Peptide Conformations. 42.[1,2] Conformation of Side Chains in Peptides Using Heteronuclear Coupling Constants Obtained by Two-Dimensional NMR Spectroscopy

Horst Kessler,* C. Griesinger,† and K. Wagner

Contribution from the Institute of Organic Chemistry, J. W. Goethe-University, Niederurseler Hang, D-6000 Frankfurt 50, West Germany. *Received August 18, 1986*

Abstract: The conformation about the $C^{\alpha}C^{\beta}$ bond of amino acid residues in peptides (χ_1 angle) can be obtained from homo- and heteronuclear coupling constants. Ambiguities in the assignment of the most stable side-chain conformation derived from homonuclear coupling constants are resolved by the inclusion of $H^{\beta}C'$ couplings. These coupling constants can be obtained quantitatively from various two-dimensional techniques, which, however, suffer from an inherently low signal to noise ratio. In this paper, it is shown that qualitative knowledge of heteronuclear coupling constants as obtained by the evaluation of a COLOC spectrum for the carbonyl carbons is sufficient to discriminate heteronuclear antiperiplanar coupling from synclinal coupling and thus assign the side-chain conformation unambiguously. This procedure is demonstrated on the example of amino acid residues in cyclic oligopeptides, in which the side-chain conformation was determined by other methods (such as stereoselective deuteriation or by NOE effects). $^3J(H^{\beta}{}^{15}N)$ coupling constants are shown to be useful for conformational analysis with this procedure too.

The shape of biologically active peptides is strongly determined by the orientation of the side chains of the individual amino acids. Hence, in trying to understand molecular recognition, the knowledge of the side-chain conformations is indispensible. In general, side-chain flexibility allows the molecule to adopt many conformations in solution, from which only one fits into the receptor.[3,4] Although conformational changes can be induced during binding,[5] it is certainly advantageous for high activity when the most stable conformation in solution is close to the conformation adopted in the receptor. Under such circumstances no additional energy would be required for conformational rearrangements of the side chains during binding.

The orientation of the side chain with respect to the backbone is mainly determined by the torsion angle χ_1 about the $C^{\alpha}C^{\beta}$ bond. Throughout the text we assume that only the three staggered conformations about this bond are populated. This approach is generally used in cases when the side chains are not included in cyclic structures. For example, in oxytocin, deviations from the staggered conformations are observed in the cystin side chains,[6] which are part of the cyclus, whereas, for the free side chains, staggered conformations are found. Although heteronuclear coupling constants are well-known to contain information about the side-chain conformation, they were seldom used because they are not easily accessible.

In this paper we demonstrate that, in conjunction with exact values of homonuclear coupling constants, a qualitative knowledge of the size of heteronuclear couplings is sufficient for the determination of the side-chain conformation.

Homonuclear coupling constants can be determined, even for molecules with complex spectra, by the E.COSY[7-9] or the DISCO[10-12] technique. The qualitative information about the size of heteronuclear coupling constants is derived from H,X-COLOC spectra.[13] We also show an example in which quantitatively measured heteronuclear coupling constants are used.[7,14]

Side-Chain Conformations from Coupling Constants in Peptides. The dihedral angle χ_1 about the $C^{\alpha}C^{\beta}$ bond is defined as the (mathematically negative) angle between the $C^{\alpha}N$ and $C^{\beta}C^{\gamma}$ bonds in the Newman projection.[15] Usually the population of the three staggered conformers I–III is assumed for amino acids with one or two β-protons[16,17] (Figure 1a,b). Coupling constants between vicinal nuclei about the $C^{\alpha}C^{\beta}$ bond obey Karplus-type equations[18-20] (Figure 2) and provide the population distribution, according to eq 1. $H^{\alpha}H^{\beta}$ coupling constants yield quantitative values for P_{I}, P_{II}, and P_{III}; however, they are not sufficient, since they cannot distinguish conformations I and II as long as the diastereotopic β-protons are not assigned.

$$J_{\mathrm{obs}} = \sum_i P_i J_i \qquad i = \mathrm{I\text{–}III} \tag{1}$$

Selective Deuteriation. Unambiguous assignment of the prochiral β-protons can be obtained by selective deuteriations,[21-32]

† Present address: Laboratorium für Physikalische Chemie, Eidgenössische Technische Hochschule, CH-8092 Zürich, Switzerland.

(1) This publication is part of the Ph.D. thesis of C.G., Universität Frankfurt, 1986.

(2) Part 41: Kessler, H.; Müller, A. *Liebigs Ann. Chem.* **1986**, 1687–1704.

(3) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512–523.

(4) Vida, J. A.; Gordon, M. *Conformationally Directed Drug Design*; ACS Symposium Series 251; American Chemical Society: Washington, DC, 1984.

(5) Eberle, A.; Geiger, R.; Wieland, T. *Perspectives in Peptide Chemisry*; Karger: Basel, Switzerland, 1981.

(6) Fishman, A. J.; Live, D. H.; Wyssbrod, H. R.; Agosta, W. C.; Cowburn, D. *J. Am. Chem. Soc.* **1980**, *102*, 2533–2539.

(7) Griesinger, C.; Sørensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1985**, *107*, 6394–6396.

(8) Griesinger, C.; Sørensen, O. W.; Ernst, R. R. *J. Chem. Phys.* **1986**, *85*, 6837.

(9) Griesinger, C.; Sørensen, O. W.; Ernst, R. R. *J. Magn. Reson.*, in press.

(10) Oschkinat, H.; Freeman, R. *J. Magn. Reson.* **1984**, *60*, 164–169.

(11) Kessler, H.; Oschkinat, H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 690–692.

(12) Kessler, H.; Müller, A.; Oschkinat, H. *Magn. Reson. Chem.* **1985**, *23*, 844–852.

(13) Kessler, H.; Griesinger, C.; Zarbock, J.; Loosli, H. R. *J. Magn. Reson.* **1984**, *57*, 331–336.

(14) Griesinger, C.; Oschkinat, H.; Kessler, H.; Sørensen, O. W.; Ernst, R. R., unpublished results.

(15) IUPAC-IUB Commission *Eur. J. Biochem.* **1984**, *138*, 9–37; *Pure Appl. Chem.* **1972**, *31*, 639–645; **1974**, *40*, 315–331.

(16) Pachler, K. G. R. *Spectrochim. Acta* **1963**, *19*, 2085–2092.

(17) Pachler, K. G. R. *Spectrochim. Acta* **1964**, *20*, 581–587.

(18) Karplus, M. J. *J. Chem. Phys.* **1959**, *30*, 11–15.

(19) Karplus, M. J. *J. Am. Chem. Soc.* **1963**, *85*, 2870–2871.

(20) Bystrov, V. F. *Prog. Nucl. Magn. Reson. Spectrosc.* **1976**, *10*, 41–82.

(21) Lichter, R. L.; Roberts, J. D. *J. Org. Chem.* **1970**, *35*, 2806–2807.

(22) Hanson, K. R.; Wightman, R. H.; Staunton, J.; Battersby, A. R. *J. Chem. Soc., Chem. Commun.* **1971**, 185–186.

(23) Kainosho, M.; Ajisaka, K.; Kamisaka, M.; Murai, A. *Biochem. Biophys. Res. Commun.* **1975**, *64*, 425–432.

(24) Kainosho, M.; Ajisaka, K. *J. Am. Chem. Soc.* **1975**, *97*, 5630–5631.

(25) Fishman, A. J.; Wyssbrod, H. R.; Agosta, W. C.; Cowburn, D. *J. Am. Chem. Soc.* **1978**, *100*, 54–58.

(26) Kobayashi, J.; Nagai, U. *Biopolymers* **1978**, *17*, 2265–2277.

(27) Kainosho, M.; Ajisaka, K.; Sawada, S.; Tanokura, M.; Miyazawa, T. *Chem. Lett.* **1979**, 395–396.

0002-7863/87/1509-6927$01.50/0

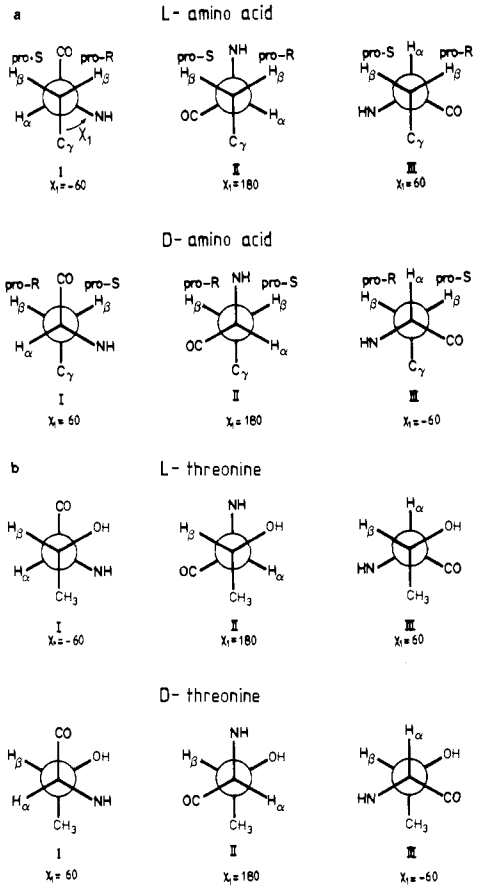

Figure 1. Newman projections of the three staggered side-chain conformations of an amino acid (a) with two β-protons and (b) with one β-proton (e.g. Thr), together with the corresponding χ_1 angles.

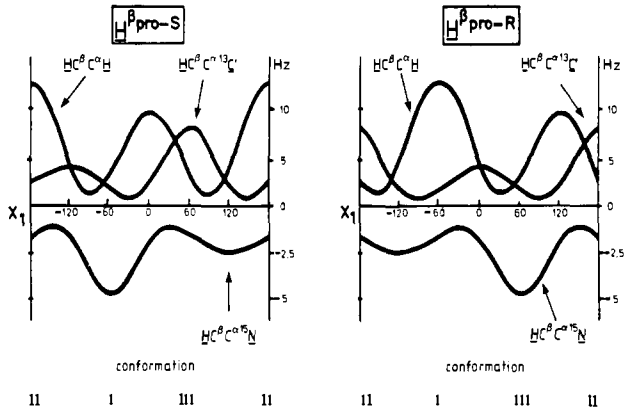

Figure 2. Homo- and heteronuclear coupling constants of $H^{\beta\ pro\text{-}R}$ and $H^{\beta\ pro\text{-}S}$ as a function of the torsional angle χ_1.[19,20]

when the two β-protons have different chemical shifts. We have stereochemically assigned the β-protons of the Phe and Trp residues in the hexapeptides *cyclo*-(-Phe[11]-Thr[10]-Lys[9](Z)-Trp[8]-Phe[7]-D-Pro[6]-) (**1**)[33-35] and *cyclo*-(-Phe[11]-Thr[10]-Lys[9](Z)-D-Trp[8]-Phe[7]-Pro[6]-) (**2**),[33] which contain the retrosequence of the highly potent somatostatin analogous MSD peptide,[36,37] by synthesis of several derivatives, in which the H^{α} and the $H^{\beta\ pro\text{-}S}$-proton was deuteriated selectively (see the Experimental Section). These compounds serve as examples to check the reliability of the method proposed here. In general, the total synthesis of deuteriated peptide derivatives is a tedious procedure, and it is highly desirable to replace it by spectroscopic methods. Obviously, for amino acids that contain only one β-proton deuteriation cannot be applied for the discrimination of rotamer I and III (Figure 1b). These rotamers exhibit a small synclinal $H^{\alpha}H^{\beta}$ coupling constant, whereas in rotamer II the antiperiplanar orientation of both protons leads to a large coupling constant.

NOE Effects. The evaluation of NOE (ROE) effects by NOESY in two[38] or one dimension[39] or ROESY (≡ CAMELSPIN)[40-45] and transformation of these values into distances (here, NH–H^{β}) may serve to discriminate alternative conformations. For example, the β-proton of Thr exhibits a strong intraresidual NOE to the NH proton only in conformation III but not in conformation I. The orientation of the Thr side chain in **1** and **2** is determined by these effects (see below).

In amino acids containing two β-protons, the intraresidual NH–H^{β} NOE cannot be used to discriminate conformation I and II (Figure 1). In both rotamers the β-proton with the larger $H^{\alpha}H^{\beta}$ coupling constant shows the larger NOE. In addition, the strong dependence of the NH–H^{β} distances on φ hampers a straightforward interpretation. On the other hand, NOE effects of C^{γ} residues to the C- or N-terminal side determine the side-chain conformation in all cases.

In antamanide, cyclo-(-Val[1]-Pro[2]-Pro[3]-Ala[4]-Phe[5]-Phe[6]-Pro[7]-Pro[8]-Phe[9]-Phe[10]-) (**3**),[46-49] rotamer I was found to be preferred in the equilibrium[50] from NOE data and other evidence for the Phe[9] and Phe[10] residue. The $H^{\alpha}H^{\beta}$ coupling constants of the Phe[5] and Phe[6] residues have similar size, indicating no strong conformational preference.

Heteronuclear Coupling Constants $^3J(HC^{\beta}C^{\alpha}C')$. Due to the Karplus relationship,[20,50,51] the three possible side-chain conformations can be unambiguously distinguished by a joint analysis of $H^{\alpha}H^{\beta}$ and $H^{\beta}C'$ coupling constants.[20,52-55] In the case of amino

(28) Kobayashi, J.; Nagai, U.; Higashijima, T.; Miyazawa, T. *Biochim. Biophys. Acta* **1980**, *577*, 195–206.
(29) Kobayashi, J.; Higashijima, T.; Nagai, U.; Miyazawa, T. *Biochim. Biophys. Acta* **1980**, *621*, 190–203.
(30) Kobayashi, J.; Higashijima, T.; Sekido, S.; Miyazawa, T. *Int. J. Pept. Protein Res.* **1981**, *17*, 486–494.
(31) Cowburn, D.; Live, D. H.; Fishman, A. J.; Agosta, W. C. *J. Am. Chem. Soc.* **1983**, *105*, 7435–7442.
(32) Kobayashi, J.; Higashijima, T.; Miyazawa, T. *Int. J. Pept. Protein Res.* **1984**, *24*, 40–47.
(33) Both peptides contain the slightly modified retrosequence of somatostatin 7–11. Therefore, the numbering of this sequence is retained here. Brazeau, P.; Vale, W.; Burgus, R.; Ling, N.; Butcher, M.; Rivier, J.; Guillemin, R. *Science (Washington, D.C.)* **1973**, *179*, 77–79.
(34) Koll, S. Ph.D. Thesis, Universität Frankfurt, 1984.
(35) Kessler, H.; Klein, M.; Müller, A.; Wagner, K.; Bats, J. W.; Ziegler, K.; Frimmer, M. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 997–999.
(36) Veber, D. F.; Holly, F. W.; Nutt, R. F.; Bergstrand, S. J.; Brady, S. F.; Hirschmann, R.; Torchiana, M. L.; Saperstein, R. *Nature (London)* **1979**, *280*, 512–514.
(37) Veber, D. F.; Freidinger, R.; Schwenk, F.; Palaveda, W.; Holly, F. W.; Strachan, R.; Nutt, R. F.; Arison, B.; Homnick, C.; Randall, W.; Glitzer, M.; Saperstein, R.; Hirschmann, R. *Nature (London)* **1981**, *292*, 55–58.
(38) Jeener, J.; Meier, B. H.; Bachmann, P.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546–4553.
(39) Kessler, H.; Oschkinat, H.; Griesinger, C.; Bermel, W. *J. Magn. Reson.* **1986**, *70*, 106–133.
(40) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811–813.
(41) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207–213.
(42) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355–360.
(43) Neuhaus, D.; Keeler, J. *J. Magn. Reson.* **1986**, *68*, 568–574.
(44) Kessler, H.; Griesinger, C.; Kerssebaum, R.; Wagner, K.; Ernst, R. R. *J. Am. Chem. Soc.* **1987**, *109*, 607–609.
(45) Griesinger, C.; Ernst, R. R. *J. Magn. Reson.*, in press.
(46) Wieland, T.; Lüben, G.; Ottenheym, H.; Faesel, J.; de Vries, J. X.; Konz, W.; Prox, A.; Schmid, A. *Angew. Chem., Int. Ed. Engl.* **1968**, *7*, 204–208.
(47) Müller, A. Ph.D. Thesis, Universtät Frankfurt, 1986.
(48) Kessler, H.; Griesinger, C.; Lautz, J.; Müller, A.; van Gunsteren, W., submitted for publication.
(49) Kessler, H.; Bermel, W.; Müller, A.; Pook, K.-H.; The *Peptides: Analysis, Synthesis, Biology*; New York, 1985; pp 437–473.
(50) Feeney, J.; Hansen, P. E.; Roberts, G. C. K. *J. Chem. Soc., Chem. Commun.* **1974**, 465–466.
(51) Hansen, P. E.; Feeney, J.; Roberts, G. C. K. *J. Magn. Reson.* **1975**, *17*, 249–261.
(52) Bystrov, V. F.; Gavrilov, Y. D.; Solkan, V. N. *J. Magn. Reson.* **1975**, *19*, 123–129.

Chart I

P_I, %	5	15	25	35	45	55	65	75	85
$H^{\beta\,pro\text{-}R}C'$	0.404	0.339	0.276	0.217	0.165	0.119	0.081	0.051	0.029
$H^{\beta\,pro\text{-}S}C'$	0.015	0.019	0.022	0.025	0.029	0.032	0.035	0.038	0.040

acids with two β-protons, only the $H^{\beta\,pro\text{-}R}$ proton in conformation II is antiperiplanar to C′, when the conformation III is weakly populated as usual. In analogy to Pachler's equations (2) for

$$P_I = \frac{J(H^\alpha H^{\beta\,pro\text{-}R}) - J_{sc}}{J_{ap} - J_{sc}}$$

$$P_{II} = \frac{J(H^\alpha H^{\beta\,pro\text{-}S}) - J_{sc}}{J_{ap} - J_{sc}}$$

$$P_{III} = 1 - P_I - P_{II} \qquad (2)$$

proton couplings in L-amino acids, similar equations (3) for $H^\beta C'$

$$P_{II} = \frac{J'(H^{\beta\,pro\text{-}R}C') - J_{sc}'}{J_{ap}' - J_{sc}'}$$

$$P_{III} = \frac{J'(H^{\beta\,pro\text{-}S}C') - J_{sc}'}{J_{ap}' - J_{sc}'}$$

$$P_I = 1 - P_{II} - P_{III} \qquad (3)$$

coupling constants were proposed.[53,56,57] For D-amino acids the labels *pro-R* and *pro-S* must be interchanged. In the literature the following values for antiperiplanar (ap) and synclinal (sc) arrangements are given.[16,17,20]

$$H^\alpha H^\beta\text{: } J_{ap} = 13.6 \text{ Hz}, J_{sc} = 2.6 \text{ Hz}$$

$$H^\beta C'\text{: } J_{ap}' = 8.5 \text{ Hz}, J_{sc}' = 1.4 \text{ Hz}$$

Slightly different values (J_{ap}' = 9.8 Hz, J_{sc}' = 1.3 Hz) have been reported[31] too.

To date, heteronuclear coupling constants in oligopeptides were difficult to obtain in natural-abundance spectra. The carbonyl carbons give weak and broad lines. The C'_i of the *i*th amino acid in a peptide chain is usually coupled to about six protons: ($H^{\beta\,pro\text{-}R}$, $H^{\beta\,pro\text{-}S}$, H^α, NH)$_i$(NH, H^α)$_{i+1}$. Thus, labeled compounds requiring laborious syntheses were used for this purpose.[26,30,32,58] However, there are some examples for measurements of compounds in natural abundance using double- and triple-resonance methods to determine $^3J(H^\beta C')$.[59,60] A heteronuclear selective 2D *J* experiment[61,62] was used in valinomycine[63] and cyclic tripeptides[64] for this purpose.

A simplification of the multiplicity patterns in cross peaks of a coupled H,C-COSY spectrum can be achieved with the heteronuclear E.COSY technique,[7,14] which is applied here for the first time. With this method, we were able to determine quantitatively the vicinal $H^\beta C'$ coupling constants of the MeLeu[4] and MeLeu[6] residues in cyclosporin A (**4**). These coupling constants, together with the rotamer populations derived from eq 2 and 3, are contained in Table I.

Table I. Homo- and Heteronuclear Coupling Constants (Hz), β-Proton Assignments, and Rotamer Populations of the Side-Chain Conformations of MeLeu[4] and MeLeu[6] of Cyclosporin A (**4**) in $CDCl_3$

	MeLeu[4]		MeLeu[6]	
	β_1[a]	β_2	β_1	β_2
$J(H^\alpha H^\beta)$	4.5	12.0	10.5	6.0
$J(H^\beta C')$	2.0	1.8	1.6	7.4
prochirality[b]	*pro-S*	*pro-R*	*pro-S*	*pro-R*

	MeLeu[4]		MeLeu[6]	
P, %	$J(H^\alpha H^\beta)$	$J(H^\beta C')$	$J(H^\alpha H^\beta)$	$J(H^\beta C')$
P_I	85 (17)[c]	88	31 (72)	5
P_{II}	17 (85)	5 (7)	72 (31)	84 (11)
P_{III}	−2	7 (5)	−3	11 (84)

[a] β_1 is the low-field β-proton and β_2 the high-field β-proton. [b] Prochirality of the β-protons as derived from the differentiation of the conformers by the analysis of homo- and heteronuclear coupling constants. [c] The four proposals for the rotamer distribution as derived by insertion of the value of the $J(H^\alpha H^\beta)$ and $J(H^\beta C')$ coupling constants into eq 2 and 3. The two inconsistent distributions are in parentheses.

While the results from homo- and heteronuclear coupling constants for MeLeu[4] agree very well, the results for MeLeu[6] differ somewhat. However, the preference of conformation I for MeLeu[4] and conformation II for MeLeu[6] is obvious. This finding is in agreement with results from NOE data.[65,66]

H,C-COLOC for the Analysis of Side-Chain Conformations. Side Chains of Amino Acids with Two β-Protons. An assignment of the side-chain rotamers is only meaningful if one of the three conformations is dominant in the equilibrium. This is checked by the easily accessible homonuclear $H^\alpha H^\beta$ coupling constants. As mentioned above, two sets of populations are obtained, which are identical except for the interchange of conformations I and II. If one of those is strongly preferred, we can proceed in the following way: For conformation I, both $H^\beta C'$ coupling constants are small (both are synclinal). In conformation II, one of the $H^\beta C'$ coupling constants is small (synclinal) and the other one is large (antiperiplanar). We show in the following that peak intensities of $H^\beta C'$ cross peaks in a COLOC spectrum essentially reflect the heteronuclear coupling constants quadratically. The general expression for the transfer amplitude I_{ij} of a COLOC peak from proton *i* to carbon *j* is given in eq 4, where *k* and *l* denote "passive"

$$I_{ij} = \sin(\pi J(H^i C^j)\Delta_1) \sin(\pi J(H^i C^j)\Delta_2) \prod_k \cos(\pi J(H^i H^k)\Delta_1) \prod_l \cos(\pi J(H^i C^j)\Delta_2) \qquad (4)$$

proton spins, which couple to the active proton H^i or to the active carbon C^j, respectively. To compare relative intenties of two $H^\beta C'$ cross peaks, we may omit all identical terms in the two transfer amplitudes and arrive at eq 5 and 6. These equations hold under

$$H^{\beta_1}C'\text{: } \cos(\pi J(H^\alpha H^{\beta_1})\Delta_1) \sin(\pi J'(H^{\beta_1}C')\Delta_1) \sin(\pi J'(H^{\beta_1}C')\Delta_2) \cos(\pi J'(H^{\beta_2}C')\Delta_2) \qquad (5)$$

$$H^{\beta_2}C'\text{: } \cos(\pi J(H^\alpha H^{\beta_2})\Delta_1) \sin(\pi J(H^{\beta_2}C')\Delta_1) \sin(\pi J(H^{\beta_2}C')\Delta_2) \cos(\pi J(H^{\beta_1}C')\Delta_2) \qquad (6)$$

(53) Toma, F.; Monnot, M.; Piriou, F.; Savda, J.; Fermandijan, S. *Biochem. Biophys. Res. Commun.* **1980**, *97*, 751–758.
(54) Wasylishen, R.; Schaefer, T. *Can. J. Chem.* **1973**, *51*, 961–973.
(55) Aydin, R.; Loux, J. P.; Günther, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 449.
(56) Espersen, W. G.; Martin, R. B. *J. Phys. Chem.* **1976**, *80*, 741–745.
(57) Martin, R. B. *J. Phys. Chem.* **1979**, *83*, 2404–2407.
(58) Fishman, A. J.; Wyssbrod, H. R.; Agosta, W. C.; Field, H. F.; Gibbons, W. A.; Cowburn, D. *J. Am. Chem. Soc.* **1977**, *99*, 2953–2957.
(59) Bystrov, V. F.; Gavrilov, Y. D.; Ivanov, V. T.; Ovchinnikov, Y. A. *Eur. J. Biochem.* **1977**, *78*, 63–82.
(60) Garbay-Jaureguiberry, G.; Marion, D.; Fellion, E.; Roques, B. P. *Int. J. Pept. Protein Res.* **1982**, *20*, 443–450.
(61) Bax, A.; Freeman, R. *J. Am. Chem. Soc.* **1982**, *104*, 1099–1100.
(62) Bax, A.; Ferretti, J. A.; Nashed, N.; Jerina, D. M. *J. Org. Chem.* **1985**, *50*, 3029–3034.
(63) Davis, D. G.; Agosta, W. C.; Cowburn, D. *J. Am. Chem. Soc.* **1983**, *105*, 6189–6190.
(64) Bermel, W. Ph.D. Thesis, Universität Frankfurt, 1983.
(65) Kessler, H.; Loosli, H. R.; Oschkinat, H. *Helv. Chim. Acta* **1985**, *68*, 661–681.
(66) Loosli, H. R.; Kessler, H.; Oschkinat, H.; Weber, H. P.; Petcher, T. J.; Widmer, A. *Helv. Chim. Acta* **1985**, *68*, 682–704.

Table II. Homonuclear α,β-Coupling Constants, Rotamer Populations, and Assignment of the β-Protons for **1** in DMSO-d_6

	J,[a] Hz		rotamer populations,[b] %			assignments[b]	
	$\alpha\beta_1$	$\alpha\beta_2$	I	II	III	β_1	β_2
Phe^7	4.5	9.0	17 (58)[c]	58 (17)	25	*pro-R*	*pro-S*
Trp^8	4.2	10.9	75 (15)	15 (75)	10	*pro-S*	*pro-R*
Phe^{11}	3.1	11.5	80 (5)	5 (80)	15	*pro-S*	*pro-R*

[a] β_1 is the low-field β-proton and β_2 the high-field β-proton. [b] Assignment via COLOC and selective deuteriation (Phe^7, Phe^{11}). [c] Without the information from the COLOC experiment, the populations given in parentheses are possible too. They are then ruled out by the COLOC experiment.

the additional assumption that the two β-protons couple by similar coupling constants to other protons (e.g. γ-protons) in the side chain. This condition is obviously fulfilled in aromatic amino acids and in most aliphatic amino acids because of the flexibility of the side chain with respect to rotation about χ_2 and bonds further away. However, this does not apply for proline. A full analysis, including all proton couplings, is recommended for this residue.

Strong coupling between the β-protons was not discussed so far. Simulations done by Dr. W. Studer, ETH Zürich, however, show that strong coupling does not invalidate our qualitative approach.

In our experience delays of $\Delta_1 = 25$ ms and $\Delta_2 = 30$ ms in the COLOC yield good results for routine peptide sequencing. We assume $P_{III} = 15\%$, which is a value frequently found in our systems. The transfer functions in (Chart I), which are proportional to the cross-peak intensities of the $H^{\beta}C'$ cross peaks, are obtained from eq 5 and 6 for $P_I(P_{II})$ varying between 5 (80)% and 85 (0)%. From this chart is is obvious that, for predominance of either of the two conformations I or II, P_I and P_{II} can be distinguished from the relative intensities of the $H^{\beta}C'$ cross peaks, which mainly depend on the heteronuclear coupling constant.

It is clear from the chart that only the $H^{\beta\,pro\text{-}R}$-proton in conformation II is expected to exhibit an intense cross peak in the COLOC spectrum. Yet the absolute intensity of this cross peak is unknown. However, empirically it is found that its intensity is comparable with that of cross peaks due to $^2J(CH)$ couplings between $(H^{\alpha})_i(C')_i$ or $(NH)_{i+1}(C')_i$. Hence, the following practical procedure is suggested for side chains with predominance of conformer I or II: Inspection of the COLOC spectrum shows whether there are *two weak* cross peaks of carbonyl carbons to β-protons or *one intense* and *one weak* cross peak. In the former case, conformation I dominates, and the *β-proton* with the large coupling to the *α-proton* is to be assigned to *pro-R*. In the latter case, the intense cross peak assigns the $H^{\beta\,pro\text{-}R}$-proton and conformation II is predominant. Thus, the two proposals for the populations obtained from homonuclear coupling constants can be distinguished.

Experimental Verification. The $H^{\alpha}H^{\beta}$ coupling constants of the three aromatic amino acids of **1**[67] and the two corresponding sets of rotamer populations are given in Table II. In the COLOC spectrum (Figure 3a) only the Phe^7 carbonyl carbon resonance shows a strong cross peak to a β-proton (at low field) whose intensity is comparable to the intensity of cross peaks across two bonds. Thus, only Phe^7 prefers conformation II, and the low-field β-proton is *pro-R*. Phe^{11} and Trp^8 prefer conformation I. The assignment of the β-protons follows from the $H^{\alpha}H^{\beta}$ couplings as described above. These assignments are in agreement with those obtained by selective deuteriation in the H^{α}- and $H^{\beta\,pro\text{-}S}$-position. The conformation of **1** is shown in Figure 3b.

Our procedure has been tested on the hexapeptide **2** too. This peptide adopts two conformations in DMSO solution, which interconvert slowly on the NMR time scale and therefore give rise to two distinct sets of NMR resonances (Figure 4; lower case letters designate the residues of the less populated conformation and capital letters those of the dominant conformation). The chemical shifts of the β-protons of the aromatic amino acids are

(67) Kessler, H.; Bats, J. W.; Griesinger, C.; Koll, S.; Will, M.; Wagner, K. *J. Am. Chem. Soc.*, in press.

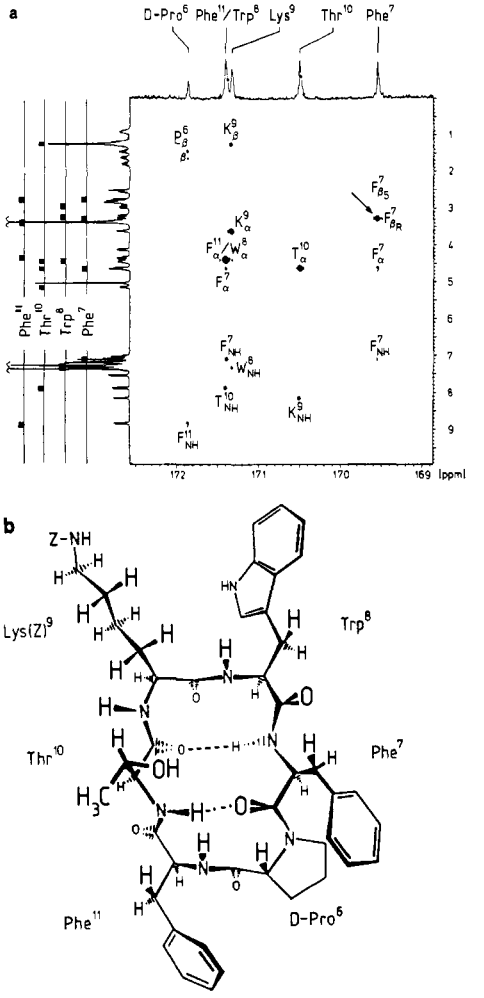

Figure 3. (a) 300-MHz, H,C-COLOC spectrum of *cyclo*-(-Phe^{11}-Thr^{10}-Lys^9(Z)-Trp^8-Phe^7-D-Pro^6-) (**1**) in DMSO. The cross peaks between Phe^7 $H^{\beta\,pro\text{-}R}C'$ (arrow) indicate predominance of conformation II. Assignments of the relevant proton signals are given on the left. The cross peaks are marked with the assignment of that proton that couples to the carbonyl. The one-letter symbols for amino acids[15] are used. (b) Proposed conformation of **1**. The conformations of the side chains of Phe^7, Trp^8, Thr^{10}, and Phe^{11} were also derived from stereoselective deuteriation and/or from the ROESY spectrum.[69] Note that the aromatic rings of both Phe residues are oriented toward the Pro residue.

Table III. Homonuclear α,β-Proton Coupling Constants, Rotamer Populations, and Assignment of β-Protons for **2** in DMSO-d_6

	J,[a] Hz		rotamer populations,[b] %			assignments	
	$\alpha\beta_1$	$\alpha\beta_2$	I	II	III	β_1	β_2
Phe^7	4.5	9.5	63 (17)[c]	17 (63)	20	*pro-S*	*pro-R*
D-Trp^8	3.7	10.7	74 (10)	10 (74)	16	*pro-R*	*pro-S*
phe^7	8.5	6.0	31 (54)	54 (31)	15	*pro-S*	*pro-R*
D-trp^8	6.6	7.7	47 (36)	36 (47)	17	*pro-R*	*pro-S*
phe^{11}	5.4	10.6	73 (26)	26 (73)	1	*pro-S*	*pro-R*

[a] β_1 is the low-field β-proton and β_2 the high-field β-proton. [b] Assignment via COLOC and selective deuteriation (Phe^7, D-Trp^8, phe^7, D-trp^8, phe^{11}). [c] Without the information from the COLOC experiment, the populations given in parentheses are possible too. They are then ruled out by the COLOC experiment.

well separated except for Phe^{11}. Hence, five residues can be used for checking our method. The $H^{\alpha}H^{\beta}$ coupling constants of these residues and the corresponding rotamer populations are given in Table III. Only the phe^7 carbonyl carbon exhibits a cross peak to a β-proton, indicating the predominance of conformation II. The other aromatic amino acids thus prefer conformation I, and the diastereotopic β-protons are assigned via the $H^{\alpha}H^{\beta}$ couplings. Selective deuteriation of the D-Trp and the two Phe residues described above independently proves the results of the COLOC

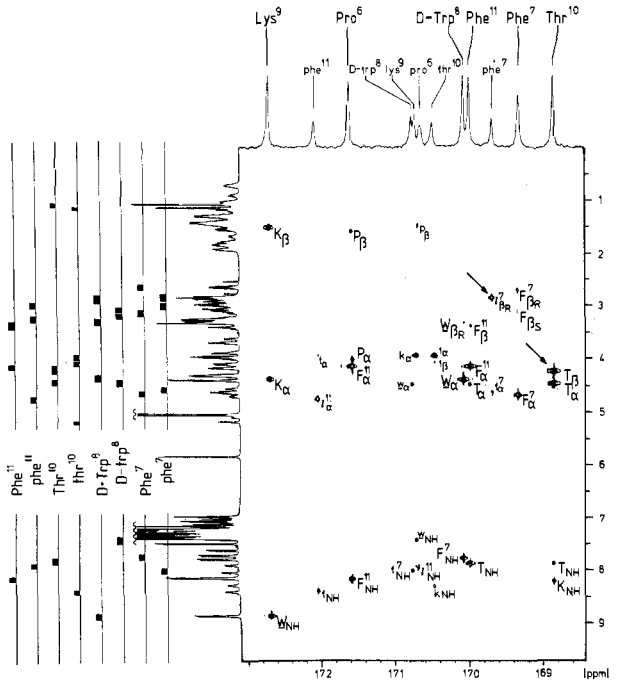

Figure 4. 500-MHz, H,C-COLOC spectrum of *cyclo*-(-Phe[11]-Thr[10]-Lys[9](Z)-D-Trp[8]-Phe[7]-Pr[6]-) (**2**) in DMSO. Capital letters mark the predominant conformation, whereas lower-case letters mark the less populated conformation. Of special importance are the cross-peaks phe[7] $H^{\beta\,pro\text{-}R}C'$ (conformation II) and Thr[10] $H^{\beta}C'$ (conformation III).

Table IV. Homonuclear α,β-Proton Coupling Constants, Rotamer Populations, and Assignment of β-Protons for Antamanide (**3**) in $CDCl_3$

	J,[a] Hz		rotamer populations,[b] %			assignments	
	β_1	β_2	I	II	III	β_1	β_2
Phe[9]	4.1	12.6	91 (14)[c]	14 (91)	−5	*pro-R*	*pro-S*
Phe[10]	11.1	4.0	78 (13)	13 (78)	9	*pro-S*	*pro-R*

[a] β_1 is the low-field β-proton and β_2 the high-field β-proton. [b] Assignment via COLOC and NOE measurements (Phe[9], Phe[10]). [c] Without the information from the COLOC experiment, the populations given in parentheses are possible too. They are then ruled out by the COLOC experiment.

analysis. The two conformations of **2** are shown in Figure 5.

Evaluation of $^3J(HC^{\beta}C^{\alpha}N)$ Coupling Constants. The conformation I so far was identified by the lack of an $H^{\beta}C'$ cross peak. Positive indicators are certainly desirable. It was pointed out in the literature[19,20] that $H^{\beta}{}^{15}N$ couplings may be used to determine side-chain conformations. Because of experimental difficulties, this coupling was only used in ^{15}N-labeled compounds.[6,25,31,58,59,68] In H,N-COLOC spectra, we may also inspect the long-range couplings to nitrogens. For predominance of rotamer I, one β-proton is antiperiplanar to nitrogen, whereas for conformation II both β-protons are synclinal with respect to ^{15}N. The following coupling constants have been determined for $^3J(NH)$ for the antiperiplanar and synclinal conformations.[69]

$$J_{ap}' = -4.8 \text{ Hz} \qquad J_{sc}' = -1.8 \text{ Hz}$$

Again the qualitative analysis can be used: One intense and one weak cross peak are expected for conformation I but two weak cross peaks for conformation II. The β-proton, which exhibits a large HN coupling constant, is *pro-S*. In the case of a preferred conformation II, the assignment follows from the vicinal $H^{\alpha}H^{\beta}$ coupling constant.

These considerations are checked for the Phe[9] residue of antamanide (**3**). The evaluation of the homonuclear and HC coupling constants of the Phe[9] and Phe[10] residues exhibits a strong preference of conformation I (Table IV). Therefore, there is no cross peak in the H,C-COLOC to the carbonyl carbons of these residues (Figure 6).

In the H,N-COLOC spectrum the nitrogen signal of Phe[10] was unfortunately obscured by instrumental artifacts. However, the cross section at the ^{15}N chemical shift of the Phe[9] nitrogen exhibits a correlation only to the low-field $H^{\beta\,pro\text{-}S}$-proton in conformation

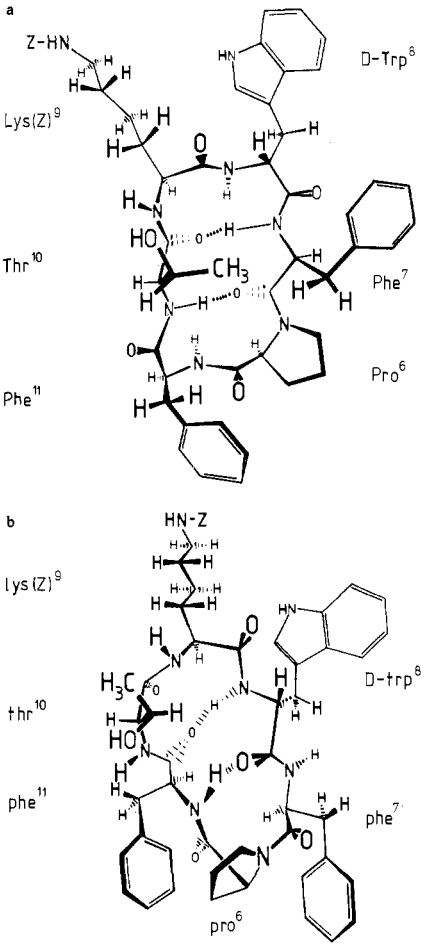

Figure 5. (a) Proposed conformation of the predominant species of *cyclo*-(-Phe[11]-Thr[10]-Lys[9](Z)-D-Trp[8]-Phe[7]-Pro[6]-) (**2**). Note the spatial proximity of the Thr methyl group and the Phe[7] $H^{\beta\text{-}pro\text{-}R}$-proton, which is proven by an ROE cross peak. (b) Proposed conformation of the minor populated species of **2**. Note the side-chain conformation of Phe[7], which is different from that of Phe[7] in the predominant conformation. The Phe[7]–Pro[6] peptide bond is cis configurated.

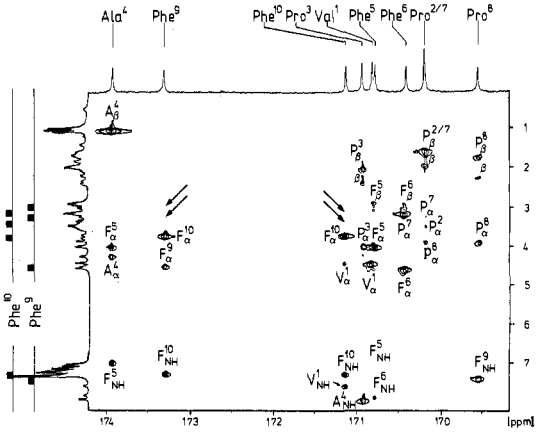

Figure 6. 400-MHz, H,C-COLOC spectrum of antamanide (**3**) in $CDCl_3$. The missing cross peaks (arrows) for the β-protons of Phe[9] and Phe[10] are in agreement with conformation I for these two side chains.

(68) Stimson, E. R.; Meinwald, Y. C.; Montelione, G. T.; Scheraga, H. A. *Int. J. Pept. Protein Res.* **1986**, *27*, 569–582.
(69) Song, J. A.; Gibbons, W. A.; Randall, E. W. *Biochemistry* **1973**, *12*, 2100–2104.

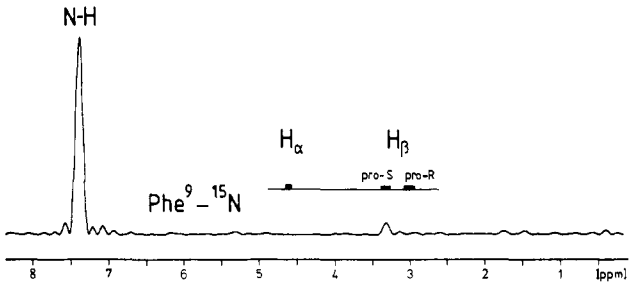

Figure 7. F1 trace extracted from a 500-MHz H,N-COLOC spectrum of **3** at the resonance of the ^{15}N of Phe^9 in $CDCl_3$. The correlation to the $H^{\beta\,pro\text{-}S}$ proves conformation I for the side chain of Phe^9.

I in addition to the correlation to the directly attached NH proton (Figure 7). This is in complete agreement with the results obtained with H,C-COLOC, homonuclear coupling constants, and NOE effects.[47,48]

Side-Chain Conformations of Amino Acids with One β-Proton. The size of the $H^\alpha H^\beta$ coupling constant of amino acids with one β-proton quantitatively yields the amount of the conformation II (Figure 2) and the sum of the populations of conformations I and III. Conformation II is distinguished by the large antiperiplanar $H^\alpha H^\beta$ coupling constant from conformation I and III. We obtain eq 7. The $H^\beta C'$ coupling serves to discriminate conformations

$$P_{II} = \frac{J(H^\alpha H^\beta) - J_{sc}}{J_{ap} - J_{sc}} P_I + P_{III} = 1 - P_{II} \qquad (7)$$

I and III. Conformation III is indicated by a large antiperiplanar $H^\beta C'$ coupling constant, whereas conformations I and II show only a small coupling between synclinal H^β and C'. We thus obtain eq 8 for the heteronuclear coupling. The qualitative measurement

$$P_{III} = \frac{J(H^\beta C') - J_{sc}'}{J_{ap}' - J_{sc}'} \qquad (8)$$

of the $H^\beta C'$ coupling in a COLOC spectrum gives only a rough estimate of the ratio P_{III}/P_I. Thus, in contrast to amino acids with two β-protons, only a quantitative measurement of the $H^\beta C'$ coupling constant suffices to determine quantitatively all three populations. To obtain an estimate of the size of the heteronuclear coupling constants, the intensity of the $H^\beta C'$ cross peaks of amino acids with one β-proton in COLOC spectra must be compared to that of other cross peaks via $^2J(CH)$ and $^3J(CH)$ coupling. This is, however, not too severe a limitation. In the examples shown so far, cross peaks via antiperiplanar heteronuclear ^3J coupling have about the same intensities as those cross peaks originating from $^2J(CH)$ couplings. This is due to the fact that, with the Δ_1 and Δ_2 delays chosen as specified above, the sizes of the cross peaks are almost independent of the passive coupling constants. Taking into account the qualitative nature of the measurement of heteronuclear coupling constants in H,C-COLOC spectra, we arrive at the following practical procedure: The analysis begins with the determination of the $H^\alpha H^\beta$ coupling. A dominating conformation II is indicated by a coupling constant of about 10 Hz. If this coupling is smaller than 4 Hz, conformation I and/or III dominate(s). Predominance of conformation III is indicated by an intense cross-peak $H^\beta C'$ in the COLOC spectrum. If the cross peak is missing, conformation I is preferred.

Experimental Verification. For the above-mentioned hexapeptide **1**, the $H^\alpha H^\beta$ coupling in the Thr residue is small (3.5 Hz). The missing cross peak in the COLOC spectrum (Figure 3) indicates conformation I, which is in agreement with the observed strong NOE effect in the NOESY and ROESY experiment[40,67] between the NH proton of Lys^9 and the Thr^{10} H^β proton. Also in the dominant backbone conformation of **2**, a small $H^\alpha H^\beta$ coupling constant (3.8 Hz) is observed for the Thr^{10} residue. In the COLOC spectrum of this peptide (Figure 4), an intense $H^\beta C'$ cross peak is observed, which is indicative of conformation III. A transannular NOE effect of the Thr^{10} methyl group to the $H^{\beta\,pro\text{-}R}$-proton of Phe^7 in a ROESY experiment provides independent evidence for this finding.

Valine residues normally prefer conformation II for steric reasons. This was found in proteins,[70] as well as in Val, containing oligopeptides by X-ray analysis.[71] We also observed a dominant conformation II for the Val side chain in three cyclic pentapeptides derived from thymopoietin[72,73] as well as for Val and *N*-MeVal in cyclosporin A.[65,66]

Conclusions

The use of vicinal proton–proton coupling constants yields qualitative and quantitative information of side-chain conformations in amino acids. However, the information is incomplete from homonuclear couplings alone. It was shown that this missing information is obtained from inspection of a routine H,C-COLOC experiment. This qualitative estimation of the relevant heteronuclear coupling constant is sufficient to provide the assignment of the conformations.

For amino acids with *two* β-protons from this kind of analysis, the quantitative population distribution resulted, the absolute amounts of the populations being derived exclusively from $H^\alpha H^\beta$ coupling constants. The discrimination of the diastereotopic β-protons is achieved simultaneously.

For amino acids with *one* β-proton, the ratio of the populations P_I and P_{III} can be obtained only qualitatively, whereas the amount of conformation II is quantitatively determined.

It is important to mention that the qualitative interpretation of a COLOC spectrum to discriminate vicinal heteronuclear coupling between gauche and trans H,X nuclei may also be applied to analyze conformations and configurations of non-peptides (e.g. sugars, macrolides, and terpenes) and the peptide backbone.[1]

In order to avoid wrong interpretations, the influence of homonuclear couplings for the cross-peak intensities in the COLOC spectrum should be taken into account. The errors inferred by neglecting the evolution of homonuclear couplings in Δ_1 and heteronuclear couplings in Δ_2 are smaller as the two Δ-delays are shortened. For application of COLOC to protonated heteronuclei, strong oscillations of peak intensities arising from evolution of $^1J(CH)$ couplings during Δ_2 should be removed by the insertion of a bilinear π-pulse (BIRD-sandwich[74]) in the middle of Δ_2. Finally, we should mention that qualitative information about heteronuclear coupling constants can be obtained not only from H,C-COLOC but also from inverse heteronuclear shift correlation via long-range couplings,[75,76] presumably with an increased signal to noise ratio.

Experimental Section

Measurement Conditions. The spectra were measured on Bruker AM 300, Bruker AM 400, and Bruker AM 500 spectrometers and were processed on a Bruker data station with an Aspect 3000 and a 160-MByte B-DS 160F disk.

H,C-COLOC pulse sequence is the following: $\pi/2(^1H)$, $t_1/2$, $\pi(^1H,^{13}C)$, $\Delta_1 - t_1/2$, $\pi/2(^1H,^{13}C)$, Δ_2, aq(^{13}C,^1H-BB).

H,C-COLOC spectrum (300 MHz) of 250 mg of **1** in 2.5 mL of DMSO-d_6: 144 experiments of 128 scans each; relaxation delay 1.8 s; acquisition time for one scan 1.8 s; Δ_1 = 25 ms, Δ_2 = 30 ms; size 1K; spectral width in F2 280 Hz, in F1 3000 Hz; zero filling in F2 to 2 K; $\cos^2$ multiplication; zero filling in F1 to 512 W, sinebell multiplication.

H,C-COLOC spectrum (500 MHz) of 480 mg of **2** in 2.8 mL of DMSO-d_6: 240 experiments of 128 scans each; relaxation delay 1 s; acquisition time for one scan 1.55 s; Δ_1 = 25 ms, Δ_2 = 35 ms; size 2K; spetral width in F2 600 Hz, in F1 4854 Hz; zero filling in F2 to 4 K; exponential multiplication (line broadening of 1 Hz); zero filling in F1 to 512 W, sinebell multiplication.

H,C-COLOC (400-MHz) and H,N-COLOC (500-MHz) spectra of **3**: experimental parameters found in ref 47 and 49.

(70) Bhat, T. N.; Sasisekharan, V.; Vijayan, M. *Int. J. Pept. Protein Res.* **1979**, *13*, 170–184.
(71) Ashida, T.; Kojima, T.; Tanaka, I.; Yamane, T. *Int. J. Pept. Protein Res.* **1986**, *27*, 61–69.
(72) Kessler, H.; Klein, A., unpublished results.
(73) Kessler, H.; Kerssebaum, R., unpublished results.
(74) Garbow, J. R.; Weitekamp, D. P.; Pines, A. *Chem. Phys. Lett.* **1982**, *93*, 504–509.
(75) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093–2094.
(76) Bermel, W.; Griesinger, C.; Kessler, H.; Wagner, K. *Magn. Reson. Chem.* **1987**, *25*, 325–326.

The hexapeptides **1** and **2** have been synthesized from their linear precursors H-Xxx-Phe[7]-Yyy-Phe[11]-Thr[10]-Lys[9](Z)-$NHNH_2$ (**1**, Xxx = Trp and Yyy = D-Pro; **2**, Xxx = D-Trp and Yyy = Pro) via the azide method.[77] The linear precursor were prepared by the solid-phase method as described previously.[78,79] For the deuteriated analogues the Boc-protected deuteriated amino acids were used.

Boc-(*S*,*S*)-[α-^{2}H,β-^{2}H]phenylalanine and Boc-(*R*,*R*)-[α-^{2}H,β-^{2}H]-tryptophane were prepared by deuteriation of *N*-acetyl-Z-dehydrophenylalanine[80,81] and *N*-acetyl-Z-dehydrotryptophane.[82] The N-acetylated racemic phenylalanine was asymmetrically hydrolyzed by the action of porcine kidney acylase[83] (Sigma Chemical Co., No. A-8376); the amino functionality was directly protected (Boc) under usual conditions[84] and separated by crystallization.

Deuteriated *N*-acetyl-*d*,*l*-tryptophane has been resolved by means of the *d*-α-phenylethylamine salt with the isolation of *N*-acetyl-*d*-tryptophane.[85,86] The acetyl group was removed by refluxing with 48% aqueous hydrobromic acid (5 equiv) in water/methanol (1/1), and again the amino functionality was protected (Boc).

Acknowledgment. We thank Dr. A. Müller for making available the spectra of **3** and for valuable discussions concerning its conformation. We are grateful to the Sandoz AG for a gift of **4** and to Dr. H. Oschkinat for helpful discussions. We thank Dr. W. Studer (ETH, Zürich) who did the computer simulations concerning strong coupling effects. We gratefully acknowledge financial support by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. C.G. also thanks the Fonds der Chemischen Industrie for a personal grant during early stages of this work.

(77) Klausner, Y. S.; Bodanszky, M. *Synthesis* **1974**, 549-559.
(78) Kessler, H.; Bernd, M.; Damm, I. *Tetrahedron Lett.* **1982**, *23*, 4685-4688.
(79) Kessler, H.; Eiermann, V. *Tetrahedron Lett.* **1982**, *23*, 4689-4692.
(80) Kirby, G. W.; Michael, J. *J. Chem. Soc., Perkin. Trans. 1* **1973**, 115-120.
(81) Herbst, R. M.; Shemin, D. *Org. Synth.* **1943**, *2*, 1-3.
(82) Hengartner, U.; Valentine, D.; Johnson, K. K.; Larscheid, M. E.; Pigott, F.; Scheidl, F.; Scott, J. W.; Sun, R. C.; Townsend, J. M.; Williams, T. H. *J. Org. Chem.* **1979**, *44*, 3741-3747.
(83) Birnbau, M.; Levintow, L.; Kingsley, R. B.; Greenstein, J. P. *J. Biol. Chem.* **1952**, *194*, 455-470.
(84) Moroder, L.; Hallet, A.; Wünsch, E.; Keller, O.; Wersin, G. *Hoppe-Seyler's Z. Physiol. Chem.* **1976**, *357*, 1651-1653.
(85) du Vigneaud, V.; Sealock, R. R. *J. Biol. Chem.* **1932**, *96*, 511-517.
(86) Overby, L. R. *J. Org. Chem.* **1958**, *32*, 1393-1394.

π-Electron Valence Bond Calculations on Benzenoid Hydrocarbons via Graphical Unitary Group Methods

S. A. Alexander[†] and T. G. Schmalz*

Contribution from the Theoretical Chemical Physics Group, Department of Marine Sciences, Texas A&M University at Galveston, Galveston, Texas 77553. *Received April 17, 1986*

Abstract: Graphical unitary group techniques are applied to the valence bond model of π-electron systems. These techniques, which have produced highly efficient solution methods for ab initio quantum chemical problems, are found to yield a corresponding increase in the efficiency with which valence bond (and other approximate model) calculations can be carried out. As a result, it becomes feasible to solve the valence bond model exactly for systems of up to about 24 π-centers. The exact valence bond ground state energy is presented for all benzenoid hydrocarbons of up to 20 carbon atoms plus selected 22 and 24 atom systems. Using these energies we show that the resonance energy predicted by valence bond theory correlates well with resonance energy predicted by Hückel theory, in spite of the very different assumptions underlying the two methods.

I. Introduction

The past decade has witnessed a dramatic increase in the number of ab initio quantum chemical studies of molecular electronic structure. The reason for this growth is due primarily to the development of efficient computational techniques, e.g., the graphical unitary group approach,[1-3] which significantly reduce the amount of computer time needed for such studies. Although seldom used, many of the same concepts that have been so fruitful in ab initio programs can also be applied to approximate and/or semiempirical calculations. These calculation lack the absolute predictive power of their ab initio counterparts, but for certain classes of systems semiempirical theories can provide useful insight into molecular behavior. In this paper we focus on one such theory—the valence bond (VB) model.

The valence bond model was introduced in the early years of quantum mechanics by Pauling[4] and others[5-7] as an extension of classical chemical bonding concepts. Indeed, most chemists today continue to think in terms of valence bond concepts when they "push electrons" in organic reactions. Because the complexity of valence bond calculations rises very rapidly as a function of the size of the chemical system, these ideas are frequently abandoned in favor of molecular orbital based ideas whenever even semiquantitative results are called for. Clearly if the VB model is to be usefully applied to moderate or large-size molecules, efficient computational techniques are essential.

One class of molecules where the valence bond model has had a number of qualitative successes is conjugated π-electron systems. To illustrate this point we examine the phenomenon of resonance stabilization in such molecules. Our discussion is outlined roughly as follows: In Section II we present the valence bond model Hamiltonian and discuss its adequacy in studies of conjugated π-electron systems. Section III discusses how ab initio techniques

[†] Robert A. Welch Foundation Fellow. Current Address: Quantum Theory Project, University of Florida, Gainsville, FL 32611.

(1) (a) Shavitt, I. *Int. J. Quantum Chem. Symp.* **1977**, *11*, 131; **1978**, *12*, 5. (b) Brooks, B. R.; Schaefer, H. F. *J. Chem. Phys.* **1979**, *70*, 5092. (c) An excellent review of this method can be found in the following: Shavitt, I. *Lecture Notes in Chemistry, No. 22*; Hinze, Jürgen, Ed.; Springer-Verlag: New York, 1981.
(2) Robb, M. A.; Niazi, U. *Comp. Phys. Rep.* **1984**, *1*, 127.
(3) Paldus, J. *Chem. Phys.* **1974**, *61*, 5321; *Int. J. Quantum Chem.* **1975**, *59*, 165; *Phys. Rev.* **1976**, *A14*, 1620; *Lecture Notes in Chemistry No. 22*; Hinze, Jürgen, Ed.; Springer-Verlag: New York, 1981.
(4) (a) Pauling, L.; Wheland, G. W. *J. Chem. Phys.* **1933**, *1*, 362. (b) Pauling, L. *The Nature of the Chemical Bond*; Cornell University Press: Ithaca, 1940.
(5) Rumer, G. *Nachr. Ges. Wiss. Gottingen, Math.-Phys. Kl.* **1932**, 337.
(6) Van Vleck, J. H.; Sherman, A. *Rev. Mod. Phys.* **1953**, *7*, 167.
(7) Eyring, H.; Walter, J.; Kimball, G. E. *Quantum Chemistry*; John Wiley and Sons: New York, 1944; Chapter 13.

0002-7863/87/1509-6933$01.50/0