Carbon-13 Magnetic Resonance Spectroscopy. The Spectrum of Proline in Oligopeptides

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The carbon-13 magnetic resonance spectra of a selection of simple proline derivatives are compared and discussed. The general features of such spectra are identified, with especial importance given to the problem of differentiation of cis and trans X-proline amide bonds. Spectra of the proline moiety in more complex oligopeptides are discussed in terms of the conclusions drawn from the spectra of simple compounds.

Because of the conformational requirements inherent in the pyrrolidine ring, proline has a unique place among the naturally occurring amino acids. Its occurrence in biologically active oligopeptides and proteins has important consequences upon the three-dimensional structures of these compounds.[1] As a result, proline and polypeptides containing proline have received extensive and detailed study by a wide variety of physical methods, including proton magnetic resonance (pmr) spectroscopy.[2]

Unlike the pmr spectrum of the proline residue, the fully proton-decoupled carbon-13 magnetic resonance (cmr) spectrum would be expected to be relatively simple. Furthermore, because of the importance of steric[3] and other proximity[4-6] effects in carbon-13 chemical shifts, one might expect that cmr spectroscopy would provide useful information regarding the conformation within and around the proline unit. We have therefore surveyed the cmr spectra of a number of proline derivatives and proline-containing oligopeptides. The purpose of the present paper is to identify and discuss the major features of the cmr spectrum of the proline unit.

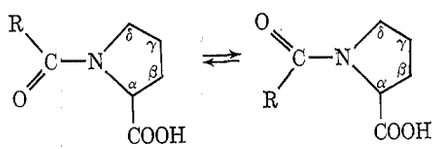
Experimental Section

The cmr spectra of the proline derivatives, all of which were commercially available, were measured as 10% (w/v) aqueous solutions, using a Varian XL-100 spectrometer adapted for Fourier transform spectroscopy.[7] Chemical shifts were measured relative to internal 1,4-dioxane, then referred to external carbon disulfide on the basis of the chemical shift of 1,4-dioxane relative to the reference (126.2 ppm).

Results

The cmr spectra of the proline derivatives studied are presented in Table I. The problem of peak assignments in such simple compounds is reduced to a minimum and can be based entirely upon the well-known correlations between carbon chemical shift and substitution.[8-10] These assignments are supported by comparisons within the series of proline deriviatives (*vide infra*), as well as by the proton-coupled ^{13}C nmr spectrum of proline.

The cmr spectra of the *N*-formyl and *N*-acetyl derivatives of proline (Table I) all show the peak doubling which would be anticipated because of the *cis–trans* isomerism of these compounds.[2,4-7,11] The major contributors to these mixtures have been clearly demonstrated by pmr spectroscopy[11] to be the trans conformers. Due to the general experience[4-6] that



trans cis

carbons syn to the carbonyl oxygen of amides are shielded relative to those which are anti, one would expect the α carbon of the trans conformer to be shielded relative to that of the cis. The δ carbon, however, would be expected to be more shielded in the cis, or minor, isomer. Such a situation should lead to a back-to-back[12] pattern for these resonances, as is observed in the case of *N*-acetylsarcosine.[6] As seen in the spectrum of *N*-acetylprolinamide (Figure 1), such is indeed the case. It is also evident from Figure 1 that a very similar pattern is observed for the β and γ resonances.[11b]

The carbons of the pyrrolidine ring throughout this series were assigned on the basis of (1) comparison to the spectrum of proline itself, and (2) the assumption that the trans conformer was the dominant species in each mixture.

The carbonyl resonances were assigned on the usual assumption that carbon chemical shift changes will be greatest near the site where substitution is altered. Comparison of the spectra of *N*-formyl- and *N*-acetylproline, for example, shows that both spectra have peaks near 16.5–17.0 ppm. Because the proline carboxyl might be expected to show only minor chemical shift changes in these two compounds, these peaks are assigned to that carbon. This leaves the peaks at about 28.5 and 20.3 ppm to be assigned to the formyl and acetyl carbonyls, respectively. These assignments are supported by other comparisons. Thus, in the acetylated proline and the analogous ester and amide, only the carboxyl carbon resonance should show large variations in chemical shift. Certainly the acetyl

(1) L. Mandelkern in "Poly-α-amino Acids," G. D. Fasman, Ed., Marcel Dekker, New York, N. Y., 1967, Chapter 13.

(2) *Cf.*, for example, F. A. Bovey, *et al.*, *Accounts Chem. Res.*, **5**, 193 (1972).

(3) D. M. Grant and B. V. Cheney, *J. Amer. Chem. Soc.*, **89**, 5315, 5319 (1967).

(4) W. McFarlane, *Chem. Commun.*, 418 (1970).

(5) G. C. Levy and G. L. Nelson, *J. Amer. Chem. Soc.*, **94**, 4897 (1972).

(6) D. E. Dorman and F. A. Bovey, *J. Org. Chem.*, **38**, 1719 (1973).

(7) H. Sternlicht and D. M. Zuckerman, *Rev. Sci. Instrum.*, **43**, 525 (1972).

(8) (a) D. M. Grant and E. G. Paul, *J. Amer. Chem. Soc.*, **86**, 2984 (1964); (b) L. P. Lindeman and J. Q. Adams, *Anal. Chem.*, **43**, 1245 (1971).

(9) W. Horsley, H. Sternlicht, and J. S. Cohen, *J. Amer. Chem. Soc.*, **92**, 680 (1970).

(10) M. Christl and J. D. Roberts, *ibid.*, **94**, 4565 (1972).

(11) (a) W. A. Thomas and M. K. Williams, *J. Chem. Soc., Chem. Commun.*, 788 (1972). (b) This feature of the cmr spectra of proline derivatives has been noted in an independent study: K. Wüthrich, A. Tun-Kyi, and R. Schwyzer, *F.E.B.S. Lett.*, **25**, 104 (1972).

(12) F. A. Bovey, J. J. Ryan, and F. P. Hood, *Macromolecules*, **1**, 305 (1968).

TABLE I

CARBON CHEMICAL SHIFTS[a] OF SELECTED PROLINE DERIVATIVES

	Registry no.	Cis						Trans					
		CO	α	β	γ	δ	Other	CO	α	β	γ	δ	Other
Proline (zwitterion)	147-85-3							18.3	131.4	163.8	168.9	146.5	
N-Formylproline	13200-83-4	16.6	133.1	163.4	170.3	148.5	CHO 28.3	17.3	135.8	163.4	169.1	145.5	CHO: 29.4
N-Acetylproline	68-95-1	16.4	131.8	161.9	170.2	146.1	CO 19.7; CH_3 171.4	16.5	133.7	163.3	168.5	144.3	CO 20.0; CH_3 171.4
N-Acetylproline, methyl ester	27460-51-1	17.9	132.1	161.8	170.4	146.1	CO 20.0; CH_3 139.9	17.9	133.8	163.6	168.4	144.4	CO 20.0; CH_3 171.5; OCH_3 139.9
N-Acetylprolinamide	16395-58-7	15.5	131.5	161.1	170.3	145.8	CO 19.8; CH_3 171.3	15.5	133.0	162.7	168.7	144.2	CO 19.8; CH_3 171.5
Glycylproline	704-15-4	14.5	131.1	161.2	170.4	145.5	Gly(CO) 27.2; Gly(α) 152.3	13.8	130.8	163.2	168.5	146.1	Gly(CO) 27.8; Gly(α) 152.1
tert-Butoxycarbonylglycylproline[b]	14296-92-5	18.1	133.4	161.5	170.6	146.0	Gly(CO); 23.2; Gly(α) 150.0; Boc(CO) 35.5; C-quat 113.1; CH_3 165.1	18.1	133.4	163.8	168.2	146.6	Gly(CO) 23.7; Gly(α) 150.0; Boc(CO) 35.5; C-quat 113.1; CH_3 165.1
"Typical generalized proline"		14.5–18.5	131.1–133.4	161.1–163.4	170.2–170.6	145.5–148.5		13.8–18.3	130.8–135.8	162.7–163.8	168.2–169.1	144.3–146.6	

[a] Relative to external carbon disulfide. [b] D. A. Torchia, unpublished results.

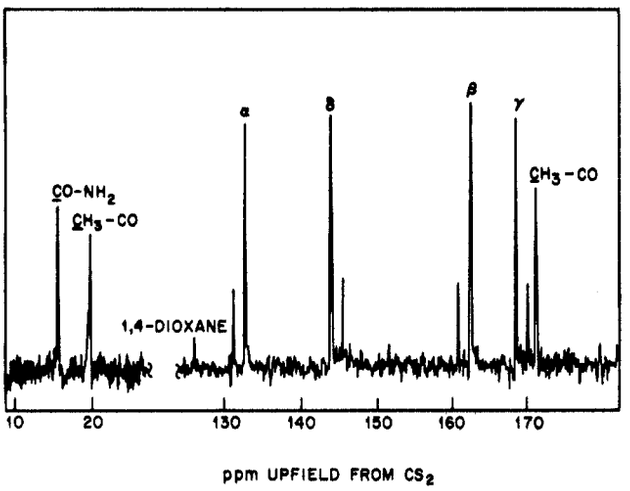


Figure 1.—The proton-decoupled cmr spectrum of *N*-acetylprolinamide. The resonances of the pyrrolidine ring carbons all show doubling due to cis–trans isomerism around the X-Pro bond. The methyl and carbonyl resonances also show this doubling, but only under conditions of higher resolution.

carbonyl resonances near 20 ppm appear to be unchanged in chemical shift throughout the series.

The acetyl methyl carbon resonance is assigned by elimination. It is evident that its chemical shift is unaffected by changes in the substitution at the carboxyl group. The assignment of the glycine resonances of the two glycylprolines is based on comparisons with earlier studies.[9,10] Peak assignments for other peptides discussed in this paper are available in the original articles.

Discussion

It is apparent from Table I that the spectra of these proline derivatives show many similarities. Most striking is that seen in the resonances assigned to the γ carbons. Throughout this series, the peak assigned to the γ-carbon resonance of the trans conformer is within the narrow range 168.6 ± 0.5 ppm. The analogous peak of the minor cis isomer occurs at 170.3 ± 0.3 ppm. This pattern in the γ-carbon resonances was noted very early in this work and formed the basis of the hypothesis that this chemical shift behavior could be used to determine the conformation about the peptide bond involving the proline nitrogen, the so-called X-Pro bond.[13]

A similar but more variable pattern is seen in the resonances of the β carbons (Table I). In the trans conformers, the chemical shifts of this carbon range from 162.7 to 163.8 ppm. With the exception of *cis*-*N*-formylproline, the β resonances of the cis conformers vary from 161.1 to 161.9 ppm. Unfortunately, the chemical shift of the β carbon is subject to conformational effects other than the cis–trans isomerism of the peptide bond (*vide infra*). For this reason this resonance is a less reliable guide in the determination of the conformation of the X-Pro bond.

On the basis of prior results with simple amides,[4–6] it is reasonable to suppose that the chemical shift differences observed at the α and δ positions (*vide supra*) would suffice to identify the peptide bond conformation. Such would certainly appear to be the case for the *N*-acetylproline derivatives (*cf.* Table I). The normal back-to-back pattern in these resonances are reversed, however, when the acyl group on the proline nitrogen is an amino acid, as is seen in the spectra of glycylproline derivatives (*cf.* Figure 2). Closer examination of Figure 2 leads to additional conclusions regarding the sources of chemical shift differences in these compounds. It is obvious that the chemical shift of the δ carbon of the cis isomer is broadly independent of the nature of R. This is as might have been expected on the grounds that R is relatively distant from this position. The δ resonance of the trans conformer, however, is seen to move upfield as the steric bulk of R increases (Figure 2). This shielding effect may result from steric perturbations.[3] The net result of this chemical shift effect, regardless of its origin, is that the δ-carbon resonances of *cis*- and *trans*-proline derivatives have very similar chemical shifts and are therefore not generally useful in the identification of the dihedral angle about the X-Pro bond.

An analogous change is observed in the α resonances. Comparison of the spectra of *N*-acetyl- and *tert*-butoxycarbonylglycyl-L-proline in Figure 2 shows that it is the α resonance of the cis conformer which moves upfield. Again, such a shift is consonant with a steric

(13) F. A. Bovey, "Proceedings of the Third American Peptide Symposium," Ann Arbor Science Publishers, Ann Arbor, Mich., 1972.

effect and leads to difficulties in interpretation of the α-carbon chemical shift in terms of conformation of the X-Pro bond. In the spectrum of glycyl-L-proline, this effect is masked by those resulting from ionization of the neighboring carboxyl group. The observed chemical shift change at the α position in the last compound is in accord with an earlier study of the effects of ionization of carboxylic acids,[14] which showed that the α carbons of even these simpler compounds were deshielded by ionization of the acid.

From these spectra we may conclude that the chemical shifts of the γ resonance provide a means by which the conformation of the X-Pro bond may be analyzed. In those spectra wherein the γ resonance cannot be observed or identified, the chemical shift of the β resonance can serve this function. To assess the reliability of these criteria, we have attempted to identify the sources of the chemical shift dependences at the β and γ positions upon the conformation of the X-Pro bond. From Table I it is seen that the γ resonances of a pair of X-Pro conformers differ in chemical shift by 1.5–2.0 ppm; similar differences are observed at the β position. In the spectra of *N*-formyl- and *N*-acetylpyrrolidine (Table II), in which

TABLE II

CARBON CHEMICAL SHIFTS OF *N*-FORMYL- AND *N*-ACETYLPYRROLIDINE

Pyrrolidine	C-2	C-3	C-4	C-5	CO	CH_3
N-Formyl	149.5	169.1	168.5	146.0	30.7	
N-Acetyl	147.0	168.7	167.5	144.9	21.0	171.6

the β and γ carbons differ in chemical shift solely by virtue of the amide group, we observe chemical shift differences of approximately 1 ppm at these positions. It therefore appears that to a large degree the dependence of these chemical shifts upon the conformation of X-Pro bonds arises from the direct influence of the peptide function.

Correlation with Other Results.—The above conclusions are based solely on the cmr spectra of relatively simple compounds. In no case, for example, is the proline carboxyl involved in a peptide bond to a second amino acid. It is possible, therefore, that some of our generalizations will fail when they are applied to more complex systems. Fortunately, there are available in the literature sufficient data to test our conclusions thoroughly. Indeed, in some cases the reevaluation of published data in the light of our generalizations leads to additional insight into the structures and conformations of these oligopeptides.

The simplest cases available for comparison are the di- and tripeptides studied by Christl and Roberts.[10] Perhaps because these authors were limited to the sensitivity available from a continuous wave spectrometer, they were unable to observe peak doubling due to cis–trans isomerism of the X-Pro bond. In the case of L-prolyl-L-phenylalanine (Table III), no such isomerism is possible. It is interesting that the chemical shifts of both the β and γ carbons of this compound occur in the ranges typical of trans X-Pro systems, as was observed for unsubstituted proline

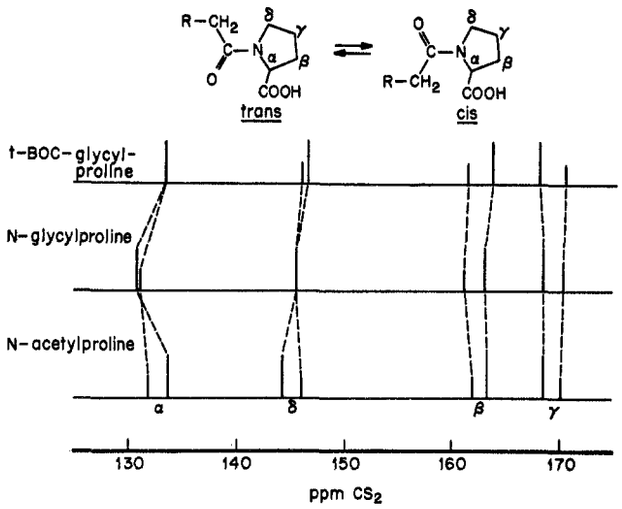


Figure 2.—Comparison of the cmr spectra of *N*-acetylproline, *N*-glycylproline, and *tert*-butoxycarbonylglycyl-L-proline. The peaks of the major trans conformer are denoted by the lines of greater height. Only the spectra of the pryrrolidine ring carbons are shown.

(Table I). This suggests that the chemical shifts of these carbons are not influenced by charge on the proline nitrogen or by substitution at the prolyl carboxyl. It is also notable that the prolyl carbonyl resonance of prolylphenylalanine comes into resonance at significantly higher field than in all previous examples, including *N*-acetylprolinamide. In previous studies of simple amides[6] it was noted that increasing substitution upon the amide nitrogen had a generally shielding effect at the carbonyl carbon. It is probable that the generally higher field position of the proline carbonyl carbon in peptides is due at least in part to this substituent effect.

A rather more interesting case is that of phenylalanyl-L-proline.[10] In acidic solutions, the spectrum of the pyrrolidine ring most closely corresponds to that of a trans X-Pro system (Table III). In neutral and alkaline solutions, however, the carbon chemical shifts suggest that the X-Pro bond is cis. Interpreted on the basis of our hypotheses, then, these results suggest that the X-Pro equilibrium in these compounds is pH dependent.

In the case of L-phenylalanyl-L-prolyl-L-arginine,[10] again only one species was observed. The chemical shifts of the β and γ resonances (Table III) are in better agreement with a trans X-Pro system. However, both these resonances appear at unusually high field. Problems in the peak assignments in this spectrum led the authors to suggest an alternative assignment by which the β and γ resonances occur at 163.0 and 168.4 ppm, respectively.[10] It will be noted that this latter assignment is in excellent agreement with a typical trans X-Pro spectrum. For this reason we consider the alternative assignment more reliable.

In pmr spectroscopy the diketopiperazines are not necessarily good models for polypeptides.[15] One might expect that ring strain and other effects associated with such structures would make their cmr spectra atypical.

(14) R. Hagen and J. D. Roberts, *J. Amer. Chem. Soc.*, **91**, 4504 (1969).

(15) F. A. Bovey, "High Resolution NMR of Macromolecules," Academic Press, New York, N. Y., 1972, p 274.

TABLE III
CARBON CHEMICAL SHIFTS[a] OF PROLINE IN SELECTED OLIGOPEPTIDES

	CO	α	β	γ	δ	Solvent	Ref
Prolylphenylalanine (pH 0.9)	22.9	132.4	162.5	168.5	145.4	H_2O	10
Phenylalanylproline (pH 0.9)	17.3	132.5	163.6	167.7	144.8	H_2O	10
Phenylalanylproline (pH 5.5)	14.4	130.4	161.2	170.2	145.8	H_2O	10
Phenylalanylproline (pH 10.5)	13.5	130.4	161.2	170.0	145.6	H_2O	10
Phenylalanylprolylarginine (pH ~7)	19.6	131.4	165.8[c]	169.2[c]	144.6	H_2O	10
Polyproline-I	20.9	133.7	161.4	171.1	144.6	H_2O	23
Polyproline-II	21.3	134.3	164.9	168.2	145.2	H_2O	23
Glycyl-L-proline diketopiperazine	23.4	134.7	164.8	170.6	146.7	DMSO	16
L-Prolyl-L-proline diketopiperazine	26.8	133.0	165.5	169.8	148.1	DMSO	16
c-(Pro-Gly)$_3$	22.4	133.2	165.9	167.1	146.4	CH_2Cl_2	24
c-(Pro-Gly)$_3$	21.0	133.1	161.8	168.8	146.2	DMSO	24
	22.0	134.1[b]	162.3	169.8	147.1[b]		
	22.8		163.7	171.2			
c-(Pro-Gly)$_3$-Na	20.3	133.9	164.3	168.8	147.2	DMSO	25
c-(Pro-Ser-Gly)$_2$	21.9[b]	131.1	163.3	168.3	145.9	H_2O	25
Gramicidin S	*d*	132.6	163.4	168.5	146.1	DMSO	19
Gramicidin S	*d*	130.3	161.6	166.4	144.3	CH_3OH	19
Antamanide	*d*	131.5	161.0	167.6	144.3	CD_3CN	22
		131.7	161.6	167.6	144.7		
		133.5	163.7	170.4	145.8		
		133.8	164.0	171.2	145.9		
Antamanide-Na	*d*	133.8	160.9	167.2	144.5	CD_3CN	22
		134.0	160.9	167.9	145.2		
		134.2	163.7	170.1	145.5		
		134.2	164.3	170.5	145.5		
Actinomycin D	*d*	133.7	161.4	169.6	145.2	1,4-Dioxane-d_8	26
Actinomycin D	*d*	134.5	161.4[e]	170.2	145.7	DMF	26
Actinomycin D–deoxyguanosine	*d*	134.4	161.5[e]	170.0	145.8	DMF	26
				170.2			

[a] Relative to external carbon disulfide. [b] Double intensity. [c] Assignment tentative. [d] Not tabulated by original authors. [e] Peak position uncertain due to overlapping solvent resonances.

It is apparent from Table III, however, that the chemical shifts of the γ resonances of glycyl-L-proline and L-prolyl-L-proline diketopiperazines are quite close to the average shift of this carbon in a cis X-Pro system.[16] In contrast, the β-carbon resonances are shielded relative to the simpler derivatives. We believe this shielding to result from the proximity effect[4-6] of the carbonyl oxygen. In normal proline systems the angle ψ[17] is approximately 320° (the so-called trans′ conformation), and in such a situation the β carbon is not particularly close to the proline carbonyl group. In the diketopiperazines, however, the β carbon can be judged from models to be very nearly coplanar with the carbonyl group. Such a conformation is expected to shield the β carbon.[4-6] In possible accord with this notion are the unusually high chemical shifts observed for the proline carboxyl carbons of the diketopiperazines. Thus, for *N*-methylformamide[5,6] the chemical shifts of both carbons were found to be upfield in the trans isomer, indicating that syn interaction between the *N*-methyl and the carbonyl oxygen led to a general shielding effect. An analogous interaction with the β carbon could shield the carbonyl nucleus of proline in diketopiperazines.

Gramicidin S, a decapeptide antibiotic, is of particular interest in that the published conformational models[18] for this compound have the ψ angle of the proline units in the cis′ conformation ($\psi \sim 120°$). Such a conformation might be expected to lead to irregularities in the cmr spectrum as, for example, in the β resonances of the diketopiperazines. This does not appear to be the case. The cmr spectra of gramicidin S[19] in methanol and dimethyl sulfoxide are qualitatively very similar, both clearly showing the existence of C_2 symmetry in the molecule. As seen from Table III, the proline spectrum of gramicidin S in dimethyl sulfoxide is entirely consistent with that of a typical trans X-Pro peptide bond.[20] Clearly additional experiments are necessary before the effects of ψ upon the chemical shifts of the β and γ resonances can be properly assessed.

The decapeptide antamanide contains no element of symmetry, and its cmr spectrum is accordingly complex.[22] However, the grouping of the β- and γ-proline carbon resonances enables one to conclude that two of the four proline residues are cis and two are trans,[22] a conclusion which cannot be drawn from the proton data alone.

Conclusions

The present results show that carbon chemical shifts alone can be instrumental in the assignment of the conformation of the X-Pro bond. Application of this principle in these laboratories has led to the clarification of details of the conformation of poly-L-proline,[23] *cyclo*-(Pro-Gly)$_3$,[24] *cyclo*-(Pro-Ser-Gly)$_2$,[25] antamanide,[22]

(16) D. J. Patel, unpublished results.

(17) For an explanation of the conventions used in this paper describing dihedral angles in peptides, see J. T. Edsall, *et al.*, *Biopolymers*, **4**, 121 (1966); *J. Biol. Chem.*, **241**, 1004 (1966); *J. Mol. Biol.*, **15**, 399 (1966). Another convention has been more recently introduced: J. C. Kendrew, *et al.*, *Biochemistry*, **9**, 3471 (1970); *J. Biol. Chem.*, **245**, 489 (1970); *J. Mol. Biol.*, **52**, 1 (1970).

(18) (a) A. Stern, W. A. Gibbons, and L. C. Craig, *Proc. Nat. Acad. Sci. U. S.*, **61**, 734 (1968); (b) Y. A. Ovchinnikov, Y. T. Ivanov, V. F. Bystrov, A. I. Miroshnikov, E. N. Shepel, N. D. Abdullaev, E. S. Efremov, and L. B. Senyavina, *Biochem. Biophys. Res. Commun.*, **39**, 217 (1970).

(19) W. A. Gibbons, J. A. Sogn, A. Stern, L. C. Craig, and L. F. Johnson, *Nature*, **227**, 840 (1970).

(20) The spectrum reported[19] for gramicidin S in methanol is in very poor agreement with all other results. Comparison of the spectra of this compound in the two solvents shows that the entire spectrum appears to have been shifted downfield in methanol. This suggests the possibility of a systematic error. In accord with this notion is Figure 2 of ref 19, in which the methanol resonance is represented as occurring at about 142 ppm. In fact, the chemical shift of methanol referred to external carbon disulfide is 144.2 ppm.[21] If a +2 ppm adjustment is made in the spectrum of gramicidin in methanol, much better correspondence for the two different solvents is obtained.

(21) J. D. Roberts, F. J. Weigert, J. I. Kroschiwitz, and H. J. Reich, *J. Amer. Chem. Soc.*, **92**, 1338 (1970). These authors report chemical shifts relative to internal carbon disulfide, and their chemical shift data must be accordingly adjusted by +0.7 ppm.

(22) D. J. Patel, *Biochemistry*, **12**, 667, 677 (1973).

(23) D. E. Dorman, D. A. Torchia, and F. A. Bovey, *Macromolecules*, **6**, 80 (1973).

(24) C. M. Deber, D. A. Torchia, D. E. Dorman, F. A. Bovey, and E. R. Blout, Proceedings of the Third American Peptide Symposium, 1972.

(25) D. E. Dorman, A. I. Brewster, F. A. Bovey, C. M. Deber, and E. R. Blout, in preparation.

actinomycin D,[26] and oxytocin.[27] Only in the case of *cyclo*-(Pro-Gly)$_3$ in methylene chloride do these rules fail to agree with previous conclusions, and this failure may be due to an unusual dihedral angle ψ.[24] We believe this application of ^{13}C nmr spectroscopy will find great importance in future studies of oligo- and polypeptides.

(26) D. J. Patel, *et al.*, unpublished results.
(27) A. I. R. Brewster, V. J. Hruby, A. F. Spatola, and F. A. Bovey, *Biochemistry*, **12**, 1643 (1973).

Registry No.—*N*-Formylpyrrolidine, 3760-54-1; *N*-acetylpyrrolidine, 4030-18-6.

Zonarol and Isozonarol, Fungitoxic Hydroquinones from the Brown Seaweed *Dictyopteris zonarioides*[1]

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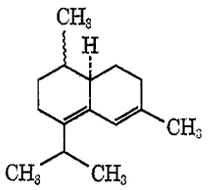
Zonarol and isozonarol, isomeric C_{21} hydroquinones, have been obtained each from separate methanol extracts of *Dictyopteris zonarioides* collected in the Pacific Ocean and in the Gulf of California. The structural assignments were made based upon spectral grounds and by degradation to dihydrotauranic acid and comparison with an authentic sample.

Previous investigations of members of the genus *Dictyopteris* (family Dictyotaceae) have led to the isolation of two new oxygenated sesquiterpenes[3,4] and a novel assortment of nonterpenoid C_{11} hydrocarbons and sulfur-containing compounds.[5-8] In an earlier paper[9] we described the structure of zonarene (**1**),



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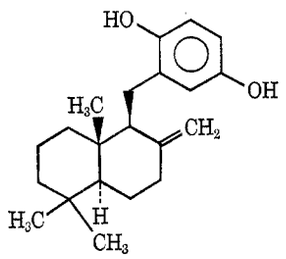
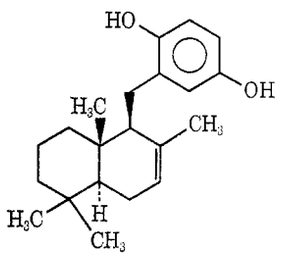
the major hydrocarbon component of the hexane extract of *D. zonarioides*, an alga indigenous to the Pacific Ocean near southern California and to the Gulf of California. We wish to report here the structures of zonarol (**2**) and isozonarol (**3**), hydroquinones obtained from the methanol extract of this alga. Zonarol was the exclusive isomer present in samples collected in the Pacific Ocean, while only isozonarol was obtained from the Gulf of California source. Both methanol extracts also contained **1** and small amounts of the corresponding quinones which are discussed below. Both **2** and **3** are moderately fungitoxic toward *Phytophthora cinnamomi*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Sclerotium rolfsii*.



2



3

Column chromatographic separation of the methanol extract of *D. zonarioides*, collected in San Diego, Calif., gave zonarol (**2**) as a noncrystalline gum. All attempts to crystallize this material failed. The infrared absorptions of this compound clearly showed the presence of hydroxyl (3400 cm^{-1}) and an exocyclic double bond (1650 and 908 cm^{-1}). While **2** did not give a positive ferric chloride test, it was recognized as a monosubstituted hydroquinone by its eventual oxidation to the corresponding quinone. In addition, its nmr spectrum showed three aromatic protons at δ 6.55 as a complex band and two hydroxyl protons at solvent-dependent chemical shifts. Two exocyclic methylene protons were recognized by broad bands at δ 4.64 and 4.75. Multiple bands from δ 1.0 to 2.8 showed the molecule to contain a variety of saturated methylene hydrogen. Overlapping sharp signals centered at δ 0.80 indicated three quaternary methyl groups to be present. The mass spectrum of **2** and the integration of the nmr bands described above were consistent (P = *m/e* 314) in indicating the molecular formula $C_{21}H_{30}O_2$. The uv spectrum[10] also suggested the hydroquinone structure, λ_{max}^{MeOH} 211 nm (ϵ 8400) and 295 (3150). Treatment of **2** with Jones reagent[11]

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