intrinsic optical activity, is not vulnerable to possible racemization and is a priori an attractive substrate for an NTA-based coupling reaction.

The synthetic sequence employed is depicted in Scheme I.

Treatment of β-alanine with methyl ethylxanthate (1.05 equiv) at 45 °C for 1 h in aqueous sodium hydroxide (1 equiv)/methanol afforded a 91% yield of thionourethane derivative **1** (mp 72–74 °C). Compound **1** was readily cyclized to NTA **2**[7,8] with PBr_3 (0.5 mol, 1.5 equiv) in ethyl acetate (25 °C, 15 min; brine quench). The yield of analytically pure material was 72% [mp 88–90 °C dec (lit.[7] mp 90–92 °C dec)]. The key coupling reaction was carried out by adding **2** to an aqueous solution of L-histidine under conditions of carefully controlled pH (see Experimental Section for specific details). A reaction of this type can be quite impractical when the resulting product is very water soluble. The necessary pH manipulations (addition of aqueous NaOH solution during the reaction and subsequent acidification with 12 N HCl) generate substantial quantities of sodium chloride. In our initial studies we found that it was virtually impossible to isolate L-carnosine that was not contaminated with varying amounts of salt. The crude, salt-containing dipeptide was difficult to purify by recrystallization; such efforts inevitably resulted in seriously diminished yields. To solve this problem, we used tetraethylammonium hydroxide and formic acid in place of NaOH and HCl. Tetraethylammonium formate, obtained as the side product, is soluble in ethanol and can easily be separated from the desired L-carnosine as follows: Water is evaporated from the reaction mixture and replaced with ethanol. The $Et_4N^+HCO_2^-$ dissolves, and L-carnosine slowly crystallizes from solution. This methodology may be of general utility in the isolation of water-soluble peptides when isoelectric point pH adjustments must be made. Using this technique, we isolated a 79% yield of crude L-carnosine which was readily purified by recrystallization.

The above route to **3** is not only a highly efficient preparation of this interesting compound but also serves to illustrate the potential value of NTA's in peptide synthesis. Further studies are in progress.

Experimental Section

General Procedures. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer Model 21 spectrophotometer. NMR spectra were obtained with a Varian XL-100 or EM 360L spectrometer with Me_4Si as an internal standard. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Microanalyses were performed by the Pfizer Analytical Department.

L-Carnosine (3). L-Histidine (6.2 g, 40 mmol) was stirred as a suspension at 0–5 °C in 60 mL of water. The pH was adjusted to 9.2 with 20% aqueous tetraethylammonium hydroxide, and **2** (10.5 g, 80 mmol) was added portionwise with vigorous stirring. Tetraethylammonium hydroxide was added as needed to maintain pH 8.7–9.2. After completion of the NTA addition, the mixture was stirred until the pH stabilized at 9.2 (1 h) and then acidified with 98% formic acid to pH 4.0–4.5.[9] The pH was adjusted to 8.2 (L-carnosine isoelectric pH) with tetraethylammonium hydroxide and the water evaporated in vacuo. The yellowish, gummy residue was stirred in 600 mL of absolute ethanol; fine white crystals formed, with were collected by filtration, washed with ethanol and then ether, and dried. The isolated yield of L-carnosine was 7.20 g (79%).

A sample of material was recrystallized from aqueous ethanol to give analytically pure **3**: mp 262 °C dec (lit.[5d] mp 260 °C dec); $[\alpha]^{25}_D$ +21.0° (*c* 1.5, H_2O) [lit.[5d] $[\alpha]^{25}_D$ +20.5° (*c* 2, H_2O)]; IR (KBr) 3174, 1639, 1575, 1563 cm^{-1}; ^1H NMR (D_2O) δ 2.64 (t, 2 H, *J* = 6 Hz), 3.04 (AB of ABX, 2 H, J_{AB} = 16 Hz), 3.20 (t, 2 H, *J* = 6 Hz), 4.44 (X of ABX, 1 H, $J_{AX} + J_{BX}$ = 14 Hz), 6.94 (s, 1 H), 7.70 (s, 1 H).

Anal. Calcd for $C_9H_{14}N_4O_3$: C, 47.78; H, 6.24; N, 24.76. Found: C, 47.32; H, 5.82; N, 24.55.

Registry No. **1**, 84040-82-4; **2**, 34653-21-9; **3**, 305-84-0; L-histidine, 71-00-1; β-alanine, 107-95-9; methyl ethylxanthate, 623-54-1.

(8) The NTA can be stored at 0 °C for indefinite periods of time; at 25 °C slow decomposition is observed.

(9) At pH 4.0–4.5 the protecting/activating group is released as COS gas.

Fluorinated Carbohydrates. Use of (Diethylamino)sulfur Trifluoride in the Synthesis of Fluorinated Sugars†

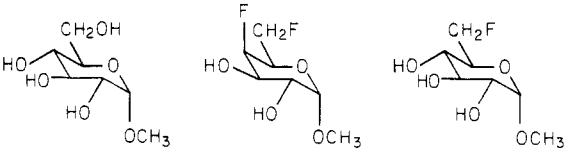
Peter J. Card

Central Research and Development Department, E. I. du Pont de Nemours and Company, Experimental Station, Wilmington, Delaware 19898

Received July 20, 1982

Fluorinated carbohydrates have been widely utilized in biochemical investigations (enzyme–carbohydrate interactions, etc.).[1-3] However, the synthesis of fluorinated sugars is both tedious and time consuming because of the requisite protection and deprotection steps.[4,5] As part of a program concerned with the synthesis of modified carbohydrates, we became interested in facile syntheses of fluorinated derivatives. In particular, we hope to synthesize specifically fluorinated carbohydrates, from unprotected or only partially protected substrates, by use of (diethylamino)sulfur trifluoride (DAST).[6] The simple and selective fluorination of methyl α-D-glucopyranoside presented here is an initial demonstration of this specificity. In addition, we also report preparations of methyl 4,6-dideoxy-4,6-difluoro-α-D-talopyranoside and methyl 2,3,6-tri-*O*-benzoyl-4-deoxy-4-fluoro-α-D-glucopyranoside.

Somawardhana and Brunngraber[7] recently reported that methyl α-D-glucopyranoside (**1**) reacts with neat DAST to



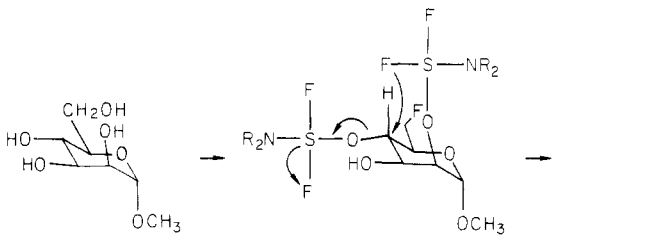
1 **2** **3**

afford methyl 4,6-dideoxy-4,6-difluoro-α-galactopyranoside (**2**) in 60% yield. Sidhu[8] has also reported a similar observation. In contrast to these reports, we have found that treatment of a suspension of **1** in dichloromethane with 6 equiv of DAST, initially at −30 °C and then 1 h at room temperature, gave the monofluorinated product methyl 6-deoxy-6-fluoro-α-D-glucopyranoside[9] (**3**) in 70–88% yield (see Experimental Section). Only a trace of **2** was detected by thin-layer chromatography and use of longer reaction times did not significantly affect the yields of **2** or **3**. However, when added to neat DAST, **3** was cleanly converted into the difluoro derivative **2**. Compound **2** was identical in all respects with material prepared as previously reported.[7] Thus, use of dichloromethane as a reaction solvent instead of neat DAST allows for the selective monofluorination of **1**.

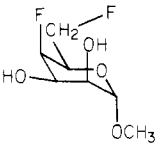
† Contribution no. 3097.

0022-3263/83/1948-0393$01.50/0

When methyl α-D-mannopyranoside (4) was allowed to



4 5

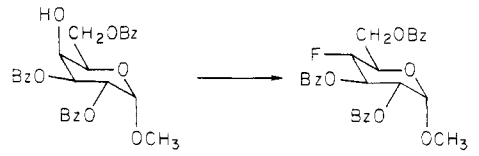


6

react with 5 equiv of DAST in dichloromethane, both the 4- and 6-hydroxyl groups readily reacted to afford methyl 4,6-dideoxy-4,6-difluoro-α-D-talopyranoside (6) in 80% yield. The structure of 6 was assigned on the basis of its ^1H, ^{19}F, and ^{13}C NMR spectra. The ^{19}F NMR spectrum of 6 reveals the presence of two fluorine atoms, one primary fluorine at ϕ −231.9 (F-6) and a secondary fluorine at δ −216.9 (F-4). The ^{13}C NMR spectrum exhibits singlets for C-1 (δ 102) and C-2 (δ 69.4), indicating that no fluorine is on C-2 or C-3. In addition, the rest of the ^{13}C chemical shifts (see Experimental Section) are as expected for a pyranoside, indicating that ring contraction to a furanoside has not occurred. The 360-MHz ^1H NMR spectrum shows H-3 at δ 3.83 ($J_{H\text{-}3,F\text{-}4}$ = 32 Hz), H-4 at δ 4.79 (d, $J_{H\text{-}4,F\text{-}4}$ = 48.6 Hz), H-5 at δ 4.07 ($J_{H\text{-}5,F\text{-}4}$ = 32, $J_{H\text{-}5,F\text{-}6}$ = 13.8 Hz), and H-6,6′ at δ 4.62 (dd, $J_{F\text{-}6,H\text{-}6}$ = 46.6, $J_{H\text{-}5,H\text{-}6}$ = 7.3 Hz). The splitting patterns of H-3, H-4, and H-5 place the secondary fluorine atom at C-4 in an axial position. As expected, substitution of the hydroxyl group at C-4 with fluorine via the DAST reagent has occurred with inversion of configuration.

S_N2 type displacement reactions at C-4 of mannose derivatives are frequently unsuccessful because of the steric hindrance exerted by the axial substituent at C-2.[10] Since under our reaction conditions 1 gave only the monofluorinated product 3, the formation of 6 from 4 suggests the possibility of an intramolecular fluoride ion delivery via an intermediate such as 5, in which an acylated axial substituent at C-2 directs fluoride ion to C-4.

The multistage nature of preparative routes to 4-fluoro derivatives of glucose severely limits their synthetic utility.[4] We have found that the readily available[11] methyl 2,3,6-tri-O-benzoyl-α-D-galactopyranoside (7) provides facile access to these structures. thus, treatment of 7 in di-



7 8

chloromethane with 1.5 equiv of DAST (room temperature, 18 h), followed by workup and chromatography, gave methyl 2,3,6-tri-O-benzoyl-4-deoxy-4-fluoro-α-D-glucopyranoside (8) in 41% yield. The structure of 8 follows from its ^{19}F NMR spectrum, which reveals a fluorine atom at ϕ −197.6, and its 360-MHz ^1H NMR spectrum, which shows H-4 at δ 4.77 (dt, $J_{F\text{-}4,H\text{-}4}$ = 51.3 Hz, $J_{H\text{-}3,H\text{-}4}$ = 9.1 Hz, $J_{H\text{-}5,H\text{-}4}$ = 10 Hz), indicating that 8 has the gluco configuration. The splitting patterns of H-3 and H-5 are also consistent with this structure.

The above methodology affords ready access to fluorinated carbohydrates that have previously been difficult to obtain. Application of this methodology to the synthesis of other fluorinated sugars is in progress.

Experimental Section

General Methods. All reactions were performed under a nitrogen atmosphere. Melting points were determined with a Hoover capillary melting point apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer 241 MC polarimeter. ^1H NMR spectra were obtained in chloroform-*d* on a Nicolet NT WB 360 spectrometer and are referenced to internal tetramethylsilane. The ^{19}F NMR spectra were obtained on a Varian XL-100 spectrometer and are referenced to internal trichlorofluoromethane. ^{13}C NMR spectra were determined on a Bruker WH-90 spectrometer and are referenced to internal tetramethylsilane.

Methyl 6-Deoxy-6-fluoro-α-D-glucopyranoside (3). To a suspension of 1 (970 mg, 5 mmol) in anhydrous dichloromethane (20 mL) at −40 °C was added DAST (3.75 mL, 30 mmol). The cooling bath was removed, and the mixture was allowed to stir for 1 h as it warmed to room temperature. The mixture was cooled to −10 °C, quenched via addition of MeOH (10 mL), and then concentrated under reduced pressure. Chromatography on silica gel (9:1 EtOAc/MeOH) afforded 690 mg (70%) of 3 as a colorless solid: mp 102–104 °C; ^1H decoupled ^{19}F NMR ϕ −234.5 (s); ^{13}C NMR δ 100.8 (C-1), 83.3 (d, C-6, $J_{C\text{-}6,F}$ = 170.6 Hz), 74.9 (C-3), 73.0 (C-2), 71.7 (d, C-5, $J_{C\text{-}5,F}$ = 17.6), 70.2 (d, C-4, $J_{C\text{-}4,F}$ = 7.35 Hz), 55.4 (OCH_3); mass spectrum, *m/e* calcd for $C_6H_{10}FO_4$ ($M^+ - OCH_3$) 165.0563, found 165.0541; $[\alpha]_D$ 148.6° (*c* 1.02, H_2O) [lit.[9] mp 103–104 °C; $[\alpha]_D$ 167° (H_2O)].

Methyl 4,6-Dideoxy-4,6-difluoro-α-D-talopyranoside (6). A suspension of 4 (4.85 g, 25 mmol) in anhydrous dichloromethane (125 mL) at −40 °C was treated with DAST (15.6 mL, 125 mmol). The cooling bath was removed, and the mixture was allowed to stir for 2 h at room temperature. The reaction mixture was subsequently cooled to −20 °C and quenched with MeOH (50 mL). Workup as above and column chromatography on silica gel (9:1 $CHCl_3$/MeOH) yielded 3.92 g (80%) of 6 as a colorless solid: mp 90–92 °C; 360-MHz ^1H NMR δ 2.5 (br d, 1 H, OH, *J* = 7.3 Hz), 3.44 (m, 4 H, OCH_3 and OH), 3.79 (m, 1 H, H-2), 3.83 dm, 1 H, H-3, $J_{H\text{-}3,F\text{-}4}$ = 32 Hz), 4.07 (dm, 1 H, H-5, $J_{H\text{-}5,F\text{-}4}$ = 32 Hz, $J_{H\text{-}5,F\text{-}6}$ = 13.8 Hz), 4.62 (dd, 2 H, H-6 + H-6′, $J_{H\text{-}5,F\text{-}6}$ = 7.3 Hz, $J_{H\text{-}6,F\text{-}6}$ = 46.6 Hz), 4.79 (d, 1 H, H-4, $J_{H\text{-}4,F\text{-}4}$ = 48.6 Hz), 4.88 (d, 1 H, H-1, *J* = 3.5 Hz); ^{19}F NMR (^1H decoupled) ϕ −216.9 (s), −231.9 (s); ^{13}C NMR δ 102.0 (s, C-1), 89.6 (dd, C-6, $J_{C\text{-}6,F\text{-}6}$ = 172, $J_{C\text{-}6,F\text{-}4}$ = 7.35 Hz), 81.9 (dd, C-4, $J_{C\text{-}4,F\text{-}4}$ = 158, $J_{C\text{-}4,F\text{-}6}$ = 7.35 Hz), 69.4 (s, C-2), 67.5 (q, C-5, $J_{C\text{-}5,F\text{-}4}$ = 22.06, $J_{C\text{-}5,F\text{-}6}$ = 17.6 Hz), 65.29 (C-3, $J_{C\text{-}3,F\text{-}4}$ = 16.1 Hz), 54.6 (s, OCH_3); $[\alpha]_D$ 102.3° (*c* 1.03, $CHCl_3$).

Anal. Calcd for $C_7H_{12}F_2O_4$: C, 42.43; H, 6.10. Found: C, 42.34; H, 5.74.

Methyl 2,3,6-Tri-O-benzoyl-4-deoxy-4-fluoro-α-D-glucopyranoside (8). To a solution of 7[11] (3.54 g, 7 mmol) in anhydrous dichloromethane (20 mL) at −25 °C was added DAST (1.25 mL, 10 mmol). After stirring overnight at room temperature, the reaction was quenched as above and then poured into 100 mL

(1) Barnett, J. E. G. *Ciba Found. Symp.* **1972**, 95.
(2) Taylor, N. F. *Ciba Found. Symp.* **1972**, 215.
(3) Bessel, E. M.; Courtenay, V. D.; Foster, A. B.; Jones, A.; Westwood, J. H. *Eur. J. Cancer* **1973**, *9*, 463.
(4) Foster, A. B.; Westwood, J. H. *Pure Appl. Chem.* **1968**, *35*, 147.
(5) Penglis, A. A. E. *Adv. Carbohydr. Chem. Biochem.* **1981**, *38*, 195.
(6) Middleton, W. J. *J. Org. Chem.* **1975**, *40*, 574. The use of DAST for the fluorination of polyhydroxy derivatives is described by Middleton (Middleton, W. J. *U.S. Patent* 3914265).
(7) Somawardhana, C. W.; Brunngraber, E. G. *Carbohydr. Res.* **1981**, *94*, C14.
(8) Klemm, G. H.; Kaufman, R. J.; Sidhu, R. S. *Tetrahedron Lett.* **1982**, *23*, 2927.
(9) Bessell, E. M.; Foster, A. B.; Westwood, J. H.; Hall, L. D.; Johnson, R. N. *Carbohydr. Res.* **1971**, *19*, 39.
(10) Capon, B. *Chem. Rev.* **1969**, *69*, 407.
(11) Reist, E. J.; Spencer, R. R.; Calkins, D. F.; Baker, B. R.; Goodman, L. *J. Org. Chem.* **1965**, *30*, 2312.

of saturated $NaHCO_3$ solution. Extraction into CH_2Cl_2 followed by drying and concentration afforded a colorless syrup. Chromatography on silica gel (CH_2Cl_2), and recrystallization from EtOH gave 1.47 g (41%) of **8** as a colorless solid: mp 139–141 °C; 360-MHz ^1H NMR δ 3.48 (s, OCH_3), 4.33 (m, H-5), 4.67 (m, H-6 + H-6′, $J_{6,6'}$ = 12.8 Hz), 4.77 (dt, H-4, $J_{3,4}$ = 9.1 Hz, $J_{H\text{-}4,F\text{-}4}$ = 51.3 Hz, $J_{4,5}$ = 10 Hz), 5.18 (m, H-2), 5.22 (d, H-1, $J_{1,2}$ = 3.5 Hz), 6.12 (dt, H-3, $J_{H\text{-}3,F\text{-}4}$ = 14.7 Hz, $J_{2,3}$ = 9 Hz), 7.35–7.63 (m, 9 H), 7.96–8.13 (m, 6 H); ^{19}F NMR (^1H decoupled) ϕ −197.6 (s); $[\alpha]_D$ 119.1° (*c* 1.0, $CHCl_3$).

Anal. Calcd for $C_{28}H_{25}O_8F$: C, 66.14; H, 4.96; F, 3.74. Found: C, 65.99; H, 5.07; F, 3.73.

Acknowledgment. We thank Dr. G. S. Reddy of this department for obtaining the 360-MHz ^1H NMR spectra and for extensive decoupling experiments.

Registry No. 1, 97-30-3; 3, 4577-39-3; 4, 617-04-9; 6, 84073-36-9; 7, 3601-36-3; 8, 84065-98-5; DAST, 38078-09-0.

Srilankenyne, a New Metabolite from the Sea Hare *Aplysia oculifera*

E. Dilip de Silva, Robert E. Schwartz, and Paul J. Scheuer*

Department of Chemistry, University of Hawaii, Honolulu, Hawaii 96822

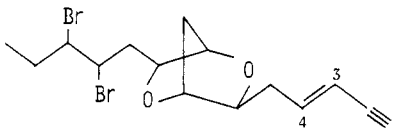
James N. Shoolery

Varian Associates, Palo Alto, California 94303

Received July 1, 1982

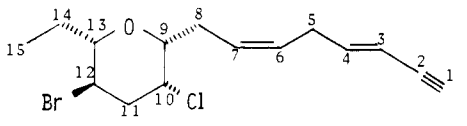
The structure of a C_{15}-tetrasubstituted tetrahydropyran, isolated from a sea hare, was elucidated by spectral analysis.

Sea hares that feed on the red algal genus *Laurencia* have been a convenient source of *Laurencia* metabolites, predominantly sesquiterpenes and derivatives of unbranched polyunsaturated C_{15} hydrocarbons bearing oxygen and halogen functions. More than 200 metabolites have been isolated to date from *Laurencia* spp. and from sea hares.[1] We recently reported the structures of the bicyclic ocellenynes (**1a,b**) from *Aplysia oculifera* collected



1 a, b (Z) 3,4

in Hawaii.[2] The same animal from Duwa, Sri Lanka,[3] contained as its principal metabolite the monocyclic srilankenyne (**2**), which is the subject of this report. Srilankenyne (**2**) is a dialkyl tetrahydropyran derivative. Surprisingly, this type has been rather uncommon among *Laurencia* constituents.[1]



2

Table I. NMR Data of 2

C	chem shift, ppm, multiplicity (*J*, Hz) ^{13}C	^1H	effect of ^1H decoupling
1	76.32 d	2.81 d (2.2)	5.48[a]
2	82.8 s		
3	109.17 d	5.48 m	same as H-7
4	143.96 d	6.23 dt (15.8, 6.8)	2.9 dd (6.4, 2.2) 5.48 ch
5	30.87 t[b]	2.90 ddd (6.8, 6.4, 2.2)	5.48[a] 6.23 d (15.8)
6	126.04 d[b]	5.48 m	same as H-7
7	128.48 d[b]	5.48 m	2.36 ch 2.81 s 2.90 d (6.8) 6.23 ch
8	31.08 t[b]	2.36 complex AB pattern	3.57 d (1.7) 5.48 ch 2.72 ch 4.05 dd (3.0, 1.75) 4.14 ch
9	78.73 d[b]	3.57 dt (7.0, 1.75)	2.36 ch 4.05 dd (3.4, 3.0)
10	60.47 d	4.05 ddd (3.4, 3.0, 1.75)	2.4 ch 2.72 dd (14.0, 4.4) 3.57 t (7.0)
11	43.85 t	2.72 ddd (14.0, 3.0, 4.4) 2.40 ddd (14.0, 12.0, 3.4)	was not decoupled similar to irradiation of 2.36
12	46.54 d	4.14 ddd (12.0, 10.2, 4.4)	2.40 ch 2.72 dd (14.0, 3.0) 3.36 dd (8.4, 2.5)
13	83.83 d[b]	3.36 ddd (10.2, 8.4, 2.5)	1.54 qd (14.5, 7.2) 2.02 qd (14.5, 7.2) 4.14 dd (12.0, 4.4)
14	26.19 t	1.54 qdd (7.2, 8.4, 14.5) 2.02 qdd (7.2, 2.5, 14.5)	0.97 d (7.2) 2.02 ch[a] 3.36 dd (10.2, 2.5) 0.97 d 7.2 1.54 ch 3.36 dd (10.2, 8.4)
15	9.28 q	0.97 t (7.2)	1.54 dd (14.5, 8.4) 2.02 dd (14.5, 2.5)

[a] Changed pattern. [b] Interchangeable with closest value.

The frozen animals were blended with acetone. The filtrate from the acetone suspension was concentrated at reduced pressure to a dark brown syrup, which was partitioned between water and dichloromethane. The organic residue was chromatographed on Bio-Sil A and then by HPLC, yielding srilankenyne (**2**) as a colorless liquid, $[\alpha]_D$ +7.14°. A molecular ion cluster at *m/z* 334, 332, and 330 suggested a formula of $C_{15}H_{20}BrClO$, and IR bands at 3300 and 2100 cm^{-1} indicated a terminal enyne function. ^1H NMR signals at δ 2.81 (H-1), 5.48 (H-3), and 6.23 (H-4), with corresponding ^{13}C NMR shifts at δ 76.32 (d, C-1), 82.8 (s, C-2), 109.17 (d, C-3), and 143.96 (d, C-4) fully confirmed the enyne tail of the molecule. A coupling constant of 15.8 Hz for H-4 showed the trans geometry of the 3,4-olefin. The chemical shifts of the acetylenic ≡CH also are characteristic of a *trans*-enyne. Corresponding values for *cis*-enyne are found at lower field.[4] The ether nature of the sole oxygen atom was seen in the IR spectrum by 1100 and 1082 (sh) cm^{-1} bands and a lack of hydroxyl and carbonyl absorption. The ^{13}C NMR spectrum exhibited six additional low-field signals. Two of these, doublets at δ 83.83 and 78.73, were assigned to carbons bearing oxygen, two doublets at δ 60.47 and 46.54 to carbons bearing

(1) Erickson, K. L. In "Marine Natural Products"; Scheuer, P. J., Ed.; Academic Press: New York, Vol. 5, in press.
(2) Schulte, G. R.; Chung, M. C. H.; Scheuer, P. J. *J. Org. Chem.* **1981**, *46*, 3870–3873.
(3) Collected by E. D. deS., Oct 1978, and identified by Professor E. Alison Kay.
(4) Howard, B. M.; Fenical, W.; Hirotsu, K.; Solheim, B.; Clardy, J. *Tetrahedron* **1980**, *36*, 171–176.

0022-3263/83/1948-0395$01.50/0