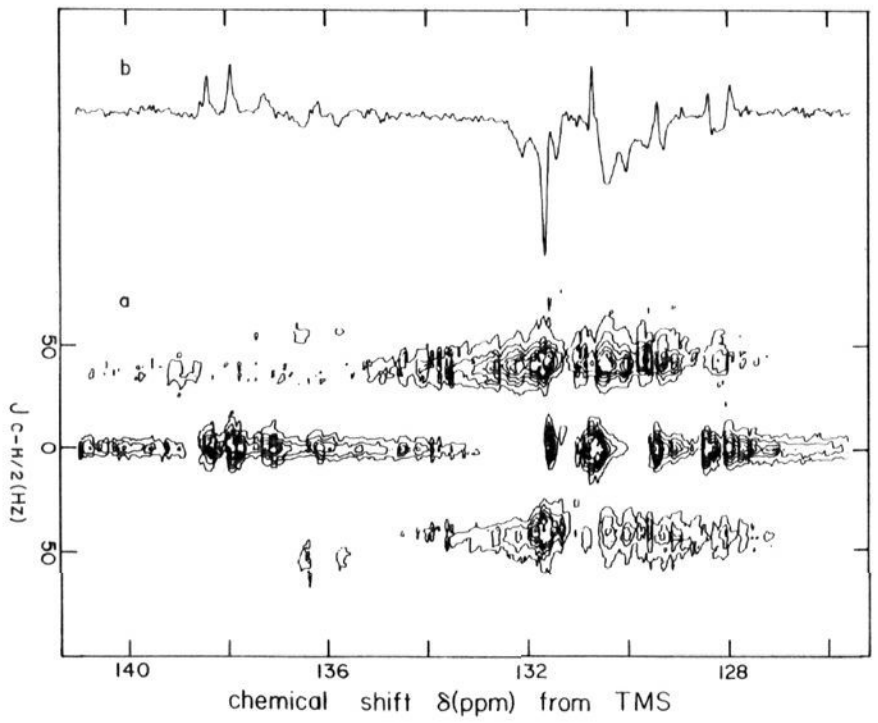

Figure 3. (a) Two-dimensional heteronuclear (^1H–^{13}C) J spectrum[15] displayed as a contour plot.[18] The chemical shifts are displayed in ppm along the horizontal axis, and the coupling constants are given by twice the vertical separation between pairs of contour spots in Hz. (b) The APT spectrum[2,3] (^{13}C NMR J-modulated spin-echo experiment) is plotted for comparison.

is then the determination of spin multiplicity, but the APT sequence is competent for this purpose as well as being much less time consuming.

These results demonstrate that the APT pulse sequence provides an efficient method for determining the multiplicity of ^{13}C NMR peaks in a small protein. The following communication[16] describes how extensive assignments of the subspectrum consisting of protonated carbons can be made by use of heteronuclear (^1H–^{13}C) two-dimensional correlation spectroscopy.

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Heteronuclear (^1H, ^{13}C) Two-Dimensional Chemical Shift Correlation NMR Spectroscopy of a Protein. Ferredoxin from *Anabaena variabilis*

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Relatively few examples of extensive assignments in ^{13}C NMR spectra of proteins are to be found in the literature. Allerhand and co-workers used the convolution difference technique to resolve resonances due to nonprotonated carbons in cytochrome *c* and lysozyme; in favorable cases, these peaks could be assigned to specific carbon atoms in the proteins.[1] Several ^{13}C resonances due to methyl groups that have well-resolved ^1H resonances were assigned by Wüthrich and co-workers in spectra of bovine pancreatic trypsin inhibitor by the use of single-frequency selective decoupling.[2] We report here the use of heteronuclear (^1H, ^{13}C) two-dimensional (2-D) chemical shift correlation NMR[3] for the assignment of resonances from protonated carbons in proteins.

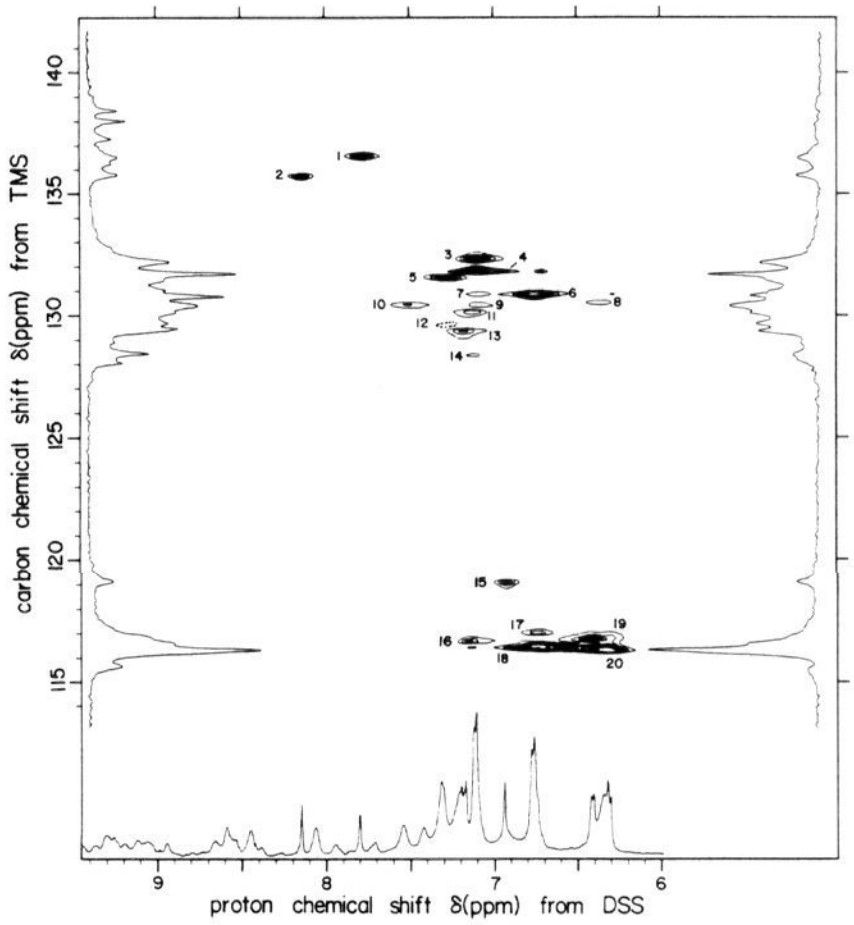

Figure 1. Cross assignment of chemical shifts in the ^1H and ^{13}C NMR spectra of the aromatic region of oxidized ferredoxin from *A. variabilis* by means of a heteronuclear two-dimensional chemical shift correlation map obtained at ^{13}C and ^1H resonance frequencies of 50.31 and 200 MHz, respectively, at 24 °C. A 470-MHz one-dimensional ^1H NMR spectrum of the protein is shown at the bottom. Two 50.31-MHz one-dimensional ^{13}C NMR spectra are plotted at the sides. That on the right-hand side is a subspectrum[7] that contains resonances from only protonated carbons, i.e., those expected to show up in the 2-D contour plot. The ferredoxin, which has two histidine, five tyrosine, and three phenylalanine residues, was enriched uniformly with ^{13}C (20% level of isotope) to increase the signal-to-noise ratio. Contour peak 12 (dashed) was observed at a lower contour than the others shown.

Proton-carbon 2-D chemical shift correlation spectroscopy has been applied recently to small molecules[4] and to a series of carbohydrates.[5] The extension of this technique to proteins is hindered by the insensitivity of ^{13}C NMR. However, this technique will be useful for small proteins or other macromolecules enriched in ^{13}C or for small unenriched macromolecules that are very soluble and can be obtained in large quantity. The increase in time required is more than offset by the additional information gained.

It is easier, in general, to obtain assignments to specific residue positions for ^1H than for ^{13}C NMR spectra of proteins. Because of the high sensitivity of ^1H NMR spectroscopy, several strategies can be used that may not be practical in ^{13}C NMR spectroscopy, for example pH titrations and comparisons of spectra from proteins having homologous sequences. Two-dimensional chemical shift correlation allows the cross assignment of ^1H and ^{13}C NMR peaks from bonded pairs of ^{13}C–^1H nuclei. Also, better resolution can be achieved by spreading the resonances in two dimensions according to their proton and carbon chemical shifts. Resonances from protonated carbons in proteins can be grouped into two regions, the aliphatic region (from about 10 to 70 ppm from tetramethylsilane) and the aromatic region (110–140 ppm). The corresponding proton chemical shifts are similarly segregated. It is desirable to obtain separate 2-D correlation spectra for each region in order to allow better digital resolution in the proton chemical shift dimension.

The contour plot of a 2-D spectrum of the aromatic region of oxidized *Anabaena variabilis* ferredoxin is shown in Figure 1. The protein is a metalloprotein of M_r 11 000 with a 2Fe·2S* cluster;[6]

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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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Total Synthesis of (±)-Panacene

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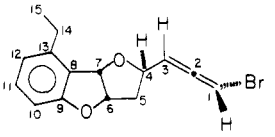
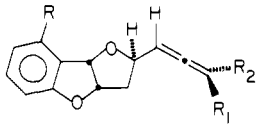
Panacene is one of several halogenated marine natural products isolated from *Aplysia brasiliana*, a large sluglike gastropod mollusk indigenous to the gulf coast of Florida.[1] These halogenated sesquiterpenes and fatty acid metabolites are believed to be sequestered from dietary sources and serve as 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]

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