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ASSIGNMENT OF THE CHEMICAL SHIFT VALUES OF *N*-TRITYL L-HOMOSERINE LACTONE

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SUMMARY: This paper reports the correct assignment of all chemical shift values of *N*-trityl L-homoserine lactone based on the ^{1}H-nmr spectra of *N*-trityl-2*S*, 3*R*-[3-^{2}H]- and 2*S*, 3*S*-[2,3-$^2H_2$]homoserine lactone.

In difference to the recent report[1] concerning the facile synthesis and assignment of the chemical shift values of *N*-trityl L-homoserine lactone (2*S*), we wish to report the correct chemical shift values for all the hydrogen atoms in *N*-trityl L-homoserine lactone based on the ^{1}H-nmr spectra of *N*-trityl-2*S*, 3*R*-[3-^{2}H]- and 2*S*, 3*S*-[2,3-$^2H_2$]homoserine lactone. During studies in our laboratories involving the chemical syntheses of various regio- and stereospecific deuteriated four-carbon amino acids and the investigation of the stereochemical reaction mechanisms of enzymes responsible for the interconversion of these four-carbon amino acids, we have synthesized 4*R*- and 4*S*-[4-^{2}H]homoserine lactone,[2,3] 3*R*- and 3*S*-[3-^{2}H]homoserine lactone,[3,4] and various regio- and stereospecific di-, tri- and tetradeuteriated homoserine lactones[3,5] as well as ^{17}O, ^{15}N, and ^{13}C-labelled homoserine lactones.[6]

Based on ^{1}H-nmr studies of the hydrochloride salt of our homoserine lactones,[2,3] we believed that the reported chemical shift assignments of the hydrogen atoms at the C-4 carbon atom were incorrect but could not ascertain if the substitution of the trityl group at the nitrogen atom had caused some unusual shifts of the two individual resonances causing a cross-over of the signals due to the C-4 pro-*R* (H-4) and pro-*S* (H-5) hydrogen atoms. The conversion of 4*R*- [4-^{2}H]homoserine lactone, available from earlier studies,[2,3] into its *N*-trityl derivative and the measurement of its ^{1}H-nmr spectrum demonstrated that this was not the case since the *S*-hydrogen atom of 4*R*- [4-^{2}H]homoserine lactone resonated at δ = 4.45 ppm with some of the residual *R*-hydrogen atom (deuteriation was 99% but enantiomeric excess was only 90%) resonating at δ = 4.61 ppm while the C-4 *S*-hydrogen atom of *N*-trityl-4*R*- [4-^{2}H]homoserine lactone resonated at δ = 3.8 ppm and the residual *R*-hydrogen atom at δ = 4.1 ppm. Therefore, it appeared either our ^{1}H-nmr assignments at the C-4 carbon were incorrect, which we felt was unlikely based on all the chemical and ^{1}H-nmr data available to us, or possibly the previous authors had mis-assigned the proton chemical shift values at the C-4 carbon atom due to the misassignment of the resonances at C-3. Since there was very little experimental ^{1}H-nmr data presented other than a decoupling table, it was difficult to

FIGURE 1. Dreiding Models of Two Conformations of *N*-trityl L-homoserine lactone.

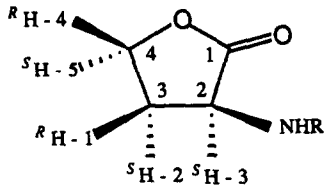
ascertain how the chemical shift values had been assigned, other than the observation that one of the C-4 hydrogen atoms was orthogonal to one of the C-3 hydrogen atoms, namely the angle formed between H-1 of C-3 and H-5 of

C-4 is ≈ 90°. The assignment of the chemical shift values based on orthogonallity of two vicinal hydrogen atoms seems to be dubious since there appear, at least in Dreiding models, to be at least two stable conformations of the *N*-trityl L-homoserine lactone (see Figure 1), both of which allow either of the C-4 hydrogens to be orthogonal with one or the other C-3 hydrogen atoms (i.e. either H-4 is orthogonal to H-2 or H-5 is orthogonal to H-1, as originally reported). Our assignment of the chemical shift values of the signals due to pro-R (δ = 2.46 ppm) and pro-*S* (δ = 2.80 ppm) hydrogen atoms at the C-3 carbon of the hydrochloride salt of homoserine lactone[3] is in "apparent" directional agreement with those reported for the pro-*R* (δ = 1.2 ppm) and pro-*S* (δ = 1.6 ppm) hydrogen atoms at the C-3 carbon of the *N*-trityl L-homoserine lactone.[1] Close examination of either of the two Dreiding models (Figure 1) of the *N*-trityl-L-(2*S*)homoserine lactone, however, strongly suggests that it is possible that a differential shift in the chemical shifts of the two proton resonances at the C-3 carbon caused a cross-over in the chemical shifts of the hydrogen atoms at C-3 in going from the homoserine lactone salts to the *N*-trityl L-homoserine lactone derivatives. Therefore, the pro-3*R* (δ = 2.46 ppm) hydrogen atom in the lactone would resonate at δ = 1.6 ppm in the *N*-trityl L-homoserine lactone and likewise the pro-3*S* (δ = 2.80 ppm) hydrogen atom resonance would shift to δ = 1.2 ppm in the trityl derivative. In order to test this hypothesis, we synthesized 2*S*, 3*R*-[3-^{2}H]aspartic acid *via* the action of the enzyme aspartase [EC 4.3.1.1] on the ammonium salt of fumaric acid in ^2H_2O as previously described.[7] The aspartase reaction has been shown by numerous researchers[7,8] to catalyze the trans-diaxial (anti-periplanar) addition of H, from the solvent, and NH_2 to fumaric acid to give an amino acid belonging to the L-family (2*S*). Therefore, the deuterium atom, from the solvent ^2H_2O, that is introduced into the 3-position of aspartic acid will generate the *R* configuration at the C-3 position. The aspartic acid thus obtained from the aspartase reaction was converted,[9,10,1] into the corresponding *N*-trityl-2*S*, 3*R*-[3-^{2}H]homoserine lactone. The ^{1}H-nmr spectra of the *N*-trityl-2*S*, 3*R*-[3-^{2}H]homoserine lactone and its homoserine lactone salt, obtained by treatment of the trityl derivative with etheral-HCl in chloroform, are shown in Figure 2, panel A.[11] In order to further confirm these assignments, 2*S*, 3*S*-[2,3-$^2H_2$]aspartic acid was synthesized from 1,2-dideuteriated fumaric acid[12] as described above except that the enzyme reaction was performed in H_2O.[7,8] The aspartic acid thus obtained was converted into *N*-trityl-2*S*, 3*S*-[2,3-$^2H_2$]homoserine lactone and its ^{1}H-nmr spectrum along with that of its homoserine lactone hydrochloride is also shown in Figure 2, panel B. Table 1 gives the chemical shifts values of both the lactone hydrochloride salt in ^2H_2O and *N*-trityl L-homoserine lactone in chloroform from our laboratory as well as the chemical shift values of *N*-trityl L-homoserine lactone in chloroform previously reported.[1]

The apparent absence of coupling, or more precisely, a J ≈ 0 Hz, between H-4 (which appears as an apparent triplet but is actually a doublet of doublets with coupling constants of $|J| \approx 9.2$ Hz for the geminal coupling with H-5 and J ≈ 8.8 Hz for the vicinal coupling with H-1) and H-2 (originally reported[1] as a J = 0 between H-1 and H-5, respectively) indicates that one of the two major conformationally stable conformers discussed above and shown in Figure 1, is more stable than the other. Based on our ^{1}H-nmr spectra it is likely that conformer I (Figure 1.) is more stable than conformer II.

Just prior to submission of this manuscript two additional reports[13,14] have appeared that deal with the assignment of the chemical shift values of various homoserine lactone derivatives. Schwab, et al.,[13] have reported a chemical synthesis of *N*-(3,5-dinitrobenzoyl)(2*S*, 3*S*, 4*S*)-[3,4-$^2H_2$]homoserine lactone and measured its ^{1}H-nmr spectrum. The chemical shift values assigned to the two C-3 hydrogen atoms of *N*-(3,5-dinitrobenzoyl)-(2*S*, 3*S*, 4*S*)-[3,4-$^2H_2$]homoserine lactone correspond in direction to the chemical shift values of our homoserine lactone hydrochloride in that no cross-over is observed in the chemical shift values of the 3*R* and 3*S* hydrogen atoms. While the assignments of Schwab, et al., are rigorous, they do <u>not</u> allow one to *a priori* assign the chemical shift values of the hydrogen atoms of *N*-trityl L-homoserine lactone. In the second paper[14] the authors who originally reported the chemical shift values of *N*-trityl L-homoserine lactone[1] have, upon a suggestion from Schwab (see reference 7 in our reference 14), re-evaluated their assignments,[14] which are now in agreement with those presented

TABLE 1. Chemical Shift Values (δ ppm) for Various Homoserine Lactones.



	R = H_2Cl*	Trityl#	Trityl‡
H-1 (pro-*R*)	2.46	1.6	1.2
H-2 (pro-*S*)	2.80	1.2	1.6
H-3 (*S*)	4.43	3.4	3.4
H-4 (pro-*R*)	4.61	4.1	3.8
H-5 (pro-*S*)	4.45	3.8	4.1

* Reference 2. # Present values. ‡ Reference 1.

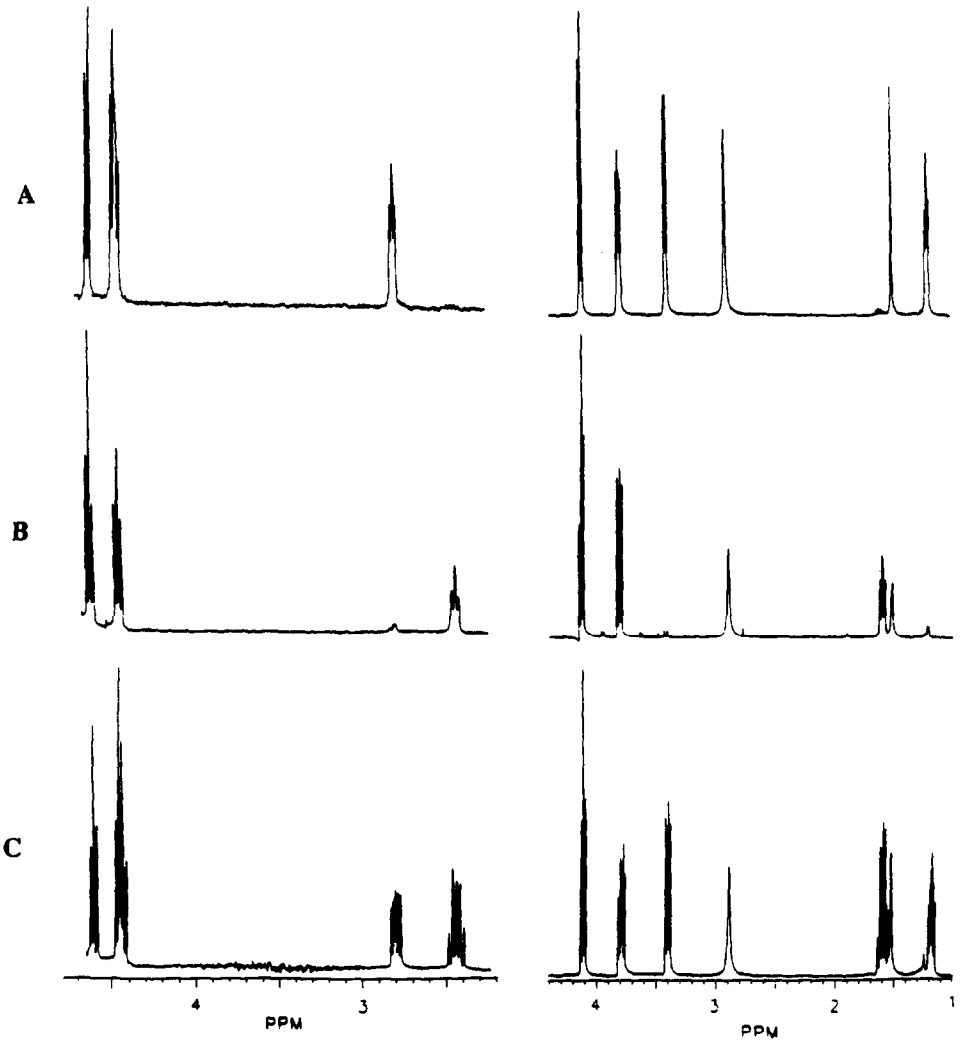


FIGURE 2. The 500 MHz ^{1}H-NMR spectra of: A. Left, 2*S*, 3*R*-[3-^{2}H]homoserine lactone hydrochloride in D_2O and right, *N*-trityl-2*S*, 3*R*-[3-^{2}H]homoserine lactone in $CDCl_3$. B. Left, 2*S*, 3*S*-[2,3-$^2H_2$]homoserine lactone hydrochloride in D_2O and right, *N*-trityl-2*S*, 3*S*-[2,3-$^2H_2$]homoserine lactone in $CDCl_3$. C. Left, homoserine lactone hydrochloride in D_2O and right, *N*-trityl homoserine lactone in $CDCl_3$. The peaks at δ ≈ 1.50 ppm in the $CDCl_3$ spectra are due to the residual H_2O in the commercial $CDCl_3$.

in this manuscript. While Baldwin's new assignments are correct, they are based on nuclear Overhauser enhancement experiments which are known to be fraught with experimental pitfalls.[15] For instance, even a minor conformational isomer may make a large contribution to the n. O. e. measured and therefore could lead to incorrect assignments of the spectrum. The chemical shift values presented herein are based solely on the measurement of the ^{1}H-nmr spectra of *N*-trityl L-homoserine lactones stereospecifically labelled with deuterium individually in either the 3*R* or 3S position and therefore are unequivocal.

The simulated ^{1}H-nmr spectrum of *N*-trityl L-homoserine lactone has generated by the Bruker software program PANIC using the various coupling constants derived from this study. The coupling constants obtained from the PANIC-simulated spectrum are $J_{1,2}$ = 12.487 Hz, $J_{1,3}$ = 11.670 Hz, $J_{1,4}$ = 8.787 Hz, $J_{1,5}$ = 11.751 Hz, $J_{2,3}$ = 7.998 Hz, $J_{2,4}$ = 0.000 Hz, $J_{2,5}$ = 5.525 Hz, $J_{4,5}$ = 9.156 Hz.

Based on assignments previously reported by our group for the chemical shift values of the hydrogen atoms at C-4 position of 4*R*- and 4*S*- [4-^{2}H]homoserine lactone hydrochloride[2,3] and the results presented in this paper for the chemical shift assignments for both the *N*-trityl-2*S*, 3*R*-[3-^{2}H]- and *N*-trityl-2*S*, 3*S*-[2,3-^{2}H$_2$]homoserine lactone as well as those presented by Schwab, et al.,[13] one must conclude that the trityl group has some unique shielding/deshielding effects on the hydrogen atoms at the C-3 position of *N*-trityl homoserine lactone. In conclusion, based on the data presented here and previously,[2,3] the absolute chemical shift values of both *N*-trityl L-homoserine lactone and homoserine lactone hydrochloride have been established unequivocally.

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