(5) T. Kamiya, T. Sulta, H. Tsutomu, T. Masashi, O. Nakagutl, O. Osamu, O. Osaka, and K. Teruo, German Offenlegungsschrift 2,358,178 (May 30, 1974).
(6) R. R. Chauvette, U.S. Patent 3,632,578 (Jan 4, 1972).
(7) F. M. Dean, J. Goodchild, and A. W. Hill, *J. Chem. Soc. C*, 2192 (1969).
(8) Combustion analyses for the indicated elements were within ±0.3%.
(9) Excellent summaries of the spectroscopic properties of β-lactam antibiotics may be found in P. V. Demarco and R. Nagarajan in "Cephalosporins and Penicillins", E. H. Flynn, Ed., Academic Press, New York, N.Y., 1972, Chapter 8.
(10) Squibb Postdoctoral Fellow.

**P. W. Wojtkowski,*[10] J. E. Dolfini**
**Octavian Kocy, Christopher M. Cimarusti**
*The Squibb Institute for Medical Research*
*Princeton, New Jersey 08540*
*Received May 12, 1975*

## Conformational Analysis of Amino Acids and Peptides Using Specific Isotope Substitution. II. Conformation of Serine, Tyrosine, Phenylalanine, Aspartic Acid, Asparagine, and Aspartic Acid β-Methyl Ester in Various Ionization States

*Sir:*

As pointed out by Roberts and Jardetzky,[1] an uncertainty in the assignment of the two protons attached to the β-carbon has been a major problem in the conformational study by NMR of amino acids in solution. Very recently Feeney et al.[2] showed that the vicinal coupling constant between the carbonyl carbon and the β-proton might be useful as a conformational index of amino acids, if one could analyze the proton NMR spectrum independently. Due to the lack of any reliable empirical relation between the vicinal carbon–proton coupling constant and the dihedral angle, this method is particularly unsatisfactory if the two β-protons are incidentally overlapping. In practice, the chemical shift difference between these two protons frequently is very small. Stereoselective deuteration, therefore, must be exploited.[3]

In the present communication, we describe a conformational study of Ser, Phe, Tyr, and Asp and its derivatives, using stereoselective deuteration. A major object of our study is to understand qualitatively the factors responsible for the conformational changes in various ionization states. We therefore used the most convenient method of analysis, developed by Pachler[4] (Figure 1).

The proton NMR spectrum of (2*S*)-Asp at pD 5.8 shows a typical ABX pattern (Figure 2a).[5] A straightforward analysis of this spectrum gives us three chemical shifts, $\delta_\alpha$, $\delta_{\beta_1}$, and $\delta_{\beta_2}$ and three coupling constants, $J_{\alpha\beta_1}$, $J_{\alpha\beta_2}$, and $J_{\beta_1\beta_2}$. The unambiguous assignment of $\beta_1$ and $\beta_2$ can be made using selectively deuterated amino acid[6] which gives an AB pattern at any pD (Figure 2b). An analysis of this

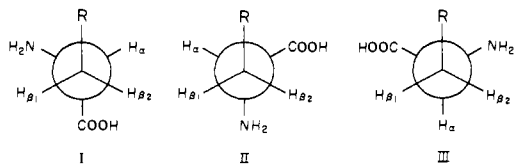


**Figure 1.** Newman projections about the $C_\alpha$–$C_\beta$ bond for the three rotamers of L-amino acids. The configurational notation for the C-2 of L-amino acids is usually *S*, except for L-Cys which is 2*R*. The configurational notation of $H_{\beta_1}$ and $H_{\beta_2}$ are pro-*S* and pro-*R* for L-(2*S*)-Asp, Asn, AspOMe, and Ser, but pro-*R* and pro-*S* for L-(2*S*)-Phe and Tyr. The fractional population $P_I$, $P_{II}$, and $P_{III}$ for each conformer can be given by the following equations: $P_I = (J_{\alpha\beta_1} - J_g)/(J_t - J_g)$, $P_{II} = (J_{\alpha\beta_2} - J_g)/(J_t - J_g)$, $P_{III} = 1 - (P_I + P_{II})$, in which $J_g = 2.60$ Hz and $J_t = 13.56$ Hz (ref 4).

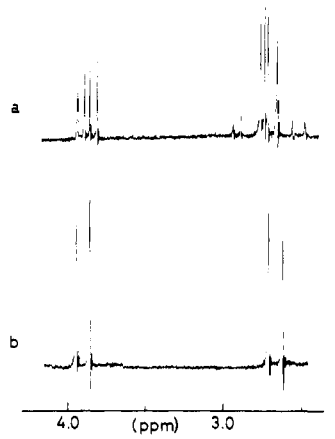


**Figure 2.** The 100-MHz $^1$H NMR spectra of (a) L-(2*S*)-aspartic acid at pD 5.8; (b) L-(2*S*,3*R*)-[3-$^2$H]aspartic acid at pD 6.6 with a deuterium decoupling. Chemical shifts refer to internal DSS.

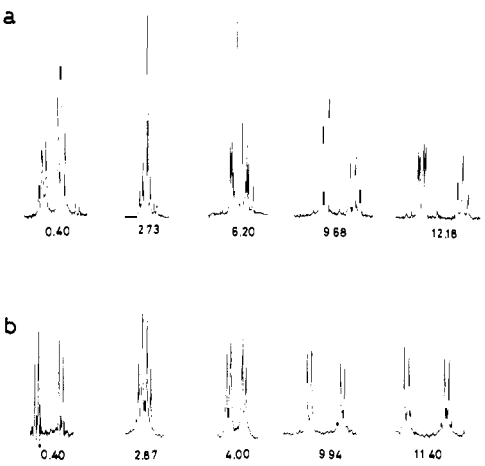


**Figure 3.** The 100-MHz $^1$H NMR spectra of (a) L-(2*S*)-serine; and (b) DL-{(2*S*,3*S*);(2*R*,3*R*)}-[3-$^2$H]serine with a deuterium irradiation. Numbers below each spectrum denote the pD's.

spectrum affords $J_{\alpha\beta_1}$, $\delta_\alpha$, and $\delta_{\beta_1}$ because in this case the $\beta_2$ proton was replaced by a deuterium. There was no observable iostope effect in the vicinal coupling constants, which indicates the population of the rotational isomers is not affected by deuteration.

The proton spectra of (2*S*)-Ser and a racemic mixture of (2*S*,3*S*)- and (2*R*,3*R*)-[3-$^2$H]Ser[7] at various pD's are shown in Figure 3. As was demonstrated by Ogura et al.[8] the spectra of (2*S*)-Ser can only be solved by an ABC treatment. At around pD 2, the chemical shifts of the three protons became very close, which makes the spectral analysis difficult. A racemic mixture of [$^2$H]Ser, instead, gives a simple AB spectrum at any pD, except pD 2.3, where the chemical shifts of α and $\beta_2$ become identical. The NMR parameters obtained by the analysis of [$^2$H]Ser therefore gives very useful information for analysis of the complex ABC spectra, and of course absolute assignment of each proton.

Proton NMR spectra of all the other amino acids we examined were analyzed in a similar way.[9] The calculated fractional populations are summarized in Table I.

The dominant form of Asp, Asn, and AspOMe at low pD, where these compounds exist in the cationic forms, is III. As this conformer seems to be the least favorable in terms of steric hindrance, there might be a compensation due to the water structure, that is a smaller free energy of the total system can be obtained by putting all large substit-

Table I. The Fractional Population of Various Amino Acids in Different Ionization States (at room temperature)

| Amino acid (β-substituent) | pD | Ionization state | $J_{\alpha\beta_1}$ | $J_{\alpha\beta_2}$ | $P_I$ | $P_{II}$ | $P_{III}$ |
|---|---|---|---|---|---|---|---|
| Ser | 0.4 | + | 4.20 | 3.40 | 0.15 | 0.07 | 0.78 |
| (OH) | 7.5 | +− | 5.70 | 3.64 | 0.28 | 0.10 | 0.62 |
| | 12.2 | − | 5.91 | 4.25 | 0.30 | 0.15 | 0.55 |
| Asp | 0.4 | + | 6.37 | 4.16 | 0.34 | 0.16 | 0.50 |
| (COOH) | 5.8 | +−− | 8.75 | 3.83 | 0.56 | 0.09 | 0.35 |
| | 12.3 | −− | 9.85 | 3.99 | 0.66 | 0.12 | 0.22 |
| Asn | 1.1 | + | 6.91 | 4.20 | 0.39 | 0.15 | 0.46 |
| ($CONH_2$) | 5.0 | +− | 8.03 | 3.97 | 0.50 | 0.13 | 0.38 |
| | 12.3 | − | 9.05 | 4.65 | 0.59 | 0.19 | 0.22 |
| AspOMe | 0.4 | + | 6.48 | 4.52 | 0.36 | 0.17 | 0.47 |
| ($COOCH_3$) | 6.9 | +− | 7.06 | 4.44 | 0.41 | 0.17 | 0.43 |
| | 12.1 | − | 7.55 | 5.37 | 0.45 | 0.25 | 0.30 |
| Phe | 0.4 | + | 7.65 | 5.65 | 0.46 | 0.28 | 0.26 |
| (phenyl) | 7.1 | +− | 7.90 | 5.20 | 0.48 | 0.24 | 0.28 |
| | 12.5 | − | 7.53 | 5.42 | 0.45 | 0.26 | 0.29 |
| Tyr | 0.2 | + | 7.65 | 5.45 | 0.46 | 0.26 | 0.28 |
| (hydroxy | 5.2 | +− | 8.01 | 4.89 | 0.49 | 0.21 | 0.30 |
| phenyl) | 11.9 | −− | 7.63 | 4.97 | 0.46 | 0.22 | 0.33 |

uents into the same spatial area.[10] This effect is clearest for Ser, in which about 80% of the molecules exist in III at low pD.[8] However, in the case of Phe and Tyr, $P_{III}$ is not the largest, presumably because the strong steric hindrance between the bulky phenyl or the hydroxyphenyl group and the carboxylate and/or amino group become more important.[11]

A marked increase of $P_I$ was observed for Asp, Asn, and AspOMe, accompanied by the decrease of $P_{III}$, at higher pD's. These changes occurred in two steps, which correspond to the ionization state changes. From a closer look at the data we might conclude that the first change corresponds to the coulombic repulsion between the α-carboxylate anion and either the β-carboxylate anion (Asp), the carboxyamide (Asn), or the carboxymethyl (AspOMe) group. A further increase of I in alkaline solutions can be explained by a favorable coulombic interaction in III between the ammonium cation and the β-substituents at neutral pD, this is eliminated by the deprotonation of the ammonium group. The appreciable differences in $P_I$ for these three compounds in alkaline solutions might indicate that the electrostatic repulsion between the α-carboxylate and the β-substituents decrease in the following order: $COO^- > CONH_2 > COOMe$.

The same arguments can be made for Ser. In this case, however, a charge repulsion between the α-carboxylate and the hydroxyl group is not strong enough to be an overwhelming factor for determining the population of each rotamer. The population profiles of Phe and Tyr are very similar, and do not greatly depend on pD's as expected for steric repulsion.

The above discussions about the factors responsible for the conformational energy in aqueous solution can be summarized as follows: (I) a tendency for large groups to be close, due to the water structure, (II) coulombic interactions (possibly including hydrogen bonding) among charged (polar) groups, either attractive or repulsive, (III) steric hindrance arising from large substituents. Which of these dominates strongly depends on the pD, temperature, ionic strength, and presumably many other parameters. Obviously the population changes must not in general be analyzed based on a single one of these factors.

This preliminary account of the conformational analysis of amino acids clearly demonstrates that by specific deuteration a better understanding of the intra- and inter (solute-solvent, solute-solute) molecular interactions which determine the structure in solution can be obtained. A full account of these and further results will be published shortly.

**Acknowledgments.** We are greatly indebted to Ms. Masako Kamisaku for her assistance throughout this work. We also wish to express our gratitude to Drs. S. Sawada and H. Kumagai for their timely gift of [$^2$H]Tyr.

## References and Notes

(1) G. C. K. Roberts and O. Jardetzky, *Adv. Protein Chem.*, **24**, 477 (1970).
(2) J. Feeney, P. E. Hansen, and G. C. K. Roberts, *J. Chem. Soc., Chem. Commun.*, 465 (1974).
(3) M. Kainosho, K. Ajisaka, M. Kamisaku, A. Murai, and Y. Kyogoku, *Biochem. Biophys. Res. Commun.*, **64**, 425 (1975).
(4) K. G. R. Pachler, *Spectrochim. Acta*, **20**, 581 (1964).
(5) NMR spectra were measured with an XL-100 FT spectrometer at room temperature in deuterium oxide solutions. pD's were direct meter readings and were adjusted by adding DCl or NaOD solution.
(6) (2*S*,3*R*)-[3-$^2$H]Asp was prepared by an aspartase catalyzed ammonium addition to fumaric acid in deuterium oxide (R. Bentley, "Molecular Asymmetry in Biology", Vol. II, Academic Press, New York, N.Y., 1970, p 152), the Asp was in turn converted to (2*S*,3*R*)-[3-$^2$H]Asn and (2*S*,3*R*)-[3-$^2$H]Asp β-methyl ester (AspOMe).
(7) A racemic mixture of (2*S*,3*S*)- and (2*R*,3*R*)-[3-$^2$H]Ser was prepared by a previously reported method for [$^{15}$N]Ser (P. Stetter, Jr., *J. Biol. Chem.*, **144**, 501 (1942)). We employed a catalytic hydrogenation for reducing the precursor olefin, of which geometry was deduced by comparing the $J_{H-^{13}C}$ (H—C═C—C) value with those from model compounds.
(8) H. Ogura, Y. Arata, and S. Fujiwara, *J. Mol. Spectrosc.*, **23**, 76 (1967).
(9) A racemic mixture of (2*S*,3*R*)- and (2*R*,3*S*)-[3-$^2$H]Phe was synthesized by a previously reported method (G. W. Kirby and J. Michael, *J. Chem. Soc., Perkin Trans. 1*, *115* (1973)). (2*S*,3*R*)-[3-$^2$H]Tyr was kindly supplied by Drs. S. Sawada and H. Kumagai, who synthesized it by the same procedure (S. Sawada, H. Kumagai, H. Yamada, R. K. Hill, Y. Mugibayashi, and K. Ogata, *Biochim. Biophys. Acta*, **315**, 204 (1973)).
(10) T. Akimoto, M. Tsuboi, M. Kainosho, F. Tamura, A. Nakamura, S. Murahashi, and T. Kajiura, *Bull. Chem. Soc. Jpn.* **44**, 2577 (1971).
(11) J. M. Purcell, J. E. Ramirez, and J. R. Cavanaugh, *J. Phys. Chem.*, **77**, 1501 (1973).

**Masatsune Kainosho,* Katsumi Ajisaka**

*Central Research Laboratories, Ajinomoto Co., Inc.*
*1-1 Suzukicho, Kawasaki, 210 Japan*

*Received June 30, 1975*

## Utilization of Excited State p*K*'s to Initiate a Ground State Chemical Reaction

*Sir:*

The physicochemical properties of molecular photoexcited states are often quite different from those of the ground state due to a change in electron distribution upon electronic excitation.[1] Acidity constants, for example, for electronically excited molecules[2-5] have been observed to vary considerably from that of the molecular ground state. In the case of 2-naphthol an increase in acidity of six orders of magnitude has been observed[4,6] as a result of electronic excitation. To date, however, the utilization of this enhanced acidity of excited state species to initiate a bimolecular ground state chemical reaction has not been reported.

We have now observed the initiation of a ground state chemical reaction as a direct consequence, we believe, of the enhanced acidity of the excited singlet state of several hydroxy aromatic compounds. Nitrosation and diazo coupling reactions of sodium 2-naphthol-6-sulfonate (1- and 2-naphthol, as well as phenols) in neutral aqueous solution (EtOH-$H_2O$ for naphthols) have been initiated photochemically in the presence of sodium nitrite. The reaction sequence (aqueous solution, pH 7.0) is presented in Scheme I. The nitrosation and diazo coupling reaction, which do not take place in the dark at constant pH, require the presence of nitrous acid (p$K$ = 3.37 (12.5°) $H_2O$) which reacts with **1** to produce **4**. Sodium nitrite serves both to increase the rate of deprotonation of the naphthol excited state[7] by means of general acid-base catalysis as well as being a reactant. In the presence of the aromatic amine (**3**) an arylazonaphthol (**5**) is formed either by the direct reaction of **3** on **4** or the