search (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO). The 500-MHz ^{1}H NMR spectra were run at the Dutch National 300/500 high field NMR facility at Nijmegen. We thank Dr. J. W. de Haan for valuable discussions and L. J. M. van de Ven and P. van Dael (Nijmegen) for the technical assistance in recording the NMR spectra.

Registry No. 1, 91237-85-3; 2, 96259-12-0; cAMP, 60-92-4; trimethyl phosphite, 121-45-9; dimethylamine, 124-40-3; dimethoxy(dimethylamino)phosphine, 20217-54-3; 2′,3′-*O*-isopropylideneadenosine, 362-75-4; 2′,3′-*O*-isopropylideneadenosin-5′-yl dimethyl phosphite, 96259-13-1.

Two-Dimensional NMR Studies of Marine Natural Products. 2.[1] Utilization of Two-Dimensional Proton Double Quantum Coherence NMR Spectroscopy in Natural Products Structure Elucidation—Determination of Long-Range Couplings in Plumericin

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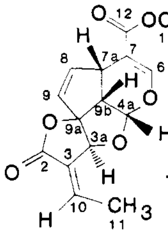
Two-dimensional NMR experiments have provided a convenient means of access to multiple quantum information,[2,3] this work leading to the development of the proton double quantum experiment recently described by Mareci and Freeman.[4] Although the proton double quantum technique has been applied to large molecule,[5-9] there have been no reported applications of the technique in natural products structure elucidation. We would therefore like to report the isolation of plumericin (1) from



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Cliona caribboea and the utilization of the proton double quantum experiment to uncover spin coupling pathways where $J \sim 0$ Hz[3,6,9] which were not observed in the much more commonly utilized COSY experiment.[10-12] The assignment of the ^{13}C NMR spectrum of 1 is also reported through the use of two-dimensional proton–carbon heteronuclear chemical shift correlation techniques.[13-15]

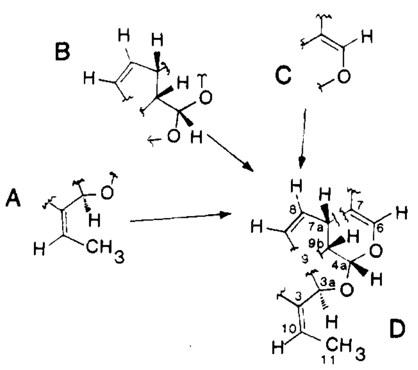

Figure 1. Structural fragments of plumericin assembled (A–C) from COSY data and from the two-dimensional proton double quantum spectrum (D) shown in Figure 2.

Results and Discussion

On the basis of the 300-MHz ^{1}H NMR spectrum and mass spectral data, 1 was tentatively identified as plumericin (3-ethylidine-3,3a,7a,9b-tetrahydro-2-oxo-2*H*,4*H*-1,4,5-trioxa-1*H*-dicyclopent[*a*,*hi*]indene-7-carboxylic acid methyl ester). The unprecedented occurrence of plumericin (1) in a marine invertebrate prompted us to initiate a carefully detailed study of the molecule. We were especially interested in the examination of the molecule for long-range spin-coupling pathways which would link together the several discrete proton spin systems contained in the structure despite the fact that no long-range coupling information was contained in the previous reports on the structure determination[16] or in the COSY spectrum. Rather than utilizing variants of the COSY experiment intended to emphasize long-range couplings,[11,17,18] we instead employed the proton double quantum experiment[4] which should also be suitable for this purpose.[3,6,9]

Structural fragments of plumericin (1) which are shown in Figure 1 were assembled from a COSY spectrum (not shown). Initial attempts at linking these components via homonuclear decoupling were unsuccessful because of the digitization employed during the survey decoupling experiments, thus representing a potential source of ambiguity in either the case of molecules of unknown structure or in those cases where the molecule is somewhat larger and the possibilities of selecting a permuted connectivity consequently are greater.

The proton double quantum coherence spectrum,[4] shown in Figure 2, did successfully link the structural components derived from the COSY experiment to afford the single large structural fragment shown in Figure 1. The final structure of the molecule follows directly from the large structural fragment. The utility of the proton double quantum experiment derives from several of its features which are worthy of further comment. First, responses in the double quantum frequency domain (ω_1 or F_1) are observed at the algebraic sum of the offsets of the coupled spins from the carrier frequency (0 Hz on the axis above

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[1] For the previous paper in this series, see: Martin, G. E.; Sanduja, R.; Alam, M. *J. Nat. Prod.*, submitted for publication.

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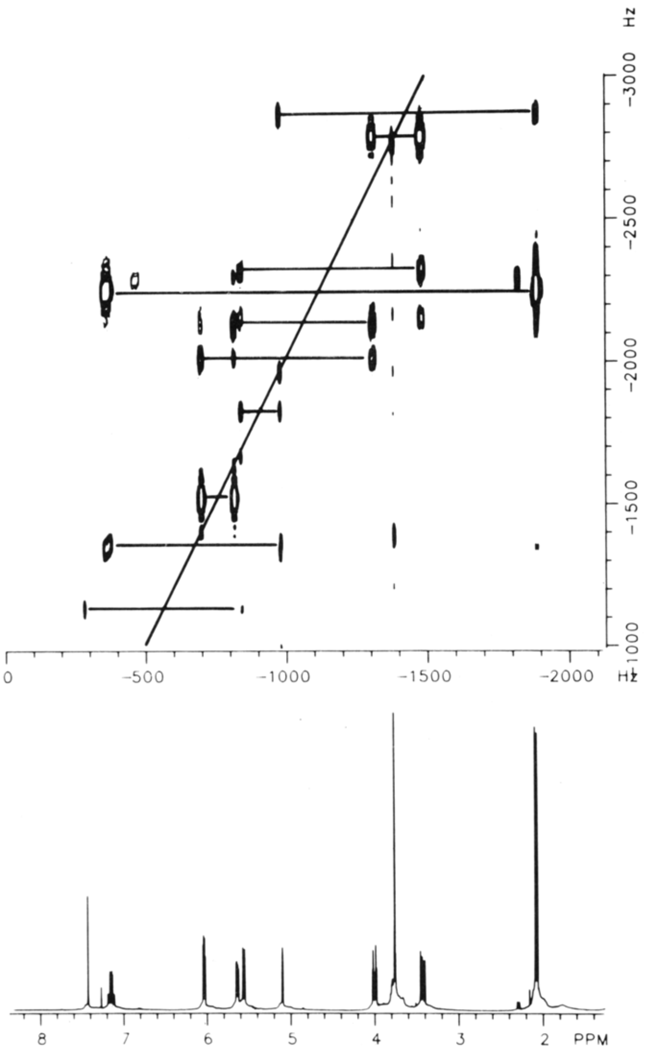

Figure 2. Proton double quantum two-dimensional NMR spectrum of plumericin (1). The conventional spectrum is shown below the contour plot. The axis below shows chemical shifts downfield from Me_4Si; that above shows shifts relative to the carrier. The spectrum correlates about the axis $F_1 = \pm 2F_2$.

the spectrum plotted below the contour plot). It should also be noted that these responses generally appear symmetrically disposed about the "skew" diagonal[3] ($F_1 = \pm 2F_2$), the coupled spins denoted by horizontal lines connecting the responses in Figure 2. Furthermore, although frequencies in the double quantum spectrum may be calculated as shown in Table I, this need not be done for the spectrum to be effectively utilized. Rather, the calculation of expected F_1 frequencies should be most beneficial when examining the spectrum for specific couplings between given resonances, for example, long-range couplings. Second, at a given response in the F_1 domain, additional responses may also be observed involving transfer to a passive member of the spin system, this constituting new information and thus providing an additional means of elucidating complex spin systems.[3,8] Third, the diagonal in the proton double quantum experiment, unlike its counterpart COSY spectrum, is not complicated by the presence of the normal spectrum, thus facilitating the detection of couplings between resonances with closely similar chemical shifts.[3]

Responses expected in the proton double quantum experiment have been rigorously treated in the comprehensive work of Braunschweiler, Bodenhausen, and Ernst.[3] Briefly, the three types of responses which may be observed are summarized as follows: type I, responses symmetrically disposed about the skew diagonal which indicated directly coupled resonances ($J_{AX} \neq 0$ Hz), the response appearing at the sum of the offsets from the carrier (these may also be accompanied by responses arising from transfer of magnetization to a passive member of the spin system, thus providing "relay" information); type II, single peak with an F_1 frequency that intersects the diagonal at the chemical shift (F_2), indicating at least two magnetically equivalent nuclei are present (e.g., the response due to the methoxyl protons on the skew diagonal at $F_1 \sim -2755$ Hz); type III, a single peak with an F_1 frequency intersecting the skew diagonal at an F_2 frequency not corresponding to the chemical shift of any of the resonances in the spectrum, this type of response arising from nuclei which are not coupled to one another but which have a common coupling partner (e.g., an AMX spin system where $J_{AX} = 0$ Hz, this information equivalent to that provided by the relayed coherence transfer experiments[8,19–21]).

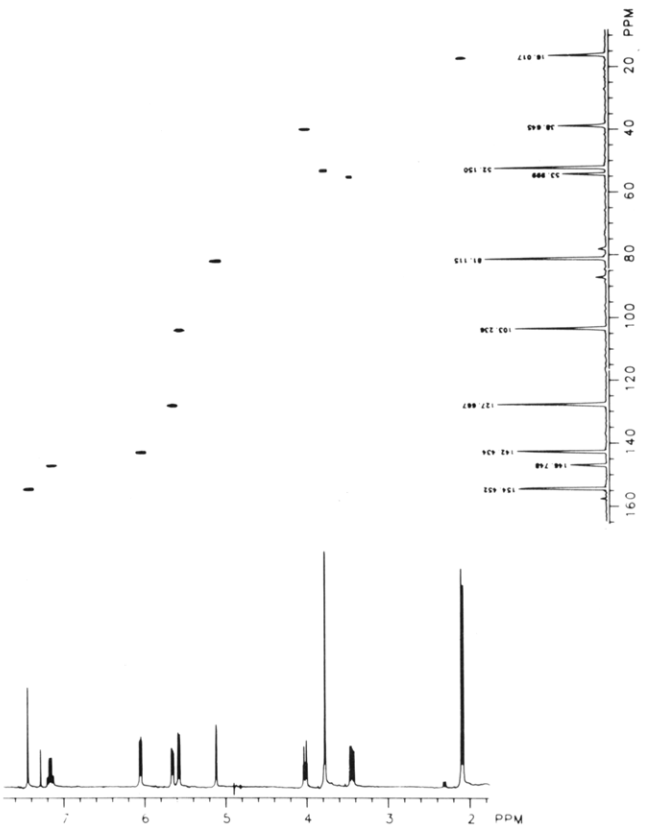

Figure 3. Heteronuclear ($^1H/^{13}C$) chemical shift correlation spectrum of plumericin at 300.068/75.457 MHz. The spectrum (1) plotted along the F_1 axis is the conventional ^1H reference spectrum while that along F_2 is a 90° projection of the two-dimensional data matrix.

The key to the assembly of the structural components of 1 which are shown in Figure 1 resides in the connectivities of the H4a resonance which was observed at δ 5.525. This resonance is readily identified as the acetal proton on the basis of the chemical shift of the directly bonded carbon (δ 103.24, Figure 3). While connectivities within the five spin system containing the H4a resonance were readily established from a COSY experiment (H4a–H9b, H9b–H7a, H7a–H8, H8–H9), there was no evidence of connectivities between H4a–H3a and/or between H4a–H6 when individual slices of the COSY spectrum were plotted.

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Table I. ^{1}H and ^{13}C NMR Spectral Data Obtained for Plumericin (1) in Deuteriochloroform at Observation Frequencies of 300.068 and 75.457 MHz, Respectively, at 20 °C

		double quantum responses					
			response (Hz)				heteronuclear coupling
	$\delta_{^1H}$	connectivity	calcd	obsd	proton coupling (J_{HH}, Hz)	$\delta_{^{13}C}$	(J_{CH}, Hz)
2						168.13	
3						127.39	
3a	5.093	3a/4a	−1793.8	−1798	dddd, J = 1.60, 0.76, 0.33, 0.42	81.16	161.0, 8.2, 7.1
		3a/10	−1316.7	−1318			
		3a/11	−2839.8	−2841			
4a	5.525	4a/3a	−1793.8	−1798	ddd, J = 5.80, 0.54, 0.64	103.24	180.2, 4.78, 4.8
		4a/6	−1130.9	−1133			
		4a/9b	−2331.8	−2331			
6	7.423	6/4a	−1130.9	−1133	dd, J = 0.50, 0.50	154.95	194.4, 5.5
7						104.55	
7a	3.948	7a/8	−2135.5	−2135	ddd, J = 9.49, 2.20, 2.20	38.65	
		7a/9	−2017.1	−2018			
		7a/9b	−2799.6	−2798			
8	5.618	8/7a	−2135.5	−2135	dddd, J = 5.49, 2.19, 0.54, 0.47	127.67	
		8/9	−1525.5	−1525			
		8/9b	−2297.7	−2303			
9	6.013	9/7a	−2017.1	−2018	dd, J = 5.49, 2.15	142.42	
		9/8	−1525.5	−1525			
9a						109.30	
9b	3.403	9b/4a	−2331.8	−2331	ddddd, J = 9.46, 5.88, 0.80, 0.47, 0.40	54.00	
		9b/7a	−2799.6	−2798			
		9b/8	−2308.0	−2315			
10	7.116	10/3a	−1316.7	−1318	dq, J = 7.21, 1.60	146.75	
		10/11	−2260.5	−2259			
11	2.034	11/3a	−2839.8	−2841	dd, J = 7.25, 0.76	16.02	
		11/10	−2260.5	−2259			
12						166.62	
13	3.753	type II	−2752.2	−2755	s	53.15	

The first of these connectivities, H4a–H3a, has a predicted location in the second frequency domain of the proton double quantum experiment at approximately $F_1 = -1793.8$ Hz. Usefully, as expected, the double quantum experiment contained a response at $F_1 = -1798$ Hz centered about the skew diagonal (type I), thus confirming the existence of the H4a–H3a connectivity. Further connectivities link H3a to the balance of the ethylidene moiety. The proton double quantum experiment also provided the connectivity between H4a and H6 expected at $F_1 = -1130.9$ Hz and observed at $F_1 = -1132$ Hz. Thus, the proton double quantum experiment successfully linked the three structural components shown in Figure 1 to provide the single large structural fragment. From these results, it is clearly demonstrated that the proton double quantum experiment provides a viable alternative to the long-range COSY experiment,[11,17,18] in some cases even providing the information which would be obtained from the performance of both a COSY and a long-range COSY (LRCOSY) experiment in a single experiment. Several other long-range couplings were also identified by the proton double quantum experiment and are summarized in Table I, although there remain several such couplings which have yet to be accounted for.

Finally, from the assigned proton resonances of 1, the carbon resonance assignments were made through the acquisition of a two-dimensional proton–carbon heteronuclear chemical shift correlation experiment[13-15] which is shown in Figure 3. These resonance assignments, selected heteronuclear spin-coupling information accompanied by data from the high resolution and proton double quantum experiments is presented in Table I.

Conclusions

Two-dimensional proton double quantum NMR spectroscopy provides the natural products chemist with a potentially powerful new adjunct to the assortment of two-dimensional NMR experiments already available for the solution of structural problems. Expansions of the scope and utilization of the proton double quantum experiment have already begun to appear in the form of proton double quantum relayed coherence transfer[8] and double quantum echo correlated spectroscopy (DECSY) experiments.[22] Despite these attractive points, there are limitations, and, indeed, the proton double quantum experiment may still fail in the strong coupling limit.[3,6] Nonetheless, we have found the proton double quantum experiment to be highly useful in the present study as well as in others which are underway in these laboratories which will form the basis of future reports.

Experimental Section

Cliona caribboea collected from the waters of Summerland Key, FL, was soaked in 2-propanol and then shipped in a 5-gal container. The 2-propanol was decanted and then evaporated to yield a residue which was sequentially extracted with hexanes, chloroform, and methanol. The chloroform extract (2.52 g of residue) was subjected to flash chromatography over silica gel (E. Merck, 70–230 mesh). Elution with hexanes/chloroform (1:1, v/v) gave a fraction (523.7 mg) which contained three major components. This residue upon rechromatography at low pressure [~50 psi silica gel 60 (E Merck, 230–400 mesh), eluting solvent methylene chloride] gave 1 (40.5 mg). Recrystallization from benzene gave rectangular plates: mp 204–206 °C (lit.[16] mp 209–212 °C); LRMS, m/z (relative intensity) 290 (M^+, 24), 272, (14), 269, (29), 250, (39), 230, (68), 193, (71), 160, (71), 139, (92), 77, (69), 53 (100).

All of the NMR spectral data on 1 were acquired on a Nicolet NT-300 wide bore spectrometer operating at 300.068 and 75.457 MHz for ^{1}H and ^{13}C observations, respectively. The instrument was controlled by a Model 293-C pulse programmer and all spectra were acquired in deuteriochloroform at 20 °C by using a 5-mm ^{1}H/^{13}C dual tuned probe.

Two-dimensional NMR spectra of 1 were acquired on a sample prepared by dissolving 35 mg of 1 in 0.4 mL of deuteriochloroform. The COSY spectrum (not shown) was acquired as 512 × 1K data points and was transformed by using 0.1 Hz sinusoidal multi-

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plication in both frequency domains to give 512 × 512 real data points, the final data matrix symmetrized[23] prior to plotting. Total data acquisition time for the COSY spectrum was 6 h. The proton double quantum spectrum was acquired by using the following pulse sequence:

$$\pi/2-\tau-\pi/2,\ \pi,\ \pi/2-\tau-\pi/2-t_1-\pi/4-t_2$$

with the transmitter located downfield of the H6 resonance and with data collection conducted using a 32-step phase cycle.[24] The spectrum was collected as 512 × 2K data points and was processed by using 0.1-Hz sinusoidal multiplication in both frequency domains with zero filling prior to the second Fourier transform to give 512 × 512 real data points in the quadrant of the spectrum finally plotted as a ten-level contour plot (Figure 2). Total acquisition time for the proton double quantum spectrum was 14 h. Finally, the heteronuclear chemical shift correlation spectrum was acquired overnight by using the modified pulse sequence described by Bax and Morris.[15] The spectrum was collected as 256 × 2K data points and was processed by using double exponential apodization prior to both Fourier transformations with zero filling prior to the second to give the final data matrix containing 512 × 1K data points which is presented as a six-level contour plot in Figure 3.

The proton reference spectrum shown in all of the two-dimensional NMR spectra was obtained by using 32K data points which was processed by using an 0.03-Hz exponential line broadening. The long-range couplings initially identified from the proton double quantum spectrum were confirmed by using a 64K data file which was processed with double exponential apodization followed by 0.02-Hz sinusoidal multiplication, the observed couplings reported in Table I.

Acknowledgment. We acknowledge the support of The Robert A. Welch Foundation through Grants No. E-792 to GEM and E-745 to M.A. as well as the support of the University of Houston—University Park which provided the funds for the acquistion and operation of the NT-300 spectrometer system. We also thank Professor A. J. Weinheimer for kindly providing the extract of *Cliona caribboea* which was collected with the support of NCI Contract No. NO1-CM-87207 and Dr. Steve Fesik of Abbott Laboratories, Abbott Park, IL, for a suggested modification of the 32-step phase cycle utilized in the performance of the proton double quantum experiment.

Registry No. 1, 77-16-7.

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[24] The experiment was performed with the delay $\tau = {}^1/_4J_{HH}$. An average vicinal coupling constant of 7 Hz was assumed, giving τ = 35.7 ms. Complete details of the 32-step phase cycling scheme employed in the performance of the proton double quantum experiment are available from the authors upon request.

In Situ Preparation of (μ-Chloro)(μ-methylene)bis(cyclopentadienyl)(dimethylaluminum)titanium (Tebbe's Reagent)[†]

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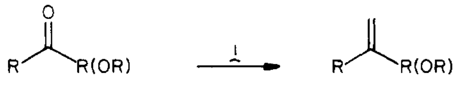
Received November 21, 1984

The titanium alkylidene 1 (Tebbe's reagent) is a versatile methylenation agent for the conversion of ketones to olefins and esters to vinyl ethers (eq 1).[1] Unfortunately, this commercially available reagent (Strem, Alfa) is expensive for large scale reactions, and currently one needs to employ vacuum line and Schlenk techniques for its synthesis.[2]

Table I. Equivalents of 1 Produced under Different Reaction Conditions[a]

runs	prep	equiv of 1 produced
1	68 °C, 11 h	0.40
2	72 °C, 11 h	0.47
3	87 °C, 11 h	0.23
4	60 °C, 11.5 h	0.30
5	50 °C, 18.5 h	0.55
6	rt, 72 h	0.65

[a] See Experimental Section for preparation of 1 and determination of equivalents produced.



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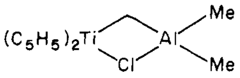

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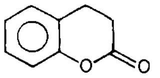
We felt there was a need to develop a facile method for the in situ generation of 1 (utilizing only an inert gas manifold and standard synthetic organic techniques) which could also be applied to large scale synthesis.

It was found that the crude reaction mixture formed by the combination of titanocene dichloride and 2 equiv of $AlMe_3$ (2.0 M in PhMe) could be used directly to effect methylene transfer. Optimization of reaction conditions were performed by allowing the crude mixture, containing 1 and ~1 equiv of $AlMe_2Cl$ in PhMe, to react with a THF solution of 2-*tert*-butylcyclohexanone (2). Workup afforded a combined quantitative yield of starting ketone and the methylenation product 3 as an oil upon which ^1H NMR analysis gave the equivalents of 1 consumed by the ketone (Table I).

On a 50-mmol scale performed using the parameters of run no. 6 (Table I) under slightly modified conditions, 1.3 equiv of 4-phenylcyclohexanone (4) were methylenated by the in situ Tebbe's reagent 1 *derived from 1 equiv of titanocene dichloride* to give an 82% isolated yield (94% crude yield) of the olefin 5.[3]

In addition, 1.0 equiv of the ester dihydrocoumarin (7) was converted to the vinyl ether 8[1b] in 76% isolated yield upon treatment with 1 generated as above while use of 1.3 equiv of ester gave unreacted ester upon workup.



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[1] (a) Clawson, L. E.; Buchwald, S. L.; Grubbs, R. H. *Tetrahedron Lett.* 1984, 5733. (b) Pine, S. H.; Zahler, R.; Evans, D. A.; Grubbs, R. H. *J. Am. Chem. Soc.* 1980, *102*, 3270.

[2] Lee, J. B.; Ott, K. C.; Grubbs, R. H. *J. Am. Chem. Soc.* 1982, *104*, 7491.

[3] This may result from regeneration of 1 during the reaction. Conversion of the methylenation byproduct [Cp_2Ti=O] (Cp = cyclopentadienyl) to $Cp_2TiMeCl$ by $AlMe_2Cl$[4] and its reaction with $AlMe_3$ could yield 1.[5a] Alternatively, $AlMe_2Cl$ may react with $Cp_2TiMeCl$ to give $Cp_2TiCH_2{\cdot}AlMeCl_2$ (6),[5a] a less reactive methylenation agent.[5b] The existence and potential use of these proposed catalytic pathways are currently being explored. Standard Wittig procedure gave 34% isolated yield of 5. Barrett, A. G. M.; Betts, M. J.; Fenwick, A. J. *J. Org. Chem.* 1985, *50*, 169.

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[†] Contribution No. 7126.

0022-3263/85/1950-2386$01.50/0