Nuclear Magnetic Resonance Nonequivalence of Diastereomeric Esters of α-Substituted Phenylacetic Acids for the Determination of Stereochemical Purity[1]

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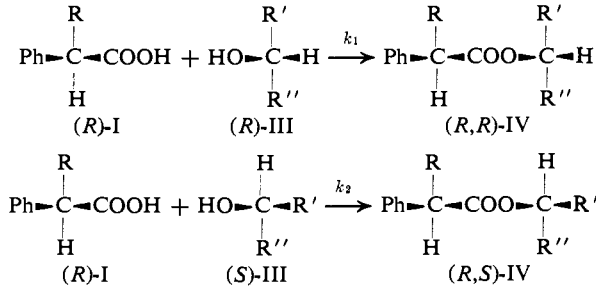
Abstract: Nuclear magnetic resonance (nmr) spectra have been determined for a series of diastereomeric esters of α-substituted phenylacetic acids with various dissymmetric secondary carbinols In nearly all cases, the nmr spectra of the *R,R* and *S,S* diastereomers were significantly different from those of the *S,R* and *R,S* diastereomers. The effects upon the spectra of α-methoxy, α-methyl, α-*t*-butyl, α-trifluoromethyl, α-hydroxy, and α-chloro groups were explored. Chemical shift differences were observed for all epimerically related groups except phenyl in the diastereomeric esters such as *R,S* and *R,R* methyltrifluoromethylcarbinyl O-methylmandelate. These chemical shift differences between diastereomers were used to determine enantiomeric purities of chiral secondary carbinols. Enantiomeric purities within 1% of the polarimetrically determined values were obtained readily with an HR-100 instrument. However, racemization and/or kinetic resolution was encountered during the synthesis of some esters, particularly when the alcohol was sterically hindered. Therefore, if this method is to be used with confidence in a specific case, it will always be necessary to establish the quantitative validity of the chemical procedure by control experiments. The presence of a trifluoromethyl group in the alcohol or acid moiety of the ester was quite advantageous since the fluorine nuclear magnetic signals are then of high intensity, occur in a region uncomplicated by overlapping signals, and show generally large chemical shift differences (0.1–0.29 ppm) between diastereomers. Furthermore, the enhanced volatility of these compounds facilitates their purification. Preliminary observations of solvent and temperature effects have been made also.

Our search for practical methods of determining the enantiomeric purity of optically active alcohols produced by asymmetric reductions,[2] which would circumvent some of the limitations of polarimetry, led to a study of gas–liquid partition chromatography (glpc) for the resolution of substituted trifluoromethylcarbinyl esters of (*R*)-(−)-O-methylmandelic acid and (*R*)-(−)-hydratropic acid.[3-5] During these studies it became apparent that the nuclear magnetic resonance (nmr) nonequivalence of signals arising from epimerically related groups[6] could also serve such a purpose. The nmr spectral nonequivalence of the methylene fluorines in substituted ethanes,[7] of the methylene hydrogens in benzyl and ethyl ethers,[8] and of the methyl hydrogens in the isopropyl side chain of lunine and lunacrine[9] indicated that the magnitude of such chemical shift differences was sufficient to be of practical value in the quantitative determination of diastereomers. Mislow and Raban[10] and Gerlach[11] have introduced the use of this technique in the determination of the enantiomeric purity of compounds which owe their dissymmetry to deuterium substitution. This nmr method for the determination of enantiomeric purity recently has been reviewed critically by Raban and Mislow.[12] Our study presents data on a variety of esters of α-substituted phenylacetic acids which were gathered to delineate the scope of the nmr method for determining both the enantiomeric purity of secondary carbinols and the optimum reagents and conditions to be utilized in specific instances.

The reliability of the nmr method for determining enantiomeric purity requires that there be neither racemization nor kinetic resolution in the synthesis of the diastereomers.[12] In the reaction of the chiral α-substituted phenylacetic acid (I) with a mixture of enantiomeric alcohols (III) the two rates will be different[13] if the process is not diffusion controlled; thus, the reaction must be conducted so that it is quantitative



(1) We acknowledge with gratitude the support of this work by the U. S. Public Health Service (NIH GM 5248) and the National Science Foundation (GP 6738).

(2) J. S. Birtwistle, K. Lee, J. D. Morrison, W. A. Sanderson, and H. S. Mosher, *J. Org. Chem.*, **29**, 37 (1964).

(3) D. M. Feigl and H. S. Mosher, *Chem. Commun.*, 615 (1965).

(4) D. M. Feigl, Ph.D. Thesis, Stanford University, 1966.

(5) D. M. Feigl, D. L. Dull, and H. S. Mosher, publication in preparation.

(6) K. Mislow and M. Raban, in "Topics in Stereochemistry," N. L. Allinger and E. Eliel, Ed., Interscience Publishers, New York, N. Y., 1966, pp 1–37.

(7) J. J. Drysdale and W. D. Phillips, *J. Am. Chem. Soc.*, **79**, 319 (1957).

(8) (a) G. M. Whitesides, F. Kaplan, and J. D. Roberts, *Proc. Natl. Acad. Sci. U. S.*, **48**, 1112 (1962); (b) G. M. Whitesides, F. Holtz, and J. D. Roberts, *J. Am. Chem. Soc.*, **86**, 2629 (1964).

(9) S. Goodwin, J. N. Schoolery, and L. F. Johnson, *ibid.*, **81**, 3065 (1959).

(10) (a) K. Mislow and M. Raban, *Tetrahedron Letters*, 4249 (1965); (b) M. Raban and K. Mislow, *ibid.*, 3961 (1966).

(11) H. Gerlach, *Helv. Chim. Acta*, **49**, 2481 (1966).

(12) M. Raban and K. Mislow, ref 6, Vol. II, 1967, pp 216–226. We wish to thank these authors for the opportunity of seeing this manuscript before publication.

(13) If a chiral reagent reacts with enantiomers, it will do so *via* diastereomeric transition states with different rates. It is, however, possible under rare circumstances to have identical rates. Assume for instance that (*S*)-I reacts with racemic III in solvent X to produce (*S,S*)-IV in slight excess but in solvent Y to produce (*S,R*)-IV in slight excess, then there will be some mixture of solvents X and Y in which the two reactions will be isokinetic.

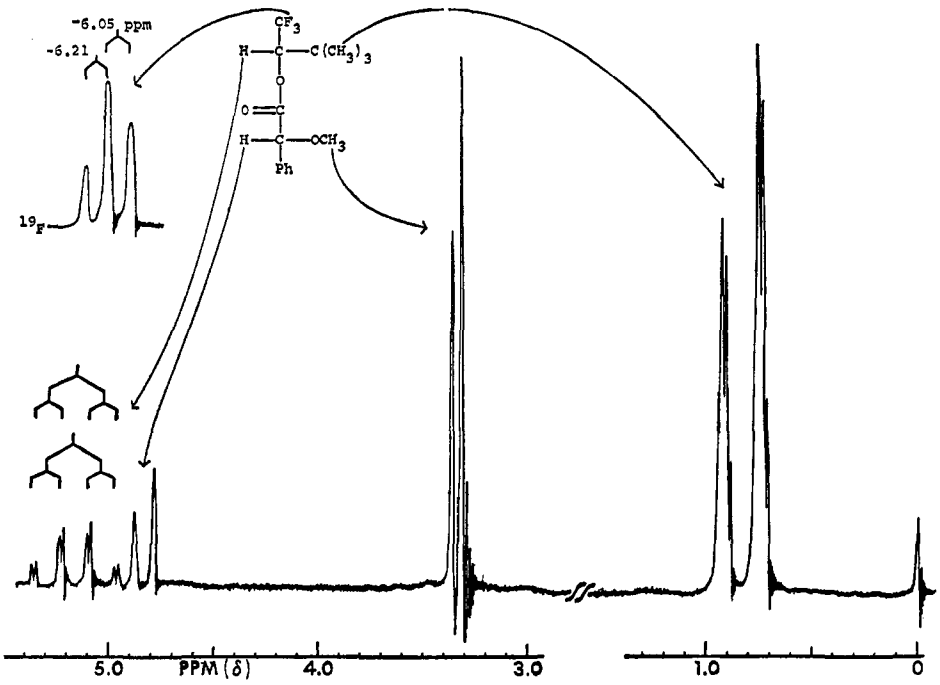


Figure 1. The 60-MHz proton (tetramethylsilane internal reference) and 56.4-MHz fluorine (trifluoroacetic acid internal reference) nmr spectra of *t*-butyltrifluoromethylcarbinyl O-methylmandelate, prepared from racemic acid and carbinol; spectra taken in α,α,α-trifluorotoluene solvent at 37°.

with respect to the alcohol in order for the diastereomer ratio to reflect exactly the enantiomer ratio. It is also necessary that there be no concentration of one diastereomer over the other in any purification step, as may occur by fractionally crystallizing the diastereomer mixture.

Our approach was the same as that used by Mislow and Raban,[10] which employed the irreversible reaction of an α-substituted phenylacetyl chloride (II) with a dissymmetric secondary carbinol (III) to give a mixture of diastereomeric esters (IV). Exploratory experiments

$$\underset{\text{II}}{\text{PhCHRCCl}} + \underset{\text{III}}{\text{HOCHR R''}} \longrightarrow \underset{\text{IV}}{\text{PhCHRCOCHR'R'}}$$

were first performed using racemic acid chloride (*RS*)-II and racemic carbinol (*RS*)-III which produced an optically inactive mixture of four diastereomers consisting of two epimeric pairs, (*R,S*)-IV and (*S,R*)-IV *vs.* (*R,R*)-IV and (*S,S*)-IV. Since nmr cannot distinguish between enantiomers,[12,14] the spectrum of the *R,S* isomer will be identical with that of the *S,R* isomer, and that of the *R,R* isomer will be identical with that of the *S,S* isomer. Thus, an equal mixture of the four diastereomers will give the same nmr spectrum as an equal mixture of the two epimers.

In Figure 1 is shown the 60-MHz proton and 56.4-MHz fluorine spectrum of the α,α,α-trifluorotoluene solution of *t*-butyltrifluoromethylcarbinyl O-methylmandelate prepared from racemic materials. This is an example in which all of the epimerically related groups except phenyl show significant chemical shift differences. The methine hydrogen signals of the mandelate moiety (H_B) show a chemical shift difference between the diastereomeric pairs of 5.5 Hz, the methyl hydrogens of the methoxy group a 3.0-Hz difference, the methine hydrogen signals of the carbinol moiety a 2.0-Hz difference, and the hydrogens of the *t*-butyl group an 11.5-Hz difference. The fluorine resonances are separated by 9.0 Hz. Although made from racemic materials, the signals do not have the same intensity, indicating either different rates of formation of the diastereomeric pairs *R,R* and *S,S vs. R,S* and *S,R* and/or the thermodynamic equilibration of these pairs after or during formation. The latter is almost certainly the case, based upon our observations of other closely related examples. Thus the chemical process used to synthesize this particular ester cannot be employed for analytical purposes. Unfortunately, this situation was encountered repeatedly with hindered carbinols.

The chemical shift separations between diastereomeric pairs are not always as clear as in Figure 1. Figure 2 represents the 60-MHz methyl region of the O-methylmandelate ester of methylisopropylcarbinol. Curve A is the spectrum, taken in carbon tetrachloride solvent, of the ester made from enantiomerically pure *S* carbinol and 90.9% enantiomerically pure *S* acid. The doublets for the predominate *S,S* diastereomer are readily distinguished. Curve B is of the same sample taken in benzene–carbon tetrachloride solution. The diastereotopic methyls of the isopropyl group in the *S,S* diastereomer now show up as nonequivalent. Such nmr nonequivalence of the methyl signals of an isopropyl group has been thoroughly documented;[9,15] the present example illustrates the importance of solvent effects (Table I) and emphasizes the complications which may arise. Curve C of Figure 2 is the nmr spectrum in carbon tetrachloride of the same ester prepared from

(14) This of course does not apply in a chiral environment as demonstrated by (a) W. H. Pirkle, *J. Am. Chem. Soc.* **88**, 1837 (1966), and (b) T. G. Burlingame and W. H. Pirkle, *ibid.*, **88**, 4249 (1966).

(15) (a) B. Halpern, J. S. Westly, and B. Weinstein, *Chem. Commun.*, 160 (1967); (b) M. Kajtax and L. Radics, *ibid.*, 784 (1967), and references therein.

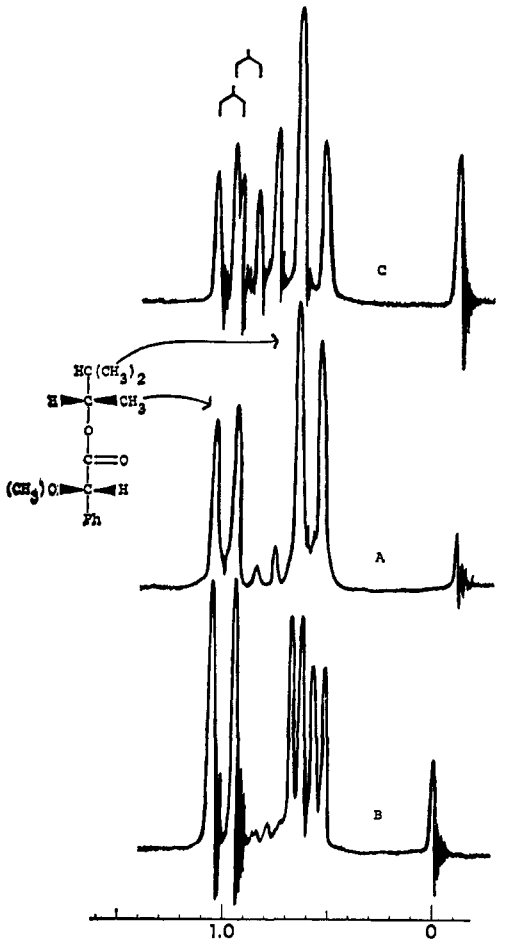
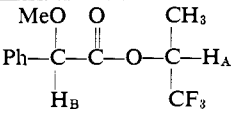


Figure 2. 60-MHz nmr spectra of methylisopropylcarbinyl O-methylmandelate: A, the carbon tetrachloride solution of ester prepared from nearly enantiomerically pure *S* carbinol and *S* acid; B, the carbon tetrachloride plus benzene solution of the same sample; C, the carbon tetrachloride solution of ester prepared from racemic acid and carbinol.

racemic carbinol and acid. It is apparent that the methyl signals in the spectrum of the *R,S* and *S,R* diastereomers appear at lower field than those of the *S,S* and *R,R* diastereomers. The signal for the isopropyl group appears to be a triplet but is clearly a set of two doublets at 100 MHz. The chemical shift differences for methyltrifluoromethylcarbinyl O-methylmandelate in various solvents are given in Table I. The chemical shift differences for various esters are given in Table II.

To test the accuracy of this nmr method for the determination of enantiomeric purity, (*S*)-O-methylmandelic acid, 90.9% enantiomerically pure (95.5:4.5 enantiomer ratio) by optical rotation, was converted to the acid chloride by refluxing with excess thionyl chloride and benzene for 1 hr; after vacuum evaporation of solvent below 100°, the unpurified acid chloride was allowed to react with a fourfold excess of *l*-menthol in excess pyridine–benzene. The reaction mixture was extracted with water and then dilute hydrochloric acid and the ester isolated by glpc. The ratio of the two nmr singlets was 95.8:4.2 for the α-hydrogen (H_B) of the O-methylmandelate moiety (275 and 277 Hz downfield of internal TMS at 60 MHz in carbon tetrachloride) when measured by machine integrations on a Varian HR-100 instrument. The same ratio was obtained also by direct comparison of the peak heights. Thus this method has correctly given the enantiomeric purity of the O-methylmandelic acid within experimental error.[16] The consistency of these results confirms the correctness of the previously assigned maximum rotation of O-methylmandelic acid[17] and corroborates the findings of Raban and Mislow.[12] Table III contains the results of other quantitative determinations in which O-methylmandelic acid is used as the standard. Accurate determination of enantiomeric composition becomes difficult when the mixture is nearly racemic since small differences in two large numbers are being compared. In addition, the accuracy of the determination is very much dependent upon the signals being clearly separated from each other and from other signals in the spectrum of the particular ester.

Table I. Solvent Dependence of Nmr Chemical Shift Differences between Diastereomers[a]



Solvent	CH_3	H_A	MeO	H_B
CS_2	7.0	1.5	...[b]	1.0
CCl_4	7.0	1.5	...	...
$CDCl_3$	11.0	2.0	...	...
CH_2Cl_2	8.5	1.5	...	...
CF_3COOH	11.0	...	...	...
$(CF_3CO_2)O$	11.0	1.5	...	...
$(CD_3)_2CO$	8.0	1.5	...	...
DMSO-d_6	7.5	1.5	...	...
DMF	7.0	1.0	...	...
Pyridine	7.5	1.5	1.0	...
Benzene-d_6	7.0	3.0	1.5	2.0
$PhCF_3$	10.0	1.5	1.5	1.5
$1,2,4\text{-}Cl_3C_6H_3$	7.0	1.5	1.0	...

[a] The values are rounded off to the nearest 0.5 Hz taken on a Varian A-60 at 37°. [b] Not readily observable: 0.0 ~ 0.5 Hz.

A major impediment to the general application of this system for the determination of enantiomeric purity of the wide spectrum of secondary carbinols is the equilibration of diastereomers encountered during the synthetic procedure, especially when sterically hindered alcohols are used. Racemization has been observed previously during the preparation of derivatives of various α-substituted phenylacetic acids.[5,18-20] We observed that the nmr spectrum of phenyl-*t*-butylcarbinyl O-methylmandelate, prepared from optically active O-methylmandelic acid (*via* the acid chloride) and optically active phenyl-*t*-butylcarbinol, was identical with that prepared from racemic O-methylmandelic acid and racemic carbinol. Similarly the nmr spectra of the O-methylmandelate esters of ethyl-*t*-butylcarbinol as well as methyl-*t*-butylcarbinol were identical when the esters were prepared from either racemic or optically active compounds. Presumably the nmr spectrum of the

(16) It was known already that there was no resolution of the diastereomers since racemic acid and pure *l*-menthol under the same conditions gave the two nmr signals of equal area and equal height. The consistency of these data rules out the possibility of racemization, which was observed when the temperature rose significantly above 100° during the removal of solvent.

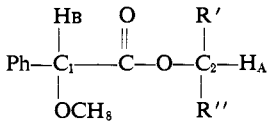
(17) W. A. Bonner, *J. Am. Chem. Soc.*, 73, 3126 (1951).

(18) C. Aaron, D. L. Dull, J. Larkin, Y. Ohashi, D Jaeger, and H. S. Mosher, *J. Org. Chem.*, 32, 2797 (1967).

(19) J. Smejkal and J. Farkas, *Collection Czech. Chem. Commun.*, 28, 481 (1963).

(20) R. Wiedman and A. Horeau, *Bull. Soc. Chim. France*, 117 (1967).

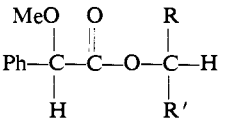
Table II. Nmr Chemical Shifts of O-Methylmandelate Diastereomers[a]



R′	R″	C_1	C_2	OCH_3	H_B[b]	H_A[b]	CH_3	t-C_4H_9	i-C_3H_7[c]	CH_2——CH_3	
CH_3	CF_3	*R*	*S*		284.5	316.5	72	...	...	...	...
				203							
		S	*S*		...	315	79	...	...	...	...
CH_3	C_2H_5	*S*	*R*			289.0	65	...	...	84.5	49
				202	277.5						
		S	*S*			287.5	70	...	...	86.0	40
CH_3	n-C_6H_{13}	*S*	*R*		277	292.5	67	...	...	...	...
				202							
		R	*R*		278	293.5	63.5	...	...	...	...
CH_3	C_6H_{11}[e]	...	...		277.5		62	...	...	...	...
				203		279					
		...	...		276		67				
CH_3	i-C_3H_7	*S*	*S*		278	278	67	...	41	...	...
				201							
		R	*S*		279.5	279.5	60	...	48	...	...
CH_3	C_6H_5	...	...				86.5				
				199	280	249.5		...	...	...	...
		...	...				81.5				
C_6H_5[d]	CF_3	*R*	*S*		370.5	281	...	...	...	...	...
				186							
		S	*S*		369.5	282	...	...	...	...	...
C_6H_5	C_2H_5	...	...								38.5
				199	280	338	...	...	...	...	
		...	...								46
C_6H_5	i-C_3H_7	...	...						39	...	...
				201	281.5	325.5	...	...			
		...	...						43.5	...	...
C_6H_5	t-C_4H_9	...	...					49	...	...	...
				201	281	324	...				
		...	...					44	...	...	...
C_2H_5	t-C_4H_9	...	...					40	...	...	45
				204	279	776.5	...				
		...	...					48	...	...	39
CH_3	t-C_4H_9	...	...		276	276	64.5	43	...	...	...
				202							
		...	...		278	278	59	49.5	...	...	...
CF_3	t-C_4H_9	...	...	205	285.5		...	47.5	...	...	...
						301.5					
		...	...	206.5	289		...	55.5	...	...	...

[a] The values are rounded off to the nearest 0.5 Hz, taken on a Varian 60-MHz spectrometer with TMS as internal standard for the carbon tetrachloride solutions at 37°. [b] The 100-MHz spectra typically show chemical differences of 1–2 Hz, which are not readily distinguishable in the 60-MHz spectra unless the signals are sharp and the instrument very well tuned. [c] The methyl signals of the isopropyl group. [d] Benzene solvent. [e] Cyclohexyl representation.

Table III. Comparison of Alcohol Enantiomeric Purities as Determined by Nmr and Other Methods[a]

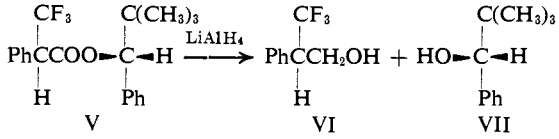


R	R′	Polarimetry	Glpc[b]	Nmr[c]
CH_3	C_6H_{11}[e]	2.0 ± 1.0	...	3.0 ± 2.0
CH_3	n-C_6H_{13}	96.1 ± 0.3	...	97.5 ± 2.0
CH_3	CF_3	...	62.3 ± 0.5	62.5 ± 0.3[b]
Ph	CF_3	100.0 ± 0.1[d]	100.0 ± 0.5	100.0 ± 0.5[b]
CH_3	i-C_3H_7	100.0 ± 1.0	...	100.0 ± 0.5

[a] Values are per cent enantiomeric purity. [b] The same diastereomeric esters as reported in ref 3 were available. [c] The 100-MHz pmr spectra of the esters in carbon tetrachloride solvent were used for analysis. See Table II for positions of signals. [d] See ref 4. [e] C_6H_{11} represents cyclohexyl.

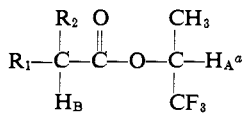
ester from trifluoromethyl-*t*-butylcarbinol also would be the same as that in Figure 1 if optically active carbinol had been used instead of racemic carbinol. Apparently we are observing the thermodynamic equilibration of the diastereomeric esters at the α position of the acid moiety. In support of this, these nmr spectra repeatedly consisted of unequal signals for epimerically related groups whether racemic or optically active

alcohol was used as the limiting reagent during synthesis. This could not be caused by prior racemization of the acid chloride since identical samples of the acid chloride gave esters with optically active methylisopropylcarbinol and methyl-*n*-hexylcarbinol whose nmr spectra accurately reflected the enantiomeric composition, known from polarimetric data, of the carbinols (Table II). Finally, lithium aluminum hydride reduction of *t*-butylphenylcarbinyl α-trifluoromethylphenylacetate (V), prepared from optically active reagents, gave alcohols (VI and VII) which showed that *only* the acid moiety had been racemized and indeed to the extent shown by the nmr spectrum.



The trifluoromethyl-containing compounds offer a real advantage from an analytical standpoint (Table IV).

Table IV. Nmr Chemical Shift Differences of Diastereomeric Methyltrifluoromethylcarbinyl Esters[a]



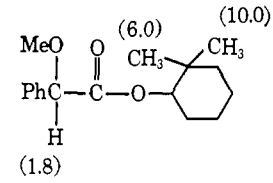
R_1	R_2	H_A[b]	CH_3	CF_3[c]
Ph	CF_3	1.0	7.0	5.9
Ph	CH_3	1.2	7.2	4.3
Ph	*t*-Bu	2.2	9.0	13.4
Ph	Cl	<0.2	6.2	9.1
Ph	MeO	1.7	7.0	7.7
o-ClPh	MeO	1.7	6.8	10.0

[a] Carbon tetrachloride solvent. [b] Proton resonances are ±0.2 Hz at 60 MHz with TMS internal standard. [c] Fluorine resonances are ±0.2 Hz at 56.4 MHz with trifluoroacetic acid internal standard.

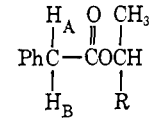
The fluorine resonance occurs in a region uncomplicated by interfering signals, the chemical shift differences between diastereomers are usually large, and the intensities of the signals are comparable to those of methyl groups; thus, integrations are more accurate. α-Methoxy-α-trifluoromethylphenylacetic acid may be an especially favorable reagent because it not only contains the trifluoromethyl group but does not have an α hydrogen and thus cannot racemize by either a carbanion or ketene mechanism.[18,19] Its further application to nmr enantiomeric purity determinations is now being studied.

The magnitude of the chemical shift differences generally seems to be greatest for the group attached to the carbinyl carbon of the alcohol moiety of the ester. The trifluoromethyl, methyl, and *t*-butyl groups all show significant chemical shift differences between the epimers when situated in the alcohol moiety, but *only* the trifluoromethyl group showed a significant shift difference between epimers when situated in the α position of the acid moiety. It is difficult to recognize any trend in the chemical shift differences between the various ester derivatives of a particular carbinol, nor is there any obvious relation to direction of the shift in going from the *R,R* to *R,S* diastereomer.[21]

It seems reasonable to assume that the steric and electronic nonbonded interactions—when combined with the anisotropy of an aromatic ring, which is sufficiently close in at least one conformation for magnetic interaction to occur—are the major contributing factors to these rather large observed chemical shift differences in diastereomers. In this connection the ester XIV was prepared from methyltrifluoromethylcarbinol and 1-indancarboxylic acid. In this ester the phenyl ring is not free to rotate with respect to the asymmetric center α to the carboxyl group. There was no observable difference in the methyl and methine proton signals between the two diastereomers, but the fluorine signals from the trifluoromethyl group showed a typical shift difference between the two diastereomers. Chemical shift differences have been measured for several epimeric polypeptides.[22] It is significant that the chemical shift differences were small (1 Hz or less) when no aromatic group was included in the peptide, but were substantial (1–8 Hz) when a phenylalanine or tyrosine unit was involved. Some other examples we have observed are represented in the formulas VIII–XV. The proton (60 MHz) and fluorine (56.4 MHz) chemical shift differences of 1 Hz or more between the epimers are indicated next to the groups; the solvent is carbon tetrachloride unless otherwise specified.

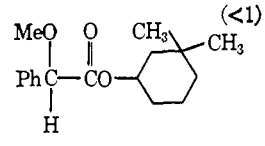


VIII

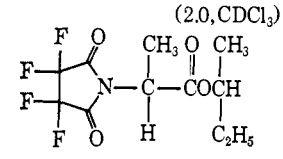


IX

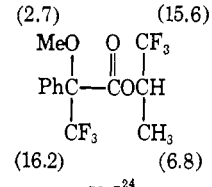
$H_A = H_B$ in C_6H_6 and CCl_4
R = CF_3, C_2H_5, Ph



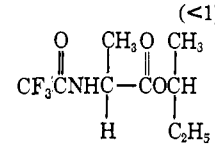
X



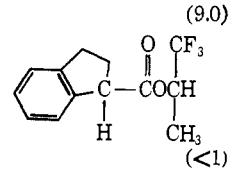
XI[23]



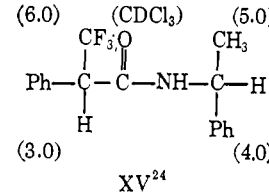
XII[24]



XIII[4]



XIV



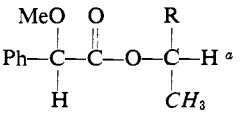
XV[24]

(21) This is not surprising in such conformationally mobile molecules as these, especially when the groups being compared are as different as alkyl, trifluoromethyl, methoxy, etc. An attempted conformational analysis based upon these spectra would be entirely speculative at this time.

(22) B. Halpern, D. W. Niteck, and B. Weinstein, *J. Am. Chem. Soc.*, **89**, 505 (1967).

A study was made of the change in nmr spectra with temperature for a series of O-methylmandelate esters as shown in Table V. The only variation was a uniform increase of the chemical shift difference as temperature was lowered from 178° (1,2,4-trichlorobenzene solvent) to −90° (vinyl chloride solvent). This argues strongly that rotamer freezing is not operative under these conditions.

Table V. Temperature Effect on Chemical Shift Differences between the Carbinol Methyl Group of Diastereomeric O-Methylmandelates[a]



Temp, °C	Solvent	Ph	*i*-Pr	*t*-Bu	CF_3
−90[b]	Vinyl chloride	6.5	12.5	13	13
−49	Vinyl chloride	6.5	10	11	11.5
−19	Vinyl chloride	6.5	9	10	10
+37	1,2,4-Trichlorobenzene	5.5	6.5	6	7
+76	1,2,4-Trichlorobenzene	5	5	5	7
+112	1,2,4-Trichlorobenzene	4	5	4	7
+178	1,2,4-Trichlorobenzene	3	4	..	6

[a] The values are rounded off to the nearest 0.5 Hz, taken on a Varian A-60 spectrometer. [b] No signal broadening.

Experimental Section

Instruments. The nmr spectra were obtained with a Varian Associates A-60 spectrometer equipped with a variable-temperature probe and controller, and with HR-60 and HR-100 instruments equipped for 56.4- and 94.1-MHz resonances, respectively. Determination of diastereomer ratios by the measurement of peak heights gave the same results as measurement of peak areas. This simplified procedure is based on the fact that the nmr signals of epimerically related groups are of nearly identical shape. Such correspondence was supported by observing equal diastereomer peak heights for esters prepared from racemic reagents, but depended, however, on tuning the machine carefully and scanning both up- and downfield. For satisfactory determinations of enantiomeric purity, the HR-100 instrument was required; the values obtained on the A-60 spectrometer, although reproducible, were consistently in error by 4–10%.

The gas–liquid partition chromatographic separations were performed with Varian-Aerograph A-90-P3 instruments; unless otherwise specified, the retention times (t_R) refer to a Varian-Aerograph 5 ft × 0.25 in. stainless steel column, packed with 20% FFAP (esterification product of polyethylene glycol and 2-nitrophthalic acid) on 60–80 DMGS-W support, at 200° with a flow rate of 60 cc/min of helium.

Carboxylic Acids. α-Chlorophenylacetic acid,[25] hydratropic acid,[26] α-methoxy-α-trifluoromethylphenylacetic acid,[27] α-methoxyphenylacetic acid (O-methylmandelic acid),[17] α-methoxy-*o*-chlorophenylacetic acid (O-methyl-*o*-chloromandelic acid),[28] α-*t*-butylphenylacetic acid,[18] and α-trifluoromethylphenylacetic acid[18] were available from published procedures.

1-Indancarboxylic acid[29] (9.0 g, mp 56–57°) was prepared by hydrogenating (30 psi, 10 min, palladium on carbon) indenecarboxylic acid (9.6 g) in ethanol (170 ml). The indenecarboxylic acid[30] (10.3 g, mp 159–160°) was obtained by adding butyllithium in hexane (90 ml, at most 12% by weight, Foote Mineral Co.) to indene (20 g, practical grade) in ether (100 ml), refluxing 30 min, bubbling carbon dioxide into the solution for 20 min, extracting with saturated sodium carbonate (50 ml), acidifying the extract with hydrochloric acid, and then filtering, vacuum subliming, and crystallizing from toluene.

Carbinols. 2,2-Dimethylcyclohexylcarbinol,[31] 3,3-dimethylcyclohexylcarbinol,[31] *l*-menthol, methyl-*t*-butylcarbinol,[32] methylcyclohexylcarbinol,[33] methyl-*n*-hexylcarbinol,[34] methylethylcarbinol,[35] methylisopropylcarbinol,[36] methyltrifluoromethylcarbinol,[3,5] methylphenylcarbinol,[37] phenyl-*t*-butylcarbinol,[37] phenylisopropylcarbinol,[37] phenyltrifluoromethylcarbinol,[5] and trifluoromethyl-*t*-butylcarbinol[5] were available from published procedures or from commercial sources.

Esters. The esters of alkylcarbinols were prepared according to the procedure given for (*R*)-menthyl (*S*)-O-methylmandelate with purification by glpc. The esters of arylcarbinols were prepared according to the procedure given for phenyl-*t*-butylcarbinyl α-trifluoromethylphenylacetate with isolation by column chromatography since, with the exception of the esters of phenyltrifluoromethylcarbinol, they could not be purified by glpc.

Key compounds were subjected to microanalysis which were satisfactory in each case. Allowing for diastereomer nonequivalence, the esters gave nmr spectra consistent, in positions and ratios of the proton signals, with their structures.

(*R*)-Menthyl (*S*)-O-Methylmandelate. A mixture of O-methylmandelic acid (0.40 g, 0.024 mol; $[\alpha]^{20}D$ +136.5 ± 0.5° (*c* 2.24, ethanol), 90.9 ± 0.3% enantiomerically pure), freshly distilled thionyl chloride (5 ml), and anhydrous benzene (50 ml) was refluxed vigorously for 1 hr and then concentrated under reduced pressure. Benzene (20 ml) was added to the residue followed by *l*-menthol (1.5 g, 0.096 mol; $[\alpha]^{20}D$ −49.1 ± 0.2° (*c* 5.26, methanol)) in pyridine (3 ml) and benzene (10 ml); heat was evolved. After the mixture had stood for 3 hr, it was extracted twice with water (20 ml) and once with hydrochloric acid (10 ml, 6 *N*) and the benzene solution vacuum-evaporated. The ester, t_R = 20.5 min, was collected directly from the gas chromatograph in an nmr tube cooled in an isopropyl alcohol and Dry Ice bath.

Anal. Calcd for $C_{19}H_{28}O_2$: C, 75.00; H, 9.26. Found: C, 74.99; H, 9.17.

Phenyl-*t*-butylcarbinyl α-Trifluoromethylphenylacetate (V), Formation with Partial Racemization. A solution of (+)-α-trifluoromethylphenylacetic acid[18] (2.50 g, 0.012 mol; $[\alpha]^{21.5}D$ +73.0 ± 0.4° (*c* 2.63, chloroform)) in benzene (10 ml) and thionyl chloride (10 ml) was refluxed together for 1 hr. After the reaction mixture was concentrated under reduced pressure, phenyl-*t*-butylcarbinol[37] (3.00 g; $[\alpha]^{24.8}D$ +30.7 ± 0.3° (*c* 3.75, acetone)), in pyridine (10 ml) and benzene (10 ml), was added to the residue. After the reaction mixture had stood for 2 days, it was extracted twice with water (20 ml) and once with hydrochloric acid (20 ml, 6 *N*), and the benzene layer concentrated under vacuum. The residue was chromatographed on 10 ml of neutral, active alumina, using carbon tetrachloride solvent, giving an initial 100-ml fraction which was concentrated and rechromatographed twice on 40 ml of alumina to give ester (1.02 g, free of hydroxyl band in the ir spectrum). The ester in carbon tetrachloride gave a proton nmr spectrum consisting of signals at 0.82 (singlet, area 4.7), 0.90 (singlet, area 4.3), 4.20 (quartet, area 1.0, J = 8.5 Hz), 5.49 (two overlapping singlets, area 1.0), and 6.8–7.5 ppm (multiplet, area 10) downfield of internal tetramethylsilane, and a ^{19}F nmr spectrum consisting of signals at 9.00 (doublet, area 1.4, J = 8.5 Hz) and 9.27 ppm (doublet, area 1.6, J = 8.5 Hz) downfield of internal trifluoroacetic acid.

(23) We are especially thankful to Mr. Devens Gust for the synthesis of this compound.
(24) D. L. Dull, Ph.D. Thesis, Stanford University, 1967.
(25) N. Rabjohn, Ed., "Organic Syntheses," Coll. Vol. IV, John Wiley and Sons, Inc., New York, N. Y., 1963, p 171.
(26) S. P. Bakshi and E. E. Turner, *J. Chem. Soc.*, 171 (1961).
(27) D. L. Dull and H. S. Mosher, *J. Am. Chem. Soc.*, **89**, 4230 (1967).
(28) (a) W. Reeve and C. W. Woods, *ibid.*, **82** 4062 (1960); (b) W. Reeve and E. L. Compere, Jr., *ibid.*, **83**, 2755 (1961).
(29) M. Tiffeneau and A. Orekhoff, *Bull. Chim. Soc. France*, 27, 783 (1920).
(30) (a) R. Weissgerber, *Ber.*, **44**, 1436 (1911); (b) H. Cromwell and D. B. Capps, *J. Am. Chem. Soc.*, **74**, 4448 (1952).
(31) E. LaCombe, Ph.D. Thesis, Stanford University, 1952.
(32) R. H. Pickard and J. Kenyon, *J. Chem. Soc.*, **105**, 1120 (1914).
(33) D. Cowan and H. S. Mosher, *J. Org. Chem.*, **28**, 204 (1963).
(34) A. I. Vogel, "Practical Organic Chemistry," 3rd ed, John Wiley and Sons, Inc., New York, N. Y., 1957, p 505.
(35) R. Macleod, H. Prosser, L. Fikentscher, J. Lanyi, and H. S. Mosher, *Biochemistry*, **3**, 838 (1964).
(36) R. H. Pickard and J. Kenyon, *J. Chem. Soc.*, **101**, 620 (1912).
(37) R. Macleod, F. J. Welch, and H. S. Mosher, *J. Am. Chem. Soc.*, **82**, 876 (1960), and references therein.

Anal. Calcd for $C_{20}H_{14}F_3O_2$: C, 68.50; H, 6.04. Found: C, 68.25; H, 6.30.

The other chromatography fractions were combined and concentrated. The residue was sublimed (50°, 1 mm, 2 hr) to give ester (1.03 g, free of hydroxyl band in the ir spectrum). This sample was reduced with lithium aluminum hydride (0.5 g) by standard procedures; the products were isolated by glpc (Dow 710, 5 ft × 0.25 in., 147°, 97 cc/min of He): phenyl-*t*-butylcarbinol (0.36 g, t_R = 9.5 min; $[\alpha]^{20.8}D$ 30.2 ± 0.3° (*c* 5.10, acetone)) and 2-phenyl-3,3,3-trifluoro-1-propanol (0.34 g, t_R = 4.3 min; $[\alpha]^{26.4}D$ −4.32 ± 0.11° (*c* 5.10, chloroform)). The maximum rotation of this latter compound is about $[\alpha]^{20}D$ −38° (chloroform).[24]

(*RS*)-Methylethylcarbinyl (*S*)-α-(Tetrafluorosuccinimido)propionate (XI).[28] Methylethylcarbinyl *l*-alaninate[38] (3.37 g) was added to tetrafluorosuccinyl chloride[39] (6 ml) in a flask, kept at 25° with a cooling bath, while a positive nitrogen pressure was maintained. The mixture was frozen, and the flask was evacuated on a vacuum line. After distilling the volatile materials (1 mm), the system was attached to a diffusion pump; on heating the residue to 100°, the product (2.69 g, 34.2%) collected in a liquid nitrogen cooled trap, t_R = 8.7 min (SE-30, 6.5 ft × 0.25 in., 200°, 60 cc/min of He). This material is very sensitive to moisture.

Anal. Calcd for $C_{11}H_{13}F_4NO_4$: C, 44.15; H, 4.38; N, 4.68. Found: C, 43.97; H, 4.59; N, 4.72.

Acknowledgment. We thank Dr. Lois Durham and Dr. Yoko Kanazawa for their unfailing efforts and cooperation in collecting the nmr spectral data.

(38) R. Rometsch and W. Kuhn, *Helv. Chim. Acta*, **29**, 1483 (1946).

(39) J. J. Padbury and E. L. Kropa, U. S. Patent 2,502,478 (1950).

Carbon-13 Magnetic Resonance Studies of Amino Acids and Peptides[1]

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Contribution from the Department of Chemistry, University of California, Berkeley, California. *Received December 29, 1967*

Abstract: The carbon-13 nuclear magnetic resonances of glycine, diglycine, triglycine, alanine, and alanylglycine have been measured as a function of pH using a double-resonance technique. The resonance positions of these amino acids and peptides can be derived from a suitable set of substituent parameters empirically obtained from carbon-13 measurements on amines and carboxylic acids. The α-carbon resonances are determined by the carbon charge density. We show that charge densities derived from the Del Re–Pullman semiempirical molecular orbital theory can be used to predict the α-carbon resonance positions in the amino acids. The carbon-13 resonances are large compared with the proton resonances. It should eventually be possible to use the large chemical shifts and narrow line widths typical of carbon-13 for macromolecular chemical and conformational studies. The pH-induced carbon-13 shifts in the amino acids are indicative that protonation of the amino group to give NH_3^+ is accompanied by transmission of negative charge from the hydrogen through the carbons to the NH_3^+ group. The carbon charge density remains essentially constant. The charge densities on the carbons may actually become slightly more negative upon protonation. A similar behavior is observed in methylamine. The opposite appears true on ionization of the carboxyl group to give COO^-. Ionization is accompanied by transmission of positive charge from the hydrogen through the carbons to the COO^- group. The carbon charge density remains essentially constant. These experimental observations are consistent with the self-consistent field predictions of charge transmission in NH_4^+ and H_3O^+ from the hydrogens in NH_3 and H_2O through the nitrogen and oxygen to the added proton. The nitrogen and oxygen *electronic* charge densities remain constant or increase upon protonation.

In recent studies of protein folding by proton magnetic resonance, Sternlicht and Wilson[2] and MacDonald and Phillips[3] have observed large upfield shifts of methyl and methylene protons in the folded enzyme. These shifted resonances arise primarily from valine, leucine, and isoleucines in the ring-current fields of neighboring aromatic residues. The detailed pattern of these upfield shifts are significant clues to the protein conformation. Unfortunately, there is no unambiguous method of assigning the resonances. If the X-ray crystal structure is known, and if the structure in solution is essentially identical with the crystal, an approximate reconstruction is possible using the Bovey–Johnson ring-current tables.[4] In the case of lysozyme[2] a reconstruction in quite good agreement with the observed spectrum was indeed achieved by this procedure. However, despite the apparent success achieved in the lysozyme study, any conclusions must be treated with caution. The proton intrinsic line widths in proteins are broad, typically 15 cps or more, while the chemical shifts are small. The methyl resonance positions of the valine, leucine, and isoleucine residues in the unfolded configuration, for example, occur within ~0.1 ppm of each other. The ring-current fields of the aromatic type residues of phenylalanine, tryptophan, and histidine are not presently known. Even if these were known accurately, the intrinsically broad proton lines and the small chemical shifts in the unfolded state make an interpretation of the folded configuration very difficult.

In view of these difficulties we have begun a program of measuring the carbon-13 (C^{13}) nmr of the amino acids and peptides. The C^{13} chemical shifts are generally more than an order of magnitude greater than

(1) This paper was presented in part at the Pacific Conference on Chemistry and Spectroscopy, Anaheim, Calif., Oct 30, 1967.

(2) H. Sternlicht and D. Wilson, *Biochemistry*, **6**, 2881 (1967).

(3) C. C. MacDonald and W. D. Phillips, "Magnetic Resonance in Biological Systems," Pergamon Press, New York, N. Y., 1967, pp 3–27; *J. Am. Chem. Soc.*, **89**, 6332 (1967).

(4) C. E. Johnson, Jr., and F. A. Bovey, *J. Chem. Phys.*, **29**, 1012 (1958).