

Two-Dimensional NMR Studies of Marine Natural Products. IV.  
Isolation of the Cembranoid Diterpene Jeunicin from the Mollusc  
*Planaxis sulcatus*: Assignment of the Proton and Carbon NMR Spectra  
by Two-Dimensional Techniques

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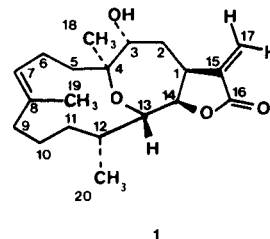
Numerous cembranoic diterpenes have been isolated from marine gorgonians and terrestrial plants. There have, however, been only a few reports of the isolation and identification of these interesting compounds from higher animal sources. The isolation of the oxo-bridged cembranoid diterpene jeunicin from the mollusc *Planaxis sulcatus* is reported. A comparative discussion of the utility of COSY, homonuclear RELAY (relayed COSY) and proton double quantum coherence techniques is presented, the latter successfully establishing the identities of all of the proton resonances. Acquisition of a two-dimensional proton-carbon chemical shift correlation spectrum provided the means of unequivocally establishing  $^{13}\text{C}$  resonance assignments.

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### Introduction.

Cembranoid diterpenes comprise an interesting class of compounds characterized by the presence of a fourteen membered carbocyclic ring. The literature from the first nearly simultaneous reports by Dauben, Thiessen and Resnick [1] and Kobayashi and Akiyoshi [2] through 1979 has been reviewed by Weinheimer, Chang and Matson [3]. Characteristic of all of these early studies was the necessity of establishing the presence of the macrocyclic ring in each case either through degradative chemical studies or through an X-ray diffraction study. More recently, a number of studies have appeared which have described partial or full  $^{13}\text{C}$ -nmr spectral assignments [4-9], these efforts providing a data base which can be employed in the elucidation of new cembrane analogs. Finally, with the advent of two-dimensional nmr techniques, it has become possible to establish the structures of these interesting compounds by spectroscopic means and there are already two reports in the literature which have employed two-dimensional techniques. Gampe and coworkers [10] have described the total assignment of the  $^{13}\text{C}$ -nmr spectrum of the oxo-bridged cembranoid eunicin; Nishida and co-workers [11] have completely established the structure of a cembranoid isolated from tobacco on the basis of two-dimensional auto-correlated (COSY) data.

Although numerous cembranoid diterpenes have been isolated and characterized from marine gorgonians and terrestrial plants there have been very few reports of the isolation of these compounds from higher animal sources; those that do appear all report the isolation of minute



quantities of cembranes from insects [7,12,13], these compounds presumably serving as pheromones [7]. In the present work, the isolation of a compound from a mollusc with properties resembling those of a cembranoid diterpene was somewhat unusual [14], prompting a careful and detailed spectral investigation of the structure. The compound which we isolated was identified as jeunicin, **1**, an oxo-bridged member of the cembranoid diterpene family. Since neither the  $^1\text{H}$ - nor the  $^{13}\text{C}$ -nmr spectra of **1** have been fully assigned, we now wish to report the total assignment of these spectra using two-dimensional nmr techniques.

One of the features of the  $^1\text{H}$ -nmr spectra of cembranes which makes the interpretation difficult is the highly congested nature of the high field region. In part, the interpretation of the proton nmr spectra of cembranes is significantly aided by two-dimensional nmr techniques such as the COSY [15,16] experiment. Difficulties in the extraction of proton connectivity network information from the high field region of the spectrum still remain, however, and are aggravated by the presence of methyl singlets which may partially obscure the very much weaker re-

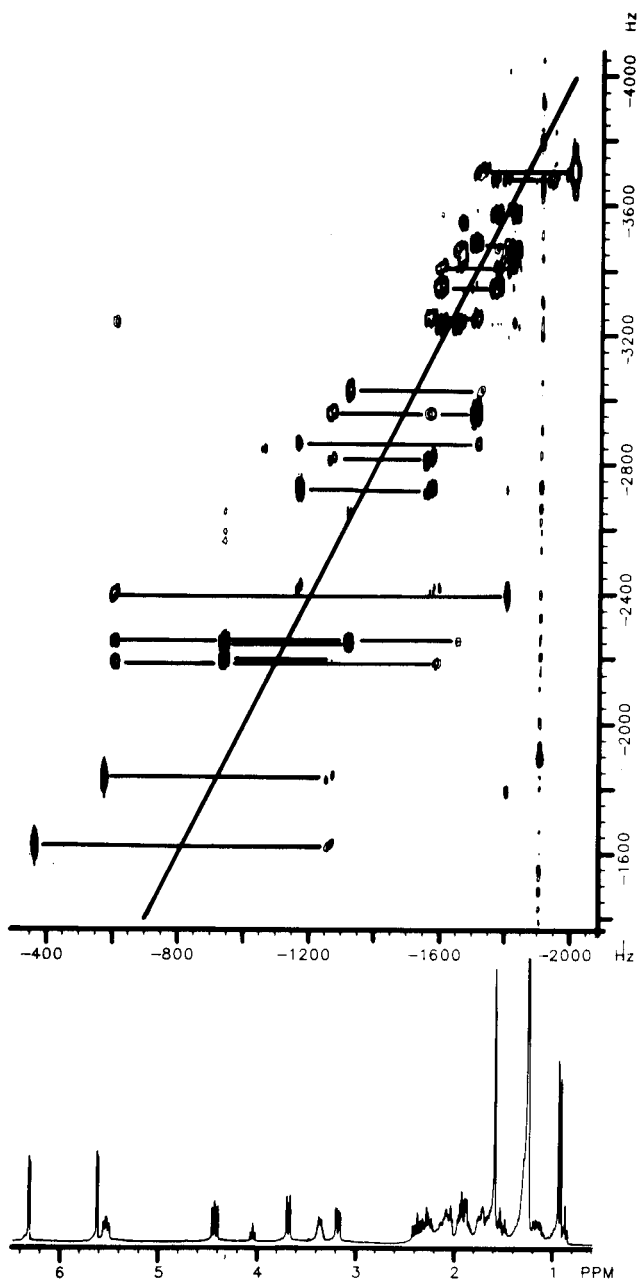


Figure 1. Proton double quantum two-dimensional nmr spectrum of jeunicin (I) recorded at 300.068 MHz in deuteriochloroform. The normal high resolution proton spectrum (32K) is plotted below the contour plot, the axis plotted beneath the spectrum showing chemical shifts referenced relative to external tetramethylsilane. The axis plotted above the spectrum gives chemical shifts relative to the transmitter (upfield and hence negative in sign) which correspond to the values shown in Table II. The double quantum frequency domain ( $F_1$  or  $\omega_1$ ) shows negative shifts in Hz which arise from the sum of pairs of resonance offsets relative to the transmitter.

sponses due to the numerous multiplets contained in this region of the spectrum. One alternative solution of these problems is provided by a double quantum filtered COSY

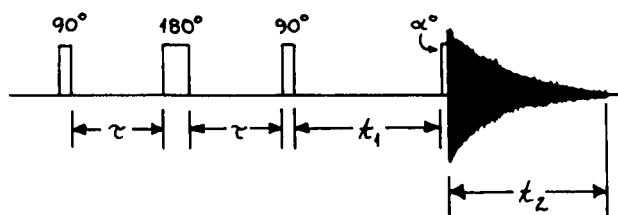


Figure 2. Proton double quantum pulse sequence. Double quantum coherence is created during the  $90^\circ$ - $\tau$ - $180^\circ$ - $\tau$ - $90^\circ$  portion of the sequence, the length of  $\tau = 1/4J_{HH} = 35.7$  msec, and evolves during the  $t_1$  interval. The final "read" pulse,  $\alpha$ , was set to  $45^\circ$  to reduce the intensity of response due to "passive transfer" of magnetization and to augment the intensity of responses in the negative  $F_1$  frequency region plotted in Figures 1 and 4 [18].

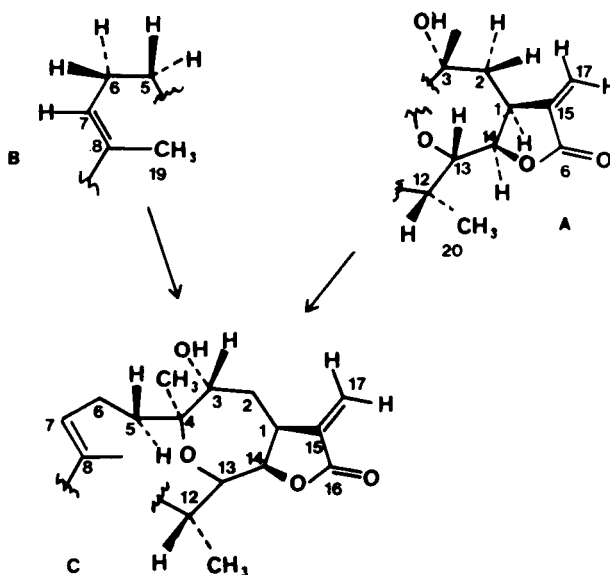


Figure 3. Structural fragments of jeunicin (I) which were assembled from the various two-dimensional nmr experiments performed in the course of the total spectral assignment.

spectrum [17]. A perhaps more elegant alternative is provided by proton double quantum coherence [18-21]. Direct proton-proton connectivity information can be supplemented with longer range structural information provided by homonuclear relayed coherence transfer (relayed COSY:RCOSY) [22] experiments. We elected to employ a combination of proton experiments which included the proton double quantum experiment for its ability to unravel connectivity pathways in congested spectral regions and the RCOSY experiment to establish longer range connectivity information. It should also be noted that the latter (RCOSY), to be fully interpreted, requires the prior acquisition of a conventional COSY spectrum. Finally a proton-carbon heteronuclear two-dimensional chemical shift correlation spectrum [23,24] was obtained to complete the assignment of the  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectra.

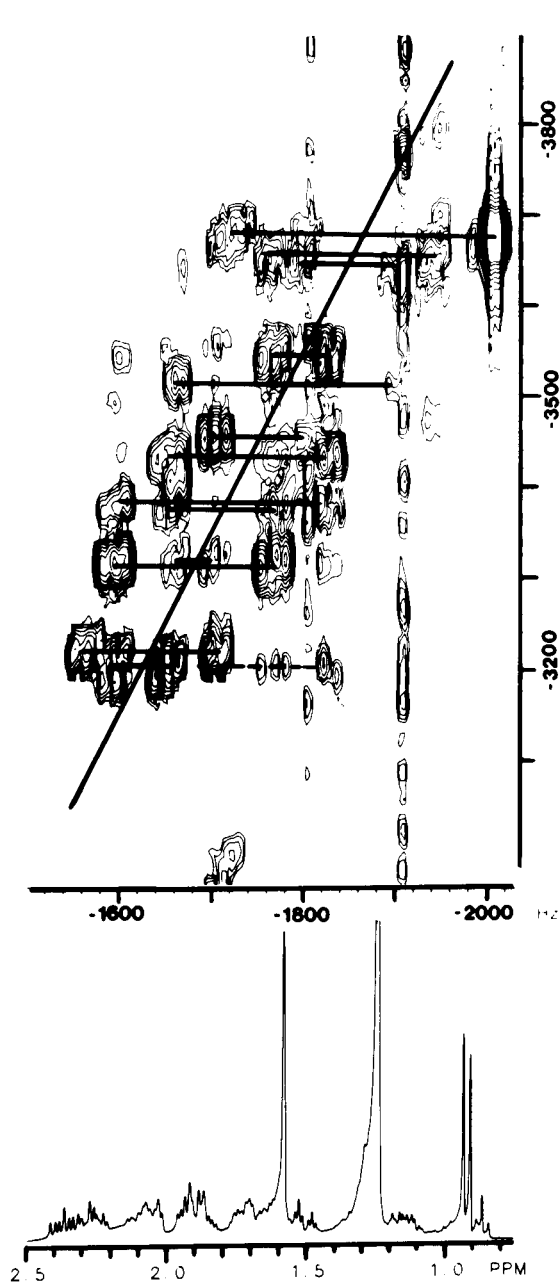


Figure 4. Expanded segment of the upfield region of the proton double quantum spectrum of jeunicin (**1**) from Figure 1 to show detail of the numerous responses in the congested upfield region. Responses in this area of the spectrum allow the assignment of the methylene bridge proton resonances which cannot be assigned from either the COSY or RCOSY experiments.

The proton double quantum spectrum of **1** is shown in Figure 1. Correlations of resonances which are directly coupled to one another are provided by responses in the double quantum frequency domain ( $F_1$  or  $\omega_1$ ) which are symmetrically disposed about the skew diagonal,  $F_1 = \pm 2F_2$ . Since considerable information can be extracted from the spectrum given a knowledge of where

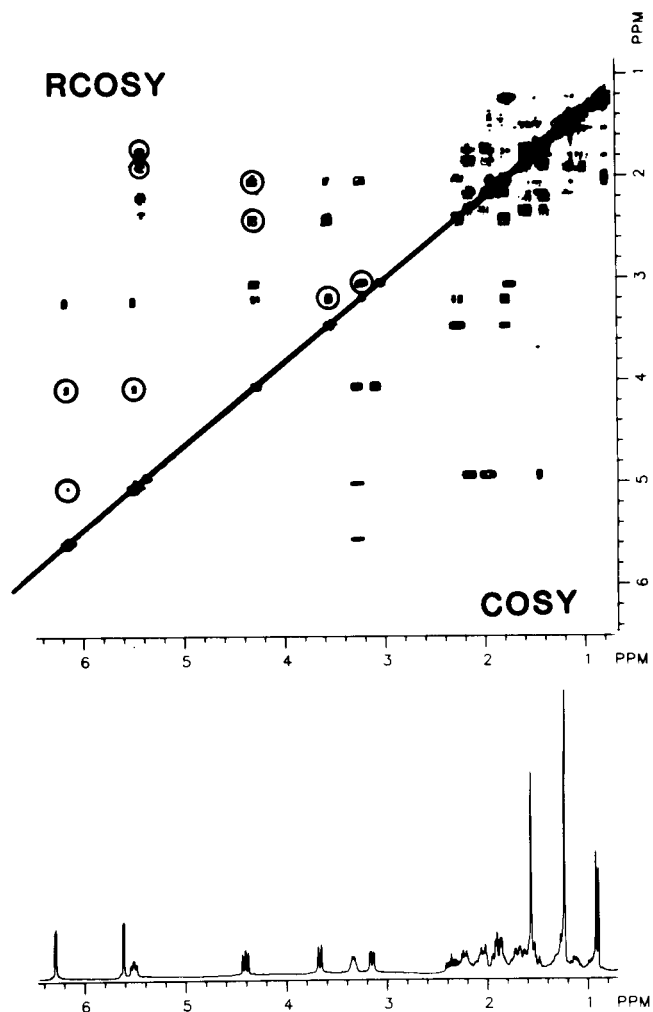


Figure 5. Combined plot of the COSY/RCOSY spectra of jeunicin (**1**) recorded in deuteriochloroform at 300.068 MHz. The COSY spectrum is shown below the diagonal while the RCOSY spectrum is shown above the diagonal. New information in the form of relay responses obtained in the RCOSY are circled to facilitate comparison of the responses obtained from the two experiments.

responses may appear, additional information on the nature and location of responses in the proton double quantum experiment is warranted. Using an AMX spin system as an example, a double quantum response which correlates A and M would consist of a pair of responses located at the  $F_2$  frequencies of the resonances at an  $F_1$  frequency which is the algebraic sum of the offsets of the A and M resonances from the carrier. (The axis ranging from 0 Hz to  $\sim -2200$  Hz plotted above the high resolution spectrum shows resonance offsets relative to the transmitter.) As expected, the coordinates of this pair of responses locate it symmetrically about the skew diagonal as specified above.

Direct proton-proton connectivity information is supplemented by passive transfer of magnetization to members

Table I

Proton and Carbon NMR Chemical Shifts of Jeunicin (I) in Deuteriochloroform at Observation Frequencies of 300.068 and 75.459 MHz Respectively

Position	<sup>13</sup> C Shift	Multiplicity	<sup>1</sup> H Shift(s)
1	40.0	CH	3.36
2	30.3	CH <sub>2</sub>	2.36,1.89
3	71.9	CH	3.67
4	80.0	C	—
5	32.3	CH <sub>2</sub>	1.71,1.50
6	22.8	CH <sub>2</sub>	2.27,2.08
7	125.4	CH	5.53
8	133.5	C	—
9	40.4	CH <sub>2</sub>	2.04,1.93
10	22.8	CH <sub>2</sub>	1.63, 1.27
11	29.6	CH <sub>2</sub>	1.72,1.14
12	36.5	CH	1.86
13	72.1	CH	3.18
14	80.0	CH	4.42
15	137.6	C	—
16	169.7	C	—
17	120.0	CH <sub>2</sub>	6.31,5.61
18	19.1	CH <sub>3</sub>	1.25
19	15.5	CH <sub>3</sub>	1.58
20	15.8	CH <sub>3</sub>	0.92

of the spin system not directly involved in a given transition. The proton double quantum pulse sequence, which is shown in Figure 2, creates double quantum coherence using a  $90_x^\circ - \tau - 180_y^\circ - \tau - 90_y^\circ$  sequence, double quantum coherence evolving during the  $t_1$  interval which follows. Responses due to passive transfer arise at the  $F_2$  frequency of the proton to which magnetization is transferred and appear at the  $F_1$  frequency of the direct connectivity response. In the case of the AMX spin system used as an example above, a passive transfer response linking the X spin to the AM transition would appear at  $F_2X$  at the  $F_1$  frequency of the AM transition. The final pulse in the sequence,  $\alpha$ , reconverts the double quantum coherence back into observable single quantum magnetization which is ultimately recorded. Mareci and Freeman [19] have shown that when  $\alpha = 90^\circ$  all responses at a given  $F_1$  frequency (both direct and those due to passive transfer) are equally populated. When the duration of  $\alpha = 45^\circ$  or  $135^\circ$ , the intensities of the passive responses are reduced thereby simplifying the spectral interpretation. It should be noted, however, that the passive responses are still detectable and can be used to provide additional, remote connectivity information in a manner analogous to that provided by the RCOSY experiments [22]. Other responses are also visible in the proton double quantum experiment and the interested reader is referred to our previous work [20] for a brief discussion of these responses or to Braunschweiler, Bodenhausen and Ernst [18] for a more rigorous discussion.

Table II

Two-Dimensional Proton Double Quantum Connectivity Frequencies of Jeunicin (I) in Deuteriochloroform at 17° and an Observation Frequency of 300.068 MHz.

Proton	$\delta^1\text{H}$	Transmitter Offset [Hz]	Connectivity	Transition Calcd.	Frequency Observed [Hz]
1	3.36	-1251	1/2a	-2795	-2783.4
			1/2b	-2930	-2927.2
			1/3	-2404	-2408.0
2a	2.36	-1544	1/14	-2181	-2176.1
			1/17a	-1616	-1612.0
			1/17b	-1822	-1818.2
2b	1.89	-1679	2a/2b	-3223	-3224.9
			2a/3	-2697	-2702.2
			2b/3	-2832	-2837.4
3	3.67	-1153	[a]	[a]	[a]
			5a/5b	-3553	-3552.0
			5a/6a	-3326	-3317.2
5a	1.71	-1748	5a/6b	-3368	-3370.3 [b]
			5b/6a	-3383	-3379.4 [b]
			5b/6b	-3425	-3433.9
6a	2.27	-1578	6a/6b	-3198	-3198.4
			6a/7	-2180	-2176.1
			6b/7	-2222	-2231.4
7	5.53	-602	7/19	-2384	-2376.4
			9a/9b	-3280	-3270.3
			9a/10a	-3389	-3392.3
9a	2.04	-1631	9a/10b	-3514	-3515.6
			9b/10a	-3407	-3405.8
			9b/10b	-3532	-3534.0
10a	1.63	-1758	10a/10b	-3641	-3642.8
			10a/11a	-3490	-3488.4
			10a/11b	-3672	-3673.1
10b	1.27	-1883	10b/11a	-3615	-3618.1
			10b/11b	-3797	-3798.0
			11a/11b	-3646	-3630
11a	1.72	-1732	11a/12	-3425	-3442.9
			11b/12	-3607	-3618.1
			12/13	-3001	-2995.6
11b	1.14	-1914	12/20	-3675	-3673.1
			13/14	-2238	-2231.4
			[a]	[a]	[a]
12	1.96	-1693	17a/17b	-936	-932.3 [c]
			[a]	[a]	[a]
			[d]	—	—
13	3.18	-1308	[a]	[a]	[a]
			[a]	[a]	[a]
			[a]	[a]	[a]
14	4.42	-930	[a]	[a]	[a]
			[a]	[a]	[a]
			[a]	[a]	[a]
17a	6.31	-365	[a]	[a]	[a]
			[a]	[a]	[a]
			[a]	[a]	[a]
17b	5.61	-571	[a]	[a]	[a]
			[a]	[a]	[a]
			[a]	[a]	[a]
18	1.25	-1782	[a]	[a]	[a]
			[a]	[a]	[a]
			[a]	[a]	[a]
19	2.58	-1982	[a]	[a]	[a]
			[a]	[a]	[a]
			[a]	[a]	[a]
20	0.92	-1982	[a]	[a]	[a]
			[a]	[a]	[a]
			[a]	[a]	[a]

[a] Connectivities and transition frequencies reported for coupling partner. [b] Observed frequency reported was a shoulder of a more intense response and may not be accurate. [c] Very weak response; not visible in contour plot of spectrum in Figure 1. [d] No response observed in proton double quantum experiment.

Importantly, the proton double quantum spectrum facilitated the assembly of two structural fragments which are shown in Figure 3. Beginning with the pivotal H1 resonance in structure A, there are extensive couplings to the neighboring protons H2 $\alpha$  and  $\beta$  (-2783 and -2927 Hz), H14(-2176 Hz) and to the more remote exomethylene protons (-1612 and -1818 Hz) and H3 (-2408 Hz - only the response at the  $F_2$  frequency of H3 is observed in the contour plot shown). From the infrared data, the presence of the  $\gamma$ -lactone ring shown in structure A could also be established. Proceeding outward from the nucleus just described, further couplings completed the balance of the structure shown by A. Relative stereochemical orientation

of pairs of protons (H3/H13 and H1/H14) was established through one-dimensional nuclear Overhauser difference spectra (nOeds). The absolute orientation shown in fragment A is taken from the X-ray structure previously reported [25]. Thus, the H3/H13 and H1/H14 pairs are oriented as shown on the basis of 11.1 and 12.7% nOe's respectively. The 20 $\alpha$ -methyl orientation was also fixed on the basis of a 1.6% nOe to the H14 resonance. Finally, the upfield exomethylene proton, 17b, resonating at  $\delta$  5.61 was assigned on the basis of the observed nOe's to the 2 $\beta$  proton ( $\delta$  2.4; 3.0%) and the 3 $\beta$  proton ( $\delta$  3.7; 1.6%).

Structural fragment B was assembled solely on the basis of the connectivity network elucidated using the proton double quantum experiment. Thus, the H7 vinyl resonance is linked to the 19-methyl "singlet" *via* the response observed at  $F_1 = -2376$  Hz and to the two H6 protons *via* the responses at  $F_1 = -2176$  and  $-2231$  Hz. The H6 protons are each linked to both of the H5 protons (see expansion of the proton double quantum spectrum shown in Figure 4). The two fragments, A and B, could not, however, be linked together through either the proton double quantum or COSY/RCOSY experiments. The observed lack of a spin coupling pathway between either H5 $\alpha$  or H5 $\beta$  and H3 is consistent with the separation of the two fragments by the C4 quaternary carbon. If this were a new compound of unknown structure, consideration of the remaining carbon resonances, three methylene carbons, a quaternary carbon resonating at  $\delta$  80.0, and a methyl carbon whose directly coupled protons comprise an isolated singlet, it would be possible to assemble structural fragment C. The quaternary carbon (C4) bearing the methyl group links fragments A and B. Simultaneously, the insertion of the oxygen bearing quaternary carbon (C4) would also allow the assembly of the oxepin ring by linking C4 to C13. Experimental evidence supporting the structure of C was also provided by small nOe's observed at both H5 $\alpha$  and H5 $\beta$  when H3 was irradiated.

Having spectroscopically demonstrated how the structure of fragment C can be established, all that remains to be assigned was the methylene bridge linking C8 and C12. If we were dealing with an unknown structure, successfully linking C8 to C12 would be necessary to complete the structure elucidation. Thus, the spectroscopic means of accomplishing this task is quite important and worthy of further comment. From our earlier work on methylene carbon chemical shift regularities [26] and the recent total assignment of the  $^{13}\text{C}$ -nmr spectrum of eunicin [10], it was clear that the C9 methylene resonance would resonate furthest downfield ( $\delta$  36.54) of the three methylene resonances remaining to be assigned. Given this entry point into the resonance assignments of the methylene bridge, the proton resonances for H9a and H9b were located from the proton-carbon heteronuclear chemical shift correlation

spectrum. The connectivity observed between the H9 protons resonating at  $-1631$  and  $-1649$  Hz relative to the carrier (Figure 4) gave a double quantum frequency response observed at  $F_1 = -3270$  Hz. Using the  $F_2$  frequencies of H9a and H9b, double quantum responses are observed at  $F_1 = -3392, -3405, -3515$  and  $-3534$  Hz which correlate the H9 resonances to the neighboring H10 resonances. The H10 resonances are, in turn coupled to one another *via* the double quantum frequency response,  $F_1 = -3642$  Hz. Finally, the remaining H11 resonances are located *via* the double quantum responses linking H10a/b to the H11a/b resonances at  $F_1 = -3488, -3618, -3673$  and  $-3798$  Hz completing the identification of the resonances of the methylene bridge.

For purposes of comparison, the COSY/RCOSY spectrum is shown in Figure 5, the COSY shown below the diagonal, the RCOSY above the diagonal to facilitate direct comparisons. As would be expected, the upfield region of the COSY spectrum was severely congested and thus of limited utility, providing none of the connectivities of the H9/H10/H11 methylene bridge which were extracted from the proton double quantum spectrum discussed above. In addition, some of the longer range connectivity information obtained from the proton double quantum spectrum, *e.g.* the H1-H3 connectivity and the connectivities between H14 and H2 $\alpha$  and H2 $\beta$ , was not provided by the conventional COSY experiment although they were observed in the RCOSY spectrum as were several other useful connectivities which included those for H1-H13, H7-H5a and H7-H5b connectivities.

#### Conclusions.

The present work, our previous work [20] and that of Fesik and co-workers [21] have demonstrated the utility of the proton double quantum nmr experiment in structure elucidation and complex proton assignment problems. Corollary information is provided by the combined acquisition of COSY and RCOSY spectra although both of these experiments have shortcomings when highly congested spectral regions must be interpreted, a difficulty circumvented by the proton double quantum experiment. Thus, the proton double quantum experiment appears to offer significant promise in the solution of complex structural problems, especially when quantities of material also permit the acquisition of a proton-carbon heteronuclear chemical shift correlation spectrum and/or one of its long range variants [27-33]. Studies on additional novel marine natural products which employ these two-dimensional nmr techniques are underway in these laboratories and will form the basis for forthcoming reports.

#### EXPERIMENTAL

##### Isolation.

The mollusc *Planaxis sulcatus* (phylum molluska; class gastropoda) was

collected off Heron Island, Australia during 1974 and soaked in 2-propanol for shipment. The 2-propanol extract upon concentration followed by lyophilization gave a residue which was triturated with hexanes followed by chloroform. The chloroform extract, after evaporation, was chromatographed over LH-20 (methanol/methylene chloride, 9:1) to give a group of fractions which were combined and evaporated to give a residue, which was further purified by hplc (silica gel column, 1% methanol in chloroform) to give pure **1**. Jeunicin crystallized from chloroform, mp 145-147° (lit mp 139-141° [25]),  $[\alpha]_D^{22} = +16.1^\circ$  (c. 6.8 in chloroform) (lit [25]  $[\alpha]_D^{22} = +12.8^\circ$ ); ms: m/z (%)  $M^+ = 334$  (23),  $C_{20}H_{30}O_4$ ; ir (carbon tetrachloride):  $\nu$  3560, 3540, 1700, 1665  $cm^{-1}$ .

#### NMR Spectroscopy.

All of the nmr experiments reported were performed using a Nicolet NT-300 wide bore spectrometer operating at frequencies of 300.068 and 75.459 MHz for  $^1H$  and  $^{13}C$  observation respectively. The instrument was equipped with a Model 293C pulse programmer and a 5 mm dual tuned  $^1H/^{13}C$  probe. All experimental observations were made in deuteriochloroform at an ambient probe temperature of 17°. The proton reference spectrum shown beneath the proton double quantum (Figures 1 and 4) COSY/RCOSY contour plots (Figure 5) was acquired as 32K points and was processed using double exponential apodization.

#### Proton Double Quantum.

The proton double quantum nmr experiment performed on **1** was conducted using a sample consisting 8 mg of analytically pure material dissolved in 0.4 ml of deuteriochloroform. The data was collected as 512 × 2K blocks of data using the pulse sequence shown in Figure 2 using the 32 step phase cycle as previously reported [20,34]. The data was processed using sinusoidal multiplication in both frequency domains with zero filling prior to the second Fourier transform. The final data matrix was also transposed to give the  $F_1F_2$  format shown in Figures 1 and 4. The conventional high resolution spectrum is plotted below the contour plot with chemical shifts referenced to residual internal protiochloroform ( $\delta$  7.24). The axis plotted above the reference spectrum gives chemical shifts relative to the transmitter which was located downfield of the chloroform resonance. The data was acquired overnight.

Observed proton double quantum frequencies reported in Table II were obtained by the 90° projection of the  $F_1F_2$  data matrix. Since the responses for the 20-methyl doublet obscures the responses of some of the other resonances in the matrix, a second 90° projection was made covering the region from -300 to -2000 Hz with the methyl double response omitted. In this fashion, it was possible to obtain accurate double quantum frequencies for all of the responses in the matrix.

#### COSY/Relayed COSY.

The COSY experiment was performed in the normal fashion using the well established pulse sequence and phase cycling of Freeman, Morris and Bax [16]. Data was acquired as a 256 × 512 matrix with zero filling in both frequency domains prior to Fourier transformation to give a final matrix consisting of 512 × 512 points which was symmetrized [35] prior to contour plotting. Performance time was approximately 2 hours.

The RCOSY spectrum was acquired using the pulse sequence of Eich, Bodenhausen and Ernst [22] with the 32 step phase cycle of Bax and Drobny [36]. The data was acquired as a 256 × 512 matrix with zero filling in both dimensions prior to Fourier transformation, giving a final matrix consisting of 512 × 512 points which was symmetrized [35] prior to contour plotting. Performance time was approximately 4 hours.

For ease of identification of the new information (relay responses) in the RCOSY spectrum, the composite of the two experiments shown in Figure 5 was prepared. Relay responses are circled.

#### Heteronuclear Proton-Carbon Chemical Shift Correlation.

The heteronuclear chemical shift correlation spectrum (not shown) was acquired using the pulse sequence of Freeman and Morris [23] modified to provide quadrature detection in the second frequency domain as de-

scribed by Bax and Morris [24]. The sample consisted of 60 mg of analytically pure material dissolved in 0.5 ml of deuteriochloroform, the data acquired as an initial 256 × 2K matrix (140 blocks of the 256 were acquired) and was processed using a 1 Hz exponential broadening prior to the first Fourier transformation and a double exponential apodization and zero filling prior to the second Fourier transformation to give a 512 × 1K data matrix.

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