1973; Siezen, 1973). In these cases oxygenation favors the associated state. DePhillips *et al.* (1969) showed a complex linkage between ligand binding and the pentamerization of the 19S component of *Loligo* hemocyanin. However, no obvious relation seems to exist between reported cooperativity of oxygen binding and ligand-dependent association–dissociation phenomena.

Acknowledgments

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Carbon-13 Magnetic Resonance Assignments of the Repeat Peptides of Elastin and Solvent Delineation of Carbonyls

D. W. Urry,† L. W. Mitchell, and T. Ohnishi

ABSTRACT: Complete assignments of the carbon-13 resonances, including the peptide carbonyls, are reported for the repeat tetrapeptide, Val_1-Pro_2-Gly_3-Gly_4, the repeat pentapeptide, Val_1-Pro_2-Gly_3-Val_4-Gly_5, and the repeat hexapeptides, Ala_1-Pro_2-Gly_3-Val_4-Gly_5-Val_6, and the cyclic permutation Val_6-Ala_1-Pro_2-Gly_3-Val_4-Gly_5 of elastin. The assignments are given in three different solvent systems, dimethyl-d_6 sulfoxide, trifluoroethanol-d_3, and D_2O. Carbonyl assignments were achieved in each solvent by chemical methods utilizing glycine-*1*-^{13}C enrichment and chemical modification of end residues. Solvent mixture titrations, which further check the carbonyl assignments, are reported and discussed relative to possible conformational information. In a manner similar to peptide NH protons, the peptide C–O carbonyls can be delineated by solvent dependence of chemical shift. In the tetrapeptide and pentapeptide the Val_1 C–O which hydrogen bonds to the Gly_4 NH and Val_4 NH, respectively, in formation of a β turn, is solvent shielded. In the two hexapeptides it is the Ala_1 C–O which hydrogen bonds to the Val_4 NH that is solvent shielded.

Solution studies on the conformation and interactions of elastin have utilized hot oxalic acid fragmentation of the elastin fiber which results in two major products, α-elastin (~70,000) and β-elastin (5–10,000) (Partridge *et al.*, 1955). The α-elastin fragment contains some 16 chains held together by four to five cross-links (Partridge and Davis, 1955).

More recently, the precursor protein, tropoelastin, has been isolated (Smith *et al.*, 1968; Sandberg *et al.*, 1969) and the amino acid sequences of some 13 peptides of tropoelastin which comprise almost one-half of the molecule have been determined (Foster *et al.*, 1973; Gray *et al.*, 1973). Gray and Sandberg and their colleagues report a repeating tetrapeptide (-Gly-Gly-Val-Pro-), a repeating pentapeptide (-Pro-Gly-Val-Gly-Val-), and a repeating hexapeptide (-Pro-Gly-Val-Gly-Val-Ala-). In previous work we have synthesized these peptides and their oligomers and have studied their conformations using proton magnetic resonance methods (Urry, 1974; Urry *et al.*, 1974; Urry and Ohnishi, 1974a,b). All three peptides exhibit a β turn in which the -Pro-Gly- sequence forms the corners, *i.e.*, positions $i + 1$ and $i + 2$, respectively, as indicated in Figure 1.

The present effort reports the carbon-13 assignment of the repeating peptides of elastin with particular emphasis on the carbonyl carbons. The assignments are given in three different solvent systems, *i.e.*, in dimethyl sulfoxide as this solvent pertains to the proton magnetic resonance studies on the repeating peptides, in trifluoroethanol as it is in this sol-

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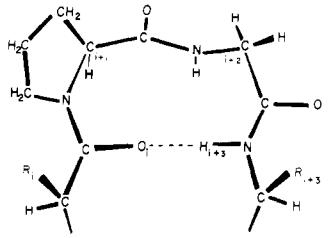


FIGURE 1: β turn of the repeat peptides of elastin as deduced from proton magnetic resonance studies (Urry *et al.*, 1974; Urry and Ohnishi, 1974a,b). In the tetrapeptide R_i = Val and R_{i+3} = Gly. In the pentapeptide R_i = Val and R_{i+3} = Val. In the hexapeptide R_i = Ala and R_{i+3} = Val.

vent that the circular dichroism pattern of α-elastin resembles that of the coacervate (Urry *et al.*, 1969; Starcher *et al.*, 1973) which is the state of reformed fibers (Cox *et al.*, 1973, 1974), and in D_2O as the elastic fiber contains about 60% water in the native state.

Materials and Methods

Carbon-13 magnetic resonance spectra were obtained on a JEOL-PFT-100 spectrometer at 25.15 MHz, utilizing an EC-100 computer system. The protons were noise decoupled at 100 MHz and a deuterium lock was used at 15.36 MHz. With this instrument a 23.5-μsec pulse width resulted in a 90° mutation of the magnetization vector. A 15-μsec pulse and a 3-sec repetition rate were found to be favorable particularly for carbonyl resonances. The free induction decay resulting from each pulse was accumulated in 8K of memory of a Texas Instrument 980A computer where it is transformed after each 100 pulses and stored in an additional 4K of memory resulting in resolution of 1.22 Hz per data point over a 5000 Hz spectral range. As the EC-100 displayed the transformed spectrum, it was possible to monitor at all times the progress of the accumulation and the experiment could be terminated when the displayed spectrum appeared satisfactory.

The 10-mm sample tube was fitted with a 0.45-ml insert (Wilmad Glass Co., Inc., Buena, N. J.). In general, 50–100 mg of peptide was used in 0.5 ml. When solubility was limiting, as, for example, with Boc-VAPGVG-OMe[1] in D_2O, the concentration was an order of magnitude less and approximately 10,000 pulses were required to obtain an adequate spectrum. Tetramethylsilane (Me_4Si) and dioxane were used as internal standards with Me_4Si taken as zero and dioxane as 67.4 ppm in D_2O (Johnson and Jankowski, 1972). The temperature in all cases was maintained at 30° with a JES-VT-3 variable temperature controller.

Dimethyl-d_6 sulfoxide (Me_2SO-d_6, 99.5% D and 99.8% D) was obtained from Diaprep Corp., Atlanta, Ga., and from Columbia Organic Chemical Co., Columbia, S. C. Deuterium oxide and 2,2,2-trifluoroethanol-d_3 were purchased from Merck, Sharp, and Dohme, Montreal, Canada.

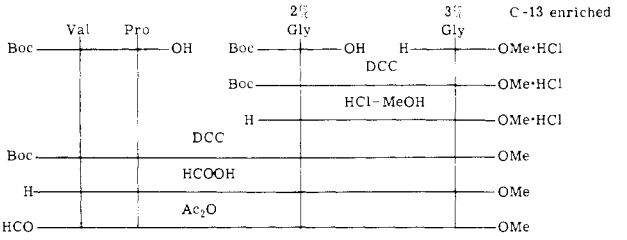
The syntheses and characterizations of nonenriched peptides have been reported previously (Urry *et al.*, 1970; Urry and Ohnishi, 1974a,b). Comparison of spectra of the C-13 enriched (see below) and the nonenriched peptides was carried out to eliminate possible error in assignments arising due to differences in longitudinal relaxation times of the carbonyl carbons.

[1] Abbreviations used are: Boc, *tert*-butyloxycarbonyl; DCC, dicyclohexylcarbodiimide; TFA, trifluoroacetic acid; TFE, trifluoroethanol. All amino acids are of the L configuration. The following one-letter abbreviations were used: A, alanine; G, glycine; P, proline; V, valine.

Synthesis of the Glycine-1-^{13}C Enriched Peptides. Glycine-*1*-^{13}C (60 atom % C-13) was obtained from Merck, Sharp, and Dohme, Canada Limited, Kirkland, Quebec, Canada. The C-13 rich glycine was divided in half, and diluted with normal glycine (Ajinomoto Co., Tokyo, Japan) to make 2 and 3 atom % glycine-*1*-^{13}C mixtures. Boc-Gly-OH was made from 2% C-13 enriched glycine by the *tert*-butyl azidoformate method, and H-Gly-OMe·HCl was made from the 3% C-13 enriched glycine by the methanol–hydrogen chloride gas method.

Peptides rich in glycine-*1*-^{13}C were synthesized by the solution method as previously reported for Boc-VPGG-OMe, Boc-VPGVG-OMe, and Boc-VAPGVG-OMe (Urry and Ohnishi, 1974a,b; Urry *et al.*, 1974) (Schemes I–III). Amino acid analyses were carried out on a Beckman Model 119H automatic amino acid analyzer using the ninhydrin method.

SCHEME I: Synthesis of Tetrapeptide.



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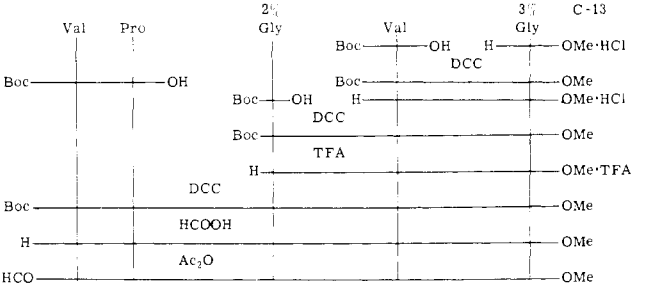

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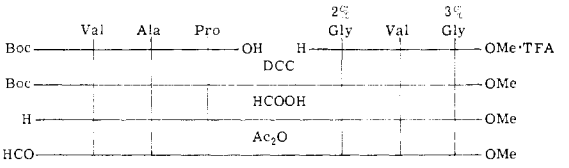

Thin-layer chromatography was performed on silica gel G, with chloroform–methanol–acetic acid (95:15:3, v/v) solvent systems.

1. Boc-Val$_1$*-Pro*$_2$*-Gly*$_3$*-Gly*$_4$*-OMe* (Gly_3 is 2 atom % and Gly_4 is 3 atom % C-13). This C-13 enriched Boc-tetrapeptide ester was made from Boc-Val-Pro-OH (1.73 g, 5.5 mmol) and C-13 enriched H-Gly-Gly-OMe·HCl (1 g, 5.5 mmol) by the DCC method as for Boc-VPGG-OMe (Urry and Ohnishi, 1974a). To remove the by-product, acylurea, the crude oil obtained from above was dissolved in ethyl acetate–benzene (9:1) and passed through a 4 × 45 cm column of 100 mesh silica gel. The main product, Boc-VPGG-OMe, was eluted with another solvent system, ethyl acetate–methanol (6:4), from the silica gel column.

After the solvent was evaporated *in vacuo*, the remaining oil was dissolved in methanol and passed through a 2.5 × 90 cm column of Sephadex LH-20 using methanol as eluent. Fractions containing pure Boc-tetrapeptide ester were collected and evaporated to dryness, giving an amorphous powder. Yield, 1.45 g, 59.5%; R_F 0.78 (single spot); mp 64–70°. Found: C, 54.28; H, 7.46; N, 12.40. Calcd for $C_{20}H_{34}N_4O_7$: C, 54.28; H, 7.74; N, 12.65. Amino acid analysis: Pro, 1.02; Gly, 2.0; Val, 1.10.

II. HCO-Val$_1$*-Pro*$_2$*-Gly*$_3$*-Gly*$_4$*-OMe* (Gly_3 is 2 atom % and Gly_4 is 3 atom % C-13). C-13 enriched Boc-VPGG-OMe (400 mg) was reacted with formic acid and acetic anhydride as outlined previously (Urry and Ohnishi, 1974a). The crude formyl tetrapeptide ester was dissolved in methanol and purified on a 2 × 20 cm column of Bio-Rad mixed bed resin, AG 501-X 8 (D), using methanol as the developing solvent. A hygroscopic, amorphous powder was obtained after removal of methanol. Yield, 250 mg, 74.6%; mp 68 ~ 73°; R_F 0.58. Found: C, 51.66; H, 6.82; N, 14.85. Calcd for $C_{16}H_{26}N_4O_6$: C, 51.88; H, 7.08; N, 15.13.

III. Boc-Val$_1$*-Pro*$_2$*-Gly*$_3$*-Val*$_4$*-Gly*$_5$*-OMe* (Gly_3 is 2 atom % and Gly_5 is 3 atom % C-13). Boc-Val-Pro-OH (1.6 g, 5 mmol) and C-13 enriched H-Gly-Val-Gly-OMe·TFA (5 mmol) were reacted by the DCC method as described previously (Urry *et al.*, 1974). This product was purified on a 2.5 × 90 cm column of Sephadex LH-20 using methanol as the developing solvent. White crystals were obtained after removing the solvent. Recrystallization was from ethyl acetate and petroleum ether. Yield, 1.66 g, 61.3%; mp 157–159°; R_F 0.76 (single spot). Found: C, 55.46; H, 7.80; N, 12.86. Calcd for $C_{24}H_{42}N_5O_8$: C, 55.54; H, 7.87; N, 12.95. Amino acid analysis: Pro, 1.04; Gly, 2.0; Val, 2.09.

IV. HCO-Val$_1$*-Pro*$_2$*-Gly*$_3$*-Val*$_4$*-Gly*$_5$*-OMe* (Gly_3 is 2 atom % and Gly_5 is 3 atom % C-13). C-13 enriched Boc-VPGVG-OMe (500 mg) was formylated and purified as for HCO-VPGG-OMe. The crystals were recrystallized from methanol and ether. Yield, 300 mg, 67.8%; mp 172–174°; R_F 0.63. Found: C, 53.43; H, 7.52; N, 14.74. Calcd for $C_{21}H_{35}N_5O_7$: C, 53.72; H, 7.51; N, 14.82.

V. Boc-Val$_6$*-Ala*$_1$*-Pro*$_2$*-Gly*$_3$*-Val*$_4$*-Gly*$_5$*-OMe* (Gly_3 is 2 atom % and Gly_5 is 3 atom % C-13). Boc-Val-Ala-Pro-OH (2 g) and 5 mmol of C-13 enriched H-Gly-Val-Gly-OMe·TFA were coupled as described previously (Urry and Ohnishi, 1974b). The gel obtained by this procedure was dissolved in methanol and purified on a column of Sephadex LH-20 using methanol as the eluent. The gel was obtained from methanol and ether. Yield, 1.8 g, 58.8%; R_F 0.72 (single spot); mp 196–198°. Found: C, 54.68; H, 7.76; N, 13.48. Calcd for $C_{28}H_{48}N_6O_9$: C, 54.88; H, 7.89; N, 13.72.

VI. HCO-Val-Ala-Pro-Gly-Val-Gly-OMe. C-13 enriched Boc-VAPGVG-OMe (400 mg) was formylated and purified as described previously (Urry and Ohnishi, 1974b). An amorphous gel was obtained from methanol and ether. Yield, 150 mg, 42.6%; R_F 0.61; mp 258–260°. Found: C, 53.13; H, 7.41; N, 15.30. Calcd for $C_{24}H_{40}N_6O_8$: C, 53.32; H, 7.46; N, 15.55. Amino acid analysis: Pro, 1.08; Gly, 2.0; Ala, 1.09; Val, 2.06.

Results

Assignment of Carbonyl Resonances in Glycine-1-^{13}C *Enriched Peptides.* The carbon-13 magnetic resonance (cmr) spectra in the carbonyl region for the three peptides and their Boc and HCO derivatives in Me_2SO-d_6 are given in Figure 2. The Boc-V_1-P_2-G_3-G_4-OMe derivative gives four lines near 170 ppm. The most intense peak is that of the ester carbonyl which was enriched to 3%.[2] The second most intense line is that of the Gly_3 C–O which was enriched to 2%.[2] The other two lines are assigned by the differences observed with the HCO-VPGG-OMe derivative. On formylation the peak near 171 ppm shifts upfield and overlaps the Gly_4 COOMe peak and the lowest field peak remains at 172 ppm. The peak which shifts upfield on formylation is assigned to the Val_1 C–O and the lowest field peak by elimination is the Pro_2 C–O resonance. The carbonyls of the Boc and HCO moieties are at higher field near 155 and 161 ppm as seen in Figure 2e and f.

The pentapeptide carbonyls in Me_2SO-d_6 are given in Figure 2c and d. The Gly_5 COOMe and Gly_3 C–O are assigned by their intensities. A peak is again observed at 172 ppm; this is the location of the Pro_2 C–O in the tetramer and is taken as the Pro_2 C–O resonance of the pentamer. On replacement of the Boc end group by HCO one resonance shifts upfield by 1 ppm while the other resonances show the same relative positions. The shifted resonance is assigned the Val_1 C–O. This leaves the remaining resonance as the Val_4 C–O.

The hexapeptide carbonyls of $V_6A_1P_2G_3V_4G_5$ in Me_2SO-d_6 are seen in Figure 2e and f. The positions of the resonances in the Boc hexapeptide are very closely the same as in the Boc pentapeptide with the exception of an added resonance (two peaks instead of one) between the Val_4 C–O and the Gly_5 COOMe peaks. On replacement of the Boc group by HCO one peak moves upfield and overlaps the Gly_5 COOMe peak. The peak that moved upfield is the Val_6 C–O (note numbering, Val_6 is the amino end); the remaining peak is the Ala_1 C–O. Also included in Figure 2e and f are the formyl and Boc carbonyls which appear at higher field.

Cmr carbonyl spectra for the same compounds are given in trifluoroethanol-d_3 in Figure 3. In general, the spectra are shifted downfield by about 4 ppm with much similarity in the relative positions of the peaks. The assignments were achieved as outlined above for Me_2SO. Replacement of the Boc amino protecting group by a formyl group again causes the carbonyl resonance of the amino terminus residue to shift upfield. The Pro_2 C–O remains essentially in the same lowest field position in all six spectra and the glycine carbonyl peaks are at the high field edge of the carbonyl resonances. In the spectrum of Boc-VAPGVG-OMe, the Val_4 and Val_6 carbonyls overlap (note that in this permutation of the hexapeptide Val_6 is the amino terminus). On the formylation one resonance, the Val_6 C–O, moves upfield to a position between the Ala_1 C–O and Gly_5 COOMe resonances. In HCO-Val-Ala-Pro-Gly-Val-Gly-OMe in TFE all carbonyl resonances are well resolved.

Delineation of Peptide and Ester Carbonyls. A useful feature which becomes apparent on comparison of the same molecule between the two different solvents, as in Figures 2 and 3, is that, relative to the peptide carbonyls, the ester carbonyl shifts downfield on going from TFE to Me_2SO. For example, in the formyl hexapeptide the chemical shift difference between the Pro_2 C–O resonance and the Gly_5 COOMe resonance is 78 Hz in TFE and 50 Hz in Me_2SO, *i.e.*, 28 Hz closer to the Pro_2 C–O resonance, whereas the chemical shift difference between the Pro_2 C–O resonance

[2] In each peptide the delineation of the glycine carbonyls was further confirmed by comparison of the spectra of the esterified and carboxyl free peptides. While the other resonances remained essentially unchanged on de-esterification the terminal carbonyl carbon resonance shifted downfield by as much as 1 ppm.

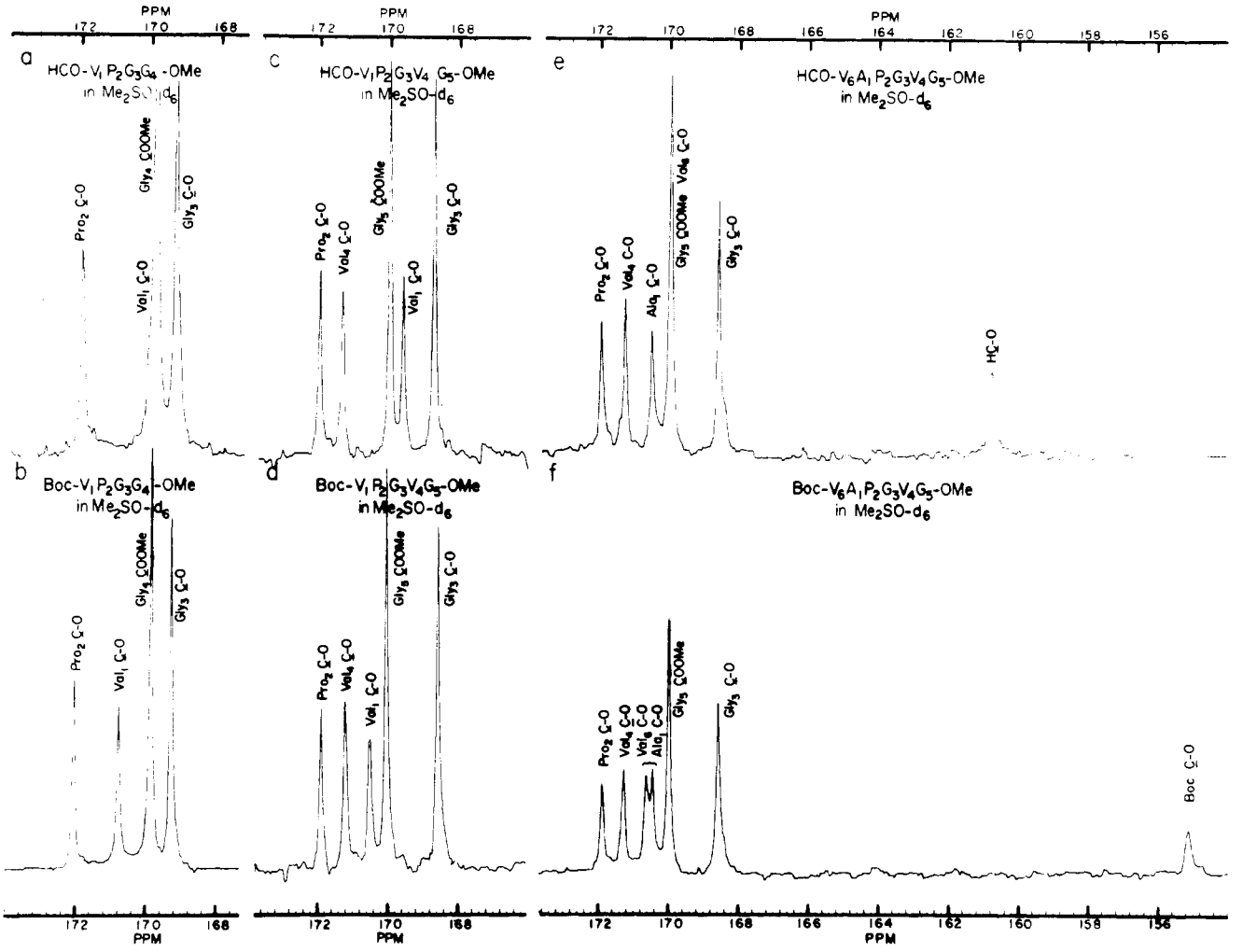

FIGURE 2: Carbon-13 magnetic resonance spectra of the glycine-*1*-^{13}C enriched repeat peptides of elastin in the carbonyl region in Me_2SO-d_6 for both the HCO and Boc derivatives. Chemical shifts given relative to an internal standard of tetramethylsilane (see text for discussion).

and the Gly_3 C–O resonance is 85 Hz in both solvents. Similarly for the formyl and Boc pentamer, the ester carbonyl moves 23 Hz closer to the Pro C–O resonance on going from TFE into Me_2SO; and in the Boc hexamer the ester carbonyl moves 30 Hz closer to the Pro carbonyl. This becomes a means of delineating peptide and ester carbonyls which will be helpful in assigning carbonyl resonances of the unenriched hexamer APGVGV. This identification must be used with caution as the peptide carbonyls themselves exhibit differential movement (see Discussion) though commonly to a lesser extent.

Assignment of Unenriched APGVGV Carbonyls. Carbonyl spectra for Boc and formyl APGVGV-OMe in TFE and Me_2SO-d_6 are given in Figure 4. Comparison of HCO-APGVGV-OMe in TFE-d_3 in Figure 4b with HCO-VAPGVG-OMe in TFE-d_3 in Figure 3e shows the Pro_2, Ala_1, and Gly_3 carbonyl resonances of Figure 3e to coincide with three resonances in Figure 4b. Overlaps are also seen for these same resonances in Figure 2e with peaks in Figure 4a. This is the basis of these three assignments in Figure 4b and a. The resonance at highest field in Figure 4b is taken to be the Gly_5 C–O as the only other possibilities for this carbonyl would be two resonances 3 ppm downfield, a position uncharacteristic of glycine carbonyls. This leaves the two peaks near 175 ppm for the Val_4 and Val_6 carbonyls. The Val_6 carbonyl, however, as an ester carbonyl, is expected to shift toward the Pro_2 C–O by some 25 Hz (1 ppm) on going into Me_2SO-d_6 (see above). This is apparent, as the lowest field peak in Figure 4a is a superposition of two resonances, the Pro_2 C–O and the Val_6 COOMe. In Figure 4b, 27 Hz separates one resonance, either the Val_4 or Val_6 carbonyl, from the Pro carbonyl resonance and 18 Hz separates the other from the Pro carbonyl carbon resonance. If the magnitude of shift is similar to that observed for the Gly COOMe in the hexamer Figures 2e and 3e then the higher field resonance of the two would be the Val_6 COOMe. The spectrum of HCO-APGVGV-OH in TFE-d_3 verified the higher field resonance to be Val_6 COOMe as the Val_4 C–O does not move and the Val_6 COOH overlaps the Pro_2 C–O. The resonance in Figure 4a which falls between the overlapping Pro_2, Val_6 peak and the Ala_1 peak is Val_4 C–O by elimination.

The Ala_1 C–O assignment is confirmed by the upfield shift on replacement of the Boc amino protecting group with a formyl moiety. Comparison of Figure 4d and b clearly shows the Ala_1 C–O appearing at higher field in the formyl derivative. A similar shift is seen on comparison of Figure 4a and c.

Assignment of Upfield Resonances. The assignments of Boc-Val-OH, Boc-Ala-OH, Boc-Pro-OH, and Boc-Gly-OH in Me_2SO have been reported (Voelter *et al.*, 1971). Using these assignments as an aid, the assignments for Boc-Val-Pro-OH, Boc-Val-Gly-OH, Boc,Val-Ala-OH, and Boc-Gly-Val-Gly-OMe were determined in Me_2SO.

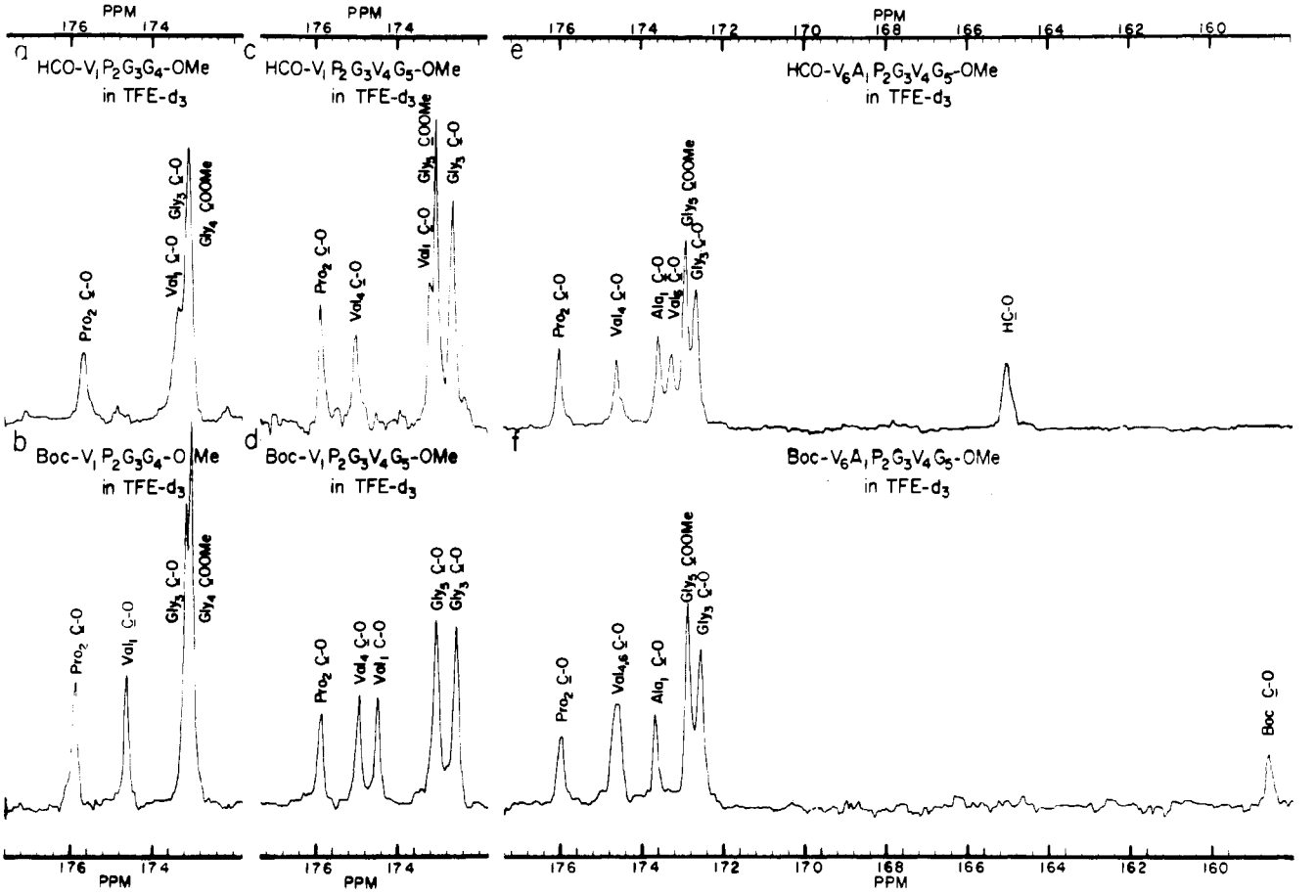

FIGURE 3: Carbon-13 magnetic resonance spectra of the glycine-*1*-^{13}C enriched repeat peptides of elastin in TFE-d_3 in the carbonyl region for both the HCO and Boc derivatives. Chemical shifts given relative to an internal standard of Me_4Si (see text for discussion).

By means of solvent titrations progressively moving from Me_2SO-d_6 to TFE-d_3 the assignments were obtained for the latter solvent. With this information assignments were made for Boc- and HCO-Val-Pro-Gly-Gly-OMe in Me_2SO-d_6 and in TFE-d_3. Assignments were then made for Boc- and HCO-Val-Pro-Gly-Val-Gly-OMe in Me_2SO-d_6 and TFE-d_3 utilizing Voelter *et al.* (1971) and the tetramer assignments. Finally, Boc- and HCO-Val-Ala-Pro-Gly-Val-Gly-OMe resonances were assigned utilizing the assignments for Boc-Val-Ala-OH and the pentamer assignments. The assignments for the formyl derivative of the three elastin peptides are given on the spectra in Figure 5d, e, and f for TFE-d_3.

A point of clarification is required in arriving at the Pro_2 αCH and Val_1 αCH assignments which are complicated by special Boc and Pro effects. In Boc-Val-OH the αCH is at 59.07 ppm and that of Boc-Pro-OH is at 58.58 ppm, *i.e.*, the Pro αCH is at higher field by about 0.5 ppm. The αCH resonances are observed at 57.03 and 58.46 ppm for Val and Pro, respectively, for Boc-Val-Pro-OH. The 2-ppm upfield shift of the Val αCH is due to the effects of the Pro residue as the Val αCH is at 59.12 and 59.46 ppm in Boc-Val-Ala-OMe and Boc-Val-Gly-OMe, respectively. However, the resonance at 59.85 ppm is assigned the Pro αCH and that at 57.18 ppm is assigned to the Val αCH in the tetramer Boc-VPGG-OMe. In the pentamer Boc-VPGVG-OMe the Pro αCH is at 59.65 ppm and the Val αCH's are at 57.71 and 57.21 ppm. This is because on replacement of the Boc group by formyl the resonance near 60 ppm remains fixed in both the tetramer and pentamer (see Figure 5) and the resonance near 57 ppm shifts upfield to 54 ppm

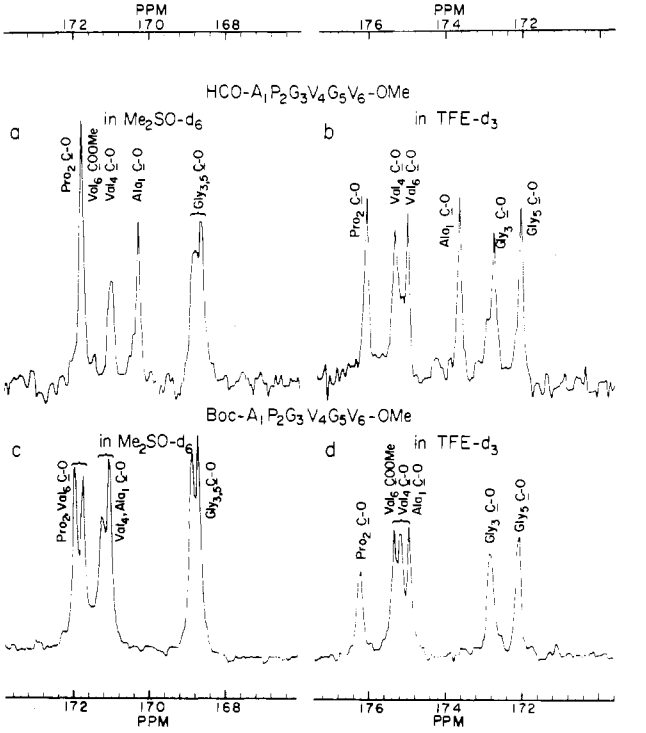

FIGURE 4: Carbon-13 magnetic resonance spectra of the HCO and Boc derivatives of the elastin repeat hexapeptide $A_1P_2G_3V_4G_5V_6$-OMe in the carbonyl region in both Me_2SO-d_6 and TFE-d_3. Chemical shifts given relative to an internal standard of Me_4Si. Note the upfield shift of the Ala_1 C–O on replacement of Boc and HCO and the movement of the Val_6 COOMe toward the Pro_2 C–O on going from TFE-d_3 to Me_2SO.

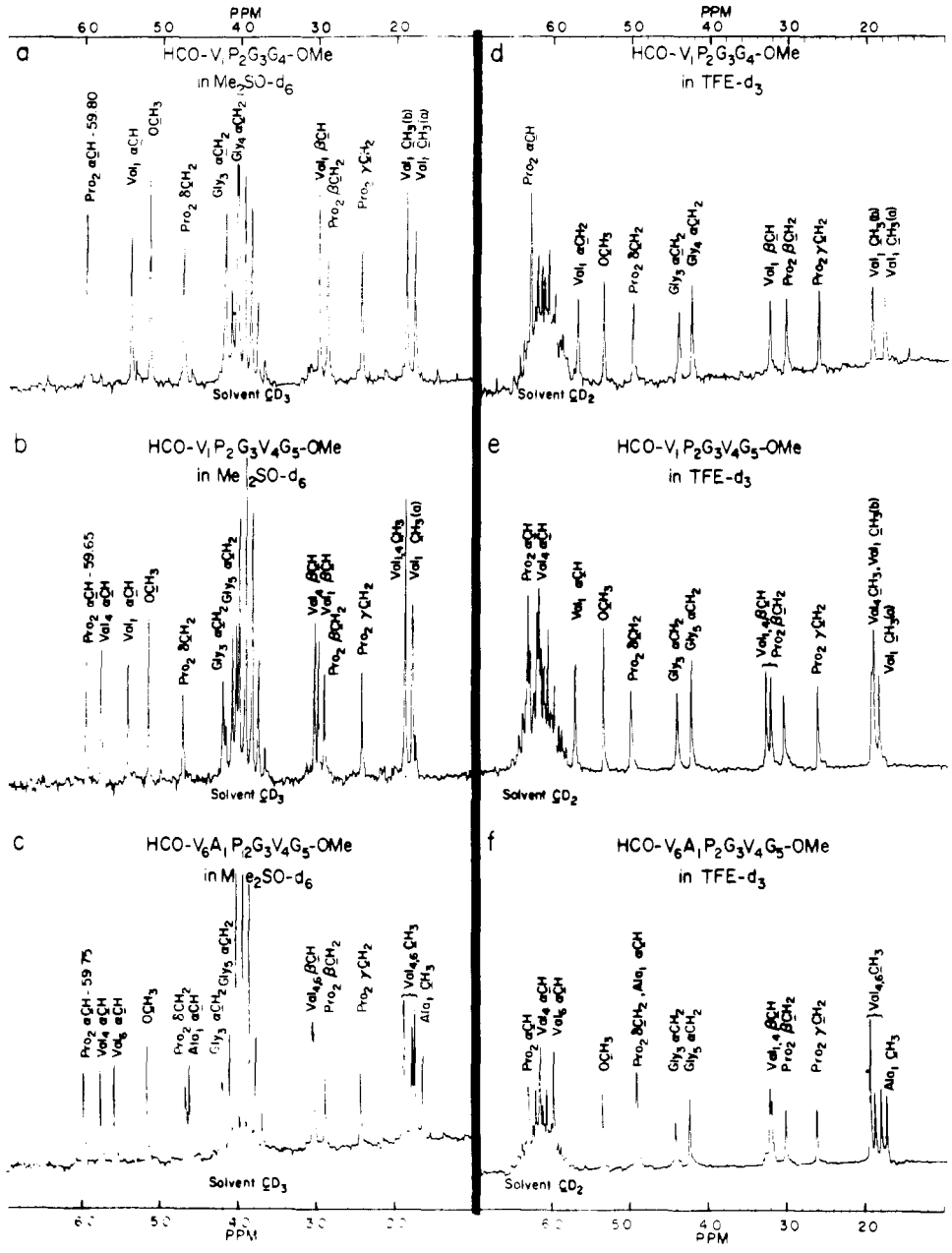

FIGURE 5: Carbon-13 magnetic resonance spectra in the upfield region in both Me_2SO-d_6 and TFE-d_3 for the repeat peptides of elastin. The chemical shifts for the Boc derivatives are given in Table I. Chemical shifts are given relative to Me_4Si as an internal standard (see text for discussion).

in the tetramer and 54.5 ppm in the pentamer. The high-field position of the Ala αCH in the hexamer, relative to the Val-Ala dimer, is explicable in part by its sequence position, *i.e.* preceding the Pro residue.

Cmr spectra in D_2O for the formylated peptides are given in Figure 6. Assignments in the high-field region, 10–65 ppm, followed directly from the assignments in Me_2SO-d_6 and TFE-d_3. Assignments in the low-field region, 172–176 ppm, utilized the enriched peptides and changes effected by replacement of the amino blocking group. Comparison of the carbonyl spectral region in D_2O, Figure 6, with that in TFE-d_3, Figure 3, shows a striking similarity. The Pro_2 C–O chemical shift is immediately below 176 ppm in TFE-d_3 and slightly above 176 ppm in D_2O whereas it is near 172 ppm in Me_2SO-d_6 (Figure 2). This demonstrates the proton (deuteron) donor capacity of trifluoroethanol to be similar in magnitude to that of water. In the comparison of spectra in D_2O with that in TFE-d_3 a minor difference is that the Val_1 and Ala_1 carbonyl carbon resonances are at slightly higher field relative to the glycine carbonyls.

Discussion

What one would like to achieve from cmr studies of peptides is additional description of the molecular system, its conformation, mobility, etc. Specifically with regard to conformation, it would be of significance to identify those carbonyl moieties which are involved in intramolecular hydrogen bonding. Proton magnetic resonance studies referred to in the introduction have been successful in delineating peptide NH protons in a manner that can, under favorable circumstances, be correlated with secondary structure (Kopple *et al.*, 1969a,b; Ohnishi and Urry, 1969; Urry and Ohnishi, 1970; Urry *et al.*, 1970; Urry and Walter, 1971; Pitner and Urry, 1972a,b; Torchia *et al.*, 1972a,b; Deber *et al.*, 1972; Pease *et al.*, 1973; Kumar and Urry, 1973a,b). Once a peptide NH becomes a candidate for intramolecular hydrogen

TABLE I: Peptide Carbonyl Solvent Shifts Relative to Residue 1 Carbonyl.[a]

Residue	(A) Me_2SO – TFE solvent pair HCO-APGVGV-OMe $\Delta\nu_j$ (Hz)	HCO-VPGVG-OMe $\Delta\nu_j$ (Hz)	Boc-VPGVG-OH $\Delta\nu_j$ (Hz)
Ala_1 (Val_1)	0	0	0
Pro_2	+23	+8	+14
Gly_3	+18	+7	+14
Val_4	+23	+3	+10
Gly_5	−2	Ester	
Val_6	Ester		

Residue	(B) Me_2SO – D_2O solvent pair HCO-VAPGVG-OMe $\Delta\nu_j$ (Hz)	HCO-VPGVG-OMe $\Delta\nu_j$ (Hz)	HCO-VPGG-OMe $\Delta\nu_j$ (Hz)
Ala_1 (Val_1)	0	0	0
Pro_2	+20	+15	+21
Gly_3	+17	+11	+15
Val_4	+13	+10	
Gly_5	Ester	Ester	
Val_6	+14		

[a] Positive numbers indicate greater relative chemical shift considered in connection with greater relative solvent exposure. $\Delta\nu_j = \nu_j$(C—O in TFE-d_3, or D_2O) − ν_j(C—O in Me_2SO) − $\Delta\nu_1$.

bonding, by the pmr methods, the problem becomes one of deciding on the peptide carbonyl moiety to which it is hydrogen bonding. To date multiple arguments involving steric requirements, αCH–NH coupling constants, chemical shifts, and chemical alteration have been used to deduce the involved peptide carbonyl. It would be more satisfactory if methods could be developed which would similarly delineate peptide carbonyls in a manner that identifies candidates for intramolecular hydrogen bonding. Then the problem is reduced to the appropriate pairing of the delineated peptide NH with the delineated peptide C–O.

With the peptide carbonyl carbons assigned and the previous pmr studies, the problem of delineating carbonyls becomes approachable using the repeat peptides of elastin. The solvent mixture method of delineating peptide NH protons was successful with gramicidin S (Pitner and Urry, 1972a) because two solvents could be used, one with good proton accepting capacity (MeOH) and the second with poor proton accepting ability (TFE). To delineate peptide carbonyls solvent pairs should involve a weak proton donor and a strong proton donor while at the same time being solvents which allow the same conformation to exist in each solvent. Dimethyl-d_6 sulfoxide is a good solvent for polypeptides allowing secondary structure to occur even though a good proton acceptor. With the CH_3 (or CD_3) groups it is a poor proton (or deuteron) donor. Trifluoroethanol is also a good solvent for polypeptides which often promotes secondary structure, as in dissolution of membrane proteins (Urry, 1972; Urry and Long, 1974), even though it is a poor proton acceptor as evidenced by the high-field shift of exposed peptide NH protons on going from MeOH to TFE (Pitner and Urry, 1972a). Trifluoroethanol is, however, a good proton donor as reflected in the lower field position of the peptide carbonyl carbon resonances in TFE-d_3 (Figures 3 and 4) than in Me_2SO-d_6 (Figures 2 and 4).

In Table I are the solvent-induced peptide carbonyl carbon chemical shifts given relative to residue 1 of the elastin peptides. The positive numbers indicate a greater solvent induced chemical shift, on going from Me_2SO-d_6 into TFE-d_3 (Table IA) and from Me_2SO-d_6 into D_2O (Table IB), than exhibited by the residue 1 carbonyl carbon resonance. Based on pmr studies indicating solvent shielding of the residue 4 NH proton, the residue 1 carbonyl is involved in the intramolecular hydrogen bond indicated in Figure 1. (It should be emphasized that solvent shielding is a statistical result and indicates an average effect that likely occurs to different extents in the different elastin peptides.) Viewing the intramolecular hydrogen bond as having the effect of shielding the carbonyl from the solvent, the positive values would indicate a greater solvent exposure of the specific carbonyls relative to the reference carbonyl. The only negative value in Table I is −2 Hz for the Gly_5 C–O of HCO-APGVGV-OMe. Following the same argument this implies essentially equivalent shielding to that of the Ala_1 C–O. Another aspect of Table I is that the shielding appears almost as large on going into D_2O. This is consistent with the similarities in the carbonyl region spectra in D_2O, Figure 6, and in TFE-d_3, Figure 3.

An obvious limitation of this approach is the possibility of conformational changes, or changes in average conformation, on changing solvent. With a change in conformation are changes in solvent and vicinal group anisotropy effects. With regard to the elastin peptides it is important to clarify whether the lesser chemical shift change, observed for the carbonyl carbon resonance of the residue immediately preceding the Pro residue, might be a primary structural effect or the secondary structural effect we are seeking. This may be checked by studying HCO-Val_1-Pro_2-Gly_3-OMe. In this sequence there is no residue 4 with a peptide NH for shielding of the Val_1 C–O by the hydrogen bonding of a β turn. For this peptide on going from Me_2SO-d_6 into TFE-d_3 the chemical shift of the Val_1 C–O is greater by a

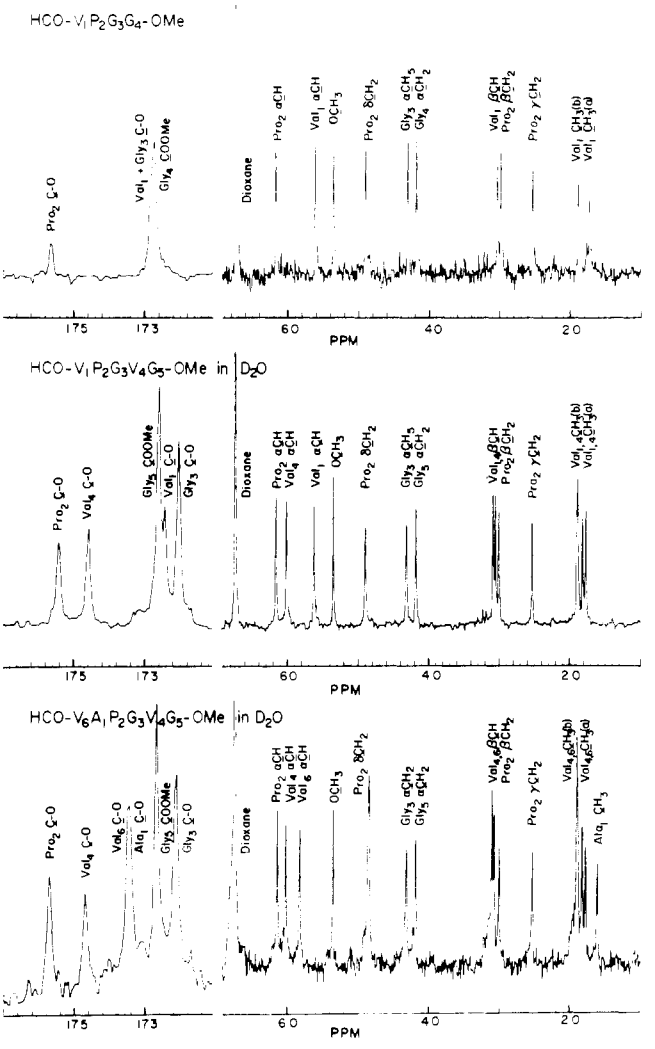

FIGURE 6: Complete cmr spectra of the formyl derivatives of the repeat peptides of elastin in D_2O. An internal reference of dioxane at 67.4 ppm was used (Johnson and Jankowski, 1972). Note the similarity, particularly in the carbonyl region (171–176 ppm), to the spectra in TFE-d_3 in Figure 3.

similar magnitude to the shielding indicated in Table IA, columns three and four. Thus it would appear that the solvent shielding of the Val_1 C–O depends on the presence of the Gly_4 NH or the Val_4 NH, *i.e.*, the solvent shielding reflects secondary structure.

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