ments in the periodical table are known to occur as heteroelements[5] in molybdenum and tungsten polyanions. The present structure is the first example of inclusion of a group 1a element into a heteropolyanion and represents a hitherto unsuspected structural type in this field. It is hoped that the present study will aid in understanding the remarkable antiviral properties of this compound.

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Supplementary Material Available: Fractional and thermal parameters and observed and calculated structure factor parameters (5 pages). Ordering information is given on any current masthead page.

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Side-Chain Substituted 2,2,5,5-Tetramethylpyrrolidine-*N*-oxyl (Proxyl) Nitroxides. A New Series of Lipid Spin Labels Showing Improved Properties for the Study of Biological Membranes

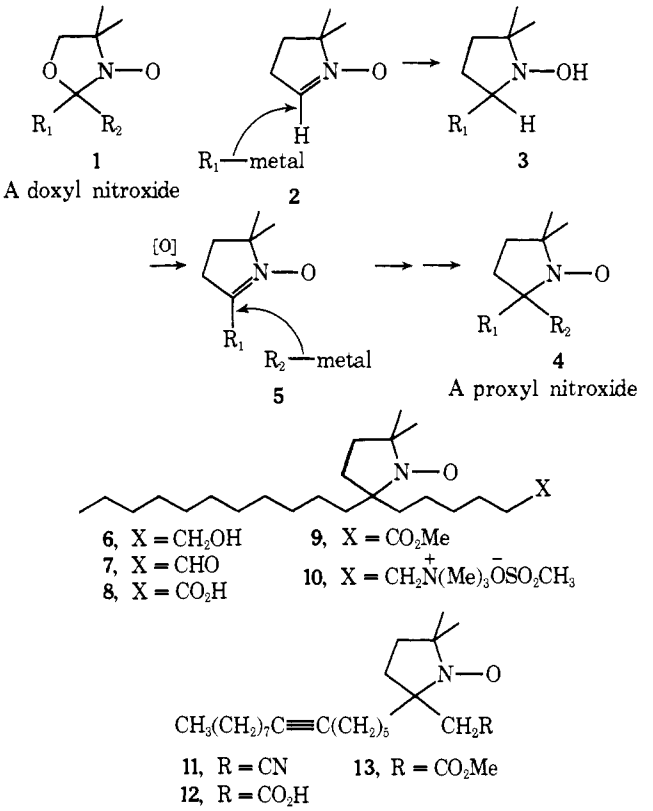
Sir:

Doxyl nitroxides **1**[2] have enjoyed wide application in the study of biological membranes by spin labeling techniques.[3] Yet in comparison with the pyrrolidine-*N*-oxyl ring system **4,** doxyl nitroxides show at times two shortcomings. Firstly, the presence of the ring oxygen atom opens up alternative pathways for decomposition, limiting the nature of subsequent chemical reactions on remote portions of doxyl containing molecules[4,5] and also giving rise to irreversible loss of ESR signal in some spin labeling studies[5] (probably via reduction to the hydrolytically unstable *N*-hydroxy derivative). Secondly, the oxygen atom within the ring renders the doxyl group quite polar, an important consideration when the label is to probe hydrophobic regions of the membrane. In response to these points we now describe the first members of a new series of nitroxide lipid spin labels based on the pyrrolidine-*N*-oxyl ring system.

Advantage is taken of the ready addition[2,6,7] of Grignard reagents to pyrroline nitrone **2** (Chart I) leading to *N*-hydroxy intermediate **3.** Since an α-hydrogen atom is present in **3,** cupric acetate-air oxidation[2,8] leads to a new nitrone **5,** itself capable of undergoing reaction with a different Grignard reagent. Oxidation then produces a stable pyrrolidine-*N*-oxyl (proxyl)[9] nitroxide **4.**

The synthesis of 7-proxylstearyl alcohol **6** is representative. To a stirred solution of 3.52 g of nitrone **2**[8] in 10 ml of THF was

Chart I



added dropwise at reflux (N_2) 45.6 ml (1.5 equiv) of a 1.0 M solution of undecylmagnesium chloride in THF. After 1 h at reflux the reaction was quenched with saturated NH_4Cl. The usual workup afforded the corresponding crude *N*-hydroxy compound which was immediately taken up with 170 ml of MeOH–concentrated NH_4OH (15:2) and stirred under air in the presence of 12.9 g of $Cu(OAc)_2 \cdot H_2O$[2,8] for 1 h, affording 7.07 g (85%, based on **2**) of nitrone **5** ($R_1 = CH_3(CH_2)_{10}$–) (bp 118–121° (0.005 mm); *m/e* 267.257). The reaction of **5** ($R_1 = CH_3(CH_2)_{10}$–) (500 mg) in THF (10 ml) at 25° (N_2) with 2.8 ml (1.5 equiv) of a 1.0 M THF solution of $THPO(CH_2)_6MgCl$ was quenched after 18 h by dropwise addition (0°) of 10 ml of MeOH and 5 ml of 3 N HCl. The quenched mixture was stirred for 1.5 h at 0° in order to hydrolyze the tetrahydropyranyl ether grouping.[10] The usual workup provided the crude *N*-hydroxy alcohol which was dissolved in MeOH (10 ml) containing 5 mg of $Cu(OAc)_2 \cdot H_2O$[2] and stirred under air for 1 h. Preparative TLC over silica gel gave 154 mg (22%) of 7-proxylstearyl alcohol **6** (*m/e* 368.354).

Reaction of nitrone **2,** on the other hand, with the Grignard reagent derived from 1-bromopentadec-6-yne[11] led, after oxidation, to nitrone **5** ($R_1 = CH_3(CH_2)_7C{\equiv}C(CH_2)_5$–) (*m/e* 319.285) (89%). Addition of lithium acetonitrile in THF to this substance (−78° → 25°) followed by oxidation afforded a mixture (47%) of nitroxide **11** (*m/e* 359.305) and the corresponding lactone (by ir). Base hydrolysis of the mixture followed by chromatography over silica gel gave 9,10-dehydro-3-proxyloleic acid (**12**) (30%, based on **5**). Esterification of **12** with diazomethane afforded ester **13** (70%) (*m/e* 392.319).

The chemical stability of the proxyl ring system in these spin labels parallels that of simple pyrrolidine-*N*-oxyl nitroxides.[5] For example, oxidation of alcohol **6** with *N*-chlorosuccinimide-dimethyl sulfide[12] afforded aldehyde **7** (78%) (*m/e* 366.338). Two phase oxidation of an ether solution of **7** with Tollen's reagent gave 7-proxylstearic acid (**8**) (*m/e* 382.330) (70%) which was also converted (CH_2N_2) to the methyl ester **9** (*m/e* 396.348). Alternatively, reaction of **6** with methan-

Table I. Summary of Proxyl and Doxyl Nitroxide ESR Spectra in Dodecane–Water

Nitroxide	A_0 water (g)	A_0 dodecane (g)	ΔA_0 (g)	K^a
 (proxyl)	16.37	14.02	2.35	7.27
 (doxyl)	16.02	14.32	1.70	3.32

$^a K$ = [nitroxide]$_{dodecane}$/[nitroxide]$_{water}$ calculated by digitalization and double integration of ESR spectra obtained from each of the two deoxygenated phases.

esulfonyl chloride–Et_3N in ether at 0° afforded the corresponding mesylate which was converted into quaternary amine salt **10**, a yellow solid, mp 88.5–89.5° (71%, based on **6**) (Found for **10**·0.5H_2O: C, 63.10; H, 11.44; N, 5.23) by heating (1 h) the mesylate in the presence of trimethylamine in THF at 110° (bomb).

In another series of experiments an egg lecithin–cholesterol–alcohol **6** mixture (molar ratio = 150:75:1) was dispersed in water. Qualitatively, the ESR spectrum of **6** in this system (A_{max} = 32 G) was the same as that where **6** was replaced by 7-doxylstearyl alcohol.[13] Most interestingly, whereas no ESR signal could be obtained with a sonicated mixture of 7-doxylstearyl alcohol–cardiolipin–cytochrome *c* (molar ratio = 1:70:20) in water (irreversible reduction, see above), with a comparable mixture containing proxyl alcohol **6** an ESR signal was readily observable. The signal intensity increased threefold when O_2 was briefly bubbled through the sample, demonstrating that while proxyl alcohol **6** does suffer some reduction in the cytochrome *c* preparation, the reaction is reversible to a large extent.

In order to assess the change in polarity resulting from substitution of the ring oxygen atom of a doxyl nitroxide by a methylene group in these new labels, both doxyl nitroxide **1** (R_1 = R_2 = Me) and proxyl nitroxide **4** (R_1 = R_2 = Me) were partitioned under identical conditions between dodecane and water. From the data shown in Table I it is apparent that a, the proxyl group is significantly less polar than the doxyl group and b, the ESR spectrum of the proxyl nitroxide is more sensitive to changes in polarity of the medium than that of the doxyl nitroxide.

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Micelle Catalyzed Reactions Are Models of Enzyme Catalyzed Reactions Which Show Positive Homotropic Interactions

Sir:

In attempting to understand the mechanisms by which enzymes catalyze reactions, chemists have expended much effort in the study of catalysis in simpler, chemical systems.[1] Catalysis of reactions within micelles has been studied extensively from this point of view[2,3] since both micelles and enzymes bind substrates in a noncovalent manner. The purpose of this report is to demonstrate an additional similarity of micelle catalysis and enzymatic catalysis: the kinetics of micelle catalyzed reactions are similar to those of many regulatory enzymes in that they show positive homotropic interactions.[4] A simple kinetic description of enzymatic homotropic interactions may be applied to analysis of the kinetics of a vast number of micelle catalyzed reactions.

The rate constants for micelle catalyzed reactions when plotted vs. detergent concentration yield approximately sigmoid-shaped curves; downward sloping sigmoid curves have also been observed for cases in which micelles inhibit reaction. The similarities in shape of these curves to the sigmoid-shaped dependencies of velocity on substrate concentration produced by many regulatory enzymes are striking. The kinetic model commonly used to quantitatively describe the relationship of rate constant to detergent concentration assumes that micelle, D_n, forms a noncovalent complex with substrate, S, before catalysis may take place.[3]

$$\begin{gathered} D_n + S \overset{K}{\rightleftharpoons} D_nS \\ D_nS \overset{k_m}{\rightarrow} \text{product} \\ S \overset{k_0}{\rightarrow} \text{product} \end{gathered} \qquad (1)$$

In this scheme K is the association constant of the micelle-substrate complex, k_m is the rate constant for micelle-catalyzed reaction, and k_0 is the rate constant for reaction in the absence of micelle. The observed rate constant at any concentration of micelle is given by

$$k_{obsd} = \frac{k_0 + k_m K\left(\frac{[D]_{total} - CMC}{n}\right)}{1 + K\left(\frac{[D]_{total} - CMC}{n}\right)} \qquad (2)$$

where CMC is the "critical micelle concentration" which is defined as that concentration of detergent at which micelles first appear, and n is the number of detergent molecules per micelle. This relationship and its equivalents describe reasonably well the dependence of the rates of very many micelle catalyzed reactions, and it has received wide acceptance.

An alternative model[5] postulates that substrate and detergent molecules aggregate to form micelles, D_nS, which may then react to yield product: