydrogens. The final crystallographic residual was 5.46%. Archival crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1E, UK. Please give a complete literature citation when ordering.
12. Still, W.C., Macromodel V 2.0, Department of Chemistry, Columbia University, New York, NY 10027.

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