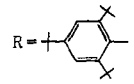
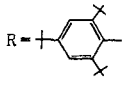
Table I. Hyperfine Splitting Constants (Gauss) for Nitroxide in Benzene Solution[a,b]



Spin adduct	a^N	a_β^H	a_m^H
RN(—O·)CH_3[c,d]	13.03	12.33 (3 H)	0.81
RN(—O·)CH_2CH_3[d]	13.46	17.99 (2 H)	0.83
RN(—O·)$CH(CH_3)_2$[d,h]	13.29	22.19 (1 H)	0.76
RN(—O·)$CH_2CH{=}CH_2$[d]	13.40	16.42 (2 H)	0.84
RN(—O·)CH_2—⟨⟩[e,g]	13.62	14.75 (2 H)	0.83
RN(—O·)CH_2OH[e-g]	13.73	13.73 (2 H)	0.95

[a] A Varian V4502-15 epr spectrometer was used with 100-kHz field modulation. [b] g value: 2.0060. [c] *tert*-BuO· → CH_3· + CH_3COCH_3. [d] Abstraction of Br or I by $(n\text{-Bu})_3$Sn·. [e] Hydrogen abstraction by *tert*-BuO· generated by photolysis or thermolysis. [f] Hydrogen abstraction by photoexcited benzophenone. [g] In solution of a substrate. [h] $a_{CH_3}^H$ = 0.38 G (6 H).

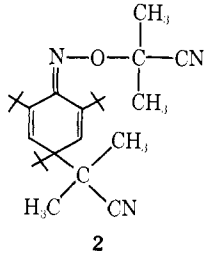
Table II. Hyperfine Splitting Constants (Gauss) for Anilino Radical in Benzene Solution[a]



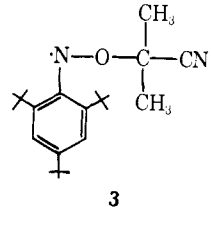
Spin adduct	a^N	a^H
RNOC(CN)$(CH_3)_2$[b]	10.01	1.98 (2 H)
RNOC$H(CH_3)_2$[c]	11.01	1.82 (3 H)
RNOC(=O)C_6H_5[d]	10.53	2.07 (2 H)
RNO-*c*-C_6H_{11}[d-f]	10.95	1.79 (3 H)

[a] g value: 2.0036–2.0040. [b] Thermolysis or photolysis of AIBN. [c] Bromine abstraction by $(n\text{-Bu})_3$Sn·. [d] Hydrogen abstraction by *tert*-BuO· generated by photolysis. [e] In solution of a substrate. [f] Only anilino radical.

oxide[8] to produce the stable anilino radical, the esr spectrum of which showed the following g value and coupling constants: g = 2.00324, a^N = 6.80 G (1 N), a_{NH}^H = 11.97 G (1 H), a_m^H = 1.90 G (2 H), $a_{t\text{-Bu}}^H$ = 0.28 G (27 H). From these results, together with the general esr character of anilino radicals,[9] and the additional fact that Hosogai, Inamoto, and Okazaki[10] isolated the dienone oxime ether **2** on heating a mixture of AIBN and **1**, we conclude that the radical described above is the *N*-(1-cyano-1-methylethoxy)-2,4,6-tri-*tert*-butylanilino radical (**3**). The *tert*-butyl radical also



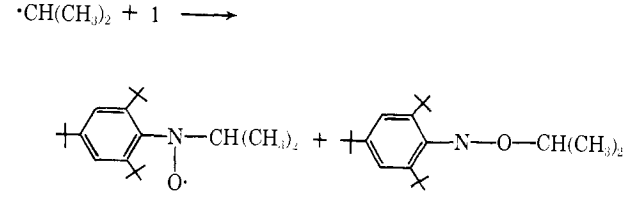
2



3

attacks the oxygen of **1** to produce an anilino radical rather than a nitroxide.

Secondary alkyl radicals attack both the oxygen and nitrogen atoms of **1**, and spectra of a mixture of a nitroxide and an anilino radical are observed. The spectrum of the spin adducts of the isopropyl radical is shown in Figure 1. The tendency to produce either a nitroxide or an anilino radical may depend on the steric hindrance of **1** for the attacking radicals. It is possible



to distinguish between attacking secondary and tertiary alkyl radicals from the spectra of the anilino radicals produced by spin trapping as shown in Table II.

The main advantages of using **1**, which is a novel "bifunctional" trap, are that information concerning the structure of the radical trapped is more easily obtained from the spectrum of the spin adduct. Moreover, being stable to light both in solution and in the solid state, **1** is useful for application to photoradical reactions. Another merit of **1** is that it is essentially a monomer and does not dimerize.[6]

Acknowledgment. The authors wish to thank Dr. K. Nishikida and Mr. S. Sakata for their assistance in obtaining the esr spectra.

Shigeru Terabe, Ryusei Konaka*
Shionogi Research Laboratory, Shionogi and Company, Ltd.
Fukushima-ku, Osaka, Japan
Received April 8, 1971

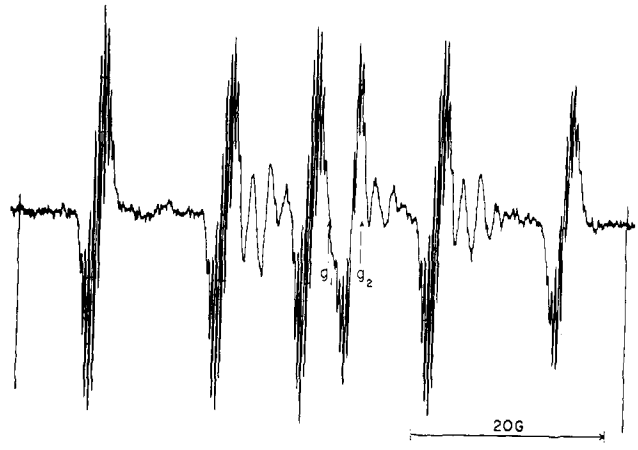


Figure 1. Esr spectrum of a mixture of 2,4,6-tri-*tert*-butylphenyl isopropyl nitroxide and *N*-isopropoxy-2,4,6-tri-*tert*-butylanilino radical in benzene solution at 2.5 hr after spin trapping. Arrows in spectrum indicate center of each: g_1 = 2.00602, g_2 = 2.00402.

Solvated Proton Mass Spectra of a Tripeptide Derivative

Sir:

Mass spectrometry has had significant but limited success for determination of peptide amino acid sequences.[1-3] It is a rapid sensitive technique but it

(8) R. Konaka, S. Terabe, and K. Kuruma, *J. Org. Chem.*, **34**, 1335 (1969).
(9) (a) N. M. Atherton, E. J. Land, and G. Porter, *Trans. Faraday Soc.*, **59**, 818 (1963); (b) A. T. Balaban, P. T. Frangopol, M. Frangopol, and N. Negoiță, *Tetrahedron*, **23**, 4661 (1967).
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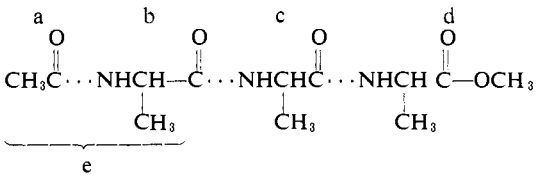
(1) G. E. Van Lear and F. W. McLafferty, *Annu. Rev. Biochem.*, **38**, 298 (1969).
(2) E. Lederer, *Pure Appl. Chem.*, **17**, 489 (1968).
(3) M. M. Shemyakin, *ibid.*, **17**, 313 (1968).

Table I. Mass Spectra of



Mass fragment		Primary ions: H_3O^+	D_3O^+	$H_5O_2^+$	$D_5O_2^+$	$H_7O_3^+$	$D_7O_3^+$	$H_9O_4^+$	$D_9O_4^+$
		Ion intensity[a]							
44		0.47	0.22	0.16	0.11	0.16	0.05	0.06	0.02
45			0.18		0.07		0.04		0.02
86	e	0.06	0.03	0.06	0.02	0.02	0.02		
87	e + D − H		0.01				0.01		
104	d + H + H	0.09	0.03	0.17	0.03	0.08	0.03	0.09	0.02
105	d + H + D		0.09		0.09		0.08		0.05
114	a + b	0.06	0.06	0.08	0.06	0.05	0.07	0.09	0.05
115	a + b + D − H		0.02		0.02		0.02		0.02
143		0.01	0.02		0.01		0.01		
144			0.03		0.01		0.01		
157		0.02	0.02		0.01		0.01		
158			0.01		0.01		0.01		
175	c + d + 2H	0.11		0.22		0.24		0.14	
176	c + d + H + D		0.11		0.20		0.20		0.18
185	a + b + c	0.10	0.08	0.18	0.17	0.20	0.13	0.09	0.10
186	a + b + c + D − H		0.05		0.11		0.09		0.07
256	P + H − (CH_3OH)	0.01	0.01	0.04	0.01		0.01		
257	P + D − (CH_3OH)		0.01		0.01		0.01		
288	P + H	0.01		0.09		0.25		0.54	
289	P + D		0.01		0.06		0.20		0.48

[a] Ion intensities are presented in units of per cent of total ions.

suffers from problems of volatility[4] and instability of parent and heavy fragment ions produced by electron impact. Heavy ion instability, ascribed to excitation generated by electron impact, can be minimized by chemical ionization techniques. Chemical ionization[5] is produced by collisions of heavy molecule peptides, present in trace concentrations, with ions made from reagent gas by electron impact and secondary ion–molecule collisions. The ion–molecule collisions between the reagent ion constituents and trace neutral heavy molecules provide a more gentle ionization mechanism which, when followed by subsequent collisions, stabilizes the parent heavy species and may build up even heavier ions.[6] Single ion impact processes in a low-pressure collision chamber effect gentle ionization without subsequent collisions.

We wish to report a study of mass spectra of *N*-acetyltrialanine methyl ester obtained by proton or deuteron transfer from hydrated species in single ion–molecule collisions. We used a tandem spectrometer system[7] consisting of a 12-in. 60° radius magnetic analyzer with a collision chamber situated at its ion exit focus and a quadrupole mass spectrometer as the second stage analyzer. Ions were generated in the primary mass spectrometer source by electron impact and detected with a Spiraltron electron multiplier. Resolved primary beams having approximately 1-eV kinetic energy in the laboratory system were introduced into the collision chamber with intensities of approximately 10^{-8} A. Ratios of primary signals at the detector to noise levels, observed several mass units displaced from the primary beam, were in the range of 10^6–10^7. Several hours of running time in the tandem system required roughly 1 μmol of material. A complete spectrum with one primary beam requires less than 5 min.

Spectra obtained using beams of solvated protons and deuterons up to and including $H_9O_4^+$ and $D_9O_4^+$ are presented in Table I. The *N*-acetyltrialanine methyl ester formula is given in the heading with small letters above and below, which are used to identify fragment ions in the table. These spectra are sensitive to primary ion kinetic energy, neutral molecule temperature, and the mass dependent transmission of the quadrupole mass analyzer which tends to discriminate against collection of higher molecular weight species. The data have been reproduced under a range of experimental conditions and represent a technique not yet developed for quantitative analysis of mixtures but sufficient for structural identification work. Spectra are presented as the per cent of total ions including all peaks with intensity greater than 1% total ions (with the possible exception of masses obscured by the primary ions or their decomposition products).

No ions were detected with molecular weight higher than isotopically substituted protonated parent molecules. Sufficient fragmentation was observed at peptide linkages to provide the information required for the sequencing of the amino acid residue in the molecule.

The striking features of these spectra are the large yields of protonated parent molecule ions which increase relative to the rest of the spectra with increasing proton hydration. The loss of alanine methyl ester fragment and proton or deuteron gives ions at mass 185 with all reagents used. The loss of the "extra" hydrogen or deuterium atom with the ester terminus indicates localized proton transfer to the ester terminus. In fragmentation which leaves the charge on the ester terminus, *i.e.*, loss of the neutral acetylated amino acid,

(4) K. Biemann, F. Gapp, and J. Seible, *J. Amer. Chem. Soc.*, **81**, 2274 (1959).
(5) W. R. Gray, L. H. Wojcik, and J. H. Futrell, *Biochem. Biophys. Res. Commun.*, **41**, 1111 (1970).
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(7) M. De Paz, J. J. Leventhal, and L. Friedman, *J. Chem. Phys.*, **57**, 3748 (1969).

a one-mass unit shift from 175 to 176 is observed in going from the proton to deuteron ion reagents, supporting a localized proton transfer ionization mechanism.

The case for localized proton or deuteron addition to the ester terminus is complicated by hydrogen atom rearrangements within the protonated molecule ion as evidenced by ions at masses 44, 104, 143, and 175 in proton transfer spectra. Internal hydrogen–deuterium exchange processes account for ions at mass 45, 87, 115, and 186 in the deuteron transfer spectra. However, if the "ionizing"deuteron were randomly attached, peaks corresponding to a + b + D at 116, and a + b + c + D at *m/e* 187 would be expected. Failure to observe these species supports the argument that proton transfer is indeed limited to the ester terminus of this molecule.

This technique utilizing protons in various states of solvation as the ionizing reagent permits controlled deposition of excess internal energy in the protonated parent molecule product. This single collision technique operates at very low pressures which may afford an advantage in treatment of relatively nonvolatile materials and which eliminates subsequent ion–molecule collisions that may perturb the final mass spectrum observed. It is clear that these secondary collisions are not required to stabilize protonated parent molecule ions when these ions are generated with very little excess energy.

The potential of this technique for sequence analysis of amino acids in peptides is demonstrated in these experiments. Experiments are in progress to test the value of the single ion impact technique on a variety of peptides including cases of polyfunctional amino acid systems.

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(8) Department of Chemistry.

R. J. Beuhler, L. J. Greene, L. Friedman*[8]
Departments of Chemistry and Biology
Brookhaven National Laboratory
Upton, New York 11973
Received May 10, 1971

Transient Effects in Excitation of Triplet States

Sir:

A number of experiments designed to demonstrate selectivity in population and depopulation of individual sublevels of photoexcited triplet states have been performed at temperatures of a few degrees Kelvin. The observations include anomalous intensities in esr lines under steady illumination[1–3] and transient effects in both electron spin resonance intensities and in intensities and polarization of phosphorescence accompanying modulation of the exciting light.[2–5] Apparently it has been presumed that low temperatures are necessary for detection of the effects in order to retard sufficiently the rates of equilibration between states so as to make them slower than or comparable to the rates of dissipation of the states.

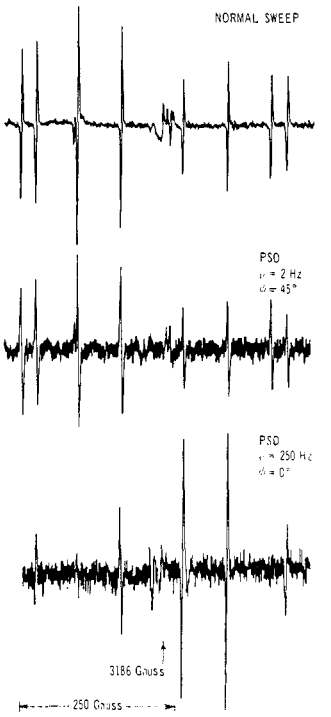

Figure 1. Esr recorded spectra of anthracene-d_{10} in crystalline benzophenone.

We report here observation of electron spin resonance carried out at 77°K which shows a variety of transient effects. The requirements for observation of the transients are simply that their amplitudes and duration, respectively, lie within the sensitivity and frequency response of the detecting instruments.

We describe as two examples among the several systems in which we have observed similar phenomena the behavior of anthracene-d_{10} dissolved in crystalline benzophenone and of phenazine dissolved in crystalline biphenyl. In each case the electron spin resonance is detected by a conventional electron spin resonance spectrometer (Varian E-3). The output of the spectrometer is recorded either in the usual way as derivative of susceptibility *vs.* field under steady illumination or with a modulated light source to yield the time variation of the signal. In the latter mode either the rise and decay of the signals at fixed fields are averaged over several thousand repetitions by means of a PAR wave form eductor or the signal is passed through a phase-sensitive detector which is referenced to the light pulse.[6] Low-frequency modulation is achieved by a rotating sector. Higher frequency modulation is produced by an electronically controlled high-pressure xenon arc (Eimac 150 XSR) which yields flat-topped pulses of variable duration and interval. The rise and decay times of the light pulses are 10–20 μsec. The resolution in time is limited to 150 μsec by the band width of the spectrometer.

The esr spectrum of anthracene-d_{10} in benzophenone excited to its triplet state by steady illumination is shown in Figure 1. Slow modulation of the light at 2 Hz and phase-sensitive detection of the esr, the light

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(3) E. H. Clarke, *ibid.*, **6**, 413 (1970).
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