k_2/k_3 between 0.5–0.1 M. This factor was used to estimate k_2/k_3 at 0.1 M total salt for the other three less reactive anions (see bromide ion data above).

The ratios of k_2/k_3 (M^{-1}), listed in Table I, were determined at 25°. At 37°, corresponding data (0.1 M total salt) for nine anions are as follows: isopropyl methylphosphonate, 2.0; fluoride, 2.1; chloride, 3.09; acetate, 3.42; bromide, 3.89 (standard); azide, 4.7; thiocyanate, 4.86; ethyl methylthiophosphonate, 5.5; iodide, 5.01.

Nmr Studies.—Nmr studies were made of the solvolysis of a sister agent, methyl 3-(trimethylammonium perchlorate)propane sulfonate,[2] in deuterium oxide, and the alkylation of sodium isopropyl methylphosphonate was studied in chloroform and dueterium oxide. All studies were carried out in an nmr tube using a Varian DP-60 operating at 60 Mcps. Hydrolysis of a saturated solution (7%) of the agent in deuterium oxide was followed by the disappearance of the signal due to protons on the $SOCH_3$ group at τ 6.1 and the appearance of the signal due to methanol at τ 6.6. Alkylation of isopropyl methylphosphonate anion was studied with the same substrate, but the substrate anion was isopropyl methylphosphonate instead of perchlorate. The chloroform solution initially showed the presence of $SOCH_3$, but, after several hours, a $POCH_3$ doublet appeared and the $SOCH_3$ peak decreased in intensity. A solid precipitated, identified as 3-(trimethylammonium)propane sulfobetaine. Methyl isopropyl methylphosphonate was isolated; the infrared and nmr spectra of the compound in carbon tetrachloride were identical with those of an authentic sample. The nmr spectrum contained a POCH multiplet centered at τ 5.35 (one proton), and a $POCH_3$ doublet at τ 6.36 (J = 11 cps). A PCH_3 doublet occurred at τ 8.66 (J = 18 cps), and a CCH_3 doublet (two methyls, six protons) appeared at τ 8.71 (J = 6 cps). This experiment was repeated in deuterium oxide at a concentration of substrate of *ca.* 20%. Although solvolysis predominated, the $POCH_3$ peak was observed; methyl isopropyl methylphosphonate was isolated and confirmed by an nmr spectrum (CCl_4). A control study showed that isopropyl methylphosphonic acid was not esterified by methanol.

Registry No.—1-Methyl-3-(methylsulfonate)pyridinium perchlorate, 21876-83-5.

Electron Spin Resonance of Trityl Alkyl Nitroxides. Spin-Labeled Amino Acids[1]

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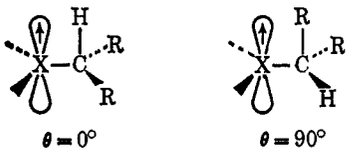
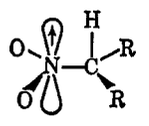
The electron spin resonance spectra of a variety of trityl alkyl nitroxides have been obtained by the reaction of *p*-nitroperbenzoic acid with the appropriate secondary amine in dioxane (or aqueous solution in some cases). Included are the nitroxides of certain trityl amino acids (or esters) and trityl dipeptides (or esters). The β-hydrogen hyperfine coupling constant is characteristic for the structure of the alkyl group. Evidence is obtained for restricted freedom of motion about the β-carbon atom. Different stable conformations are detected in the nitroxides of certain trityl dipeptides. Trityl alkyl (but no aryl) nitroxides with substituted β-carbon atoms dissociate to produce trityl radicals. Preliminary evidence indicates that this reaction may be reversible.

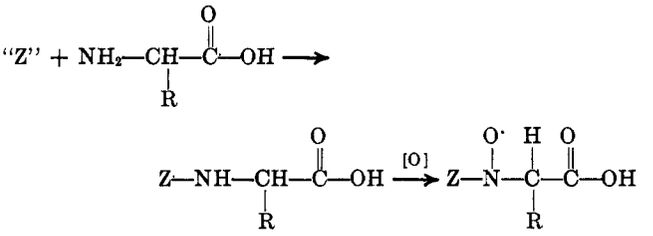
The angular dependence of β-hydrogen coupling in alkyl-substituted free radicals is well known to follow a $\cos^2\theta$ relationship[2] where θ is the dihedral angle between the orbital containing the unpaired electron (normally a p orbital) and the carbon–hydrogen bond of the attached alkyl group. Maximum coupling is



observed when θ = 0° and minimum coupling when θ = 90°. β-Hydrogen coupling thus depends on substitution since bulky groups restrict the conformations of the radical to those conformations with minimum steric crowding.[3] In most cases the β-hydrogen coupling decreases with increased substitution. For example in nitromethane, nitroethane, and 2-nitropropane radical anions, the β-hydrogen coupling is 11.4, 9.75, and 4.60 G for the methyl, methylene, and methine hydrogens, respectively.[4] Some variation in coupling is also observed in differently substituted radicals with methylene or methine hydrogens; *e.g.* for the methylene and methine hydrogens of 1-nitro- and 2-nitrobutane the coupling is 9.98 and 3.19 G, respectively.[4]



The object of this work is to investigate whether the β-hydrogen coupling in a suitably substituted radical is sensitive enough to a change in the bulk of the β-alkyl group to serve as a diagnostic tool for structure determination. Specifically, it is our aim to see if individual amino acids or peptides can be identified from the esr spectrum of a suitably substituted amino acid nitroxide produced by the oxidation of the N-substituted amino acid. The most convenient and only



nitrogen substituent (Z) which gave the desired results was the triphenylmethyl (trityl) group (see Experimental Section for other attempts). This group is frequently used as a "blocking" group in peptide synthesis. Many of the N-trityl amino acid esters used in this work were kindly supplied to us by Professor

(1) This work was supported by the Atomic Energy Commission, Contract AT-(40-L)-2851. Grateful acknowledgment is hereby made.

(2) C. Heller and H. M. McConnell, *J. Chem. Phys.*, **32**, 1535 (1960); see also M. C. R. Symons, *J. Chem. Soc.*, 277 (1959).

(3) For a review of conformational studies in electron spin resonance spectroscopy, see D. H. Geske, *Prog. Phys. Org. Chem.*, **4**, 125 (1967).

(4) L. H. Piette, P. Ludwig, and R. N. Adams, *J. Amer. Chem. Soc.*, **84**, 4212 (1962).

TABLE I
HYPERFINE SPLITTING CONSTANTS OF TRITYL NITROXIDES WITH β-METHYLENE HYDROGENS[a]

$(C_6H_5)_3C-\underset{\displaystyle O\cdot}{\underset{|}{N}}-X$

X	Registry no.	A^N	A_β^H	A_γ^H	R value[b]
CH_3	21746-55-4	15.33	9.96		
CH_3CH_2	21746-56-5	15.18	8.82	0.50	0.89
$CH_3CH_2CH_2$	21746-57-6	15.18	8.95	0.53	0.90
$CH_3CH_2CH_2CH_2$	21746-58-7	15.13	8.88	0.48	0.89
$(CH_3)_2CH-CH_2$	21746-59-8	15.12	9.68	0.46	0.96
$CH_3O-\overset{\displaystyle O}{\overset{\|}{C}}-CH_2-CH_2$	21746-60-1	15.28	10.15	0.43	1.02
$CH_3O-\overset{\displaystyle O}{\overset{\|}{C}}-CH_2$	21746-61-2	15.27	7.61		0.76
$HO-\overset{\displaystyle O}{\overset{\|}{C}}-CH_2$	21746-62-3	14.85	7.36		0.74
$C_6H_5CH_2$	21746-63-4	15.16	7.78		0.78
$HO-\overset{\displaystyle O}{\overset{\|}{C}}-CH_2-NH-\overset{\displaystyle O}{\overset{\|}{C}}-CH_2$	21746-64-5	15.00	6.85, 7.48, 8.03		
$HO-\overset{\displaystyle O}{\overset{\|}{C}}-\underset{\displaystyle CH_3}{\underset{\|}{CH}}-NH-\overset{\displaystyle O}{\overset{\|}{C}}-CH_2$	21736-16-3	15.04	6.97, 7.48, 8.03		
$CH_3O-\overset{\displaystyle O}{\overset{\|}{C}}-\underset{\displaystyle C_6H_5-CH_2}{\underset{\|}{CH}}-NH-\overset{\displaystyle O}{\overset{\|}{C}}-CH_2$	21736-17-4	15.03	8.30		0.83

[a] In gauss in dioxane at room temperature. [b] Ratio of β-hydrogen hfsc to methyl hydrogen hfsc.

C. H. Stammer's group in these laboratories.[5] In order to better understand the spectra obtained and the conformational problems involved, numerous alkyl trityl nitroxides were also made. In this investigation it was found that the trityl group is a severely hindering group and that only straight-chain alkyl groups appear to have complete freedom of motion in the trityl alkyl nitroxides. Some groups may be "frozen" into more than one conformation by steric crowding. In certain cases severe crowding leads to homolytic scission of the carbon nitrogen bond to produce the trityl radical.

Results

Method.—In each case the tritylalkylamine or N-tritylamino acid was synthesized by known procedures and oxidized *in situ* to the nitroxide. A variety of oxidizing agents and solvents were tried (see Experimental Section). The most generally successful method was the use of *p*-nitroperbenzoic acid in dioxane. Presumably the hydroxylamine is formed first and is oxidized to the nitroxide. The standard procedure is to

$$(C_6H_5)_3C-NH-R \xrightarrow{NO_2(C_6H_5)CO_3H} (C_6H_5)_3C-\underset{\displaystyle OH}{\underset{|}{N}}-R \xrightarrow{[O]} (C_6H_5)_3C-\underset{\displaystyle O\cdot}{\underset{|}{N}}-R$$

dissolve an excess of the amine and the *p*-nitroperbenzoic acid in separate portions of approximately 2 ml of solvent in separate arms of an inverted U-tube fitted with rubber septums.[6] After purging the solutions with purified nitrogen through hypodermic needles, for 20 min, the solutions are mixed and run into a flat aqueous solution sample cell already attached to the purging and mixing chamber. Frequently an esr signal could be detected immediately which increased in intensity with time. If weak signals were obtained the addition of more *p*-nitroperbenzoic acid sometimes increased the signal intensity.

Trityl Methyl Nitroxide.—The oxidation of tritylmethylamine readily produces trityl methyl nitroxide (I). The spectrum consists of twelve sharp lines in three groups each having a 1:3:3:1 intensity sequence. The radical appears to be quite stable. The nitrogen and β-hydrogen hyperfine splitting constants (N hfsc and β-H hfsc) are 15.33 and 9.96 G, respectively (see Table I). The methyl group is freely rotating.

$$(C_6H_5)_3C-NH-CH_3 \xrightarrow{[O]} (C_6H_5)_3C-\underset{\displaystyle O\cdot}{\underset{|}{N}}-CH_3$$
I

Trityl Nitroxides with β-Methylene Hydrogens.—Tritylethylamine gives a strong signal due to trityl ethyl nitroxide (II). The spectrum consists of three

$(C_6H_5)_3C-\underset{\displaystyle O\cdot}{\underset{|}{N}}-CH_2-CH_3$ (II)

$(C_6H_5)_3C-\underset{\displaystyle O\cdot}{\underset{|}{N}}-CH_2-CH_2-CH_3$ (III)

(5) C. H. Stammer and R. G. Webb, *Tetrahedron Lett.*, 4895 (1966); R. G. Webb, M. W. Haskell, and C. H. Stammer, *J. Org. Chem.*, **34**, 576 (1969).

(6) The apparatus for routine experiments of this nature has been diagrammed and described before: G. A. Russell, E. G. Janzen, and E. T. Strom, *J. Amer. Chem. Soc.*, **86**, 1807 (1964); G. A. Russell, E. G. Janzen, A. G. Bemis, E. J. Geels, A. J. Moye, S. Mak, and E. T. Strom, Advances in Chemistry Series, No. 51, American Chemical Society, Washington, D. C., 1965, p 162.

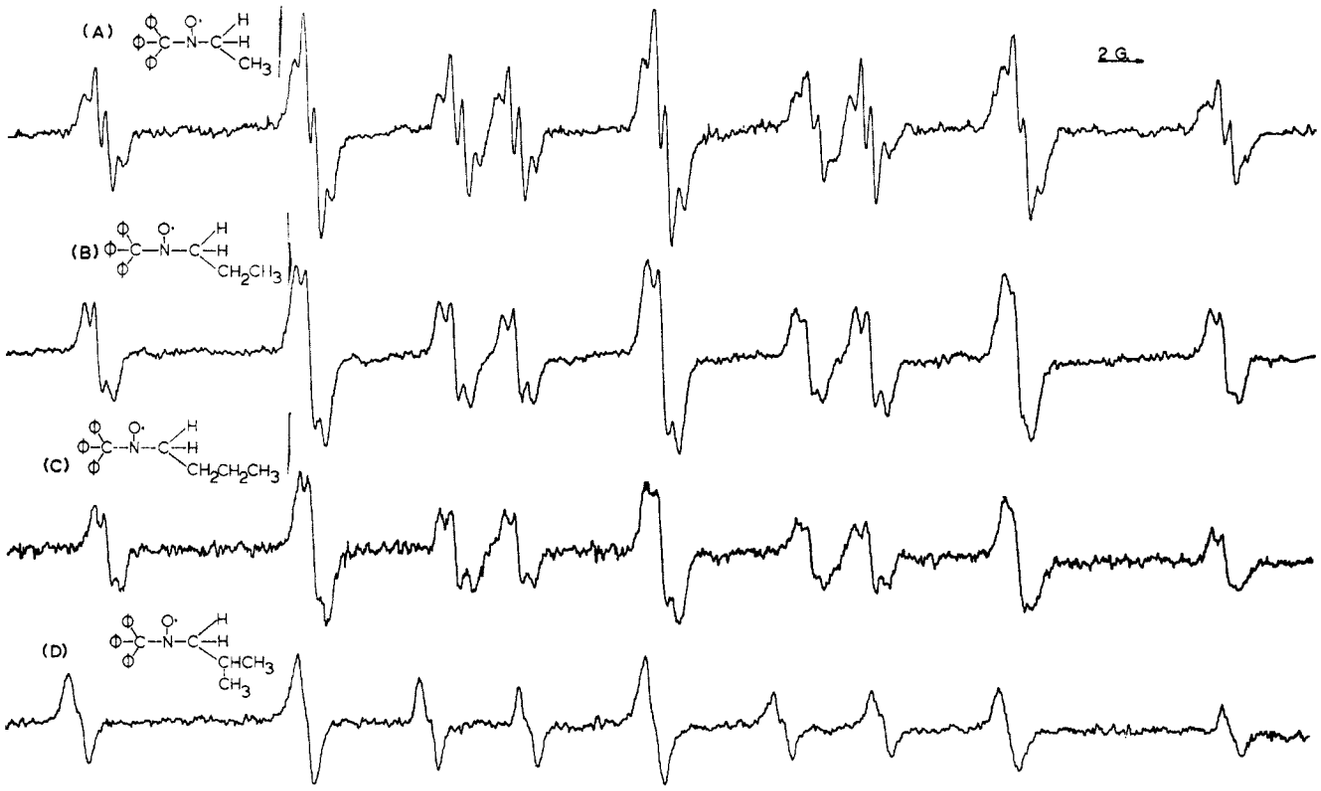
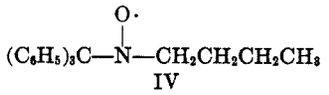


Figure 1.—(A) The esr spectra of trityl ethyl nitroxide; (B) trityl *n*-propyl nitroxide; (C) trityl *n*-butyl nitroxide; (D) trityl isobutyl nitroxide in dioxane at room temperature. Scan rate, 4 G/min.

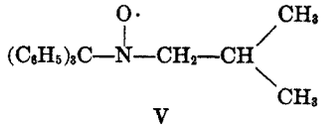
triplets of approximately 1:2:1 intensity with each line further split into four lines. The quartet splitting appears to have a 1:3:3:1 intensity pattern at slow scan rates (Figure 1A). The triplet and quartet splitting is assigned to β-methylene and γ-methyl coupling. At very slow scan rates the quartet lines show marked differences in line width and spacing. This result may be due to slight nonequivalence of the β and/or γ hydrogens. Perhaps conformations are favored wherein a W-plan alignment of the γ hydrogens with the p orbital of the nitroxide nitrogen is possible. In any event the effect is not fully understood at this time.

Tritylpropylamine oxidizes readily to trityl *n*-propyl nitroxide (III). The spectrum consists of three triplets owing to the β-methylene hydrogen coupling, with further splitting due to γ-hydrogen coupling (Figure 1B). From the intensity sequences of the lines the β-methylene hydrogens appear essentially equivalent although it is not clear whether this is true for the γ-methylene hydrogens. At very slow scan rates differences in line width and spacing are again observed.

In trityl *n*-butyl nitroxide (IV) and trityl isobutyl nitroxide (V) the γ-hydrogen coupling becomes progressively more difficult to resolve (Figure 1C and D). The β-methylene hydrogens are essentially equivalent



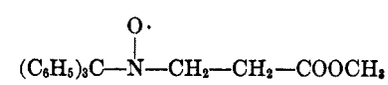
IV



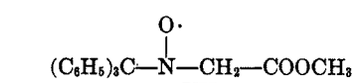
V

in IV but in V the triplet intensity sequence is approximately 1:1.4:1 instead of 1:2:1 indicating restricted freedom of motion about the β-methylene group. After an extended period of time (approximately 2 hr) the spectrum of trityl radical appears along with the spectrum of V. In a 1:1 water–dioxane mixture trityl *n*-butyl nitroxide gives the same spectrum as in dioxane except that the small γ-hydrogen coupling is not resolved. The N hfsc is larger in aqueous solutions (15.57 G) owing to a protic solvent effect.[4,7] The β-H hfsc is correspondingly larger also (9.14 G).

Trityl-β-alanine methyl ester gives a spectrum due to VI which is very similar to the one obtained from trityl *n*-butyl nitroxide (Figure 1C). Tritylglycine methyl



VI



VII

ester on the other hand gives a spectrum of the nitroxide (VII) which shows extensive broadening of the middle line of the β-methylene hydrogen triplet (Figure 2A). This is the result of restricted freedom of motion at the β carbon.

Tritylglycine gives a very weak signal due to VIII in dioxane very much like the spectrum in Figure 2A.

(7) P. Ludwig, T. Layloff, and R. N. Adams, *J. Amer. Chem. Soc.*, **86**, 4568 (1964); J. Gendell, J. H. Freed and G. K. Fraenkel, *J. Chem. Phys.*, **37**, 2832 (1962); see also ref 3, p 160.

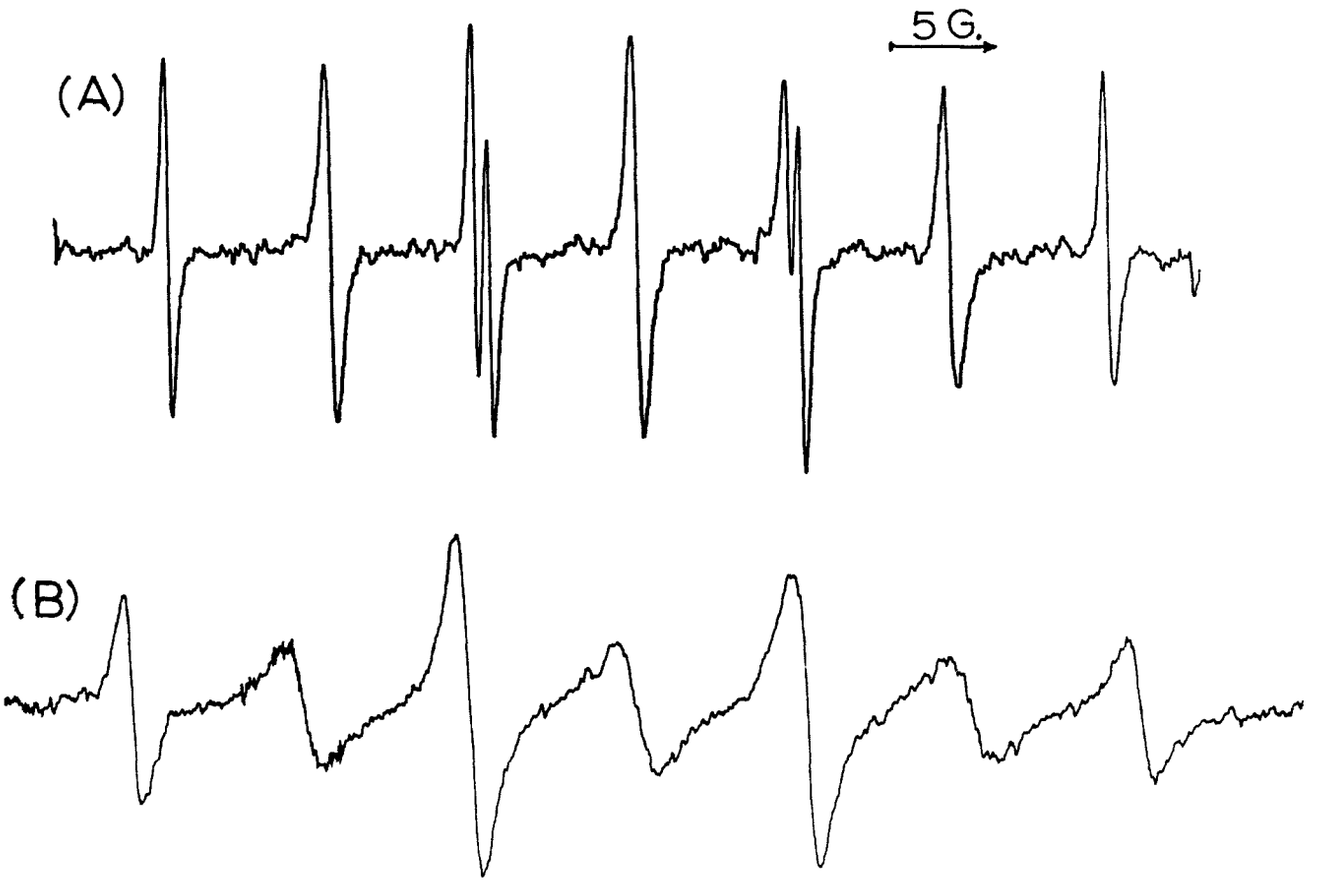


Figure 2.—The esr spectra of (A) the nitroxide of tritylglycine methyl ester in dioxane and (B) tritylglycine in aqueous sodium hydroxide (pH 8) at room temperature.

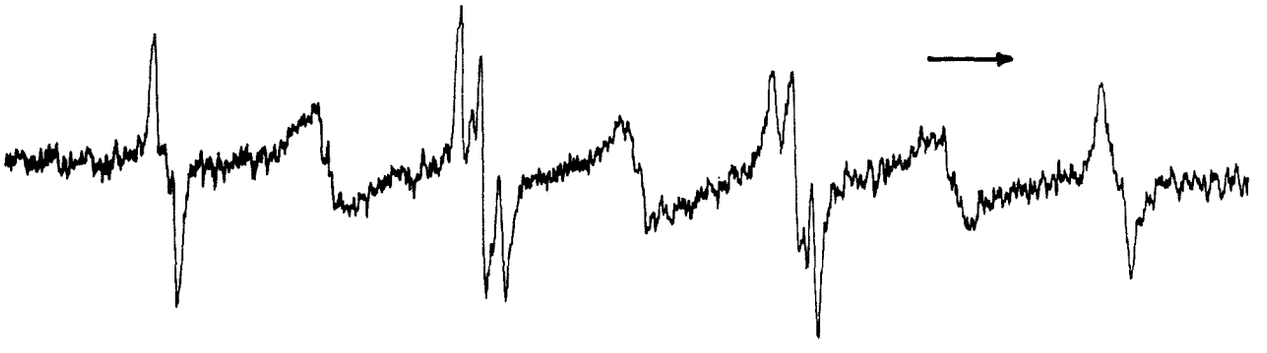
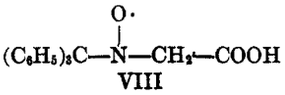
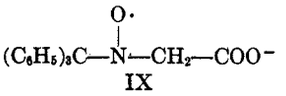


Figure 3.—The esr spectrum of the nitroxide of tritylglycylglycine in dioxane at room temperature. Scan rate, 4 G/min. Length of arrow is 4 G.

In tetrahydrofuran a higher concentration of radicals produces a similar spectrum. In aqueous basic solution (pH ~8) the nitroxide anion of tritylglycine (IX) gives a spectrum with very broad lines (Figure



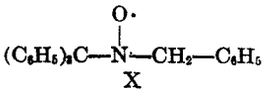
VIII



IX

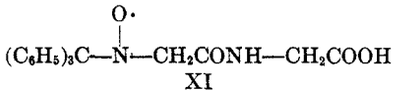
2B). In addition the β-methylene coupling is severely broadened.

Essentially the same spectrum as Figure 2A due to X was obtained from the oxidation of tritylbenzylamine.



X

Tritylglycylglycine was oxidized to the corresponding nitroxide (XI). A spectrum similar to that of the



XI

nitroxide of tritylglycine (Figure 2B) was obtained except that lines which should be singlets were split probably into triplets and lines which should be doublets consisted of at least four peaks (Figure 3). The only obvious explanation is that a number of conformations (three?) are stable on the esr time scale for this nitroxide in solution. Two possibilities arise, either different conformers exist with (1) slightly different N hfsc's or (2) slightly different β-H hfsc's. If we accept that the first line of the spectrum in Figure 3 is split into

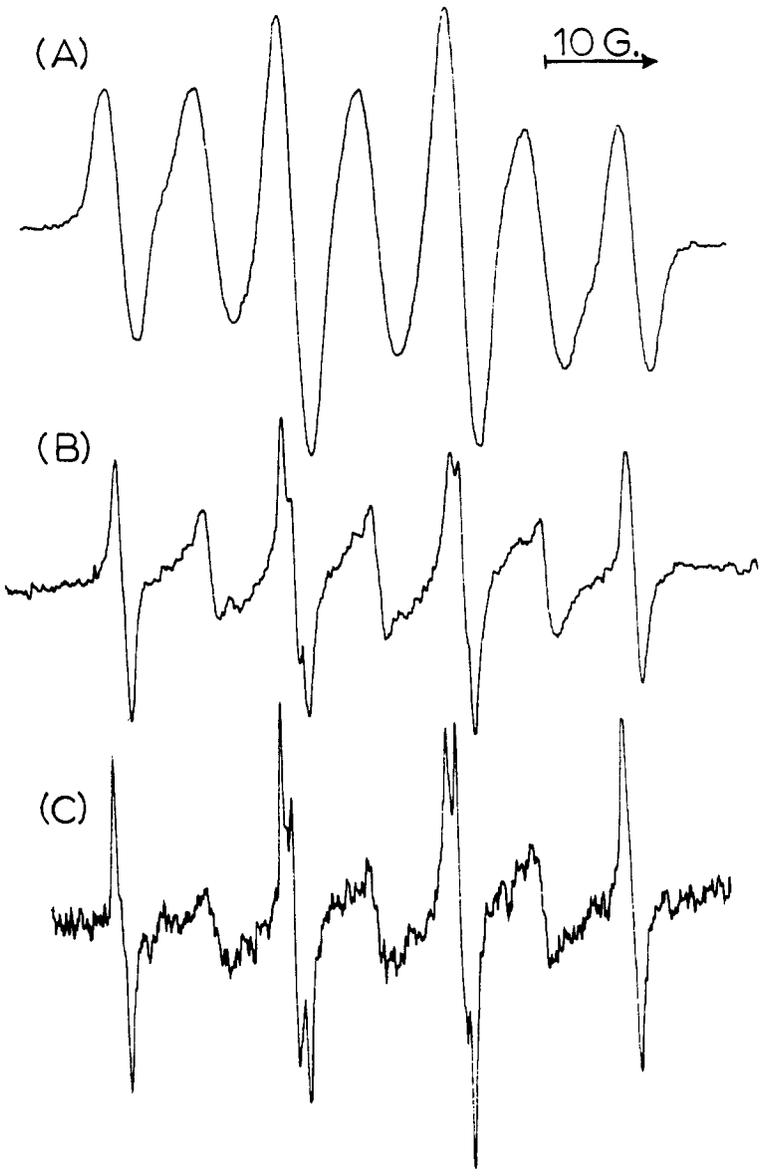
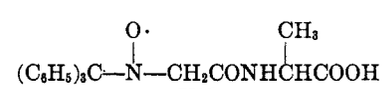


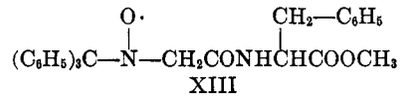
Figure 4.—The esr spectrum of the nitroxide of tritylglycylalanine nitroxide in dioxane at room temperature at different modulation amplitudes: 25:5:1 for A, B, and C.

three lines owing to the presence in solution of three conformers with slightly different N hfsc's, the third group of lines should have only one additional line since the position of the center multiplet of the nitrogen triplet will be unaffected (unless the g values of the conformers are different). If the first line (triplet) is overlapped onto the third group of lines it is visually apparent that there are at least two more lines in the multiplet. This rules out possibility 1. If the H hfsc's of the methylene hydrogens are different and three such conformers exist, the third and fifth group of lines should have six lines each. It is apparent that six lines could overlap to give the multiplet observed. If this interpretation is correct, the three conformations have β-H couplings of 6.85, 7.48, and 8.03 G, respectively. However in spite of the fact that stable conformers exist the β-methylene proton splittings are broadened. This phenomenon is the well recognized line width alternation effect[8a] which is strongly influenced by the modulation amplitude used[8b] (Figure 4).

Tritylglycylalanine oxidizes to a nitroxide (XII)



XII



XIII

(Figure 4) with a spectrum almost identical with Figure 3. The nitroxide of tritylglycylphenylalanine methyl ester (XIII) gave very broad lines. Splitting of peaks as described for XI and XII could not be discerned.

The free carboxyl function in XI and XII not present in XIII may play an important role in the conformational picture of these nitroxides (see Discussion). It should be pointed out that in no case have we been successful in oxidizing an amide nitrogen to a nitroxide function directly. The additional lines in the spectra

(8) (a) See ref 3, p 162; (b) I. Bernal and G. K. Fraenkel, *J. Amer. Chem. Soc.*, **86**, 1671 (1964); E. G. Janzen and J. L. Gerlock, *ibid.*, **89**, 4902 (1967).

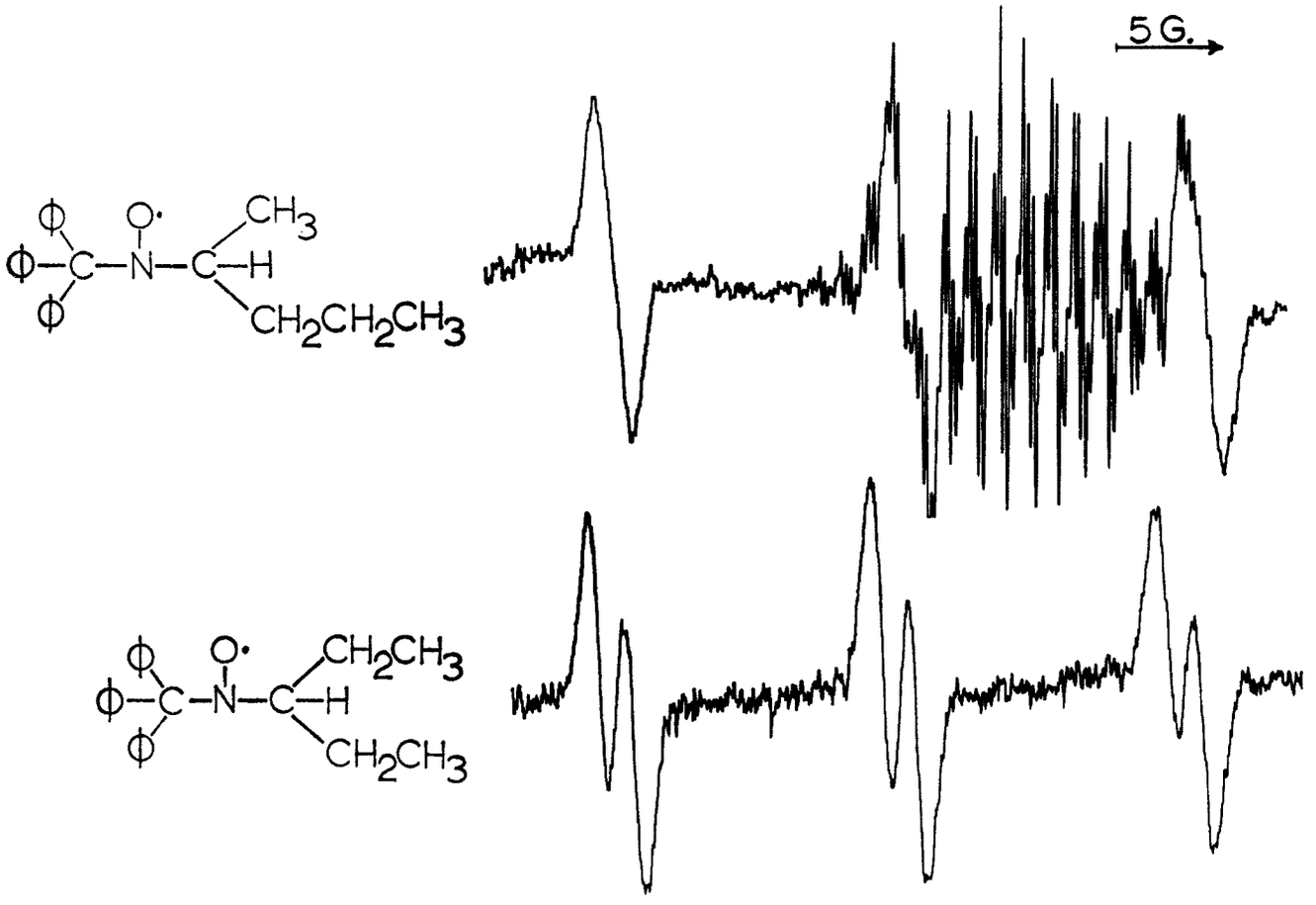


Figure 5.—Esr spectra of (A) trityl 2-pentyl nitroxide and (B) trityl 3-pentyl nitroxide in dioxane at room temperature.

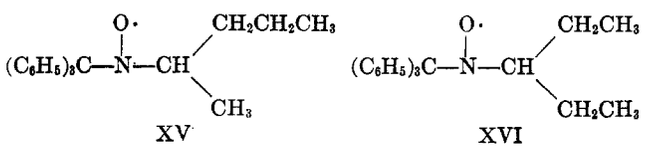
of peptide nitroxides are not due to a nitroxide produced by this oxidation pathway.

Trityl Nitroxides with β-Methine Hydrogens. Tritylisopropyl amine oxidizes to trityl isopropyl nitroxide (XIV). This radical is quite unstable and trityl radical

$$(C_6H_5)_3C{-}\underset{\text{XIV}}{\overset{O\cdot}{N}}{-}CH(CH_3)_2 \longrightarrow (C_6H_5)_3C\cdot + \overset{O}{\overset{\|}{N}}{-}CH(CH_3)_2 \quad (?)$$

was detected even in the first scan. Only three peaks were obtained which resolved into approximately eight maxima separated by 0.4 G. This splitting is assigned to coupling from the β-methine and γ-methyl hydrogens.

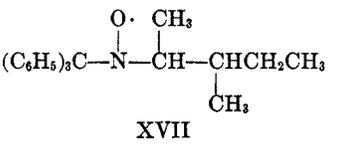
Trityl-2-pentylamine and trityl-3-pentylamine are oxidized to the nitroxides of corresponding structure (XV, XVI). The spectrum of the former nitroxide



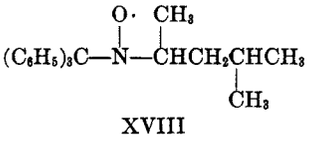
XV XVI

(Figure 5) simply consists of three broad lines (line width ≃2 G). The latter nitroxide gives three narrowly spaced doublets (Figure 5). In both cases the trityl radical spectrum is detected almost immediately and increases in intensity at the expense of the nitroxide spectrum. In the 3-pentyl derivative the nitroxide spectrum finally disappears completely and a fairly strong trityl radical spectrum remains. The half-lives are 30 and 17 min for trityl 2-pentyl and trityl 3-pentyl nitroxides, respectively, at room temperature (~29°).

Trityl-2-(3-methylpentyl)amine gave a weak spectrum owing to the nitroxide XVII consisting of three



XVII

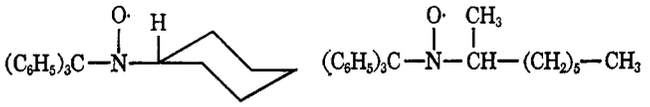


XVIII

doublets. Trityl-2-(4-methylpentyl)amine gave a rapidly decaying (half-life ~3 min) spectrum of nitroxide XVIII consisting of three broad lines. Six peaks are partially resolved with a 0.47-G spacing. This splitting is due to small β-methine and γ-methyl hydrogen coupling. The trityl radical spectrum was present almost immediately and remained as the major signal after the nitroxide radical spectrum had almost disappeared. However, the nitroxide radical spectrum did not completely disappear within the period of observation (2 hr).

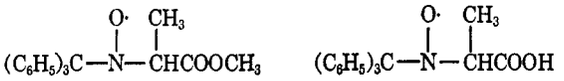
Tritylcyclohexylamine and trityl(2-octyl)amine give nitroxides XIX and XX upon oxidation. Both gen-

erate spectra consisting of three broad lines (1.3- and 2.0-G line width, respectively).



XIX XX

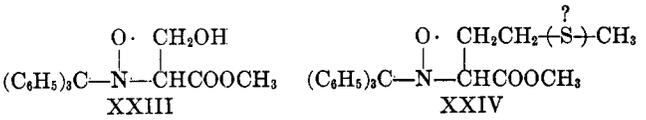
Trityl-D-alanine methyl ester oxidized readily to the nitroxide (XXI) which gives a spectrum of three dou-



XXI XXII

blets. Similar spectra were obtained in tetrahydrofuran (A^N = 13.86; A_β^H = 2.31 G). In methylene chloride each line is further split into 1:3:3:1 quartets (A^N = 13.97; A_β^H = 0.33 G). On standing in this solvent (2–3 hr) the trityl radical spectrum completely replaced the nitroxide spectrum. Trityl-DL-alanine gives a spectrum due to the nitroxide XXII which consists only of three doublets. A weak trityl radical spectrum was also detected. The anion of the same nitroxide could also be obtained in aqueous sodium hydroxide (pH ~8). The line widths are larger in this solvent (A^N = 15.51; A_β^H = 2.51 G).

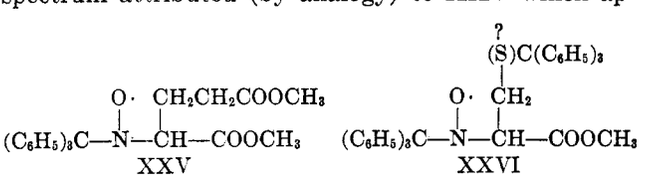
Trityl-*dl*-serine methyl ester oxidized to a nitroxide (XXIII) with a spectrum consisting of three pairs of relatively sharp lines. Tritylmethionine methyl ester required a large excess of *p*-nitroperbenzoic acid to give the spectrum of a nitroxide (XXIV). The spectrum



XXIII XXIV

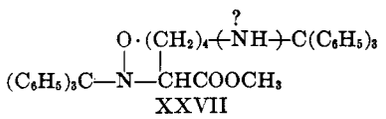
consists of three pairs of relatively sharp lines. The fate of the methyl sulfide function is not known but presumably the sulfone is produced before the amine is oxidized.

Tritylglutamic acid methyl ester gives a weak esr spectrum attributed (by analogy) to XXV which ap-



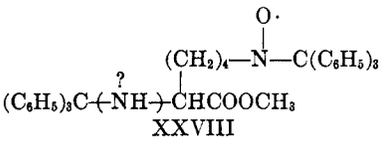
XXV XXVI

pears to consist of nine lines but there is some uncertainty about the origin of the nonnitrogen triplet. The values in Table II assume a proton doublet and a singlet presumably due to two conformers of the nitroxide. Ditritylcysteine methyl ester gave an esr spectrum only after a large excess of *p*-nitroperbenzoic acid was used. The spectrum can be analyzed in terms of A^N = 15.15 and A_β^H = 18.92 G. If this spectrum is due to XXVI the dihedral angle must be essentially zero (see Discussion). Ditritryllysine methyl ester gave a short-lived spectrum of nine lines with a 1:2:1:1:2:1:1:2:1 intensity sequence indicative of structure XXVIII. Coupling constants could not be



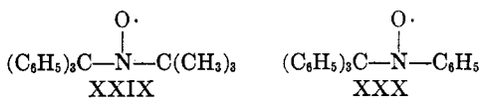
XXVII

obtained for XXVIII. No evidence for XXVII was obtained.



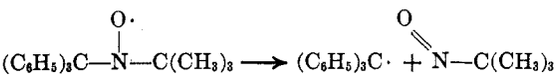
XXVIII

Other Trityl Nitroxides.—The solution of trityl-*t*-butylamine in dioxane turned a fleeting yellow when mixed with *p*-nitroperbenzoic acid and a weak triplet of lines was obtained probably due to trityl *t*-butyl nitroxide XXIX. Upon further addition of peroxide



XXIX XXX

the solution turned green and a weak trityl radical spectrum was observed. The short-lived yellow color is probably due to the nitroxide and the green color may be due to 2-nitroso-2-methylpropane.



Tritylaniline oxidized to trityl phenyl nitroxide with a spectrum consisting of three groups of twelve lines. The groups resolve into four 1:2:1 triplets of approximate 1:3:3:1 intensity. The spectrum is consistent with the structure XXX. No trace of trityl radical was observed.

Discussion

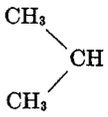
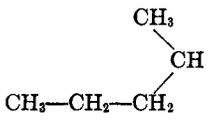
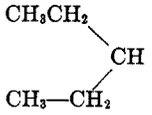
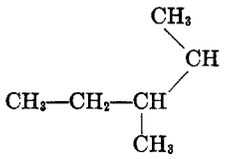
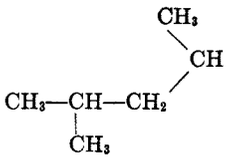
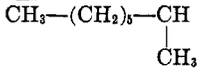
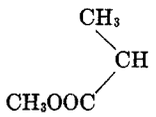
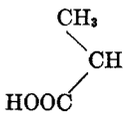
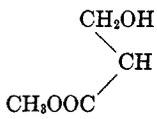
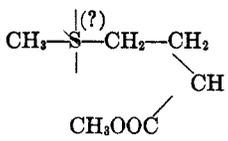
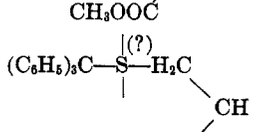
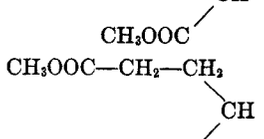
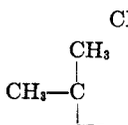
Analytical Applications.—The analytical applications of spin labeling techniques in the determination of the structure of amines, amino acids, and peptides appear promising. For example, the spectra of trityl 2-pentyl nitroxide and trityl 3-pentyl nitroxide are clearly different, the former consisting simply of a triplet of lines whereas the latter consists of three doublets. Thus with spectra of nitroxides of known structure available for comparison, the spectra of nitroxides of amines of unknown structure could serve as a diagnostic tool for differentiating isomeric primary amines.

Although not all tritylamino acid nitroxides have been obtained to date, enough have been studied to indicate that certain features of the esr spectrum can be unique for any given amino acid. The tritylglycine or the methyl ester gives a unique spectrum derived from three 1:2:1 triplets due to the coupling of the β-methylene hydrogens. The esr spectra of peptides having glycine as the terminal amino acid also retain this feature (at least for the three dipeptides studied). In the dipeptide nitroxide an added splitting of the peaks presumably due to stable conformers makes it possible to differentiate between glycine or a peptide having glycine as the terminal amino acid.

Tritylamino acid nitroxides with β-methine hydrogens are a group of radicals which give spectra consisting of three sets of doublets depending on the magnitude of the β-hydrogen coupling. Since the nature of the groups attached to the β carbon effect both the nitrogen and β-hydrogen coupling constants (Table II), these values are a unique set of parameters for any amino acid.

TABLE II
HYPERFINE SPLITTING CONSTANTS OF TRITYL NITROXIDES WITH β-METHINE HYDROGENS[a]

$(C_6H_5)_3C-\underset{\displaystyle O\cdot}{\underset{|}{N}}-X$

Registry no.	X	A^N	A_β^H	A_γ^H	R value
21746-65-6		14.67		0.4	
21746-66-7		14.46			
21744-67-8		13.91	1.77		0.18
21746-68-9		14.01	1.81		0.18
21746-69-0		14.31		0.47	
21746-70-3		14.45			
21746-71-4		14.46			
21746-72-5		13.89	2.36		0.24
21746-73-6		14.05	1.68		0.17
21746-74-7		13.55	3.78		0.38
21746-75-8		13.47	3.46		0.35
21746-76-9		15.15	18.92		1.9
21746-77-0		13.35	3.58, <0.8		0.36
21746-59-8		14.63			
			ortho–para	*meta*	
21746-79-2		10.93	2.16	0.80	

[a] In gauss in dioxane at room temperature.

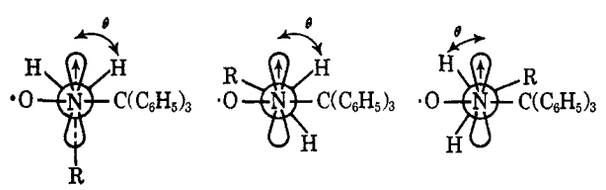
Proline oxidizes to a characteristic nitroxide (unpublished work); thus the determination of proline as the terminal amino acid is also possible by this method.

It would be desirable to circumvent the steps involved in the synthesis and isolation of the tritylamino acid prior to oxidation to the nitroxide by an *in situ* synthesis and oxidation. Preliminary experiments have been encouraging. Thus, when triphenylmethyl chloride (0.1 *M*) was refluxed with *n*-butylamine (0.5 *M*) in triethylamine (0.5 *M*)–dioxane for 10 min and poured over *p*-nitroperbenzoic acid after cooling, the esr spectrum of trityl *n*-butyl nitroxide was obtained.

$$(C_6H_5)_3CCl + BuNH_2 \xrightarrow{Et_3N}$$

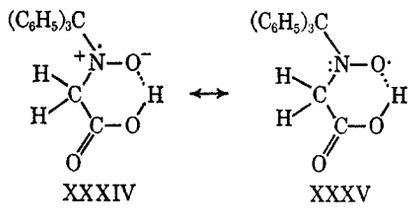
$$(C_6H_5)_3C{-}NH{-}Bu \xrightarrow{[O]} (C_6H_5)_3C{-}\overset{O\cdot}{N}{-}Bu$$

Conformational Analysis.—In discussing conformational implications based on the magnitudes of the β-hydrogen coupling constant, it is helpful to use the ratio of the coupling constants of the β hydrogen and the methyl hydrogen in question. This ratio has been used by previous authors[9a] and is called the *R* value. The methyl group is freely rotating. If any other group is also rotating freely, it is assumed that the same β-hydrogen coupling should be obtained. Deviations from this value are taken as evidence for conformational preference of groups at the β carbon. For trityl nitroxides with β-methylene hydrogens three different limiting conformations exist. In the absence of a bulky substituent (trityl), conformation XXXI should be most favored since no atoms or groups are in eclipsing positions. In the trityl nitroxides, XXXII should compete with XXXI for the lowest energy conformation whereas XXXIII is a highly unlikely conformation. The *R* values observed when R = ethyl, *n*-propyl, *n*-butyl, isobutyl, and 2-carbomethoxyethyl are essentially constant, varying from 0.9 to 1.0. This value is indicative of an average conformation for these groups between XXXI and XXXII. It is of interest to note that dialkyl nitroxides obtained in carbon tetrachloride and the amine[9b] show very similar *R* values although somewhat lower, around 0.8. The benzyl, carboxy, and carbomethoxy groups decrease the *R* value for the trityl nitroxides slightly. These groups might be considered "flat" groups and able to avoid steric interaction with the trityl group. The *R* value of dibenzyl nitroxide is similarly relatively low.



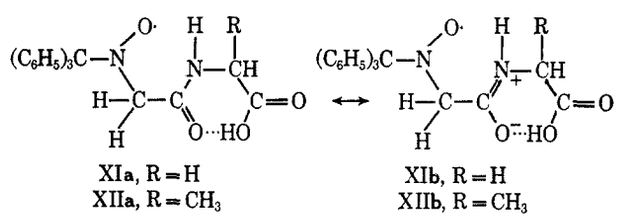
XXXI, $\theta = 60°$, *R* value = 0.5 — XXXII, $\theta = 30°$, *R* value = 1.5 — XXXIII, $\theta = 30°$, *R* value = 1.5

The nitrogen hyperfine coupling for tritylglycine nitroxide is anomalously low. Hydrogen bonding with the nitroxide should be possible but would be expected to *increase* the nitroxide nitrogen coupling instead of decreasing it very much as protic solvents do (*i.e.*, XXXIV should be favored over XXXV).

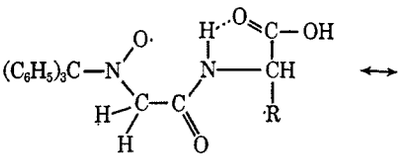


XXXIV ⟷ XXXV

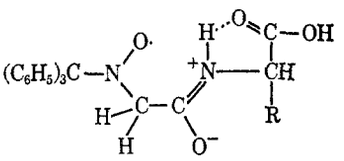
In spite of the large bulk of the dipeptide chain the conformational picture about the methylene carbon does not appear to change drastically. The reason for different conformations in the radical is not obvious.[10] Perhaps hydrogen bonding plays a role. From nmr studies it is known that the amide bond is not freely rotating because of significant double-bond character in the carbon–nitrogen bond. Two hydrogen-bonded conformations can be visualized. It is tempting to assign the conformer of XI and XII with the β-H hfsc equal to 8.03 to the nonhydrogen-bonded conformer since the tritylglycylphenylalanine methyl ester nitroxide XIII has a β-H hfsc equal to 8.30 G. Hydrogen-bonding to the nitroxide oxygen would appear to be ruled out since the analysis of the spectrum does not accommodate nitroxides with different N hfsc's.



XIa, R = H; XIIa, R = CH_3 ⟷ XIb, R = H; XIIb, R = CH_3

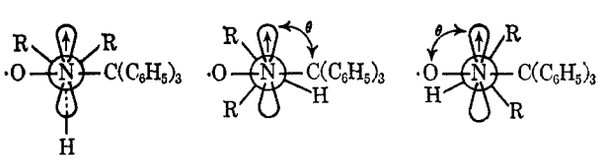


XIa, R = H; XIIa, R = CH_3



XIb, R = H; XIIb, R = CH_3

For trityl nitroxides with β-methine hydrogens the three limiting conformations are shown below. XXXVI



XXXVI, $\theta = 0°$, *R* value = 2.0 — XXXVII, $\theta = 90°$, *R* value = 0 — XXXVIII, $\theta = 90°$, *R* value = 0

(9) (a) Reference 3, p 179. (b) J. Q. Adams, S. W. Nicksic, and J. R. Thomas, *J. Chem. Phys.*, **45**, 654 (1966).

(10) Conformational isomers of 2,3,5,6-tetraisopropylnitrobenzene radical anion have been detected: ref 3, p 157; T. M. McKinney and D. H. Geske, *J. Chem. Phys.*, **44**, 2277 (1966).

TABLE III
PROTON CHEMICAL SHIFTS OF TRITYLALKYLAMINES[a]
$(C_6H_5)_3C—NH—R$

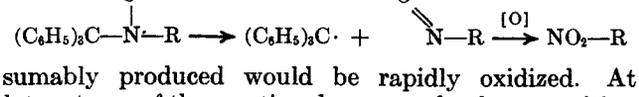
Registry no.	R	N-H	Aryl	Alkyl
2538-41-2	CH_3	1.65	7.3, 7.5	2.12 (CH_3)
7370-34-5	CH_3CH_2	1.50	7.3, 7.5	1.08 (CH_3), 2.18 (CH_2)
7370-50-5	$CH_3CH_2CH_2$	1.50	7.3, 7.5	0.95 (CH_3), 1.4 (CH_2), 2.05 (CH_2)
21746-83-8	$CH_3CH(CH_3)$	1.40	7.3, 7.6	0.75 (CH_3), 2.70 (CH)
7370-51-6	$CH_3CH_2CH_2CH_2$	1.48	7.3, 7.5	0.85 (CH_3), 1.35 (CH_2), 2.13 (CH_2)
3416-17-9	$CH_3CH(CH_3)CH_2$	1.48	7.3, 7.5	0.93 (CH_3), 1.93 (CH_2), 2.4 (CH)
21736-25-4	$CH_3CH_2CH(CH_3)CH_3$	1.53	7.3, 7.6	0.65 (CH_3), 0.7 (CH_3), 1.05 (CH_2), 2.43(CH)
21736-26-5	$(CH_3)_3C$	1.70	7.2, 7.6	0.83 (CH_3)
21736-27-5	$CH_3CH_2CH_2CH(CH_3)$	1.50	7.2, 7.6	0.65 (CH_3), 0.70 (CH_3), 1.2 (CH_2), 2.5 (CH)
21736-28-7	$(CH_3CH_2)_2CH$	1.52	7.2, 7.6	0.64 (CH_3), 0.9–1.3 (CH_2), 2.3 (CH)
21736-29-8	$CH_3CH_2CH(CH_3)CH(CH_3)_2$	1.58	7.2, 7.6	0.4–1.2 (CH_3 and CH_2), 1.5 (CH), 2.5 (CH)
21736-30-1	$CH_3CH(CH_3)CH_2CH(CH_3)_2$	1.52	7.2, 7.6	0.68 (CH_3), 0.75 (CH_3), 1.1 (CH_2), 2.5 (CH)
21736-31-2	$CH_3(CH_2)_5CH(CH_3)$	1.50	7.2, 7.6	0.67 (CH_3), 0.83 (CH_3), 1.0–1.3 (CH_2), 2.5 (CH)
20360-17-2		1.42	7.2, 7.6	0.9–1.5 (CH_2), 2.3 (CH) 3.38 (CH_2)
3378-73-2	$C_6H_5CH_2$	1.80	7.3, 7.4, 7.6	
4471-22-1	C_6H_5	5.05	6.5–7.0, 7.4	

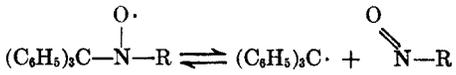
[a] Chemical shifts are in parts per million in deuteriochloroform from internal tetramethylsilane. Ratios of areas were in agreement with assignments.

and XXXVIII should be undesirable conformations. The small or unresolvable β-hydrogen hyperfine coupling observed (R = 0.2–0.4) indicates that conformation XXXVII is most stable, not an unexpected result.

Homolytic Cleavage.—The spontaneous cleavage of certain trityl alkyl nitroxides to trityl radical is an interesting side reaction in this study. The cleavage is clearly aided by sterically bulky groups. Almost every β-disubstituted alkyl nitroxide gave trityl radical whereas isobutyl nitroxide was the only β-monosubstituted nitroxide where trityl radical could be detected. Moreover t-butyl trityl nitroxide was quite unstable at room temperature. These observations point to a rather weak carbon–nitrogen bond in the trityl alkyl nitroxide. Surprisingly there was no sign of cleavage of trityl phenyl nitroxide to trityl radicals.

During initial scans the decay of the nitroxide signal was used to calculate the half life of the nitroxide radical. These values are approximate at this time. Not enough points were obtained to check the order of the decay reaction. Initially while p-nitroperbenzoic acid was still present the nitroxide decay was undoubtedly irreversible. The nitroso compound presumably produced would be rapidly oxidized. At later stages of the reaction, however, the decomposition appears to stop in some cases at a stage where both the nitroxide and the trityl radical are detected. Probably the cleavage reaction is reversible (in the absence of perbenzoic acid). Further work is needed in this area.





Experimental Section

Methods A and B of Zervas and Theodoropoulos[11] were employed to prepare the tritylamino acids, trityl dipeptides, tritylamino acid methyl esters, and tritylamines. Most of the tritylamines do not appear to have been reported. The nmr spectra (Table III) and combustion data[12] are in agreement with the structures given.

The majority of tritylamino acid methyl esters explored in this work were gifts from Dr. C. H. Stammer.[5]

Method A.[11]—To a solution of the amino acid or dipeptide (0.01 mol) in a mixture of 4 ml of water, 3 ml of diethylamine, and 8 ml of isopropyl alcohol was added portionwise 3.6 g (0.013 mol) triphenylmethyl chloride with stirring. Addition of triphenylmethyl chloride required 1 hr at room temperature. Triphenylcarbinol and trityldiethylamine were precipitated by adding 25 ml of water and removed by filtration. The filtrate was acidified with acetic acid which caused the trityl adduct to precipitate.

(11) L. Zervas and D. M. Theodoropoulos, *J. Amer. Chem. Soc.*, **78**, 1359 (1956).

(12) Satisfactory combustion analytical data were obtained for these compounds (±0.4%).

Method B.[11]—To the amino acid methyl ester hydrochloride or the amine (0.01 mol) in 15 ml of chloroform was added triethylamine (0.022 mol) and triphenylmethyl chloride (0.01 mol). The pale yellow reaction mixture was stirred for 4 hr at room temperature. The solution, containing some precipitated triethylamine hydrochloride, was washed three times with water and dried over anhydrous sodium sulfate. The solvent was removed under vacuum utilizing a rotating flask ("Rotovac"). Methanol was added and subsequently removed under vacuum.

N-Tritylmethylamine.—This low melting derivative was prepared according to method B. The reaction gave a 51% yield, recrystallized from *n*-pentane as white prisms, mp 45–47° (lit.[13a] mp 73°).

N-Tritylethylamine.—This compound was synthesized as described in method B. Recrystallization from *n*-pentane gave colorless cubes in 82% yield, mp 69–71°.[12]

N-Trityl-*n*-propylamine.—Method B was employed to prepare this compound in 75% yield. It crystallized in large colorless cubes, mp 68–70°, from *n*-pentane.[12]

N-Tritylisopropylamine.—Method B gave this product in 47% yield on crystallization from 50:50 (30–60) petroleum ether–absolute alcohol in white prisms on refrigeration, mp 54–55°.[12]

N-Trityl-*n*-butylamine.—This compound was obtained in 39% yield using method B. It was crystallized from absolute methanol in white needles, mp 52–53° (lit.[13b] mp 52.5–53.5°).

N-Tritylisobutylamine.—A 57% yield was realized on synthesis of this compound *via* method B. Recrystallization from methanol yielded white prisms, mp 66–67°.[12]

N-Trityl-*sec*-butylamine.—Method B was employed to prepare this compound in 47% yield. A thick colorless syrup was obtained and purified by dissolving in a minimum amount of methanol and freezing out the product using a Dry Ice–acetone bath.[12]

N-Trityl-*t*-butylamine.—This compound was prepared in 41% yield from *t*-butylamine and triphenylmethyl chloride according to method B. It is a colorless syrup which was purified with several freezing operations from methanol.[12]

N-Trityl-2-pentylamine.—A colorless syrup was obtained in 67% yield *via* method B. It was purified by freezing from a minimum amount of methanol.[12]

N-Trityl-3-pentylamine.—Method B was used to synthesize this amine in 58% yield. A viscous liquid was obtained and purified as above.[12]

N-Trityl-3-methyl-2-pentylamine.—Method B was employed to prepare this trityl adduct in 49% yield. A colorless syrup was obtained and purified by freezing from methanol.

N-Trityl-4-methyl-2-pentylamine.—This trityl derivative was obtained in 46% yield by method B. Again a colorless syrup resulted which was purified by freezing from methanol.[12]

N-Trityl-2-octylamine.—Method B was used to obtain this compound in 46% yield as a colorless syrup. Freezing from methanol was the means of purification as in the other cases where a syrup was obtained.

N-Tritylbenzylamine.—This amine derivative was obtained in 59% yield using method B. White needles resulted on recrystallization from ether (30–60)-petroleum ether, mp 92–93 (lit.[12,14] mp 163–165°).

N-Tritylphenylamine.—This compound was synthesized in 73% yield according to method B. It crystallized in white prisms from chloroform–methanol, mp 151–152° (lit.[13] mp 149–150°).

N-Tritylcyclohexylamine.—Method B was used to prepare this tritylamine. It was recrystallized from methanol as white plates in 64% yield, mp 122–124°.

N-Tritylglycylglycine.—This dipeptide was obtained by method A in 85% yield after recrystallizing from absolute methanol, mp 179–180° (lit.[11] mp 180°).

N-Tritylglycyl-*dl*-alanine.—Method A was used to prepare this dipeptide in 79% yield. The white product was recrystallized from absolute methanol, mp 171–172°.

N-Tritylglycyl-*dl*-phenylalanine Methyl Ester.—Method B was employed to prepare this compound from triphenylmethyl chloride and glycylphenylalanine methyl ester hydrochloride. This product was obtained as a white solid from methanol, mp 115–117°.

The melting points are uncorrected and were obtained using a Mel-Temp apparatus. In general, the tritylalkylamines having β-methylene hydrogens are solids, but those having β-methine hydrogens form syrups. The exceptions are N-trityl-iso-propylamine and N-tritylcyclohexylamine. More branching at the α carbon in these tritylamines apparently decreases the melting point.

Materials.—The *p*-dioxane employed in this esr study was an Eastman White Label chemical distilled from calcium hydride and stored over molecular sieves. Tetrahydrofuran was a Mallinkrodt chemical similarly distilled from calcium hydride.

A variety of oxidants were explored in the esr experiments in an attempt to oxidize the tritylamines to their corresponding nitroxides. The most reliable oxidant was *p*-nitroperbenzoic acid.[15] It was useful in a wide spectrum of solvents, although most of this work employed dioxane for purposes of comparing tritylamino acid derivatives to tritylamines. Other solvents used were tetrahydrofuran, methylene chloride, ethanol, water, and (50:50) water–dioxane. In some instances, phosphotungstic acid,[16] in the presence of *p*-nitroperbenzoic acid, enhanced the conversion of the tritylamines into trityl nitroxides. Oxidizing systems involving hydrogen peroxide,[17] *t*-butyl hydroperoxide, or *m*-chloroperbenzoic acid[4] were either too slow or ineffective. These oxidants were used on amines bearing a variety of amino blocking groups. Attempts to oxidize *o*-nitrosulfenylbenzylamine, *o*-nitrosulfenyl-*l*-glutamic acid, or *o*-nitrosulfenyl-*dl*-alanine to the corresponding nitroxides with any of the above methods gave no detectable radicals. Similarly, negative results were obtained in the *p*-nitroperbenzoic acid oxidation of either N-benzyl benzenesulfonamide or acetanilide in 50:50 dioxane–water at pH 8. No esr signal was evident on treating carbobenzyloxyglycine with *p*-nitroperbenzoic acid in dioxane. On dissolving trimethylsilylglycine (Pierce Chemical Co.) in ether and adding *p*-nitroperbenzoic acid, only glycine and trimethylsilanol were realized. In this attempted oxidation, no paramagnetic substance was observable.

In preparing the trityl derivatives, reagent grade chloroform from the Fisher Scientific Co., was used as solvent. Triethylamine (Eastman White Label) and triphenylmethyl chloride (Columbia Chemicals) were used directly. 4,4′-Dimethoxytrityl chloride (Aldrich Chemical Co.) was used as the N-blocking group on glycine. The quality of the derivative was poor and further attempts with this tertiary halide were abandoned.

Registry No.—Nitroxide of tritylglycylalanine nitroxide, 21736-18-5; N-tritylglycyl-*dl*-alanine, 21736-19-6; N-tritylglycyl-*dl*-phenylalanine methyl ester, 21736-20-9.

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