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Ene-yne Tetrahydrofurans from the Sponge *Xestospongia muta*. Exploiting a Weak CD Effect for Assignment of Configuration

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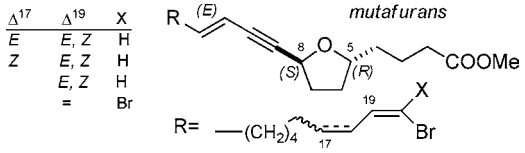
ABSTRACT



Seven new brominated ene-yne tetrahydrofurans (THFs), mutafurans A–G, were isolated from the Bahamian sponge *Xestospongia muta*. The absolute configuration of the natural products was assigned as (5*R*,8*S*) by interpretation of the Cotton effect arising from weak perturbation of an ene-yne chromophore by a propargylic THF ring.

Natural products from species of the tropical marine sponge *Xestospongia*, family Petrosidae, are of interest because of a documented association of these sponges with large populations of symbiotic bacteria[1] and the possibility that bacterial flora may contribute to secondary metabolism of the sponge. Nucleic acid analysis showed that as much as 56% of rRNA in *X. testudinaria* from Western Australia is constituted by bacterial 16S rRNA.[1] Brominated polyunsaturated fatty acids (BPUFAs) are hallmark metabolites of *X. muta*, a common sponge found throughout the Caribbean and *X. testudinaria* which is found in the Indo-Pacific.[2] For convenience, BPUFAs have been characterized often as their methyl esters. The first example, **1** (Figure 1), was described by Schmitz et al.[2a] from *X. muta* in 1978, and subsequently, the dehydro-free fatty acid **2** was characterized by the Scheuer group.[2f]

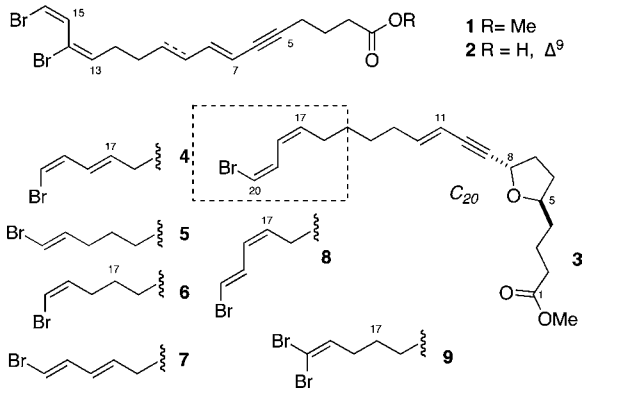


Figure 1. Known brominated polyunsaturated fatty acid **2**, ester **1**, and mutafurans A−G (**3**−**9**).

(1) (a) Brantley, S. E.; Molinski, T. F.; Preston, C. M.; DeLong, E. F. *Tetrahedron* **1995**, *51* (28), 7667−7672. (b) Montalvo, N.; Mohamed, N.; Enticknap, J.; Hill, R. *Antonie van Leewenhoek* **2005**, *87*, 29−36.

(2) (a) Schmitz, F. J.; Gopichand, Y. *Tetrahedron Lett.* **1978**, *19*, 3637−3640. (b) Bourguet-Kondraki, M. L.; Rakotoarisoa, M. T.; Martin, M. T.; Guyot, M. *Tetrahedron Lett.* **1992**, *33* (2), 225−226. (c) Carballeira, N. M.; Shalabi, F. *J. Nat. Prod.* **1993**, *56* (5), 739−746. (d) Fusetani, N.; Li, H.-y.; Tamura, K.; Matsunaga, S. *Tetrahedron* **1993**, *49* (6), 1203−1210. (e) Hirsh, S.; Carmely, S.; Kashman, Y. *Tetrahedron* **1987**, *43* (14), 3257−3261. (f) Ichiba, T.; Scheuer, P. J.; Kelly-Borges, M. *Helv. Chim. Acta* **1994**, *76*, 2814−2816. (g) Patil, A. D.; Kokke, W. C.; Cochran, S.; Francis, T. A.; Tomszek, T.; Westley, J. W. *J. Nat. Prod.* **1992**, *55* (9), 1170−1177. (h) Quinn, R. J.; Tucker, D. J. *Tetrahedron Lett.* **1985**, *26* (13), 1671−1672. (i) Quinn, R. J.; Tucker, D. J. *J. Nat. Prod.* **1991**, *54* (1), 290−294.

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Subsequent reports by us and other investigators described additional ene-yne and polyene-yne fatty acids of common carbon chain lengths C_{16} or C_{18} containing complex combinations of brominated 1,3-dienes and ene-ynes, diynes, ω-brominated acetylenes, and even C_{14} ω,ω-dibromovinylidene terminal groups.[1a] Here, we describe our recent investigations into antifungal compounds from *Xestospongia muta* that uncovered a new family of chiral antifungal brominated ene-yne 2,5-disubstituted tetrahydrofurans which we have named mutafurans A−G (**3**−**9**). Compounds **1**−**6** were found to be active against *Cryptococcus neoformans*, an opportunistic fungus commonly linked to the pathologies of HIV patients.

Compounds **3**−**9** are the first tetrahydrofuranyl BPUFAs from marine sponges. The configurational assignment of **3**−**9** provided a considerable challenge for two reasons: to the best of our knowledge, the (2′-furanyl)-ene-yne group has not been described from nature, and the compounds were available in very low yield (~0.06−1.5 mg) due to their exceedingly low concentrations in the sponge. The structural characterization of **3**−**9** is described along with an assignment of absolute configuration that exploits very weak Cotton effects associated with the ene-yne chromophore asymmetrically perturbed by a propargylic tetrahydrofuran ring.

Four collections of the sponge *Xestospongia muta* from different sites in the Bahamas in 2004 were immediatley frozen and kept at −20 °C until extraction. The CH_2Cl_2-soluble fraction from a MeOH−CH_2Cl_2 (2:1) extract of one specimen (04-15-042) was treated with TMS−diazomethane, and purification of the mixture by filtration through silica followed by reversed-phase HPLC gave the methyl esters of the known carboxylic acids **1** and **2**, a new optically active compound mutafuran A (**3**, Figure 1), $[\alpha]_D^{23}$ −19.4 (*c* 0.248, MeOH), and related mutafurans B−G (**4**−**9**).

The molecular formula of **3** was established as $C_{21}H_{29}O_3Br$ by HREIMS (*m*/*z* 408.1262, M^+, Δ −3.2 mmu). Six of the seven double bond equivalents (DBEs) in the structure of **3** could be accounted for by three C=C double bonds, one COOMe group (δ 173.2, s), and a C≡C triple bond based on interpretation of the ^1H and ^{13}C NMR (C_6D_6), DEPT gHSQC, gHMBC, and gCOSY experiments (Table 1). A strong UV band (λ_{max} 238 nm, log ϵ = 4.56) suggested conjugation which was assigned by COSY to a terminal *Z*,*Z*-20-bromo-17,19-diene (δ 5.37, dt, J = 9.9, 7.7 Hz, H17; 6.46, t, J = 10.6 Hz, H18; 6.51, dd, J = 10.6, 6.6 Hz, H19; 5.87, d, J = 6.2 Hz, H20) and an ene-yne: ^{13}C NMR revealed a triple bond (δ 89.6, s, C9; 83.2, s, C10) conjugated to the remaining *E*-double bond (δ 5.52, d, J = 15.8 Hz, H11; 6.06, dt, J = 15.8, 7 Hz, H12). Two oxymethine signals were observed, CH−O (δ 3.99, quint, J = 6.2 Hz, H5) and a low-field propargylic ether CH signal (δ 4.78, t, J = 5.5 Hz, H8), which were mutually correlated by an HMBC cross-peak (H8 to C5).

Additional HMBC cross-peaks were observed from H8 to diastereotopic CH_2 groups at C6 and C7 (Table 1) and the sp hybridized carbons C9 and C10 and the sp^2 carbon C11. Because the FTIR spectrum of **3** lacked OH stretching bands, the remaining oxygen was placed within a tetrahydrofuran ring, which also accounted for the seventh DBE. HMBC correlations from H5 to C3 and H2 to C3 and C4 supported the 2,5-disubstituted tetrahydrofuran ring located four carbons removed from the COOMe terminus (Table 1). Placement of the remaining CH_2 groups in a chain between C12 and C17 completed the structure of **3**.

Table 1. NMR Data for Mutafuran A (**3**)[a]

no.	δ_C[b]	δ_H (mult, J (Hz))	HMBC[c] (H→C)	gCOSY
1	173.2 (C)	-		
2	33.9 (CH_2)	2.11 (t, 7.3)	1, 3, 4	3
3	22.0 (CH_2)	1.61 (m)	1, 2, 4	2, 4
		1.72 (m)[c]		
4	35.1 (CH_2)	1.30 (m)	3, 6	3, 5
		1.43 (m)		
5	78.5 (CH)	3.99 (quint, 6.2)	3	4, 6
6	31.4 (CH_2)	1.10 (m)	3, 4, 6	5, 7
		1.74 (m)[c]		
7	33.8 (CH_2)	1.84 (m)	6, 8, 9	6, 8
8	68.6 (CH)	4.78 (t, 5.5)	5, 6, 7, 9, 10, 11	6
9	89.6 (C)	–		
10	83.2 (C)	–		
11	110.2 (CH)	5.52 (d, 15.8)	9, 10, 12, 13	12
12	144.3 (CH)	6.06 (dt, 15.8,7)	10, 13, 14	11,13
13	32.9 (CH_2)	1.72 (m)		
14	28.3 (CH_2)	1.02 (m)		
15	28.7 (CH_2)			
16	28.1 (CH_2)	1.78(m)		17
17	136.3(CH)	5.37 (dt, 10.6, 7.7)	19	16, 18
18	124.6 (CH)	6.46 (t, 10.6)	20	17, 19
19	127.7 (CH)	6.51 (dd, 10.6, 6.6)	17, 20	18, 20
20	109.1 (CH)	5.87 (d, 6.6)	18, 19	19
OMe	50.9 (CH_3)	3.33 (s)		

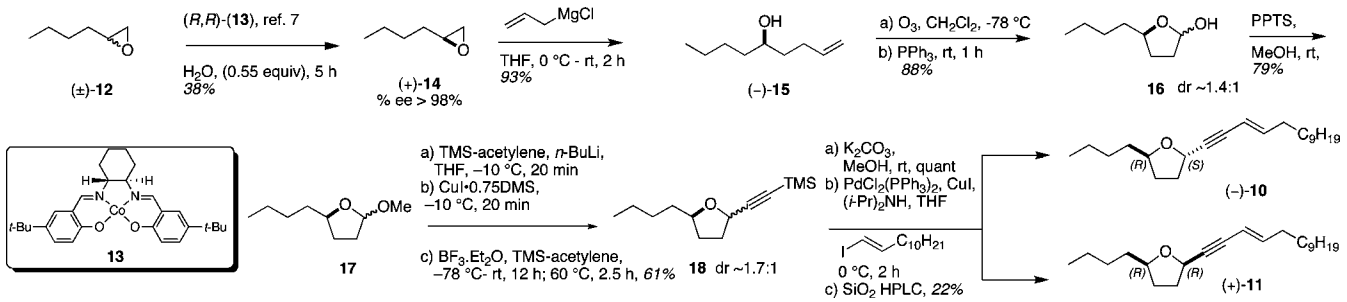
[a] C_6D_6, 400 MHz. [b] 100 MHz, multiplicities assigned from gHSQC and DEPT. [c] 600 MHz, optimized J_{CH} = 8 Hz.

Mutafurans B (**4**), E (**7**), and F (**8**) are isomeric with **3** (HRMS) and are shown by COSY analysis to be double bond isomers at the 20-bromodiene terminus. Compounds **5** and **6** are the 19-*E*- and 19-*Z*-dihydro derivatives of **3** corresponding to reduction of the penultimate double bond C17−C18 of **3**. Mutafuran G (**9**) was isolated in very small amounts (60 μg), sufficient only for HRMS and ^1H NMR. The HRESI-TOFMS mass spectrum of **9** showed an isotope pattern consistent with the presence of two Br atoms and provided a formula of $C_{21}H_{29}O_3Br_2$ (*m*/*z* 489.0616 $[M + H]^+$, Δ −1.8 mmu) which required substitution of an H atom for Br in the formula of **5** or **6**. The ^1H NMR spectrum showed the signals of H2−H12 found in **5** and replacement of the vinyl proton signals with a single olefinic triplet (δ 6.04, t, J = 7.6 Hz, H19). The chemical shift of this signal was identical with that of a terminal ω,ω-dibromovinylidene group in a synthetic long-chain analogue,[3] so we assigned the structure **9** as shown (see Supporting Information for complete details).

The relative configuration of the tetrahydrofuran ring in **3** was assigned by 1D NOESY. Selective spin inversion of

(3) Cf. 1,1-dibromonon-1-ene (^1H NMR, C_6D_6, δ 6.06, t, J = 7.6 Hz, H8), prepared from octanal (CBr_4, PPh_3, CH_2Cl_2).

Scheme 1



the H8 signal gave a weak transannular NOE to the methylene proton signal at C4 but not H5. This suggested a *trans*-disubstituted ring; however, the low S/N from the limited sample compromised a definitive assignment. Confirmation of the assignment was secured by comparison of **3** with model compounds of defined configuration prepared through synthesis (vide infra).

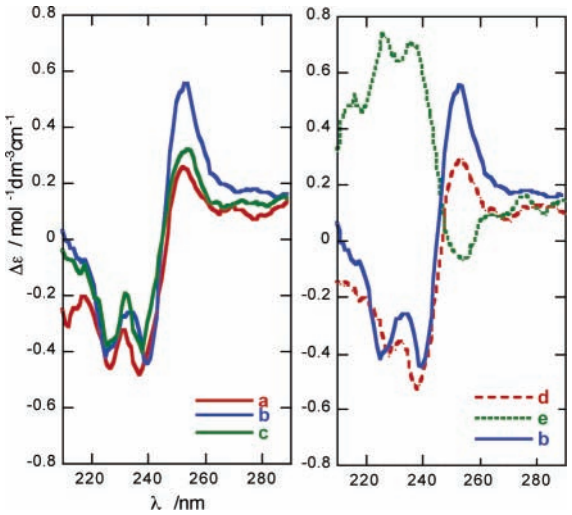


Figure 2. CD spectra for compounds (a) **3**, (b) **5**, (c) **6**, (d) (−)-**10**, and (e) (+)-**11** (25 °C, hexane).

The CD spectrum of **3** (Figure 2) and congeners **5** and **6** were similar, as each displayed a negative Cotton effect (CE) with weak double minima at short wavelengths and a maximum at higher wavelengths (e.g., **3**: λ_{max} 227 ($\Delta\epsilon$ −0.46), 237 ($\Delta\epsilon$ −0.5), 252 (+0.26)). The absolute configuration of **3** was secured from analysis of the CE associated with the ene-yne tetrahydrofuran moiety as follows. Although compound **3** contains two conjugated systems, only the 9-ene-11-yne, and not the terminal diene, is expected to be active in its CD spectrum because of its proximity to the chiral element. No empirical sector rules have been established for determinations of this system. In principle, qualitative MO methods may apply,[4] or the CD spectrum of **3** could be calculated from density functional theory methods (DFT); however, difficulties were anticipated from these approaches due to the presence of rotamers about the C8−C9 bond.

The C8−C9 bond links the axially symmetric triple bond to the tetrahydrofuran ring (first sphere of asymmetry); however, the former is essentially a "free rotor" with a very small barrier to rotation (ΔE < 0.5 kcal mol^{-1}, MM2). Free rotation is expected to average contributions to the CD from all rotamers and give a small but nonzero CE at room temperature; however, *accurate* calculation of the CD spectrum (e.g., DFT) would be made difficult by uncertainties in predicting accurate Boltzmann distributions of C8−C9 rotamers.[5]

To circumvent these ambiguities, two simple optically active model compounds, (−)-**10** and (+)-**11** embodying the conjugated ene-yne tetrahydrofuran ring, were prepared by stereoselective synthesis (Scheme 1). Kinetic resolution of the racemic epoxide (±)-**12** using (*R*,*R*)-**13** (Jacobsen's catalyst[6]) gave the expected diol and unreacted (+)-**14** epoxide [>98% ee, $[\alpha]^{22}$ +19.7° (*n*-hexane), lit.[7] for (*S*)-(−)-1,2-epoxyhexane −18.7° (pentane)]. Addition of allyl magnesium bromide to (+)-**14** gave (*R*)-non-1-en-5-ol [(−)-**15**] which underwent ozonolysis with concomitant ring closure to provide a ~1.4:1 mixture of cyclic acetals **16** (88%).[8]

The corresponding *O*-Me acetals **17** were homologated to a mixture of diastereomeric propargylic THF ethers **18** (~1.7:1 dr) using carefully optimized conditions. Cu(I)-promoted acetylide addition in the presence of BF_3•Et_2O (61%)[9] gave the diastereomeric mixture **18**. Protiodesilylation of the mixture gave volatile acetylenes which were transformed without further purification by Sonogoshira coupling with *E*-1-iodo-1-decene (*E*/*Z* = 4:1)[10] to provide *trans*-(−)-**10** and *cis*-(+)-**11** after separation by normal-phase HPLC. The relative configuration of **10** and

(4) Snatzke, G. *Angew. Chem. Int., Ed.* **1979**, *18*, 363−377.
(5) Even small computation errors for rotamer populations would compound larger errors in the predicted CD spectra.
(6) Tokunaga, M.; Larrow, J. F.; Kakiuchi, F.; Jacobsen, E. N. *Science* **1997**, *277*, 936−938.
(7) Weijers, C. A. G. M.; Botes, A. L.; van Dyk, M. S.; de Bont, J. A. M. *Tetrahedron: Asymmetry* **1998**, *9*, 467−473.
(8) Taber, D. F.; Green, J. H.; Geremia, J. M. *J. Org. Chem.* **1997**, *62*, 9342−9344.
(9) Rychnovsky, S. D.; Dahanukar, V. H. *J. Org. Chem.* **1996**, *61*, 7648−7649.
(10) Miller, M. W.; Johnson, C. R. *J. Org. Chem.* **1997**, *62*, 1582−1583.

11 was secured from 1D NOESY experiments as for **3**. Conversely, irradiation of H8 in *cis*-(+)-**11** gave a moderate NOE of H5 (mutafuran numbering).

The CD spectra of the models *trans*-(−)-**10** and *cis*-(+)-**11** corresponding to (8*S*) and (8*R*) epimers of **3**, respectively (Figure 2), showed weak CEs of similar magnitude at the expected λ_{max} for the ene-yne chromophore. The vibronic fine structures present in these CD spectra are typically seen in the UV spectra of conjugated ene-ynes.

The CEs of diastereomers (−)-**10** and (+)-**11** are essentially equal in magnitude but opposite in sign, respectively, which confirms that *only the C8 configuration* governs the asymmetric perturbation of the tetrahydrofuran-2-yl ene-yne chromophore and the sign of the CE. The sign of the CD spectrum of the natural product **3** matches that of (−)-**10**; therefore, we may assign the (5*R*,8*S*) configuration to **3**, **5**, and **6**. The compounds **7**−**9** likely share the same configuration.

The ene-yne CEs (but not $[\alpha]_D$) appear to be generally correlated with the configuration of the propargylic carbon and relatively independent of the substituents at C5 as shown by (−)-**20a** and (−)-**20b** (Figure 3). The latter compounds

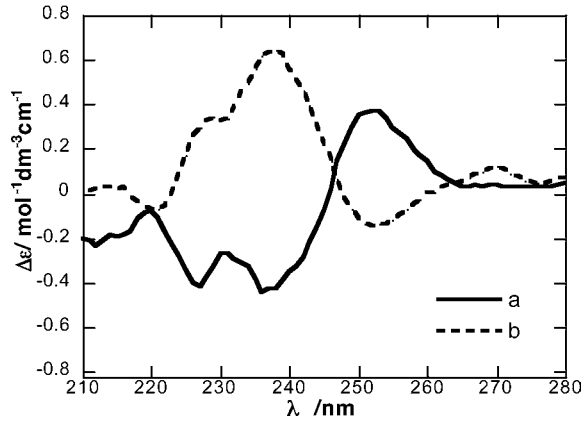
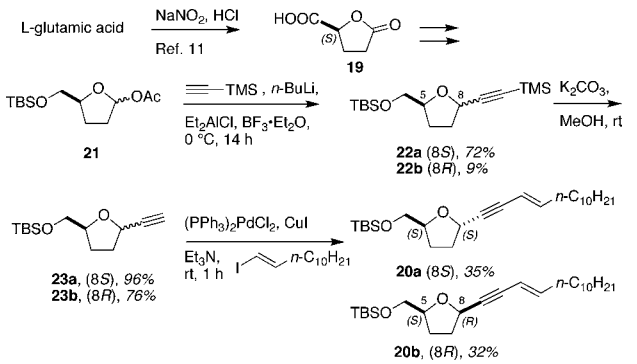


Figure 3. CD spectra of model compounds (hexane, 25 °C) (a) (5*S*,8*S*)-(−)-**20a** and (b) (5*S*,8*R*)-(−)-**20b** (mutafuran numbering).

were prepared from the known (*S*)-lactone **19** derived from (2*S*)-glutamic acid (Scheme 2).[11] Although **20a** and **20b** were both levorotatory, the CD spectra of (5*S*,8*S*)-(−)-**20a** and (5*S*,8*R*)-(−)-**20b** were almost equal and opposite with the CE, again, dictated solely by the configuration of the propargylic carbon in the tetrahydrofuran ring. The similar magnitudes of CEs in (−)-**10**, **3**, **5**, and **6** also suggest that

(11) Numbering of **20a** and **20b** corresponds to that of **3**. Note change of CIP priorities at C5. (a) Okabe, M.; Sun, R. C.; Tam, S. Y. K.; Todaro, L. J.; Coffen, D. L. *J. Org. Chem.* **1988**, *53*, 4780−4786. (b) Paquette, L. A.; Brand, S.; Behrens, C. *J. Org. Chem.* **1999**, *64*, 2010−2025.

Scheme 2 [a]



[a] Locant numbers correspond to that of **3**.

the natural products are optically pure, at least within the experimental error of CD measurement (estimated as ±20%). This would suggest that **3**−**9** are likely the products of biosynthetic enzyme-mediated transformations rather than artifacts that could arise from autoxidation and spontaneous intramolecular cyclization. The biosynthesis of BPUFAs, particularly the origin of the ω-brominated terminus, is presently unknown.

Compounds **1**−**6** showed moderate antifungal activity against the pathogenic fungus *Cryptococcus neoformans var. grubii*. The MICs for compounds **1**−**6** were 8, 16, 8, 8, 8, and 4 μg/mL, respectively. Compounds **1**−**6** were inactive against *Candida albicans* ATCC14503 and the fluconazole-resistant strains *Candida albicans* 96-489 and *Candida glabrata*.

In conclusion, the weak Cotton effects observed in propargylic ene-yne ethers **3**−**9** are correlated with the configuration at the propargylic ether center and may be useful in assignments of this chiral element in similar natural products as they are uncovered.

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Supporting Information Available: Full experimental procedures, characterization of synthetic intermediates, and selected ^{1}H and ^{13}C NMR spectra (42 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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