pounds.[32,33] These particular dipolar repulsions should disappear with departure of the C_1 leaving group, and this factor could be partly responsible for the greater reactivity of methyl pyranosides which have an equatorial rather than an axial methoxy group.[32] The anomeric stabilization of the α over the β anomer should be less important in the furanosides than in the pyranosides, and therefore part of the greater reactivity of α-ribofuranose over α-glucopyranose 1-phosphate could be caused by the differences in the dipolar repulsions in the ground states.

In glycoside hydrolysis the furanoside is approximately 200 times as reactive as the corresponding pyranoside, at 95°.[32] For the acid hydrolysis of ribose 1- and glucose 1-phosphates the relative reactivities are ~400 at 25° in 1.5 *M* acid and 200 for the spontaneous hydrolysis at pH 2.2 at 82°. Because of differences in the activation energies for these reactions the relative reactivities depend on temperature, but none the less they show the similarity of structural effects upon the rates of glycoside and phosphate ester hydrolysis.

The entropy of activation for the acid hydrolysis of ribose 1-phosphate is 7.5 eu less positive than for glucose 1-phosphate (see Results). The entropy differences are very large for the acid hydrolyses of alkyl furanosides and pyranosides, where the values have led some workers to suggest that A2 mechanisms are involved in the hydrolysis of the furanosides,[34,35] although it is generally believed that both sets of compounds are hydrolyzed by A1 mechanisms.[32]

An A2 mechanism cannot be involved in the acid hydrolysis of α-ribose 1-phosphate, because it would be much too slow to be observed under our reaction conditions,[4] and we believe that the differences in activation entropy arise because of differences in the ground state entropies. The furanose ring, like a cyclopentane, should be "entropy rich" because of the low energy barriers to small conformational changes,[33] but much of this freedom will be lost in the transition state because of the rigidity imposed by delocalization of charge into the ring oxygen atom. The pyranose ring is more rigid, and the loss of entropy due to changes in the flexibility of the ring in going to the transition state should be less serious here. The more effective overlap in the furanosides should make the activation energy lower, and these effects should also be important in glycosidic hydrolysis.

Registry No.—II, 59-56-3; IV, 18646-11-2.

(33) C. T. Bishop and F. P. Cooper, *Can. J. Chem.*, **41**, 2743 (1963).

(34) W. G. Overend, C. W. Rees, and J. S. Sequeria, *J. Chem. Soc.*, 3429 (1962).

(35) B. Capon and D. Thacker, *J. Amer. Chem. Soc.*, **87**, 4199 (1965).

A Nuclear Magnetic Resonance Method for Distinguishing α-Amino Acids from β and γ Isomers

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The methyl ester hydrochlorides and N-trityl methyl esters of 24 amino acids have been prepared and their nmr spectra determined. The methyl ester peak of each α-N-trityl ester appeared 0.27–0.97 ppm upfield of the corresponding peak in the untritylated amino ester. Methyl ester peaks in the β and γ relationship to the N-trityl function were shifted only 0.03–0.20 ppm upfield. This difference can be used diagnostically for adjacent amine and ester functions. A discussion of the size of this effect as a function of structure is presented.

In an earlier report[3] we described the preliminary results of an nmr method for distinguishing α-amino acids from isomers having amino groups β or γ to the carboxyl function. It was established that the methyl proton signal of a methyl ester function shifted upfield some 0.2–0.9 ppm when an α-amino group was tritylated. This report extends this work to 24 amino acids and more firmly establishes the necessary spacial relationships between the nitrogen substituent and the ester function.

Table I shows the results of this investigation. Clearly, α-amino ester peaks undergo a much larger diamagnetic shift[4] than β- and γ-amino ester peaks; compare amino acids **1–21** with **22–24**. The more distant ester functions shift upfield from 0.03 for γ-aminobutyric acid (**23**) to 0.21 ppm for anthranilic acid (**24**) including the β-ester group of aspartic acid (**6**), glutamic acid (**13**) and those of the hydroxyaspartic esters (**4** and **8**). Actually, anthranilic ester is a special case in which the benzene ring forces the β-amino group to be in the same plane as the ester function. The shielding effect of the N-trityl group was therefore enhanced because of its greater proximity to the ester. The largest diamagnetic shift of β-carbomethoxyl protons in a free-rotating[5] system was that of β-alanine (0.10 ppm).

The α-amino acid esters can be arranged in two distinct groups—those showing upfields shifts of 0.25–0.27 ppm and those having shifts of 0.59–0.97 ppm. The first and smaller group consists of glycine, sarcosine and proline.[6] All of the remaining esters fall into the

(1) Abstracted from the Master of Science Thesis (1967) and the Ph.D. Dissertation (1968) submitted to the graduate school of the University of Georgia by M. W. Haskell and R. G. Webb, respectively.

(2) To whom inquiries about this paper should be sent.

(3) C. H. Stammer and R. G. Webb, *Tetrahedron Lett.*, 4895 (1966).

(4) The upfield shift was due to tritylation and not just a neutralization of charge on the amino group or to a change in solvent (D_2O to $CDCl_3$). When alanine methyl ester hydrochloride was converted into the free amino ester in $CDCl_3$ a shift of only 0.10 ppm occurred. The methyl ester peak positions of leucine methyl ester hydrochloride were identical when measured in D_2O and $CDCl_3$.

(5) The α-hydroxy-β-amino esters (**4** and **8**) can not be considered entirely free-rotating systems due to H bonding.

(6) An impure sample of N-methylalanine ester also showed a $\Delta\nu$ of 0.30 ppm on tritylation.

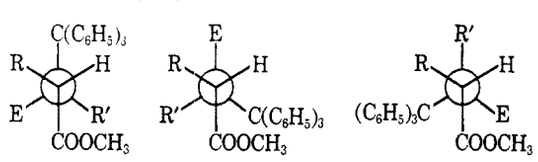
TABLE I

CHEMICAL SHIFT VALUES OF CARBOMETHOXYL PROTONS[a]

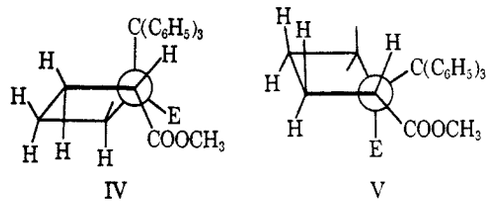
No.	Amino acid	Methyl ester[b] hydrochloride α	Methyl ester[b] hydrochloride β	N-Trityl-[c] methyl ester α	N-Trityl-[c] methyl ester β	Δν α	Δν β
1	Glycine	3.87[d]		3.60		0.27	
2	Sarcosine	4.00		3.75		0.25	
3	Proline	3.88		3.62		0.26	
4	*erythro*-HAA[e]	3.83	3.83	3.26	3.66	0.57	0.17
5	Serine	3.87		3.23		0.64	
6	Aspartic acid	3.86[f]	3.76	3.20	3.60	0.66	0.16
7	Alanine	3.85		3.17		0.68	
8	*threo*-HAA[e]	3.91[f]	3.87	3.22	3.80	0.69	0.07
9	Cystine	3.90		3.20		0.70	
10	Methionine	3.87		3.17		0.70	
11	Cysteine	3.87		3.20[g]		0.70	
12	Leucine	3.84		3.13		0.71	
13	Glutamic acid	3.88[f]	3.75[h]	3.15	3.67[h]	0.73	0.08[h]
14	Lysine	3.84		3.08[g]		0.76	
15	Arginine	3.87		3.10		0.77	
16	Valine	3.88		3.10		0.78	
17	Isoleucine	3.85		3.06		0.79	
18	Histidine	3.90		3.06[g]		0.84	
19	Phenylanine	3.90		3.05		0.85	
20	Tryptophan	3.67[i]		3.00		0.92	
21	Tyrosine	3.90		2.93		0.97	
22	β-Alanine		3.77		3.67		0.10
23	γ-Aminobutyric acid		3.70[h]		3.73[h]		−0.03[h]
24	Anthranilic acid		4.12[h]		3.91[h]		0.21[h]

[a] All spectra were determined on a Varian A-60 spectrometer using the usual side-band modulation technique for calibration. [b] Spectra determined in D_2O. [c] Spectra determined in $CDCl_3$. [d] Chemical shifts are given in parts per million (δ). In D_2O, 3-(trimethylsilyl)propanesulfonic acid sodium salt was used and in $CDCl_3$, tetramethylsilane was used as internal standard. [e] HAA is an abbreviation for β-hydroxy-DL-aspartic acid. [f] The most downfield methyl peak was chosen as that of the α-amino function. The β- and γ-methyl esters of **6** and **13** were synthesized to confirm this assignment. [g] This a dityrityl compound. [h] For γ-carbomethoxyl protons. [i] Tryptophan methyl ester hydrochloride was insoluble in D_2O and its nmr spectrum was determined in DMSO-d_6. When the nmr spectra of phenylalanine and tyrosine methyl esters were determined in DMSO-d_6, the ester peaks were diamagnetically shifted 0.25 ppm. This correction was applied to tryptophan ester before calculation of Δν.

second group with nine having Δν values within the narrow range 0.68–0.77 ppm. This sharp separation into two groups can be rationalized by examining Newman projections of the tritylated esters drawn about the Cα-N bond. The average distance between the trityl group and carbomethoxyl protons will determine the magnitude[7] of Δν. If we assume that the bonds to nitrogen and the orbital containing the nonbonded electron pair[8] (E) are situated tetrahedrally, conformations I, II, and III can be drawn. The trityl

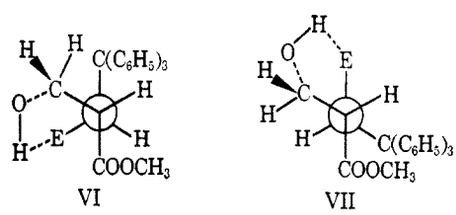


group, having the largest volume, will determine the stabilities of these conformers. We should therefore expect the stability order to be I ≃ II ≫ III when R′ = H. Conformer III will be extremely unstable when R is larger than hydrogen since the trityl group incurs two *gauche* interactions in this case. In the case of glycine (**1**), where R = R′ = H, conformer I represents the most stable form. Thus we can infer that the Δν for glycine (0.27 ppm) is approximately the minimum diamagnetic shift possible since it corresponds to a conformation in which the average distance between the carbomethoxyl and trityl groups is a maximum.[9] Proline, where R and R′ are constrained in a five-membered ring, shows a similarly small Δν which is not so easily explained. Dreiding models show clearly that the massive trityl group must exist in a pseudoequatorial conformation (V) where interactions with the pseudoaxial hydrogen atoms becomes small (IV). Consequently, the trityl and carbomethoxyl



groups cannot be *anti*, but must be at least pseudo-*gauche* (V). Since, however, the configuration about nitrogen is not fixed, angle distortion may allow a greater distance between the groups in question than models can show.[10]

The second and largest group of α-amino acid esters show diamagnetic shifts of 0.56–0.97 ppm upon tritylation. Each of these must be an equilibrium mixture of conformers I and II, where R ≠ H, R′ = H. The size of R will determine the composition of the conformer mixture, since the larger the *gauche* interaction energy between R and the trityl group in conformation I, the greater will be the population of conformation II. Increasing the population of II decreases the distance between trityl and carbomethoxyl groups and should increase the Δν value. The actual volume of R, however, may not be the only consideration to be taken into account. The β-substituted amino esters, **4** and **5**, show somewhat smaller and esters **16–21** show somewhat larger Δν values than the β-unsubstituted esters. Some rationale for the low values obtained from serine and *erythro*-β-hydroxy-DL-aspartic acid can be offered by assuming hydrogen bonding between the β-hydroxyl group and the nonbonded nitrogen electrons as shown for serine (VI and VII). Actually, the H



bonding should have little effect on the Δν value except that it might effectively reduce the size of the hydroxymethyl group by restricting its rotation. Cysteine, although it has a β-mercapto group, does not have a similarly small Δν value because the mercapto function is also tritylated by our procedure.

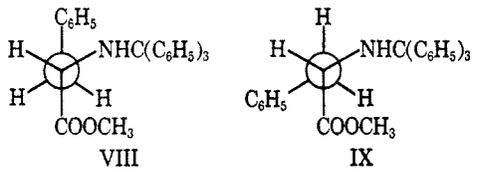
(7) (a) L. M. Jackman, "Application of NMR Spectroscopy in Organic Chemistry," MacMillan, New York, N. Y., 1959, p 125; (b) A. A. Bothner-by and J. A. Pople, *Ann. Rev. Phys. Chem.*, **15**, 43 (1964); (c) C. E. Johnson and R. W. Bovey, *J. Chem. Phys.*, **29**, 1012 (1958).

(8) For the sake of clarity we have assumed that the electron pair is configurationally fixed. This is, of course, not true, but this error does not affect the subsequent arguments.

(9) Using the isoshielding curves of Johnson and Bovey,[6c] we can estimate that the average distance between the interacting groups should be about 6.5 Å. This distance is quite compatible with measurements of conformer I made on Dreiding models.

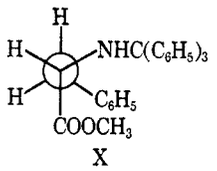
(10) Attempts to tritylate pipecolic acid methyl ester, the six-membered ring homolog of proline, were unsuccessful even under forcing conditions.

All of the other β-substituted amino acids, **16–21**, have $\Delta\nu$ values larger than the β-unsubstituted compounds. Certainly, the large size of R (isopropyl, *sec*-butyl, etc.) in conformers I and II (R′ = H) due to branching should indeed cause a decrease in the population of I and a corresponding increase in the population of II thereby increasing $\Delta\nu$. The aromatic amino acids, **18–21**, have the largest $\Delta\nu$ values of all the compounds examined. The size of the β substituent is undoubtedly partly responsible for this fact, but very probably the β substituent is also exercising a shielding effect[11] on the α-carbomethoxyl protons. Newman projections (VIII–X) about the C_α–C_β bond exemplify



VIII

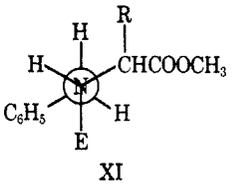
IX



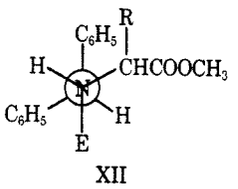
X

this clearly. If we assume that interaction with the hugh trityl amino group is the determing factor, conformer IX, where this function is flanked by hydrogen atoms, should be the most stable. The β-phenyl substituent is than *gauche* to the ester function and can exert a shielding effect. This argument explains nicely the considerable difference in $\Delta\nu$ of phenylalanine and tyrosine where the β substituents are phenyl and *p*-hydroxyphenyl. Steric differences here are negligible, but the shielding effect of the hydroxylated phenyl group should be somewhat larger than that of the unsubstituted phenyl because of the enhanced electron density in the hydroxylated ring. We hope to investigate further the effect of ring substituents on shielding.

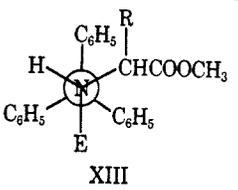
In order to establish the number of benzene rings necessary for the diamagnetic shift of the ester protons to be observable, N-benzyl- and N-benzhydrylaspartic acid dimethyl esters were synthesized and their nmr spectra were examined. It is clear from the data shown in Table II that a benzhydryl group is sufficient to distinguish between α- and β-carbomethoxyl protons, but that the triphenylmethyl group gives a much more definitive answer. Newman projections about the N–$C(C_6H_5)_3$ bond show clearly why this is true. The N-benzyl compound can exist as conformer XI with the shielding benzene ring *anti* to the acetic acid side chain, whereas both benzhydryl (XII) and trityl (XIII) groups cause the side chain to be flanked by at least one benzene ring.



XI



XII



XIII

All of the amino esters and N-alkylated esters described in this work were isolated and redissolved in the appropriate solvents for nmr determination. This, however, is completely unnecessary. When only small quantities of an unknown are available, it can be esterified and tritylated on a small scale and the nmr spectra can be determined after each operation. Such a procedure using alanine as the substrate gave the same $\Delta\nu$ value as obtained when the intermediates were isolated.

The general proposition of substituting a highly shielding group for a replaceable hydrogen atom and examining its effect on the nmr spectrum of a compound can be of considerable value in structure determination. Work toward the development of more effective shielding groups which can be used with several functional groups is underway.

Experimental Section

The nuclear magnetic resonance spectra were taken on a Varian Associates Model A60 spectrometer, calibrated by the side band modulation technique, with tetramethylsilane as an internal standard. Melting points were taken on a Nalge hot stage and are corrected. Infrared spectra were recorded on a Perkin-Elmer Model 137 Infracord. The amino acids were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, unless otherwise stated.

Esterification of the Amino Acids.—A typical esterification involved 10 mmol of amino acid and 10 mmol of thionyl chloride. The thionyl chloride was added to 25 ml of methanol slowly with cooling and the amino acid was added to the solution. The solution was refluxed 4 hr and the solvent was evaporated giving the crude methyl ester hydrochloride which was triturated with ether at 0° until excess dimethyl sulfite was removed (*ca.* 4 hr). The resulting solid product was collected and dried under high vacuum to yield 85–99% crude methyl ester hydrochloride. The crude material was recrystallized from 25 ml of hot methanol by slow addition of 100 ml of ether followed by cooling 0°. The crystals were collected, washed twice with a 5:1 ether–methanol

TABLE II

CARBOMETHOXYL CHEMICAL SHIFT VALUES FOR DERIVATIVES OF ASPARTIC ACID

Methyl ester·HCl[a]		N-Benzyl- methyl ester[b]				N-Benzhydryl- methyl				N-Trityl-methyl ester			
HCl in D_2O		in $CDCl_3$		Δν		ester in $CDCl_3$		Δν		in $CDCl_3$		Δν	
α	β	α	β	α	β	α	β	α	β	α	β	α	β
3.86	3.76	3.76	3.68	0.10	0.08	3.62	3.66	0.24	0.10	3.20	3.60	0.66	0.16

[a] In D_2O, δ in parts per million downfield of 3-(trimethylsilyl)propanesulfonic acid sodium salt. [b] In $CDCl_3$, δ in parts per million downfield of tetramethylsilane.

(11) The shielding effect of aryl groups has been invoked to explain the differences in nmr spectra of various diastereomers; cf. G. M. Whitesides, D. Holtz, and J. D. Roberts, *J. Amer. Chem. Soc.*, **86**, 2628 (1964); D. Y. Curtin and S. Dayagi, *Can. J. Chem.*, **42**, 867 (1963), M. Barbieux and R. H. Martin, *Tetrahedron Lett.*, 2919 (1965); J. B. Hyne, *J. Amer. Chem. Soc.*, **81**, 6058 (1959).

solution, once with ether and dried under high vacuum. Yields ranged from 75 to 85% of theoretical.

Tritylation[12] of the Amino Acid Methyl Ester Hydrochlorides.—Methyl ester hydrochloride (5 mmol) was dissolved in 15 ml of chloroform (dried over CaH_2) and neutralized with 10 mmol of triethylamine. To this mixture, 4.9 mmol of chlorotriphenylmethane was added and the solution was refluxed 12 hr. The solution was then washed twice with water, dried with anhydrous magnesium sulfate and the solvent was removed *in vacuo*. Absolute ethanol (10 ml) was added and the solution was again evaporated to dryness. The resulting crude material was crystallized from a minimum of warm (40°) methanol. Yields ranged from 50 to 70% of theoretical. See Table III for analytical data.

TABLE III

ANALYTICAL DATA ON NEW N-TRITYLAMINO ACID METHYL ESTERS[a]

Trityl-methyl ester	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	Mp, °C
Sarcosine	79.97	6.71	4.05	80.12	6.94	4.10	112–114
L-Proline	80.83	6.78	3.77	81.43	6.89	4.14	118–120
DL-Aspartic acid	74.42	6.25	3.47	74.83	6.68	2.96	Oil
γ-Aminobutyric acid	80.19	7.01	3.90	80.53	7.22	3.43	74–76
L-Histidine	82.66	6.01	6.43	82.00	6.03	6.59	120–124
L-Valine	80.40	7.29		80.34	7.32		Oil[b]
L-Isolencine	80.59	7.54	3.61	80.61	7.72	3.63	Oil[b]
L-Leucine	80.59	7.54	3.61	80.39	7.66	3.64	Oil[b]
DL-Serine	76.43	6.41	3.88	76.43	6.43	3.86	144–146
DL-Glutamic acid	74.79	6.52	3.36	74.70	6.50	3.41	94–96

[a] All other esters and trityl derivatives reported in this paper had melting points in agreement with those found in J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2, John Wiley & Sons, Inc., New York, N. Y., 1961, pp 909, 929. [b] These were purified by gas chromatography on a 5% silicone on firebrick column.

Direct Procedure for Determination of Δν without Isolation of Intermediates.—To a solution of 124 mg (1.12 mmol) of thionyl chloride in 5 ml of methanol, 100 mg (1.12 mmol) of DL-alanine was added. This solution was refluxed for 2 hr and the solvent was evaporated *in vacuo*. The remaining oil was triturated with several 10-ml portions of cold ether until the methyl ester solidified. The product was filtered and dried under high vacuum. The resulting dry DL-alanine methyl ester hydrochloride was divided into 50- and 100-mg portions. The 50-mg portion was dissolved in 0.4 ml of deuterium oxide and the nmr spectrum determined: $COOCH_3$, 231 cps (δ 3.85). The 100-mg portion was dissolved in 5 ml of chloroform (dried over CaH_2) and 144 mg (1.44 mmol) of triethylamine and 201 mg (0.72 mmol) of trityl chloride were added to the solution. This mixture was refluxed for 4 hr and the solvent evaporated *in vacuo*. Absolute ethanol (2 ml) was added to the residue and the mixture was again evaporated. The residue was partially dissolved in 5 ml of ether and the insoluble triethylamine hydrochloride was filtered. The filtrate was evaporated *in vacuo* and 70 mg of the residue was dissolved in 0.3 ml of deuteriochloroform and the nmr spectrum determined: $COOCH_3$, 190 cps (δ 3.17). Compare the chemical shifts found for the methyl ester protons in the two spectra; *i.e.*, $\Delta\nu = \nu_{ester} - \nu_{trityl\ ester}$.

Dimethyl N-Benzyl-DL-aspartate Hydrochloride.[13]—A solution containing 49 g (0.5 mol) of maleic anhydride and 107 g (1.0 mol) of benzylamine in 200 ml of water was refluxed for 24 hr. The water was removed *in vacuo* and the solid residue was triturated with ether and crystallized from methanol. This product was dissolved in 400 ml of methanol and dry hydrogen chloride was passed through the solution for 15 min without cooling. After standing overnight at room temperature the solution was refluxed for 4 hr and evaporated to dryness *in vacuo*. The crude product was crystallized from a minimum of hot methanol yielding 33.37 g (25%): mp 149–153°; nmr (D_2O) δ 4.07 (s, α-$COOCH_3$), 3.80 (s, β-$COOCH_3$), 4.43 (s, $C_6H_5CH_2N$–); ir (Nujol) 5.75 (C=O) and 2.9 μ (NH).

Anal. Calcd for $C_{13}H_{18}ClNO_4$: C, 54.27; H, 6.30; N, 4.87. Found: C, 54.46; H, 6.42; N, 4.83.

Dimethyl N-Benzhydryl-DL-asparate Hydrochloride.[14]—A solution of 5.39 g (54 mmol) of maleic anhydride in 15 ml of absolute methanol was refluxed for 30 min. The solvent was removed *in vacuo* and the residue immediately treated with 15 ml of dry pyridine and 9.2 g (50 mmol) of benzhydrylamine. This solution was refluxed for 4 hr and the pyridine was removed *in vacuo*. The red, oily residue was triturated with 100 ml of ether at 0°, the solid product filtered and dried under high vacuum: yield 10.96 g (70%); mp 192–194°. The crude product, 1.47 g (5 mmol), was esterified using diazomethane in 100 ml of methanol. After removal of the solvent *in vacuo*, the crude product was dissolved in 25 ml of dry ether and the solution was saturated with dry hydrogen chloride. The precipitated solid was collected, dried under vacuum and crystallized from 5 ml of chloroform by slow addition of an equal volume of ether and cooling to 0°. The product, dimethyl N-benzhydryl-DL-aspartate hydrochloride, was collected: yield 1.26 g (72%); mp 135° dec; ir (Nujol) 3500 (NH), 1755 and 1740 cm^{-1} (C=O); nmr ($CDCl_3$) δ 3.77 (s α-$COOCH_3$), 3.63 (s, β-$COOCH_3$) and 5.75 [s, $(C_6H_5)_2$-CHN–].

Anal. Calcd for $C_{19}H_{22}ClNO_4$: C, 62.71; H, 6.09; N, 3.85. Found: C, 62.79; H, 5.93; N, 3.83.

***erythro*-β-Hydroxy-DL-aspartic Acid Dimethyl Ester Hydrochloride.**[15]—A solution of dimethyl sulfite in methanol was prepared by the addition of 34 ml (0.48 mol) of thionyl chloride to 230 ml of ice-cold methanol (stirred magnetically). To this solution was added 23.0 g (0.15 mol) of *erythro*-β-hydroxy-DL-aspartic acid,[15] and the mixture was refluxed 6 hr. The solution was evaporated on a rotary evaporator, and the residue was redissolved in *ca.* 100 ml of methanol and the solution again evaporated to dryness. This process was repeated twice to remove excess hydrogen chloride. The resulting solid was pumped mechanically for 2 hr and dissolved in the minimum amount of hot methanol. The solution was cooled and diluted with ether until crystals appeared. Slow dilution with ether as crystallization proceeded gave 25.0 g (76%) of crude *erythro*-β-hydroxy-DL-aspartic acid dimethyl ester hydrochloride: mp 148–151°; ir (KBr) 3240 (NH_3^+), 1750 cm^{-1} (C=O); nmr (D_2O) δ 3.83 (s, 6, $-OCH_3$), 4.75 and 4.85 ppm (m, 2, J = 2.8 Hz, C_β-H, C_α-H. A sample recrystallized three times from methanol–ether for analysis melted at 152–153°.

Anal. Calcd for $C_6H_{12}NO_5Cl$: C, 33.73; H, 5.66; N, 6.56. Found: C, 33.49; H, 5.78; N, 6.71.

***threo*-β-Hydroxy-DL-aspartic Acid Dimethyl Ester Hydrochloride.**[15]—The procedure for synthesis of the *threo* ester hydrochloride was the same as that described for the *erythro* isomer: mp 134–136°; ir (Nujol) 3.05 (OH), 3.20 (NH_3^+), and 5.75 μ (C=O); nmr (D_2O) δ 3.91 (s, 3, $-OCH_3$), 3.87 (s, 3, $-OCH_3$), 4.48 and 4.91 ppm (m, 2, J = 3.0 Hz, $-C_\beta$-H, C_α-H). Recrystallization of the crude product from methanol–ether gave an analytical sample, mp 134–136°.

Anal. Calcd for $C_6H_{12}NO_5Cl$: C, 33.73; H, 5.66; N, 6.56; Cl, 16.60. Found: C, 33.95; H, 5.69; N, 6.85; Cl, 16.53.

N-Trityl-*erythro*-β-hydroxy-DL-aspartic Acid Dimethyl Ester.[15]—A solution containing 7.5 g (35 mmol) of *erythro* ester hydrochloride, 10.5 ml (76 mmol) of triethylamine, and 9.6 g (34 mmol) of trityl chloride in 60 ml of pyridine was stirred at room temperature for 6.5 hr. It was diluted with 450 ml of water, extracted with three 150-ml portions of chloroform, and the combined extracts were washed once with 100 ml of water and dried over anhydrous magnesium sulfate. Evaporation of this solution gave a viscous oil, a sample of which was triturated with cyclohexane giving a solid product: mp 93–96°; ir (KBr) 2.90 (OH), 2.95 (NH), and 5.75 μ (C=O); nmr ($CDCl_3$) δ 3.26 (s, $-OCH_3$), 3.66 (s, $-OCH_3$), 3.40 (s, 1, –OH) and 7.33 ppm [m, 15, $(C_6H_5)_3$]. Only half the AB quartet expected for Hα and Hβ was resolvable even at a sweep width of 50 cps. This signal appeared at 4.27 ppm; $J_{\alpha\beta}$ = 3.0 cps.

Anal. Calcd for $C_{25}H_{25}NO_5$: C, 71.58; H, 6.01; N, 3.34. Found: C, 70.26; H, 5.65; N, 3.16.

We have encountered considerable difficulties in obtaining acceptable elemental analyses of esters of N-trityl amino acids. Spectral analysis and conversion of the above trityl ester to the O-mesylate, which had acceptable analytical values, confirmed the structure of the *erythro*-N-trityl ester.

N-Trityl-*threo*-β-hydroxy-DL-aspartic acid dimethyl ester[15] was

(12) This is a modification of the method of Zervas, *cf.* L. Zervas and D. M. Theodoropoulos, *J Amer. Chem. Soc.*, **78**, 1359 (1956).

(13) This is a modification of the method of F. H. McMillan and N. F. Albertson, *ibid.*, **70**, 3779 (1948).

(14) An adaptation of the method of A. Zilkha and M. D. Bachi, *J. Org. Chem.*, **24**, 1096 (1959).

(15) Prepared by C. W. Jones, III, in these laboratories.

prepared by the same procedure described for the *erythro* isomer; yield 11.1 g (97%), mp 173–175°; ir (Nujol) 3.80 (OH), 3.95 (NH), 5.75 and 6.25 μ (C=O); nmr ($CDCl_3$) δ 3.22 (s, 3, $-OCH_3$) 3.80 (s, 3, $-OCH_3$), and 7.34 ppm [m, 15, $(C_6H_5)_3$]. Again, only half the AB quartet was visible at 4.27 ppm, $J_{\alpha\beta}$ = 2.2 cps.

Anal. Calcd for $C_{25}H_{25}NO_5$: C, 71.58; H, 6.01; N, 3.34. Found: C, 71.41; H, 5.89; N, 3.32.

Registry No.—**1** Me ester HCl, 5680-79-5; N-trityl **1** Me ester, 10065-71-1; **2** Me ester HCl, 13515-93-0; N-trityl **2** Me ester, 13515-73-6; **3** Me ester HCl, 2133-40-6; N-trityl **3** Me ester, 13515-74-7; **4** Me ester HCl, 18598-50-0; N-trityl **4** Me ester, 17267-82-2; **5** Me ester HCl, 5619-04-5; N-trityl **5** Me ester, 13515-76-9; **6** Me ester HCl, 14358-33-9; N-trityl **6** Me ester, 13515-78-1; **7** Me ester HCl, 2491-20-5; N-trityl **7** Me ester, 18598-57-7; **8** Me ester HCl, 13515-98-5; N-trityl **8** Me ester, 13515-79-2; **9** Me ester HCl, 18598-59-9; N-trityl **9** Me ester, 18598-60-2; **10** Me ester HCl, 2491-18-1; N-trityl **10** Me ester, 18598-62-4; **11** Me ester HCl, 18598-63-5; N-trityl **11** Me ester, 18598-64-6; **12** Me ester HCl, 7517-19-3; N-trityl **12** Me ester, 18598-66-8; **13** Me ester HCl, 13515-99-6; N-trityl **13** Me ester, 13515-80-5; **14** Me ester HCl, 13515-95-2; N-trityl **14** Me ester, 18598-70-4; **15** Me ester HCl, 18598-71-5; N-trityl **15** Me ester, 18598-72-6; **16** Me ester HCl, 6306-52-1; N-trityl **16** Me ester, 18598-73-7; **17** Me ester HCl, 18598-74-8; N trityl **17** Me ester, 18598-75-9; **18** Me ester HCl, 18684-16-7; **19** Me ester HCl, 7524-50-7; N-trityl **19** Me ester, 18598-80-6; **20** Me ester HCl, 7524-52-9; N-trityl **20** Me ester, 18598-78-2; **21** Me ester HCl, 3417-91-2; N-trityl **21** Me ester, 18621-06-2; **22** Me ester HCl, 3196-73-4; N-trityl **22** Me ester, 13515-81-6; **23** Me ester HCl, 13031-60-2; N-trityl **23** Me ester, 14470-68-9; **24** Me ester HCl, 13516-02-4; N-trityl **24** Me ester, 14357-95-0; dimethyl N-benzyl-DL-aspartate hydrochloride, 18598-81-7; dimethyl N-benzhydryl-DL-aspartate hydrochloride, 18598-82-8.

New Structures from the Enzymic Dehydrogenation of Lignin Model *p*-Hydroxy-α-carbinols

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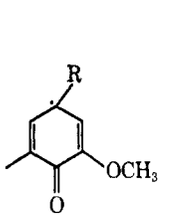
The dehydrogenation of α-ethylvanillyl alcohol (**2**) in aqueous solution with peroxide and peroxidase produced the novel dibenzo[*d*,*f*][1,3]dioxepin **3** with the side chain being expelled as propionaldehyde. The important lignin model guaiacylglycerol β-guaiacyl ether (**12**) undergoes the same type of reaction. Indications are that other *p*-hydroxybenzyl alcohols and some ethers react in a similar fashion. This reaction is significant in consideration of the structural features of the lignin macromolecule and also serves to indicate that the cyclohexadienone forms of phenoxy radicals **1** may be important contributors in the biosynthesis process.

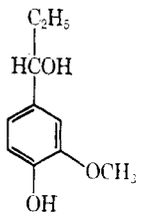
We have reported that on enzymic dehydrogenation *p*-hydroxypropiophenones give rise to the formation of novel *o*,*p*′-biphenyls as well as side chain transfer reactions with formation of esters of aliphatic acids or the free acids themselves. These reactions are thought to come about through coupling of *p*-cyclohexadienone radicals **1** with other mesomeric radicals which rearomatize through side chain transfer or expulsion to form biphenyl and polyphenyl compounds.[2]

This study has now been expanded to some *p*-hydroxy-α-carbinol compounds and preliminary results showing the involvement of radical **1** and side chain expulsion and dioxepin formation have been reported.[2a] Reviews on the importance and detection of α-carbinol groups in lignin with both free and etherified *p*-hydroxyl groups have been published.[3-5]

When α-ethylvanillyl alcohol (**2**) was dehydrogenated in aqueous solution using hydrogen peroxide and peroxidase, a distinctive odor of propionaldehyde could be detected after 2–3% of the peroxide had been added. By using 2.1 equiv (1.05 mol) of peroxide per mole of phenol, the novel dibenzo[*d*,*f*][1,3]dioxepin **3** was formed in a yield corresponding to 67% of the theoretical.



1



2

The proposed mechanism of formation of the dioxepin **3** is through the formation first of the *o*,*o*′-dihydroxybiphenyl **4** of α-ethylvanillyl alcohol followed by further dehydrogenation of **4** to give the phenoxy radical **5** of the biphenyl which couples with the cyclohexadienone mesomeric form of the same radical **6** to give the dienone tetramer **7**. This then rearomatizes through loss of propionaldehyde to **8**, which on further dehydrogenation gives phenoxy and *p*-cyclohexadienone radicals. These through intramolecular radical coupling give dioxepin **3** (Scheme I).

(1) Maintained at Madison, Wis., in cooperation with the University of Wisconsin.

(2) (a) J. C. Pew and W. J. Connors, *Nature*, **215**, 623 (1967); (b) J. C. Pew and W. J. Connors, *J. Org. Chem.*, **34**, 585 (1969).

(3) E. Adler, *Paperi Puu*, **11**, 634 (1961).

(4) E. Adler, H. D. Becker, T. Ishihara, and A. Stamvick, *Holzforschung*, **20**, 3 (1966).

(5) J. Marton and E. Adler, *Tappi*, **46**, 92 (1963).