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A NEW METHOD FOR DISTINGUISHING α-AMINO ACIDS FROM THEIR β- AND γ-ISOMERS

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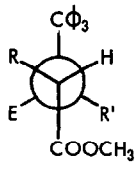
In the past, color tests (1) and various paper chromatography systems (2) using cupric ion have been used to distinguish between α-amino acids and various other amines and amino acids. These methods are qualitatively useful, but to our knowledge no simple procedure (3) is presently available to determine unequivocally that a given amino group is α- to a carboxyl function. During our recent work with threo-β-hydroxy-DL-aspartic acid (threo-HAA) and its derivatives, we observed that the protons of the α-carbomethoxyl group were shifted upfield some 42 c.p.s. when the amino function was tritylated, whereas the β-carbomethoxyl protons were essentially unaffected. On the basis of this observation, we have prepared (4) the methyl esters and N-trityl methyl esters of twelve amino acids and examined their PMR spectra. Throughout this series of compounds, α-carbomethoxyl protons were diamagnetically shifted 15-44 c.p.s. while β- and γ-carbomethoxyls shifted upfield only 1-6 c.p.s. Thus, the upfield shift may be considered truly diagnostic for α-amino acids (5).

Table I shows carbomethoxyl proton chemical shift positions for the methyl esters and their respective trityl derivatives. Examination of the $\Delta\nu$ values for the α-carbomethoxyl protons shows that the series can be divided into two groups. The first (group A) includes glycine, sarcosine and proline which have a $\Delta\nu$ of 15-16 c.p.s. and the second group (group B) contains the remaining α-esters which show a 27-44 c.p.s. $\Delta\nu$ value. This sharp division into two groups of esters can be rationalized by considering Newman projections drawn about the C_α-N bond. If we assume the nitrogen bonds to be situated tetrahedrally and give the unbonded electron pair (E) a fixed position (6), conformations I, II, and III are possible. In the case of Gly (R=R'=H), conformation I is most probable since steric interaction between the

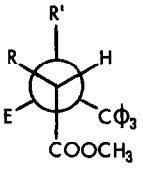
Table I[a]

Amino Acid[b]	Methyl Ester[c]		Trityl Deriv.[d]		$\Delta\nu$ (c.p.s.)		
	α	β	α	β	α	β	
Glycine	232 c.p.s.		216		16		Group A
Sarcosine	240		225		15		
L-Proline	233		217		15		
L-Lysine	233		206		27		Group B
DL-Serine	233		197		36		
DL-Alanine	231		190		41		
DL-Aspartic Acid	231	226	197	221	34	5	
threo-DL-HAA[e]	235	232	193	228	42	4	
DL-Glutamic Acid	233	225	189	220	44	5	
β-Alanine		226		220		6	
γ-Aminobutyric Acid		222		223		1	
Anthanilic Acid		247		235		12	

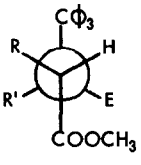
(a) All spectra were run on a Varian A-60 Spectrometer using the usual side-band modulation technique. Chemical shifts are given in c.p.s. downfield from TMS; (b) All esters and their trityl derivatives have been previously described or gave acceptable elemental analyses; (c) Hydrochloride in D_2O; (d) In $CDCl_3$; (e) threo-β-hydroxy-DL-aspartic acid.



I



II



III

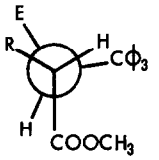
trityl and carbomethoxyl groups probably outweighs the electronic repulsion between the unbonded electron pair (E) and the carbonyl dipole (7). Sarcosine (R = H, R' = CH_3) must also exist primarily in conformation I since its $\Delta\nu$ is also only 15 c.p.s. Proline would necessarily exist in a slightly skewed form of conformation III in which R and R' are linked in the pyrrolidine ring; consequently proline ester also shows a small $\Delta\nu$ on tritylation. Group A then exists in those conformations (I and III) in which trityl and carbomethoxyl are anti.

The second group of amino acids are those which have a hydrogen atom on nitrogen and a group larger than hydrogen on the α-carbon atom. These show a 12 to 29 c.p.s. larger diamagnetic shift than those in group A indicating a closer proximity of trityl and carbomethoxyl groups in this series. An increase in the population of conformer II at the expense of conformer I is reasonable since a methyl group (as in Ala, R = CH_3, R' = H) can be considered larger than a carbomethoxyl (8) and thus the trityl group prefers gauche interaction with the latter rather than the former. The separation of these α-amino acid derivatives into two distinct groups appears to depend upon whether R is hydrogen or alkyl (except proline) and this can be explained by assuming a change of the most highly populated species from conformer I to conformer II.

It is interesting to note that carbomethoxyl protons β to the tritylamino group are shifted 4-6 c.p.s. upfield with respect to the untritylated compound. The N-trityl derivative of methyl anthranilate, which is also a β-amino ester, showed a carbomethoxyl diamagnetic shift (12 c.p.s.) twice that shown by any of the other β-amino esters examined. This is undoubtedly due to the coplanarity of the amino and carbomethoxyl groups imposed by the benzene ring. Overlap of the unbonded pair of electrons on nitrogen and the π-electron cloud of the benzene ring might cause a further decrease in the distance between the carbomethoxyl and trityl groups.

There are numerous examples of long-range shielding by benzene rings (9) and the size of the diamagnetic shift has been used to calculate the distance between the interacting groups (10). In the present system, however, it is difficult to measure the average distance of the carbomethoxyl protons from the benzene rings since there are so many possible conformations about the six single bonds between these groups. Using the "isoshielding" curves of Johnson and Bovey (11), we can estimate that among group A amino acids ($\Delta\nu$, 15 c.p.s.) the average distance between the interacting groups

should be about 6.5 Å and in group B ($\Delta\nu$, 40 c.p.s.) it is close to 4.5 Å. Measurements made on Dreiding models (12) indicate that group A amino acids are predominantly in conformation I since 6.5 Å is a likely distance between the trityl and carbomethoxyl groups in I. However, group B amino acids might be best described as existing more nearly in an eclipsed conformation (IV) in which the trityl group has moved closer to the carbomethoxyl but has not completely rotated into conformation II.



Conformer II would allow an average trityl-carbomethoxyl distance to approach 2 Å (13). This is a case in which an eclipsed conformation may actually be more stable than a staggered one. The extremely large space requirements of the trityl group apparently causes it to avoid gauche interaction with any group larger than hydrogen.

We are investigating this long-range effect of the triphenylmethyl group in other amino acids and in O- and S-trityl compounds. Diamagnetic shifts caused by trityl may be very useful in determination of configurations and conformations of amino, hydroxyl, and sulfhydryl groups in other kinds of molecules also (14).

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3. S. Ruhemann [J. Chem. Soc., 99, 792, 1306, 1486 (1911)] made the original observation that ninhydrin liberates CO_2 from α-amino acids and a quantitative method has recently been described in "Micromanometric Analyses" by D. D. van Slyke and J. Plozin, Williams and Wilkins Co., Baltimore, 1961. We feel that the method described in this work is simpler and of broader scope than the ninhydrin method.

4. The methyl esters were prepared using dimethyl sulfite in methanol and N-tritylations were carried out in chloroform by the method of G. Amiard, R. Heymes, L. Vellus, Bull. Soc. Chem., 191 (1955).

5. The upfield shift was due to tritylation and not just to neutralization of the charge on the amino group (or to a change in solvent from D_2O to $CDCl_3$). When alanine methyl ester hydrochloride was converted to the free amino ester in $CDCl_3$ an upfield shift of only 6 c.p.s. occurred.

6. The configuration about nitrogen is not fixed, but this does not affect our argument with respect to the distance between the carbomethoxyl protons and the trityl group.

7. For glycine and sarcosine conformers I and III are enantiomers and consequently have equivalent NMR spectra.

8. The conformational energies for CH_3- and -$COOCH_3$ are 1.9-2.1 and 1.15 kcal/mole in cyclohexanes; cf., E. L. Eliel, N. L. Allinger, S. J. Angyal and G. A. Morrison, "Conformational Analysis," Wiley, N. Y., 1965, p. 140.

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11. C. E. Johnson and R. E. Bovey, J. Chem. Phys., 29, 1012 (1958).

12. We assume that the ester function exists predominantly with the methyl group eclipsing the carbonyl (cf. reference 8, p. 21) and that the carbonyl is coplanar with the C_α-H bond [cf. H. van Bekkum, P. E. Verkade and B. M. Wepster, Tet. Let., No. 13, 1401 (1966)] for purposes of measuring distances on Dreiding models.

13. Johnson and Bovey (ref. 11) believe it quite unlikely that any group could approach the face of a benzene ring closer than $\sim$ 2.8 Å. Our work is consistent with this.

14. We acknowledge the financial assistance of NIAID Grant AI-05539.