Table I. Summary of Heteronuclear 2-D Chemical Shift Correlation NMR Results for the Aromatic Region of Oxidized Ferredoxin from *A. variabilis*

2-D peak no. from Figure 1	chemical shifts ^{13}C, δ	chemical shifts ^{1}H (±0.02), δ	assignments (ref 8)
1	136.5	7.80	His-93 C^{ϵ}
2	135.7	8.16	His-16 C^{ϵ}
3	132.2	7.11	Tyr-99 $C^{\delta 1}$ and $C^{\delta 2}$
4	131.6	7.11	Tyr-83 $C^{\delta 1}$ and $C^{\delta 2}$
5	131.5	7.30	Tyr-76 $C^{\delta 1}$ and $C^{\delta 2}$
6	130.8	6.77	Tyr-35 $C^{\delta 1}$ and $C^{\delta 2}$
7	130.9	7.10	
8	130.6	6.35	
9	130.4	7.09	
10	130.4	7.55	Phe and Tyr-25 $C^{\delta 1}$ and $C^{\delta 2}$
11	130.1	7.11	
12	129.5	7.25	
13	129.3	7.20	
14	128.3	7.10	
15	119.1	6.94	His-16 C^{δ}
16	116.6	7.17	His-93 C^{δ}
17	116.9	6.76	Tyr-76 $C^{\epsilon 1}$ and $C^{\epsilon 2}$
18	116.3	6.76	Tyr-99 $C^{\epsilon 1}$ and $C^{\epsilon 2}$
19	116.7	6.41	Tyr-83 $C^{\epsilon 1}$ and $C^{\epsilon 2}$
20	116.2	6.31	Tyr-35 $C^{\epsilon 1}$ and $C^{\epsilon 2}$

it was isolated from cyanobacteria grown on CO_2 (20% ^{13}C) as the sole carbon source. ^{13}C NMR spectra were taken with a Nicolet NT-200 wide-bore spectrometer with ^{13}C and ^{1}H resonance frequencies of 50.31 and 200 MHz, respectively. The sample consisted of a 2.5-mL solution containing 150 mg of [$U^{20\%}$-^{13}C]ferredoxin dissolved in $^{2}H_2O$ in a spherical cell inside a 20-mm o.d. NMR tube. A total of 128 sets of free induction decays, each of 1K data points, was obtained in a total time of 50 h. The pulse sequence used was adapted from that of Morris and Hall.[5] The carbon carrier frequency was located at the center of the aromatic region (127 ppm), and quadrature detection was used. The proton frequency was set at 6 ppm from 4,4-dimethyl-4-silapentane-1-sulfonate, sodium salt (DSS); a 700-Hz proton window was covered (3.5 ppm), which gave 5.5-Hz resolution in the proton frequency domain. The ^{1}H NMR spectrum obtained at 470 MHz with a Nicolet Model NT-470 is plotted at the bottom. In general, one must exercise care in comparing ^{1}H NMR spectra obtained at two frequencies; we have determined that the chemical shift patterns of the aromatic region of ferredoxin are similar at 200 and 470 MHz. Two ^{13}C NMR spectra are plotted on the sides. The one on the left-hand side is the normal ^{13}C NMR spectrum, and the one on the right-hand side is a subspectrum[7] that contains resonances only from the protonated carbons in the region. The chemical shifts of the peaks in both dimensions are given in Table I. The assignments of the histidine and tyrosine residues in the proton spectrum will be published later.[8] This 2-D spectrum allows the assignment of the two resolved ϵ carbons of His-16 and His-93 and the δ carbon of His-16. It also locates the δ carbon of His-93 and the δ and ϵ carbons of the four tyrosine residues, which are not well resolved in the one-dimensional carbon spectrum. The 2-D spectrum also assists in the assignment of ^{1}H NMR peaks; for example, one can readily determine whether a resonance in the proton spectrum is due to an aromatic residue such as phenylalanine or to an unexchanged amide proton. Environmental shifts are relatively less important for ^{13}C than for ^{1}H NMR so that carbon chemical shifts are more characteristic of amino acid type.

The spectrum presented here represents the first application of heteronuclear chemical shift correlation 2-D NMR to a protein. Spectra of similar quality can be obtained for the aliphatic region. This technique provides a powerful tool for the assignment of resonances in ^{1}H and ^{13}C spectra of macromolecules and hence for solution studies of their structure and function.

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(7) Chan, T.-M.; Westler, W. M.; Santini, R. E.; Markley, J. L., *J. Am. Chem. Soc.*, preceeding paper in this issue.
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Total Synthesis of (±)-Panacene

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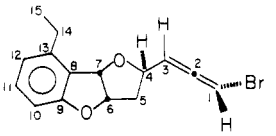
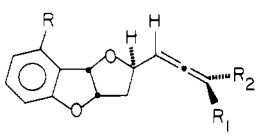
Panacene is one of several halogenated marine natural products isolated from *Aplysia brasiliana*, a large sluglike gastropod mollusk indigenous to the gulf coast of Florida.[1] These halogenated sesquiterpenes and fatty acid metabolites are believed to be sequestered from dietary sources and serve as potent feeding deterents to sharks and other predatory fish.[1-3] The gross structure of panacene was deduced from spectral data, although no assignment of the relative configuration of the bromoallene moiety could be made.[1]

Herein we report the stereoselective total synthesis of (±)-panacene (**1a**) and (±)-1-epibromopanacene (**1b**) as well as the analogous 13-desethyl species **2a** and **2b**. From comparison of the spectral data of these compounds with that of natural panacene, we can suggest that the relative configuration of panacene is as shown in **1a**.



1a



1b R = C_2H_5, R_1 = Br, R_2 = H
2a R = H, R_1 = H, R_2 = Br
2b R = H, R_1 = Br, R_2 = H

Since the relative stereochemistry was undetermined at the outset, our synthetic strategy embodied two stereochemical considerations: (1) a predictable manner of transmitting stereochemical information already present in a precursor molecule to the bromoallene fragment; (2) flexibility to permit the synthesis of both bromoallene epimers for comparison purposes. Both these considerations were initially probed in a model system that started with methyl salicylate and resulted in the synthesis of the 13-desethyl compounds **2a** and **2b**. The route employed was identical with that described for the synthesis of panacene in Scheme I, so reference will be made to this model system only where additional stereochemical information might result.

Ethyl 6-ethylsalicylate, **3**,[4] was converted to the 3(2*H*)-benzofuranone **4** by modification of known methods.[5] Allylation and decarbethoxylation led to an allyl ketone, which then was transformed into the strictly *cis*-dihydrobenzofuran **5** upon K-Selectride reduction.[6,7]

(1) Kinnel, R. B.; Duggan, A. J.; Eisner, T.; Meinwald, J. *Tetrahedron Lett.* **1977**, 3913.
(2) Kinnel, R. B.; Dieter, R. K.; Meinwald, J.; van Engen, D.; Clardy, J.; Eisner, T.; Stallard, M. O.; Fenical, W. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 3576.
(3) Dieter, R. K.; Kinnel, R.; Meinwald, J.; Eisner, T. *Tetrahedron Lett.* **1979**, 1645.
(4) Ethyl 6-ethylsalicylate was readily prepared by modifiction of the method of Hauser: Hauser, F. M.; Pogany, S. A. *Synthesis* **1980**, 814.
(5) Schroeder, D. C.; Corcoran, P. O.; Holden, C. A.; Mulligan, M. C. *J. Org. Chem.* **1962**, *27*, 586.

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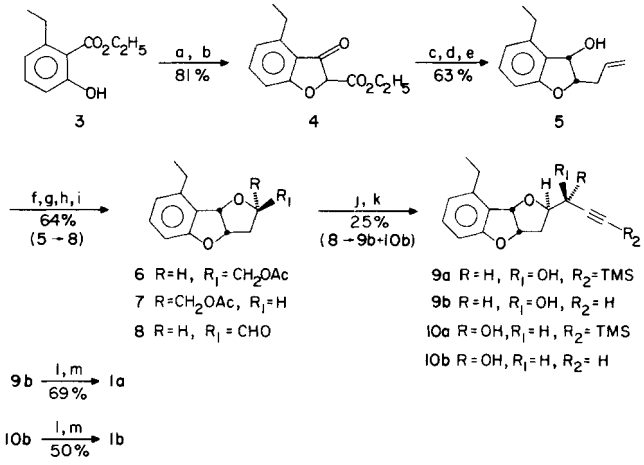
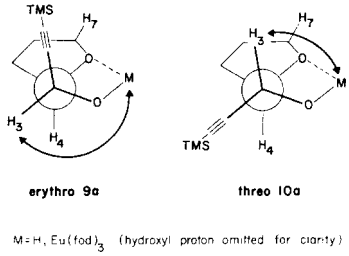
Scheme I[a]



[a] NaH, $BrCH_2COOEt$, DMF. [b] NaH, benzene. [c] NaH, $BrCH_2{=}CH_2$, DMF. [d] 3 M HCl, CH_3OH. [e] K-Selectride, THF. [f] NBS, CH_3CN. [g] KOAc, DMF. [h] $NaOCH_3$, CH_3OH. [i] $(COCl)_2$, Me_2SO, TEA, CH_2Cl_2. [j] TMSC≡CLi, THF. [k] Bu_4NF, THF. [l] MsCl, TEA, CH_2Cl_2. [m] $LiCuBr_2$, THF.

Scheme II



M=H, Eu(fod)$_3$ (hydroxyl proton omitted for clarity)

Oxidative cyclization with *N*-bromosuccinimide followed by acetate displacement afforded a 3:1 mixture of *exo*- and *endo*-tetrahydrofurfuryl acetates which were chromatographically separable (MPLC, silica gel, 40% ether/hexene). The stereochemical assignments of **6** and **7** follow from ^{1}H NMR data (primarily H(C-7)[8] and upon subsequent conversion of **6** to the aldehyde **8**.[9] This exo aldehyde, in fact, has been prepared by the ozonolysis of panacene as part of the original structure elucidation.[1,10]

The tetrahydrofurfural **8** proved exceedingly labile in our hands, and attempted epimerization or addition of a metal acetylide resulted in decomposition. However, addition of lithio- or magnesio(trimethylsilyl)acetylide in THF or ether led to varying amounts of the *erythro*-**9a** and *threo*-**10a** alcohols.[11] The stereochemical course of addition of organometallics to tetrahydrofurfurals results from the interplay of several factors and has been discussed in the literature.[12] Assignment of configuration to **9** and **10** is based upon two observations. First, *erythro*-tetrahydrofurfurols exhibit a 2–4 Hz coupling constant (**9a**, J_{34} = 3.4 Hz) while threo isomers exhibit a 6–8 Hz coupling constant (**10a**, J_{34} = 5.4 Hz). Second, treatment of a mixture of **9a** and **10a** with 5 mol % Eu(fod)$_3$ resulted in a downfield shift of 0.2 ppm for H(C3) in **9a** but a 1.3-ppm downfield shift for H(C-3) in **10a**, suggesting that europium exerts a greater influence on H(C-3) in **10a** relative to **9a**.[13] This is consistent with the assignments and conformations shown in Scheme II.[14]

The diastereomers **9b** and **10b** were obtained isomerically pure only after desilation, benzoylation, HPLC separation of the benzoates (Whatman partisil psx 10/25, 5% ether/hexane), and hydrolysis with methanolic hydroxide.[15] The conversion of the derived mesylates to the bromoallenes **1a** and **1b**[16] is presumed to follow an anti stereochemical course based upon the following experiment: In a manner analogous to the conversion of **9b** into **1a**, 17α-ethynylestradiol 3-methyl ether was converted to a bromoallene and then on to a methylallene with lithium dimethylcuprate. This methylallene exhibited ^{1}H NMR data (C18 δ 0.86) identical with 21α-methylestra-17,20-dienol 3-methyl ether, a compound whose structure was unambiguously determined by X-ray analysis.[17] Since methylation of vinyl bromides with lithium dimethylcuprate occurs with retention of configuration, bromoallene formation must then occur with inversion.

The bromoallenes were obtained isomerically pure and appeared indistinguishable by MS, IR, and ^{13}C NMR spectral comparisons. Fortunately, ^{1}H NMR (300 Mhz) revealed that H(C-3) of **1a** was a triplet (δ 5.50), J = 6.0 Hz) while H(C-3) of **1b** was a doublet of doublets (δ 5.46, J = 5.8, 6.6 Hz). Since H(C-3) of authentic panacene appears as a triplet (δ 5.46, J = 5.88 Hz), we can assign **1a** as the relative stereochemistry of panacene. Comparison of spectral data (high-resolution MS, ^{1}H NMR, ^{13}C, NMR, IR) between synthetic panacene and the authentic material indicated identity.[18]

Acknowledgment. We thank Dr. E. E. van Tamelen for his support during this work, NIH Grant 2 R01 GM 10421 for financial support, and NSF Grant CHE-77-08810 for NMR facilities.

Registry No. (±)-**1a**, 66389-39-7; (±)-**1b**, 82110-07-4; (±)-**2a**, 82064-76-4; (±)-**2b**, 82110-08-5; **3**, 82064-77-5; (±)-**4**, 82064-78-6; (±)-**5**, 82064-79-7; (±)-**6**, 82064-80-0; (±)-**7**, 82110-09-6; (±)-**8**, 82110-10-9; (±)-**9a**, 82064-81-1; (+)-**9b**, 82064-82-2; (±)-**10a**, 82110-11-0; (±)-**10b**, 82110-12-1.

(6) Reduction of the desethyl model allylbenzofuranone with other reducing agents led to varying amounts of *cis*- and *trans*-dihydrobenzofurans. These could be distinguished by ^{1}H NMR: $CH_2CH{=}CH_2$ δ (cis) 2.65, (trans) 2.41.

(7) Satisfactory spectral data (MS, IR, ^{1}H NMR) were obtained for all new compounds. **5**: ^{1}H NMR (10 MHz, $CDCl_3$) δ 7.20 (t, J = 7.8 Hz, 1 H, H-11), 6.74 (t, J = 7.7 Hz, 2 H, H-10, H-12), 4.41 (dt, J = 5.5, 7.2 Hz, 1 H, H-6).

(8) **6**: ^{1}H NMR (100 MHz, $CDCl_3$) δ 7.17 (t, J = 7.8 Hz, 1 H, H-11), 6.75 (d, J = 7.3 Hz, 1 H, H-10 or H-12), 6.62 (d, J = 8.0 Hz, 1 H, H-10 or H-12), 5.81 (d, J = 6.0 Hz, 1 H, H-7), 5.27 (t, J = 5.8 Hz, 1 H, H-6), 4.19 (d, J = 4.8 Hz, 2 H, H-3), 3.95 (m, 1 H, H-4), 2.31 (dd, J = 4.5, 13.4 Hz, 1 H, H-5), 2.06 (s, 3 H). **7**: ^{1}H NMR (100 MHz, $CDCl_3$) δ 5.60 (d, J = 6.3 Hz, 1 H H-7), 1.97 (s, 3-H).

(9) **8**: ^{1}H NMR (100 MHz, $CDCl_3$) δ 9.71 (d, J = 1.8 Hz, 1 H, H-3), 7.21 (t, J = 7.8 Hz, 1 H, H-11), 6.78 (dd, J = 0.4, 7.3 Hz, 1 H, H-10 or H-12), 6.65 (d, J = 8.0 Hz, 1 H, H-10 or H-12), 5.89 (d, J = 5.8 Hz, 1 H, H-7), 5.30 (t, J = 5.7 Hz, 1 H, H-6), 4.17 (ddd, J = 1.7, 5.4, 10.7, Hz, 1 H H-4), 2.55 (dd, J = 5.7, 13.7 Hz, 1 H, H-5), 2.12 (ddd, J = 5.7, 10.7, 13.7 Hz, 1 H H-5); IR (neat oil) 1731 cm^{-1}; mass spectrum (70 eV) m/e 218, 189, 147.

(10) In the model desethyl series, both the major and minor acetates were converted to diastereomeric aldehydes, and examination by ^{1}H NMR revealed the following: –CHO major, δ 9.71 (d, J = 1.7 Hz); minor, δ 9.20 (s); exo aldehyde from ozonolysis of panacene, δ 9.70 (d).

(11) The following data were observed (metal counterion, solvent, yield, erythro/threo): Li, THF, 38%, 60/40; MgBr, THF, 10%, 60/40; Li, ether, 24%, 86/14.

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(13) For reference, H(C-4) is shifted ca. 0.3 ppm downfield while H(C-7) is shifted ca. 0.2 ppm downfield in both **9a** and **10a**.

(14) Other examples of diastereomer differentiation using substrate bidentate ligation to europium can be found in the following: (a) Schuttler, R.; Hoffman, R. W. *Tetrahedron Lett.* **1973**, 5109. (b) Higgs, M. D.; Faulkner, D. J. *J. Org. Chem.* **1978**, *43*, 3454.

(15) The yields for the conversion of **9b**/**10b** to **1a**/**1b** in Scheme I include this purification sequence. **9b**: ^{1}H NMR (100 MHz, $CDCl_3$) δ 5.83 (d, J = 5.9 Hz, 1 H, H-7), 5.29 (m, 1 H, H-6), 4.60 (m, 1 H, H-3), 4.01 (ddd, J = 3.2, 6.2, 12.4 Hz, 1 H, H-4), 2.45 (d, J = 2.2 Hz, 1 H, H-1). **10b**: ^{1}H NMR (100 MHz, $CDCl_3$) δ 5.82 (d, J = 6.0 Hz, 1 H, H-7), 5.28 (t, J = 5.9 Hz, 1 H, H-6), 4.35 (m, 1 H, H-3), 3.94 (quint, J = 5.2 Hz, 1 H, H-4), 2.44 (d, J = 2.2 Hz, 1 H, H-1).

(16) Montury, M.; Gore, J. *Synth. Commun.* **1980**, *10*, 873.

(17) Hunt, D. J.; Jackson, S. D.; Moyes, R. B.; Wells, P. B.; Whyman, R. *J. Chem. Soc., Chem. Commun.* **1982**, 84. For a related case, see: Muscio, O. J., Jr.; Jun, Y. M.; Philip, J. B., Jr. *Tetrahedron Lett.* **1978**, 2379.

(18) We are most grateful to Dr. J. Meinwald, Cornell University, for conducting 300-MHz ^{1}H NMR, high-resolution MS, TLC, and coinjection VPC comparisons between our synthetic material and a sample of natural panacene.