nium bromide in 60% yield. The product was recrystallized from ethanol–acetone and collected under N_2, mp 167–168°. The pmr spectrum in D_2O showed one proton at τ 1.90, a two-proton multiplet at 3.20 ± 0.20, an eight-proton multiplet at 6.05 ± 0.20, a six-proton singlet at 6.40, a nine-proton singlet at 6.50, a three-proton singlet at 7.25, and a two-proton multiplet at 7.25 ± 0.25.

Anal. Calcd for $C_{17}H_{32}N_4Br_2O_2 \cdot H_2O$: C, 40.64; H, 6.82. Found: C, 40.89; H, 7.04.

N,N,N-Trimethyl-N¹,N¹-dimethyl-N¹-(γ-3-methyl-4-nitroanilinopropyl-1,3-diammoniumpropane dibromide (24) was prepared from the reaction of the amine **18** and N,N,N-trimethyl-N-3-bromopropylammonium bromide in 20% yield, mp 88–89°. The pmr spectrum in D_2O showed one proton at τ 1.90, a two-proton multiplet at 3.15, an eight-proton multiplet at 6.00 ± 0.25, a fifteen-proton singlet at 6.30, a four-proton multiplet at 7.25, and a three-proton singlet at 7.40.

Anal. Calcd for $C_{18}H_{34}N_4O_2Br_2 \cdot 2H_2O$: C, 40.45; H, 7.16. Found: C, 40.11; H, 7.25.

N,N,N-Trimethyl-N¹,N¹-dimethyl-N¹-(β-4-nitronaphthylaminoethyl)-1,3-diammoniumpropane dibromide (25) was prepared from the corresponding amine **19** and N,N,N-trimethyl-N-3-bromopropylammonium bromide in 63% yield, mp 75°. The pmr spectrum in D_2O showed a six-proton multiplet at τ 2.76 ± 1.20, an eight-proton multiplet at 5.95, a six-proton singlet at 6.33, a nine-proton singlet at 6.57, and a two-proton multiplet at 7.10.

Anal. Calcd for $C_{20}H_{32}N_4O_2Br_2 \cdot H_2O$: C, 44.61; H, 6.32. Found: C, 44.23; H, 6.90.

N,N,N-Trimethyl-N¹,N¹-dimethyl-N¹-(γ-4-nitronaphthylaminopropyl)-1,3-diammoniumpropane dibromide (26) was prepared from the corresponding amine **20** and N,N,N-trimethyl-N-3-bromopropylammonium bromide in 65% yield, mp 202°. The pmr spectrum in D_2O showed a six-proton complex multiplet at τ 7.00 ± 0.33, an eight-proton multiplet at 6.67, a nine-proton singlet at 6.80, a six-proton singlet at 6.86, and a four-proton multiplet at 7.75.

Anal. Calcd for $C_{21}H_{34}O_2Br_2 \cdot 2H_2O$: C, 44.21; H, 6.67. Found: C, 43.69; H, 6.92.

Native (N) calf thymus and salmon testes DNA are Worthington products lot no. 642, and 6CFA, respectively. Yeast RNA (lot no. 6234) and torula RNA (lot no. 55711) were obtained from Worthington and Calbiochem, respectively. These preparations of RNA are largely of ribosomal origin and are partially degraded. Denatured (D) nucleic acids were obtained by heating in a boiling water bath for 15 min and then immediate quenching in ice water.

Acknowledgment. This work was supported by Grants GM13597, GM15308, and GM15309 from the U. S. Public Health Service. The author wishes to thank Mr. David Warshawsky, Misses Valerie Cook and Kathy Seminara, and Mrs. Lee Mitschele for able technical assistance. He also wishes to thank Miss Betty Hays for her artistic talents in drawing Figures 6 and 12.

Optically Active Solvents in Nuclear Magnetic Resonance Spectroscopy. IX. Direct Determinations of Optical Purities and Correlations of Absolute Configurations of α-Amino Acids

W. H. Pirkle and S. D. Beare

Contribution from the Department of Chemistry and Chemical Engineering, University of Illinois, Urbana, Illinois 61801. *Received March 26, 1969*

Abstract: The nuclear magnetic resonance spectra of the enantiomers of a given α-amino acid methyl ester are found to differ appreciably in optically active 2,2,2-trifluorophenylethanol (**2**) solvent. It is demonstrated that this spectral nonequivalence is widely applicable to the absolute determination of the optical purities and the correlation of the absolute configurations of monosubstituted glycines. The nmr method is also shown to be applicable to optical purity determinations of disubstituted glycines and β-amino acids. A conformational model capable of explaining the origin and senses of the nmr spectral nonequivalence of the enantiomeric amino acid methyl esters is suggested.

We have recently demonstrated that enantiomers, although exhibiting identical properties in achiral media, may be readily distinguished by nuclear magnetic resonance spectroscopy in appropriate optically active solvents.[1-7] As a result of this phenomenon, optical purities and absolute configurations of a variety of alcohols,[1-4] α-hydroxy acids,[5] amines,[6] and sulfoxides[7] may now be directly determined from the relative peak areas and senses of nonequivalence[8] of the resonances of enantiotopic nuclei in chiral solvents.[9]

Other workers have also observed the nmr spectral nonequivalence of enantiomers in optically active solvents. Their studies have included racemic alkylarylcarbinols in asymmetric sulfoxides,[10] diastereomeric interactions between dissymmetric nickel(II) complexes and (+)-α-pinene,[11] spectral dissimilarities of enantio-

(1) W. H. Pirkle, *J. Amer. Chem. Soc.*, **88**, 1837 (1966).
(2) T. G. Burlingame and W. H. Pirkle, *ibid.*, **88**, 4294 (1966).
(3) W. H. Pirkle and T. G. Burlingame, *Tetrahedron Lett.*, 4039 (1967).
(4) W. H. Pirkle and S. D. Beare, *J. Amer. Chem. Soc.*, **89**, 5485 (1967).
(5) W. H. Pirkle and S. D. Beare, *Tetrahedron Lett.*, 2579 (1968).
(6) W. H. Pirkle, T. G. Burlingame, and S. D. Beare, *ibid.*, 5849 (1968).
(7) W. H. Pirkle and S. D. Beare, *J. Amer. Chem. Soc.*, **90**, 6250 (1968).
(8) The term "sense of nonequivalence," used for the sake of brevity, refers to the relative field position of a resonance of one isomer relative to the corresponding resonance of its enantiomer (in a given chiral solvent). If the solute is partially resolved, the relative field position of the larger of the two sets of unequally intense resonances will denote the sense. Should the solute be optically pure, the sense of nonequivalence can be determined through comparison of the spectrum of the solute in the *S* solvent enantiomer with its spectrum in the *R* solvent enantiomer.
(9) We shall use the terminology of Mislow (see M. Raban and K. Mislow in "Topics in Stereochemistry," Vol. 1, N. L. Allinger and E. L. Eliel, Ed., Interscience Publishers, New York, N. Y., 1967, pp 1–38) to describe nuclei according to their spatial relationships. Accordingly, enantiotopic nuclei in chiral environments are diastereotopic and may exhibit different nmr spectra.
(10) F. A. L. Anet, L. M. Sweeting, T. A. Whitney, and D. J. Cram, *Tetrahedron Lett.*, 2617 (1968).
(11) R. E. Ernst, M. J. O'Conner, and R. H. Holm, *J. Amer. Chem. Soc.*, **90**, 5305 (1968).

merically pure (−)-cocaine in the two enantiomers of methylphenylcarbinol,[12] and finally, the nonequivalence of the nmr spectra of epoxides in an optically active nematic phase.[13] Also relevant are the reports of the use of optically active solvents for enhancing spectral differences between diastereomers.[7,14,15]

From prior work[1-6] it seemed likely that the methyl esters of α-amino acids would show enantiomeric nmr spectral nonequivalence in a suitable chiral alcohol. This expectation has been fulfilled and the present paper reports a general method for the direct determination of the optical purities of α-amino acids as well as a reliable means of assigning absolute configurations to these compounds. The present method has precedent in the report that the enantiomers of N-acetyl-*p*-fluorophenylalanine are clearly distinguishable by ^{19}F nmr spectroscopy in the presence of chymotrypsin.[16] While the observed fluorine chemical shift differences were quite large (0.1–0.2 ppm), it is not evident that the enzymatic method would be generally applicable to determinations of optical purities of amino acids. Likewise, the elegant glpc method introduced by Gil-Av,[17] in which enantiomeric N-trifluoroacetyl-*n*-butyl esters of α-amino acids may be separated on columns coated with an optically active stationary phase, has not yet been applied to optical purity determinations and appears to be of still limited generality.

In contrast to these two direct methods of obtaining optical purities of α-amino acids, the indirect nmr[18] and glpc[19] methods employed by Halpern and coworkers appear to be relatively general. In each method the methyl esters of α-amino acids are converted to diastereomers, which differ in their nmr spectra and glpc behavior, by reaction with an appropriate chiral reagent. However, these determinations of optical purities are relative in the sense that the chiral reagent used must be optically pure, and there must be neither kinetic resolution in the synthetic scheme nor optical fractionation in subsequent work-up. These latter points, not demonstrated by the authors, have been critically discussed.[20]

Results

We have found that the enantiomers of type **1** α-amino acid methyl esters exhibit readily observable nmr chemical-shift differences in optically active 2,2,2-trifluorophenylethanol (**2**).[21] In addition to permitting the direct determination of the optical purities of partially resolved type **1** α-amino esters, the nmr method allows the expeditious assignment of their absolute configurations based upon the senses of nmr spectral nonequivalence in (−)-**2**.[8]

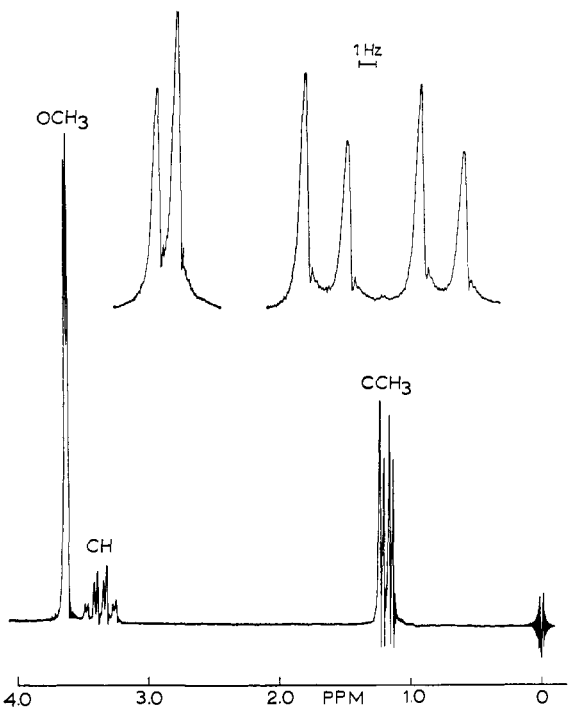
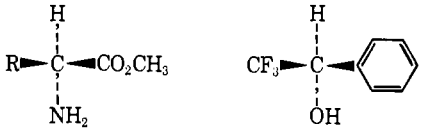


Figure 1. Portions of the 100-MHz nmr spectrum of partially resolved (*S*)-methyl alanate in (*R*)-(−)-**2**. The upper traces are scale expansions of the O-methyl and C-methyl resonances.

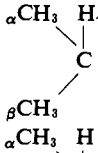
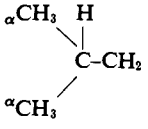
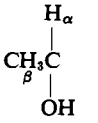
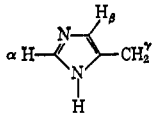
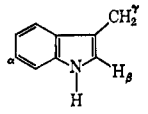


S-**1** (R = alkyl, aryl) *R*-(·)-**2**

Partially resolved methyl alanate (**1**, R = CH_3), prepared by Fischer esterification[22] of alanine enriched in the *S* enantiomer[23] and subsequent reaction of the amino ester hydrochloride with sodium methoxide, exhibits an nmr spectrum consonant with its structure in achiral solvents. However, when (−)-alcohol **2** is used as solvent, a doubling of the enantiotopic carbinyl proton, C-methyl, and O-methyl resonances is clearly observed at 100 MHz and 29° (Table I and Figure 1). A measurement of the relative peak heights of the expanded diastereotopic C-methyl or O-methyl resonances (Figure 1) affords a direct assessment of the enantiomeric *S*:*R* ratio. The experimentally determined value of 1.43:1.00 represents an optical purity of 17.8%, which compares favorably with the initial value of 20.0%, calculated from weighed portions of racemic and optically pure (*S*)-alanine. In principle, peak areas

(12) J. C. Jochims, G. Taigel, and A. Seeliger, *Tetrahedron Lett.*, 1901 (1967).
(13) E. Sackmann, S. Meiboom, and L. C. Snyder, *J. Amer. Chem. Soc.*, **90**, 2183 (1968).
(14) H. Kaehler and K. Rehse, *Tetrahedron Lett.*, 5019 (1968).
(15) In at least one report of this phenomenon [see E. W. Thomas, *Biochem. Biophys. Res. Commun.*, **24**, 611 (1966)] the author was apparently unaware of the explanation.
(16) T. McL. Spotswood, J. M. Evans, and J. H. Richards, *J. Amer. Chem. Soc.*, **89**, 5052 (1967).
(17) E. Gil-Av and B. Feibush, *Tetrahedron Lett.*, 3345 (1967).
(18) B. Halpern, L. F. Chew, and B. Weinstein, *J. Amer. Chem. Soc.*, **89**, 5051 (1967), and references cited therein.
(19) J. W. Westley and B. Halpern, *J. Org. Chem.*, **33**, 3978 (1968), and references cited therein.
(20) M. Raban and K. Mislow in "Topics in Stereochemistry," Vol. 2, N. L. Allinger and E. L. Eliel, Ed., Interscience Publishers, New York, N. Y., 1967, pp 199–230.
(21) (a) For the absolute configuration of **2** see H. M. Peters, D. M. Feigl, and H. S. Mosher, *J. Org. Chem.*, **33**, 4245 (1968). (b) For the resolution of **2** see D. M. Feigl and H. S. Mosher, *ibid.*, **33**, 4242 (1968). (c) A more convenient resolution of **2** has been reported. See W. H. Pirkle, S. D. Beare, and T. G. Burlingame, *ibid.*, **34**, 470 (1969).
(22) E. Fischer and U. Suzuki, *Chem. Ber.*, **38**, 4173 (1905).
(23) Although the absolute configurations of α-amino acids are commonly denoted as belonging to either the D or L series, we shall adhere to the *R*,*S* convention[24] for clarity. It is convenient that the D and *R* configurations and the L and *S* configurations are equivalent for the α-amino acids and esters described in this study, with the exception of L-cysteine, which has the *R* configuration.
(24) R. S. Cahn, C. K. Ingold, and V. Prelog, *Experientia*, **12**, 81 (1956).

Table I. Chemical Shifts of Type **1** α-Amino Esters in $CDCl_3$[a]

Amino ester	R	NH_2[b]	H	CO_2CH_3	R[c]
PhGly	C_6H_5	1.93	4.64	3.69	7.4
Ala	CH_3	1.88	3.65	3.80	1.39
Val		1.44	3.34	3.77	0.93, 0.98, 2.00
Leu		1.78	3.52	3.76	0.94, 1.84, 1.50
	$C_6H_5\overset{\alpha}{C}H_2\overset{\beta}{C}H_2\overset{\gamma}{}$	1.82	3.49	3.74	7.26, 2.7, 2.0
Phe	$C_6H_5\overset{\alpha}{C}H_2\overset{\beta}{}$	1.60	3.76	3.74	7.28, 2.98
Met	$CH_3S\overset{\beta}{C}H_2\overset{\gamma}{C}H_2\overset{\alpha}{}$	1.68	3.65	3.78	2.14, 2.68, 2.00
Asp	$CH_3O\overset{\alpha}{C}(=O)\overset{\beta}{C}H_2$	2.02	3.87	3.77	3.73, 2.77
Glu	$CH_3O\overset{\alpha}{C}(=O)\overset{\beta}{C}H_2\overset{\gamma}{C}H_2$	1.74	3.57	3.79	3.75, 2.5, 2.0
Cys-SH	$HSCH_2$	1.73	3.71	3.78	2.86
Ser	$HOCH_2$	2.94	3.62	3.79	3.83
Thr		2.36	3.34	3.82	3.98, 1.25
Tyr	$p\text{-}HO\overset{\alpha}{C}_6H_4CH_2$	4.04	3.74	3.75	6.92, 2.95
Lys	$H_2N\overset{\alpha}{C}H_2\overset{\beta}{C}H_2\overset{\beta}{C}H_2\overset{\gamma}{C}H_2$	1.69	3.50	3.78	2.74, 1.5, 1.70
His		5.49	3.88	3.77	7.64, 6.92, 3.05
Trp		1.86	3.88	3.75	7.2, 8.80, 3.17

[a] All values are tabulated as parts per million (ppm) downfield from tetramethylsilane as internal reference. [b] Average chemical shift of all rapidly exchanging protons. [c] Chemical shifts are for the α, β, γ protons, respectively. For those esters exhibiting complex multiplets, chemical shifts are given as the center of the multiplet.

rather than heights ought to be compared. However, the two measurements give comparable results and the height measurement is the more convenient.

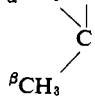
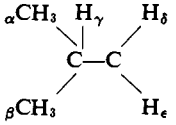
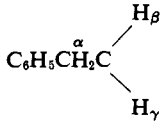
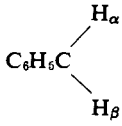
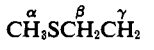
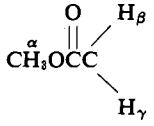
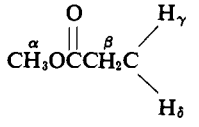
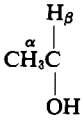
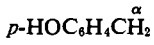
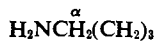
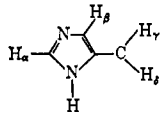
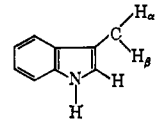
In order to ascertain the generality and reliability of this method, the nmr spectra of 15 partially resolved type **1** α-amino acid methyl esters in (−)-carbinol (**2**) have been examined and the results are summarized in Table II. It is evident (see Table II) that sufficient magnitudes of enantiomeric nonequivalence for the direct determination of optical purity are observed for the large majority of the type **1** α-amino esters investigated and that this is also true for those cyclic α-amino esters, disubstituted α-amino esters, and β-amino esters subsequently investigated (Table III).

Discussion

Nmr Spectral Determinations of Optical Purities. Although α-amino acid solutes contain two "handles" suitable for hydrogen bonding with an optically active solvent, it is well known that amino acids exist predominantly in the zwitterionic form in neutral solution and are hence rather insoluble in most organic solvents. Thus, it appeared desirable to functionalize either the amino group or the carboxylic acid group to avoid undue intra- and intermolecular solute–solute interaction, which would compete detrimentally with the desired intermolecular solute–solvent association. Based upon the previous observation that nmr spectral nonequivalence is quite general for the enantiotopic carbomethoxy groups in methyl esters of α-hydroxy acids in optically active amines,[5] the methyl esters of amino acids seemed logical derivatives. Aside from the obvious improvement over the solubility characteristics of amino acids, the methyl esters provide relatively sharp carbomethoxy resonances from which optical purities may be conveniently measured. The disadvantages associated with the methyl esters are that they are subject to base-catalyzed racemization[25] and that the free

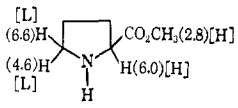
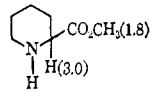
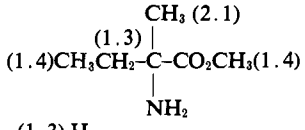
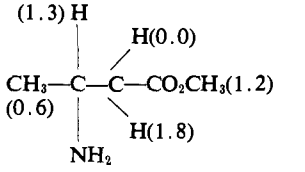
(25) M. Brenner and W. Huber, *Helv. Chim. Acta*, **36**, 1109 (1953).

Table II. Correlation of Nmr Senses of Nonequivalence with Absolute Configuration for Some Partially Resolved Type 1 α-Amino Methyl Esters[a]

Amino ester[b]	R	CO_2CH_3	Δδ, Hz[c] (nonequivalence sense[d]) H	R	% optical purity Calcd	Obsd[e]	Abs config
PhGly	C_6H_5	4.1(L)	0.0		23.6	20.1	*R*
Ala	CH_3	1.3(H)	2.3(H)	2.8(L)	20.0	17.8	*S*
Val		4.1(L)	4.1(L)	1.1, 0.9, 0.9(H)	19.2	16.2	*R*
Leu		2.4(H)	2.9(H)	1.6, 1.5, 2.0, 3.6, 2.4(L)	19.4	18.3	*S*
		1.8	2.2	1.4, 2.8, 1.9	0.0		
Phe		2.3(H)	2.2(H)	2.5, 2.2(L)	21.9	21.4	*S*
Met		1.0(H)	1.0(H)	0.6, 1.5, 2.4, 2.9(L)	20.6	19.6	*S*
Asp		0.2(H)	1.6(H)	1.0, 1.4, 2.1(L)	18.4	(19.2)	*S*
Glu		1.3(H)	0.8(H)	0.5, 1.0, 1.6, 2.0(L)	22.0	(21.6)	*S*
Cys-SH		0.0	0.0	0.0	18.9		*R*
Ser		0.4(H)	0.0	0.0	20.0		*S*
Thr		1.2(H)	2.6(H)	0.0, 0.0	19.2	(19.6)	*S*
Tyr		1.9(H)		2.0(L)	21.2	21.6	*S*
Lys		1.5(H)	3.0(H)	1.3(L)	19.4	(19.2)	*S*
His		0.0	0.0	0.0, 0.0, 1.7, 2.3(L)	16.4	*Ca.* 15	*S*
Trp		2.5(H)	1.3(H)	3.8, 1.2(L)	19.2	18.4	*S*

[a] Nmr spectra were measured on a Varian HA-100 spectrometer at 29° using samples composed of 2:1:*ca.* 3 mol ratios of alcohol:amino ester:fluorotrichloromethane, respectively, except in the case of tyrosine methyl ester, where twice the normal concentration of carbinol was used. [b] Common abbreviations are given for the parent amino acids. [c] Enantiomeric chemical shift differences (±0.1 Hz) are for the α, β, γ, δ protons, respectively. A Δδ of 0.0 indicates that the enantiomeric resonances were unresolvable under the conditions employed, while no Δδ values are recorded for protons obscured by other resonances. When necessary for interpretation of complicated multiplets, nmr spectra were measured both in racemic and optically active **2**. [d] H and L refer to high- and low-field senses of nonequivalence. In each case where more than one proton in the R group of the type **1** amino esters exhibit chemical-shift differences, the senses of nonequivalence are the same for all of the protons within the group. [e] Optical purities (±1%) were calculated from the relative peak heights of the enantiomeric carbomethoxy (or C-methyl) resonances using sweep widths of 50 Hz. Values in parentheses are those obtained by graphically correcting for peak overlap.

Table III. Enantiomeric Chemical Shift Differences for Several Nontype 1 α- and β-Amino Esters in (−)-Alcohol 2[a]

Amino ester ($\Delta\delta$, Hz) [nonequivalence sense]	% optical purity Calcd	Obsd	Config
	18.9	20.0	*S*
	0.0		
	0.0		
	0.0		

[a] See footnotes in Table II for the experimental conditions and the definition of symbols.

bases react slowly at room temperature to form diketopiperazines.[22,26]

The choice of optically active **2** as an nmr solvent for amino esters was prompted by its success in promoting spectral nonequivalence of alkyarylcarbinylamines,[6] its ready availability,[21c] lack of interfering protium resonances, and its appreciable acidity.[27] This latter property overcomes the disadvantages of employing the esters of amino acids, since alcohol **2** markedly stabilizes concentrated solutions of the free bases. For example, neat leucine methyl ester (**1**, R = isobutyl) decomposes to the solid diketopiperazine in a period of 1–2 days at room temperature, as do most of the amino esters used in this study. However, when dissolved in (−)-**2**, no evidence for diketopiperazine formation was found after 1 week at room temperature. Moreover, the optical purity (by nmr) of the ester remained unchanged in this period of time, showing that racemization of amino esters in (−)-**2** is extremely slow.

While Fischer esterification of amino acids is known to proceed without measurable racemization,[28] the free methyl esters have been shown to racemize in refluxing alcohol containing alkoxide.[25] Although our reaction conditions were much milder than this, we cannot presently exclude minor racemization in the conversion of the methyl ester hydrochlorides to the free esters. In view of the close agreement between the observed and calculated optical purities for the partially resolved amino esters listed in Table II, the nmr method will often be the method of choice for the direct determination of optical purities of amino acids, particularly for amino acids having small specific rotations[29] or contaminated with optically active impurities.

(26) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2, John Wiley & Sons, Inc., New York, N. Y., 1961, p 716.

(27) The pK_a of **2** is 11.9 [R. Stewart and R. Van der Linden, *Can. J. Chem.*, *38*, *399* (1960)].

(28) See ref 26, pp 925–931.

(29) See, *e.g.*, W. S. Chilton, G. Tsou, L. Kirk, and R. G. Benedict, *Tetrahedron Lett.*, 6283 (1968).

Nmr Spectral Correlations of Absolute Configurations. Upon examination of the data given in Table II a striking conclusion is reached. In each case where nonequivalence is observed for partially resolved type **1** α-amino esters, it is possible to correlate the absolute configurations[30] of the predominant enantiomers with the senses of spectral nonequivalence.[8] Thus, in (−)-carbinol (**2**), the α-carbomethoxy and the α-carbinyl proton resonances of type **1** esters of the *S* configuration appear at higher field, and the resonances in the R group appear at lower field than do the corresponding resonances in the enantiomers of the *R* configuration.

Because of the constancy seen in the senses of nonequivalence for the partially resolved amino esters listed in Table II, it is felt that this method may be confidently extended to the determination of the absolute configurations of other amino esters, similar to those in Table II, whose configurations are presently unknown. Furthermore, this method does not depend upon those factors, *e.g.*, interference of phenyl transitions[32,33] and marked solvent and pH dependencies,[32] which limit the scope of various ORD methods in correlating absolute configurations of α-amino acids.

Previously advanced[4,6] explanations of enantiomeric spectral nonequivalence in chiral solvents have invoked strong solvent–solute interactions (*i.e.*, hydrogen bonding) to form transitory diastereomeric solvates whose spectra are caused to be observably dissimilar through the population of conformers which place enantiotopic nuclei in nonidentical magnetic environments. Similarly, it is tacitly assumed that the spectral nonequivalence observed for α-amino esters in (−)-alcohol **2** obtains as a result of the formation of short-lived[34] diastereomeric solvates, presumably *via* association between the basic amino group and the acidic alcohol function. In support of this hypothesis are the observations that the enantiomeric chemical shift differences may be enhanced by lowering the temperature or by increasing the carbinol concentration.

The presence of an aromatic group in the amino ester is not a prerequisite for the nonequivalence phenomenon to obtain. Thus, it is not surprising that there appears to be no correlation between the senses of nonequivalence seen for configurationally similar α-amino esters and alkylarylamines[6] in optically active **2**. This also suggests that the conformational model advanced to explain nmr senses of nonequivalence for amines[6] must be somewhat modified to accommodate amino esters. Common to the rationalization of both amine and amino ester nonequivalence is the underlying assumption that only those conformations which place the enantiotopic nuclei of the solute near the anisotropic phenyl ring of the solvent will contribute appreciably to observable spectral nonequivalence.

(30) The absolute configurations of the partially resolved amino esters used in this study have been previously assigned by physical techniques, with the exception of tryptophan, which has been assigned by biological methods.[31] The configuration found by the present nmr method is in agreement with the biological assignment.

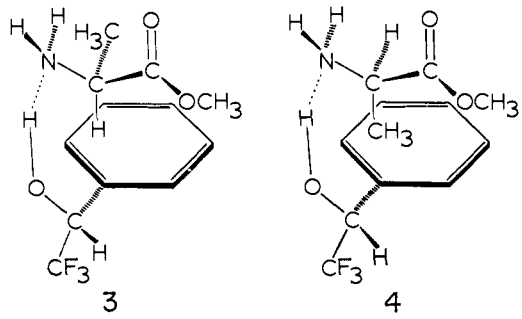
(31) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 1, John Wiley & Sons, Inc., New York, N. Y., 1961, pp 46–244.

(32) K. M. Wellman, S. Bogdansky, W. Mungall, T. G. Mecca, and C. R. Hare, *Tetrahedron Lett.*, 3607 (1967), and references cited therein.

(33) C. J. Hawkins and P. J. Lawson, *Chem. Commun.*, 177 (1968), and references cited therein.

(34) Amino ester nonequivalence is not observed in racemic **2**, indicating that exchange of hydrogen-bonding partners must be relatively fast on the nmr time scale.

The data can best be accommodated by assuming that, in addition to association between the amino group of the solute and the hydroxyl group of the solvent, there is a secondary interaction between the positive end of the dipolar group of the amino ester and the π cloud of the phenyl ring in **2**.[35] An *a priori* inspection of Dreiding models indicates that **3** is a relatively strain-free conformation for the diastereomeric solvate formed between (*R*)-**2** and (*S*)-methyl alanate, while **4** shows the corresponding conformer for the diastereomeric solvate derived from (*R*)-**2** and (*R*)-methyl alanate. It is clear that the *R,S* solvate (**3**) allows closer approach of the carbomethoxy group and the carbinyl proton to the shielding region of the phenyl ring than does the *R,R* solvate (**4**). While this model is undoubtedly an oversimplification, it does correctly account for the observed shielding of the carbomethoxy group and the carbinyl proton and for the observed deshielding of the C-methyl group of (*S*)-methyl alanate relative to the corresponding nuclei in the *R* isomer (Figure 1 and



3 4

Table II).[36] Moreover, this model is successful in predicting the senses of nonequivalence for all of the partially resolved type **1** α-amino esters studied (Table II), even to the inclusion of proline methyl ester, a cyclic type **1** amino ester (Table III).

From an examination of the magnitudes of nonequivalence seen for the mono- and difunctional amino esters in Tables II and III, there appear to be three principal factors contributing to spectral nonequivalence. These are difference in steric bulk of the two α-carbon substituents,[37] the ability of the carbonyl to interact with the π electrons of the solvent,[38] and the strength of the hydrogen bond between the α-amino group and the hydroxyl group of the solvent.[39]

(35) Interactions involving either the π electrons or the positive dipole (see T. Ledaal, *Tetrahedron Lett.*, 1683 (1968), for spectral evidence supporting this latter view) of the carbonyl group with the π electrons of the phenyl ring lead to the same predictions of nonequivalence senses.

(36) These chemical shift differences are superimposed on the upfield shifts of 0.29, 0.17, and 0.21 ppm observed for the carbinyl proton, carbomethoxy group, and C-methyl group, respectively, in going from $CDCl_3$ to racemic **2** as solvent.

(37) For example, the two methylene protons of methyl glycinate are imperceptibly anisochronous in (−)-**2** at 100 MHz and 29°.

(38) When optically active 2,2,2-trifluoro-(1-naphthyl)ethanol (**5**) is used as solvent for (*S*)-methyl alanate, the magnitudes of nonequivalence are (at 100 MHz and 29°) 3.5, 4.4, and 5.3 Hz for the H, OCH_3, and CCH_3 groups, respectively.

(39) Consistent with this are the findings that (−)-methylphenylcarbinol (**6**) is only about half as effective in promoting spectral nonequivalence as is the more acidic alcohol **2**, and that the hydrochloride of methyl alanate exhibits no observable spectral nonequivalence in (−)-**2** at 100 MHz and 29°.

Experimental Section

Spectral Methods. Nmr spectra were recorded on either Varian HA-100, A-60A, or A-56/60 spectrometers at 29, 42, or 44°, respectively. Optical rotations of the resolved amino acids were measured at the sodium D line on a Carl Zeiss visual polarimeter in a 2-dm cell at 24 ± 1° in either water or 6.0 *M* hydrochloric acid. The specific rotations obtained were in agreement with those reported[40] for enantiomerically pure materials.

Materials. All of the amino acids used in this study are commercially available and were used without further purification. Partially resolved amino acids were prepared by mixing weighed portions of racemic and optically active components in a *ca.* 4:1 ratio, respectively, and the calculated optical purities are given in Table II.

Amino Acid Methyl Esters. The methyl ester hydrochlorides of the amino esters used in this study, with the exception of (*S*)-serine methyl ester hydrochloride, which was obtained commercially, were prepared by methanolic hydrogen chloride esterification of the corresponding amino acids using standard techniques.[41] The products were shown to be ≥90% pure by integration of their nmr spectra in D_2O and were used directly in the next step, avoiding purification procedures[41] which might have caused optical fractionation of the enantiomers.

The free methyl esters were generated by a modification of the procedure of Fischer and Suzuki,[22] which will be described for the preparation of methyl alanate.

To a room temperature solution of 1.40 g (0.01 mol) of alanine methyl ester hydrochloride in 3 ml of absolute methanol was added portionwise 0.54 g (0.01 mol) of powdered sodium methoxide. The mixture was swirled for several seconds and 30 ml of ether[42] was added to precipitate salts, which were collected (filtration through Celite) and washed with ether. The filtrates were concentrated under reduced pressure (bath temperature ≤35°) and the residual liquid was again treated with ether.[43] After a second filtration–evaporation sequence, there remained 0.77 g (75%) of methyl alanate, identified by its nmr spectrum (Table I). Even when the ester is stored under nitrogen at 10°, an ether-insoluble solid, presumably the diketopiperazine,[22,26] forms after a few days.

The remaining amino acid methyl esters used in this study were similarly prepared with the exception of (*S*)-threonine methyl ester, which was obtained commercially. Crude yields ranged from 50 to 100% for the free bases, whose identities were checked by their nmr spectra (Table I). Spectral intensities and multiplicities were consistent with those expected, and no extraneous peaks were observed other than those attributable to traces of residual solvent.

Because the neat methyl esters slowly condense to form diketopiperazines,[26] solutions of the esters in optically active carbinol **2** were made up immediately after preparations of the esters.

Optically Active Carbinols. (*R*)-(−)-2,2,2-Trifluorophenylethanol (**2**), prepared as previously described[21c] and molecularly distilled before use, gives $\alpha^{25}D$ −40.7° (neat, *l* 1) [lit.[21b] $\alpha^{25}D$ −41.18° (neat, *l* 1)], corresponding to 99% enantiomeric purity.

2,2,2-Trifluoro(1-naphthyl)ethanol (**5**) was resolved similarly and an nmr determination of its optical purity and absolute configuration[4] showed this sample to be enriched in the (*R*)-(−) enantiomer and to have an optical purity of 79%.[44]

(*S*)-(−)-Methylphenylcarbinol (**6**) was resolved by the procedure of Downer and Kenyon[45] and has $\alpha^{25}D$ −38.0° (neat, *l* 1) [lit.[29] $\alpha^{20}D$ −44.2° (neat, *l* 1)], corresponding to an optical purity of 86%.

Acknowledgments. This work was supported by the U. S. Public Health Service (Grant No. GM 14518) and by a National Institutes of General Medical Sciences Fellowship. We wish to thank Dr. H. E. Carter for generous samples of many of the amino acids used in this study.

(40) J. S. Fruton and S. Simmonds, "General Biochemistry," John Wiley & Sons, Inc., New York, N. Y., 1953, p 77.

(41) Reference 26, pp 925–931.

(42) It was necessary to use 1,2-dimethoxyethane for the methyl esters of serine and tyrosine.

(43) This procedure effectively removes residual amino acid, its hydrochloride, or the methyl ester hydrochloride as well as inorganic salts.

(44) We are grateful to Mr. Ronald Muntz for the preparation, resolution, and optical purity determination of **5**.

(45) E. Downer and J. Kenyon, *J. Chem. Soc.*, 1156 (1939).