Title
SYNTHESIS OF DINITROXIDES

Authors
Joss, Urs R.
Calvin, M.

Publication Date
1971-09-01

Submitted to Journal of the
American Chemical Society

SYNTHESIS OF DINITROXIDES

Urs R. Joss and M. Calvin

September 1971

AEC Contract No. W-7405-eng-48

DISCLAIMER

SYNTHESIS OF DINITROXIDES

Urs R. Joss[1] and M. Calvin[2]

Contribution from the Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, and Department of Chemistry, University of California, Berkeley, California, 94720.[3] Received

ABSTRACT: The synthesis of seven stable nitroxide biradicals has been completed. Five of these compounds, namely, N-(1-oxyl-2,2,6,6-tetramethylpiperidyl)-N'-(1-oxyl-2,2,6,6-tetramethyl-4-methoxycarbonylpiperidyl) urea; 1-oxyl-2,2,5,5-tetramethylpyrrolyl-4-N(-1-oxyl-2,2,5,5-tetrameth pyrrolidyl-3-methylene)carboxyamide; 1-oxyl-2,2,5,5-tetramethylpyrrolidine-3-N-(1-oxyl-2,2,6,6-tetramethylpiperidyl-4)carboxyamide; 1,2-bis(1-oxyl-2,2,6,6-tetramethyl-4-methoxycarbonylpiperidyl-4)oxalic acid diamide; and 1,2-bis(1-oxyl-2,2,6,6-tetramethylpiperidyl-4)succinic acid diamide, fulfill the two conditions which are postulated for their application as a flexible strain gauge in biological material: a distance of 7 to 11 A between the two radical units in order to guarantee an interaction between the two unpaired electrons and a certain rigidity in the connecting chain in order to achieve a high resolution of the e.s.r. spectrum.

In this paper we describe the synthesis of new stable biradicals in the class of nitroxides of pyrrolines, pyrrolidines and piperidines. Stable biradicals have been proposed as a flexible strain gauge, which would be attached to a biological sample (membrane or macromolecule) at two points, deform together with the support and transduce the strain into the inter action-dependent features of the e.s.r. spectrum.[4,5]

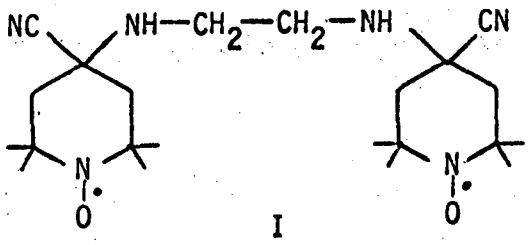
According to earlier results[5], we are concluding that such a biradical should fulfill two conditions regarding its e.s.r absorption:

1. It should have a distance of 7 to 11 A between the two radical units (nitroxide groups) to guarantee an interaction "J" in the order of magnitude of "a_N" where J is the exchange interaction between the two unpaired electrons and a_N is the isotropic hyperfine coupling constant in the biradical. The e.s.r. spectrum of such a biradical can then be accounted for in terms of the spin-Hamiltonian proposed by Reitz and Weissman[6] and applied by R. Briere and coworkers[7] as well as by Glarum and Marshall.[8]

2. The chain, which connects the two radical units, should have a certain rigidity in order to experience only a small fractional change in J, expressing itself in longer lifetimes, narrower lines, and thus result in better resolution and higher sensitivty of the e.s.r. spectrum.

SYNTHESES

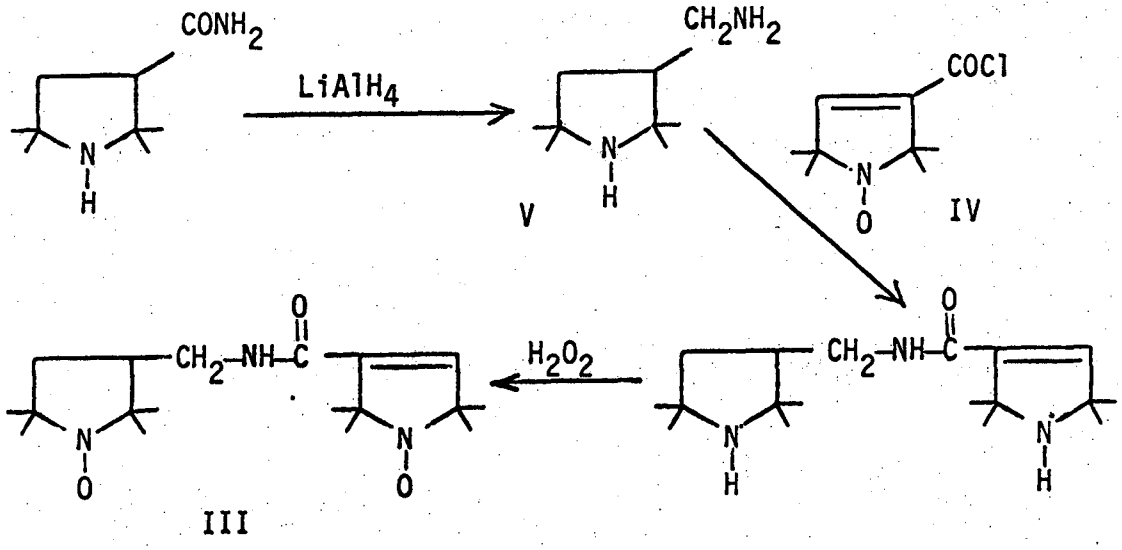
1,2-Bis(1-oxyl-2,2,6,6-tetramethyl-4-cyano-4 diaminoethane(I). This biradical in the class of the bis(α-iminoacid nitriles) was obtained by a modified synthesis according to Strecker with 1-oxyl-2,2,6,6-tetramethyl-4-piperidone and ethylenediamine.



I

I is very easily hydrolyzed back to its starting materials and is, therefore, not useful for biological applications.

1-Oxyl-2,2,5,5-tetramethylpyrrolyl-4-N(-1-oxyl-2,2,5,5-tetramethyl-pyrrolidyl-3-methylene)carboxyamide (III) This compound was prepared by acylation of 2,2,5,5-tetramethyl-3-aminomethylpyrrolidine (V) with 1-oxyl-2,2,6,6-tetramethyl-3-chloroformylpyrroline (IV), following oxidation. V is obtainable by reduction of 2,2,5,5-tetramethylpyrrolidine-3-carboxyamide with lithium aluminum hydride.



III

Derivatives of 2,2,6,6-tetramethyl-4-amino-4-carboxypiperidine (VI). This amino acid (VI), which has been described by Rassat and coworkers[9], seemed

to be a useful precursor for the synthesis of biradicals with functional groups, which could easily be transformed into highly active groups such as maleimides, acid anhydrides, isothiocyanates, or iodoacetamides in order to be anchored to the corresponding functional group, such as sulfhydryl or amino groups, in biological material. Figure 1 gives a summary of the derivatives of VI.

We prepared three biradicals from VI, namely, the amides IX, X and XII, using the methyl ester of VI, (VII), as a key intermediate.

1,2-Bis(1-oxyl-2,2,6,6-tetramethyl-4-methoxycarbonylpiperidyl-4)oxalic acid diamide (IX) was prepared by acylation of VII with oxalyl chloride, which yields the diamide VIII. It is converted to IX by oxidation with hydrogen peroxide. Mild alkaline hydrolysis yields 1,2-bis-(1-oxyl-2,2,6,6-tetramethyl-4-carboxypiperidyl-4)oxalic acid diamide (X).

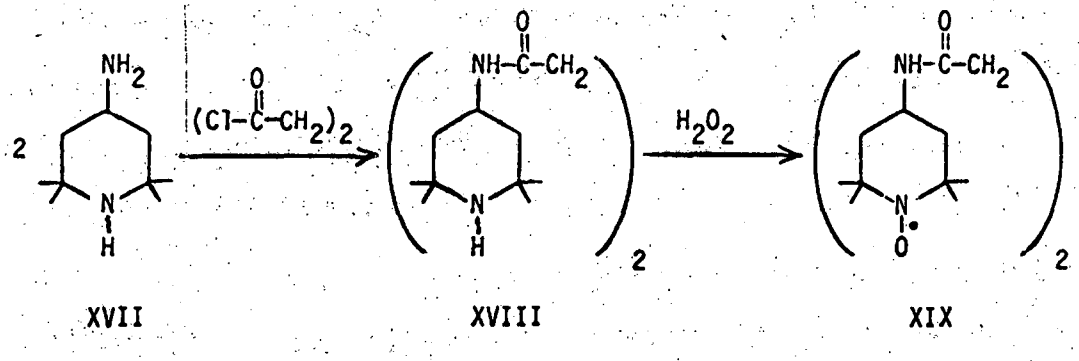
N-(1-oxyl-2,2,6,6-tetramethylpiperidyl)-N'-(1-oxyl-2,2,6,6-tetramethyl-4-methoxycarbonylpiperidyl)urea (XII). We obtained XII by selective acylation of VII with the carbamic acid chloride of 2,2,6,6-tetramethyl-4-aminopiperidine and oxidation of the unsymmetrical N,N'-disubstituted urea XI with hydrogen peroxide. With this acylation procedure, we obtained 60% XI and 40% spiro[hydantoine-5,4'(tetramethyl-2',2',6',6'-piperidine)] (XIII). The inverse procedure, namely, the reaction of the carbamic acid chloride of VI with 2,2,6,6-tetramethyl-4-aminopiperidine gave only 30% XI and 70% hydantoine (XIII).

We obtained the hydrantoin XIII exclusively in our attempts to prepare the symmetrical urea XIV by reaction of 2 moles of VII and one mole of phosgene, by the method of Schotten-Baumann, as well as under water-free conditions in chloroform-pyridine. XIII was also obtained by melting together 2 moles of VII and one mole of urea. We did not get the urea which

could be expected from the reaction of 2 moles of 2,2,6,6-tetramethyl-4-amino-4-hydroxymethylpiperidine (XV) with one mole of phosgene or urea, but we did obtain the oxazolidon XVI in good yield.

Derivatives of 2,2,6,6-tetramethyl-4-aminopiperidine (XVII)

1,2-bis(1-oxyl-2,2,6,6-tetramethylpiperidyl-4)succinic acid diamide (XIX). We obtained XIX by condensation of 2 moles of XVII with one mole of succinyl chloride to XVIII and oxidation of XVIII with hydrogen peroxide.

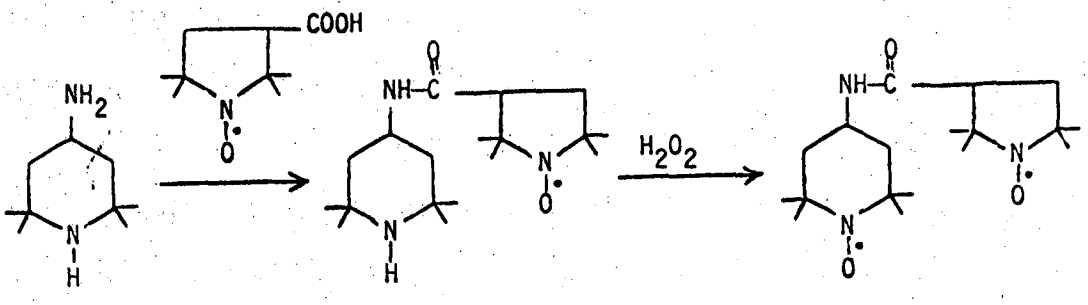


XVII XVIII XIX

Rozantsev obtained XIX by condensing succinyl chloride with 1-oxyl-2,2,6,6-tetramethyl-4-aminopiperidine[10]. This method has the disadvantage that the latter compound has to be prepared from XVII over its acetate, which adds two steps to the synthesis of XIX.

1-Oxyl-2,2,5,5-tetramethylpyrrolidine-3-N-(1-oxyl-2,2,6,6-tetramethylpiperidyl-4)carboxyamide (XX). We prepared XX by acylation of XVII with

1-oxyl-2,2,5,5-tetramethylpyrrolidine-3-carboxylic acid and 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide, and oxidation with hydrogen peroxide.



XVII XX

EXPERIMENTAL

Biradical I: Ethylenediamine (0.02 moles) is almost neutralized with HCl (pH 8) and 2.20 g (0.046 moles) NaCN added. After mixing, 5 ml ethanol is added and the mixture cooled at -10°. Within 2 hr a saturated solution of 6.84 g (0.04 moles) of 2,2,5,5-tetramethylpiperidine(1)oxyl in 90% ethanol is added, while the reaction mixture is kept at -10° to 0° and stirred. The resulting clear orange mixture is stirred for 1/2 hr with the same volume of ice, whereby compound I precipitates. After the addition of 50 ml of water, I is separated by filtration and washed with water. The product is almost pure and has a m.p. of 126° after drying over P_2O_5 in vacuo at room temperature. It can be recrystallized in benzene.

Anal. Calcd: C, 63.15; H, 9.1; N, 20.1. Found: C, 63.14; H, 9.13; N, 20.24.

IR: $-NH_2$: 3300, C N: 2219 cm^{-1}.

Biradical III. Five grams of 2,2,5,5-tetramethyl-4-carbamidopyrrolidene and 2.5 g $LiAlH_4$ are refluxed for 2 days in absolute ether. Water

and solid KOH are added, and the solvent evaporated from the filtered reaction mixture. the residue is fractionated in vacuo. The yield was 3 g of a colorless liquid at 90-92°/12 Torr.

IR: 3365, 3305, 3180, 1583 cm^{-1}.

III, a mixture of 2,2,5,5-tetramethyl-4-carboxypyrroline and 0.1 ml pyridine in 3 ml benzene, is cooled to 0° and 0.09 ml thionyl chloride are slowly added. After standing at room temperature for 1 hr, the solution of the acid chloride is separated from the pyridine-HCl with a filter pipette and concentrated in the argon stream, until the mixture does not smell of $SOCl_2$ anymore. The material is cooled at 0°, and 155 mg of 2,2,5,5-tetramethyl-4-aminomethylpyrrolidine are added. After stirring for 2 hr at room temperature, the precipitated hydrochloride is brought into solution by adding 1 M NaOH. The organic layer is washed with water and the solvent evaporated. The residue is oxidized with hydrogen peroxide without purification[11]. Yield: 250 mg raw product, yellow needles, m.p. 182.5 (cyclohexane-benzene).

IR: 3448, 3360, 1664, 1615 (Double bond) cm^{-1}.

Anal. Calcd: C, 64.14; H, 9.20; N, 12.51. Found: C, 64.15; H, 9.22; N, 12.60.

Amino acid ester (VII). Two g of VI are dissolved in 20 ml absolute methanol and the mixture is saturated with HCl gas. Methanol is removed in vacuo after 10 hr and the same procedure is repeated. The residue of the amino acid methyl ester hydrochloride is made alkaline with 15% KOH at 0°, and the free ester is extracted with chloroform. After washing with water, drying and evaporating the chloroform, the residue crystallizes in large, colorless crystals, m.p. 88-89°. After recrystallization in cycloehxane-petroleum ether, m.p. 91.5°. Yield: 60% overall.

Anal. Calcd. C, 62.0; H, 9.88; N, 13.13. Found: C, 62.08; H, 10.04; N, 13.02.

IR: -NH: 3375, $-NH_2$: 3300, C=O, 1725.

Biradical IX: To a solution of 107 mg amino acid ester (VII) in 2 ml of chloroform, 0.021 ml oxalyl chloride are added under argon at -5°. After 2 hr at 0° and 24 hr at 20° the white precipitate is separated by filtration and recyrstallized in petroleum ether and cyclohexane. Yield: 70-80%. m.p. 179.5°.

Anal. Calcd: C, 59.9; H, 8.9; N, 11.65. Found: C, 60.03; H, 8.96; N, 11.77.

IX is obtained by the usual oxidation procedure, according to Rozantsev[10]. m.p. 231°.

IR: 3390, 1741, 1658 cm^{-1}.

Biradical X. A solution of 70 mg diester (IX) in 2.5 ml of 0.5 M NaOH and 2 ml methanol is kept at 40° for 3 hr and then at 20° for an additional 3 hr. The reaction mixture is acidified very carefully to pH 3 to 4 with HCl. The bright yellow diacid X precipitates and is collected by filtration and recrystallized in acetone under pressure at 100°. Yield: 70%. m.p., dec. at 230°.

Anal. Calcd: C, 54.55; H, 7.48; N, 11.58. Found : C, 54.50; H, 7.39; N, 11.37.

IR: 3200, 2500, 1720, 1670 cm^{-1}.

Biradical XI. To a mixture of 155 mg XVII in 2 ml chloroform under argon, 0.8 ml of a 12.5% solution of phosgene in benzene are added at -20°. The mixture is warmed up at 0° and after 10 min 214 mg of amino acid ester VII in 1 ml chloroform are slowly injected. The reaction mix-

ture is stirred for 2 hr at 0° and for 30 min at 70°. Chloroform is evaporated in vacuo, the residue is dissolved in 2 ml of 1 M HCl, kept at 60° for 30 min, cooled to 0° and made alkaline to pH 12 with conc. KOH. The white precipitate is filtered and washed with water. Yield: 200 mg with a m.p. of 260-270°. Oxidation with H_2O_2 and recrystallization of the crude biradical in methanol-cyclohexane and benzene yields 120 mg of red needles, m.p. 242°.

Anal. Calcd: C, 59.1; H, 8.95; N, 13.15. Found: C, 59.58; H, 8.73; N, 13.50.

IR: 3460, 3430, 3360, 1763, 1700 (s), 1507 cm^{-1}.

Biradical XIX. To 1.56 g XVII in 20 ml chloroform, 0.78 g succinoyl chloride in 5 ml chloroform is added slowly. After 1 hr, the white precipitate is filtered and 10% aqueous NaOH solution is added. The free base is extracted with chloroform, the extract washed with saturated sodium chloride solution and dried with sodium sulfate. After recrystallization in isopropanol, the white amide dihydrate melts at 204°. Oxidation with hydrogen peroxide yields 90% XIX, m.p. 180° (lit: 178.5-180°).

Anal. Calcd: C, 62.7; H, 9.50; N, 13.25. Found: C, 62.75; H, 9.61; N, 13.37.

Biradical XX. A mixture of 186 mg (1 mM) of 2,2,5,5-tetramethyl-4-carboxypyrrolidine-1-oxyl, 200 mg (1.05 mM) of 1-ethyl-3-(dimethylaminopropyl)-carbodiimide HCl and 155 mg (1 mM) XVII in 5 ml chloroform were kept overnight at 40° under argon. After evaporation of the solvent the residue was oxidized by the usual method overnight with hydrogen peroxide without any purification. The crude red biradical was recrystallized once in benzene-cyclohexane : Yield: 30% overall, m.p. 179.5°

Anal. Calcd: C, 62.9; H, 9.54; N, 12.93. Found: C, 63.00; H, 9.60. N, 12.94.

IR: 3425, 3332, 1667 cm^{-1}

Hydantoin XII by a substitution with urea. Two moles of VII and 1 mole of urea are mixed together and heated to 160-170° in 15 min. At 140° the mixture starts evolving NH_3 and becomes turbid. After 30 min at 170° the mixture is a white solid. After cooling down, about 120 mg of starting material VII are extracted with toluene. The residue consists of pure hydantoin XIII (identified by comparison of its IR spectrum with the IRspectrum of hydantoin obtained by a Strecker synthesis with 2,2,6,6-tetramethyl-4-oxo-piperidine).

The oxazolidon XVI is made in an analogous procedure with amino alcohol XV.

Oxazolidon XVI by phosgenation of amino alcohol XV

Amino alcohol XV. Amino acid ester VII, 214 mg, and 115 mg $LiAlH_4$ are stirred in 5 ml ether for 15 min. Water (0.8 ml) is added, and then 30 ml of ether. Filtration and evaporation of the solvents yields 200 mg XV. m.p. 121.5° (petroleum ether-benzene).

Anal. Calcd: C, 64.5; H, 11.8; N, 15.05. Found: C, 64.85; H, 11.98; N, 15.04.

IR: 3620, 3340, 3160, 1580 cm^{-1}.

Oxazolidon. To a solution of 186 mg of XV in 1 ml chloroform, are added 0.4 ml of a solution of 12.5% phosgene in benzene at 0°. After 1 hr at room temperature, the reaction mixture is made alkaline with 2 ml of 0.5 M NaOH. The white precipitate is filtered and recrystallized in benzene at 120° under pressure. Additional oxazolidon can be obtained by extracting the water phase with ethyl acetate. Yield: 80 mg., m.p. 213°.

Anal. Calcd: C, 62.3; H, 9.45; N, 13.2. Found: C, 62.05; H, 9.66; N, 13.29.

IR (in Nujol): 1740, 1630, 1580, 1560 cm^{-1}

E.s.r. spectra. The spectra described here have been taken at x-band in a Varian E-3 spectrometer. Some preliminary studies were done with different solvents. The solutions were degassed and sealed off in a vacuum line. Radical concentrations were sufficiently low to eliminate intermolecular exchange broadening. The spectra were taken at 20°.

A selection of spectra of biradicals III, IX, XIX and XX is shown in Figure 2. Figure 3 shows the spectra of biradical XI in five solvents of different polarities. Biradicals I and X showed three sharp lines and two broad lines in between. This type of spectrum has been discussed by Ferruti and coworkers[5].

DISCUSSION

Except in the cases of biradicals I and X we were able to observe e.s.r. spectra with more than five lines in water, which indicates a certain rigidity in the backbone of the molecule (which might eventually stem from interaction of the biradical with water). In the case of biradical XI, we were able to observe the "forbidden" S-resonances. It is remarkable how sensitively the surrounding medium (solvent) is signalized by the position and shape of the S-resonances. The theory of the type of spectra shown in Figures 2 and 3 is extensively discussed by several authors[6-8].

A more comprehensive discussion of the significance of these complex spectra with respect to the geometrical relationships between the radicals in each of the biradical molecules and the changes in the

vectorial relationships between these radicals induced by solvent, temperature and molecular binding, and their use as probes, will be given in a succeeding theoretical discussion.

REFERENCES

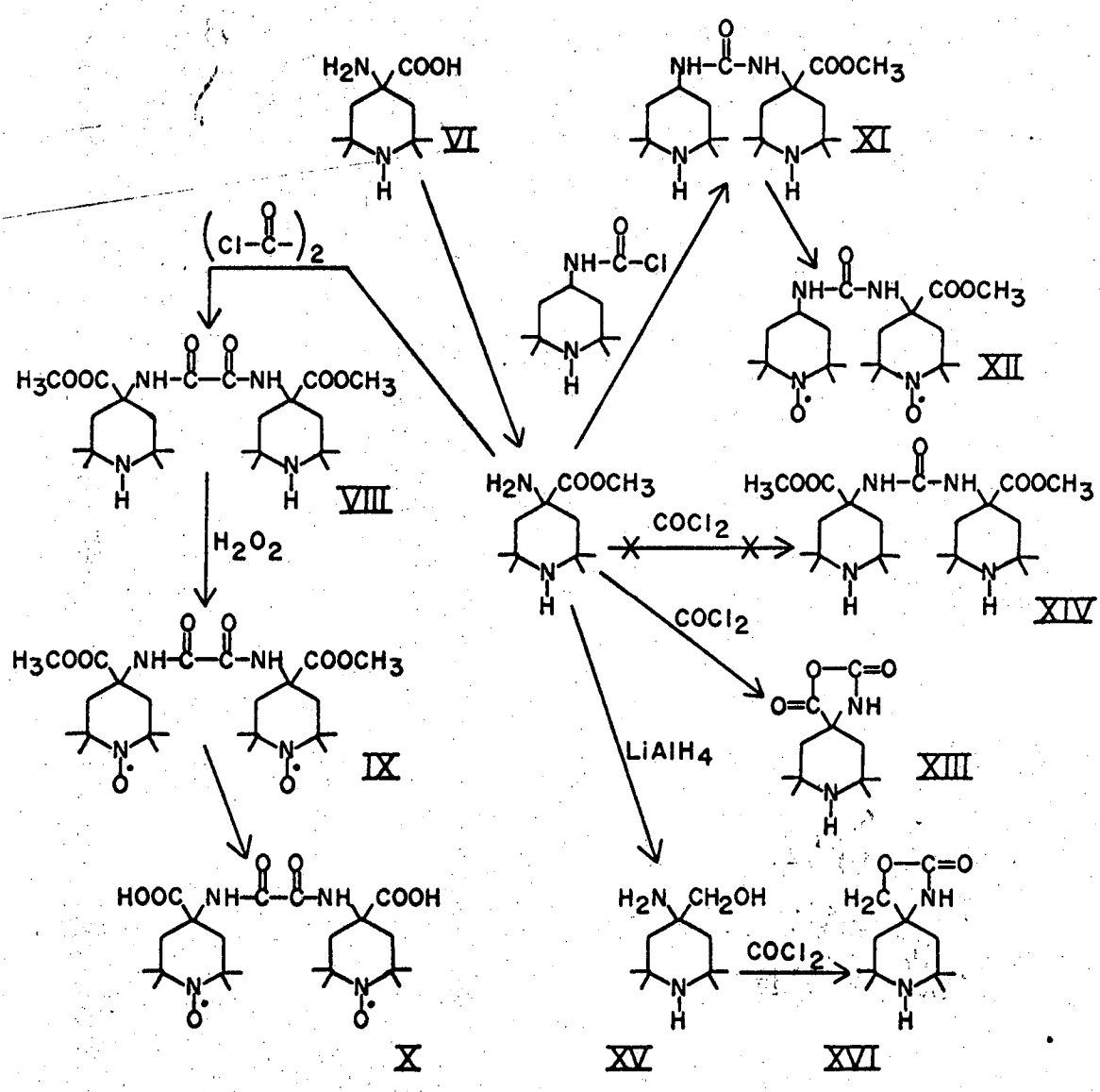
1. Fellow of the Stiftung für Stipendien auf dem Gebiete der Chemie, Basle, Switzerland.
2. To whom correspondence should be addressed.
3. The work described in this paper was sponsored, in part, by the U.S. Atomic Energy Commission.
4. M. Calvin, H. H. Wang, G. Entine, D. Gill, P. Ferruti, M. A. Harpold and M. P. Klein, Proc. Nat. Acad. Sci. U.S. 63, 1 (1969).
5. P. Ferruti, D. Gill, M. P. Klein, H. H. Wang, G. Entine and M. Calvin, J. Am. Chem. Soc. 92, 3704 (1970).
6. D. C. Reitz and S. I. Weissman, J.Chem. Phys. 33, 700 (1960).
7. R. Briere, I M. Dupeyre, H. Lemaire, C. Morat, A. Rassat and P. Rey, Bull. Soc. Chim. France 1965, 3290.
8. S. H. Glarum and J. H. Marshall, J. Chem. Phys. 47, 1374 (1967).
9. A. Rassat and P. Rey, Bull.Soc. Chim.France 1967, 815.
10. E. G. Rozantsev and V. I.Suskina, Acad. Nauk. SSSR (Ser. Khim.), 9, 2148 (1968
11. E. G. Rozantsev, "Free Nitroxyl Radicals", Plenum Press, New York, N.Y., 1970.

FIGURE CAPTIONS

Figure 1 Synthetic scheme for derivatives of 2,2,6,6-tetramethyl-4-carboxypiperidine VI.

Figure 2 First derivative e.s.r. spectra of biradicals III, IX, XIX and XX in different solvents. IX (a) in water and (b) in chloroform; IX, (c) in water and (d) in chloroform; (e) III and (f) XX in water. The spectra of oxygen-free solutions of III and XX in chloroform show the same resolution.

Figure 3 First derivative e.s.r. spectra of biradical XI in (a) water, (b) chloroform, (c) benzene, (d) carbon tetrachloride and (e) n-hexane. The J resonances ("side bands") are recorded at 10 times higher gain.



XBL718-5292

Fig. 1

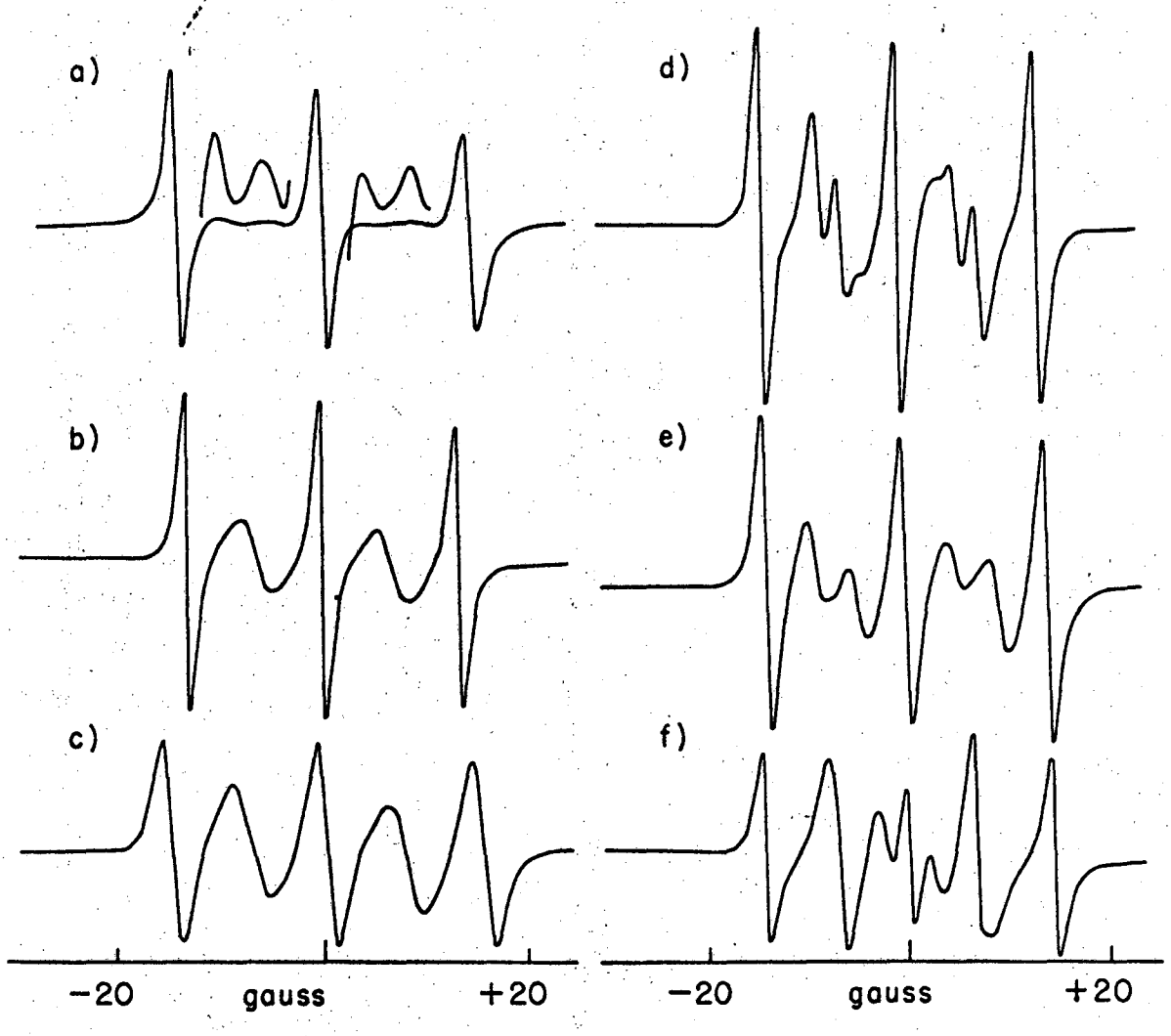


Fig. 2

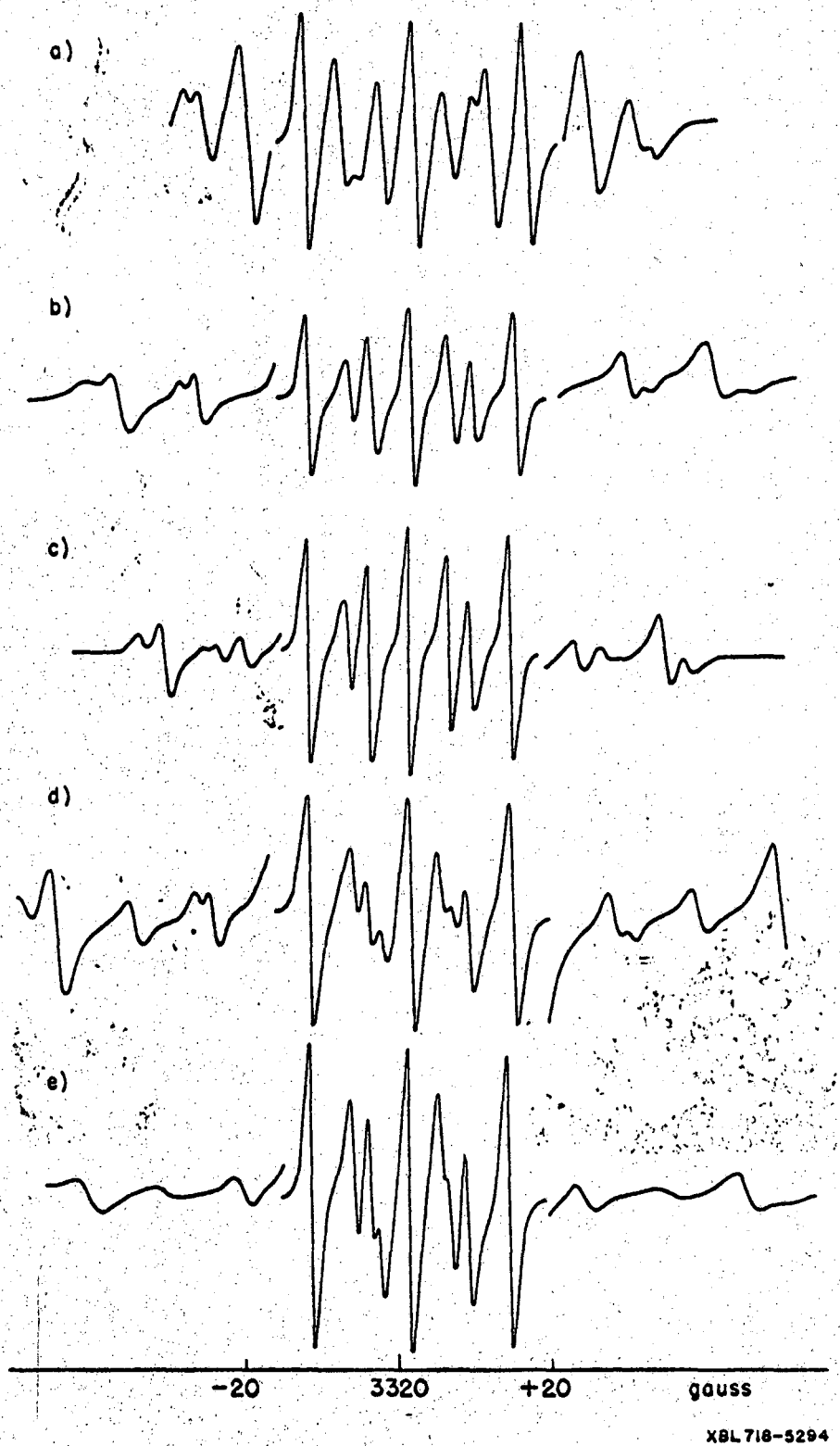


Fig. 3

TECHNICAL INFORMATION DIVISION
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720