cation of Fourier transform techniques to NMR spectrometry has made the direct observation of silicon-29 spectra—at the 4.7% natural abundance of the isotope—practicable. Since no values of spin-lattice relaxation times have been reported for perhalosilanes, and T_1 can be quite long for organosilanes not containing hydrogen directly bonded to silicon,[22] we felt it advisable to include the shiftless paramagnetic "relaxation reagent" tris(acetylacetonato)chromium(III)[23] in our NMR samples.

The ^{29}Si NMR spectrum[24] of a $CDCl_3$ solution of the photolytically generated crystals along with added $Cr(acac)_3$ is displayed in Figure 1. The spectrum consists of two single lines at chemical shifts (relative to external TMS) of 3.7 and −80.0 ppm, respectively.[25] The relative integrated intensities of the two lines, averaged over several spectra, is very close to 4.0 to 1, with the more intense line appearing at lower field. Crystals of Si_5Cl_{12} were also prepared as described in ref 17 and silicon spectra taken under conditions closely approximating those for the photolytically generated crystals. The main spectral features in the two sets of spectra are, within instrumental limits of resolution, identical. We must conclude that the pentasilane common to both systems does in fact possess the neo structure, $Si(SiCl_3)_4$.

Two mechanistic pathways to the observed series of perchloropolysilanes produced by the $SiCl_3H$ photolysis seem plausible to us. The first involves disproportionation of $SiCl_3$ radicals on recombination and the subsequent intermediacy of $SiCl_2$.[26] An alternative mechanism involves facile chlorine atom abstraction from silanes by various silyl radicals, with the polysilane products resulting from radical recombinations. We have at present no direct evidence in support of either mechanism; however, the abstraction route is consistent with our preliminary observation that mercury-sensitized cophotolysis of SiF_3H and $SiCl_4$ generates SiF_3Cl ($SiCl_4$ itself is unaffected by $Hg(^3P)$ under the conditions employed). Further work related to mechanistic pathways and additional silicon NMR studies on perchloropolysilanes is in progress.

Acknowledgment. Two of us (K.G.S. and P.A.S.) gratefully acknowledge the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this work, and the pioneering contribution of W. C. Ishii.

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(24) ^{29}Si spectra were run on JEOL Models FT-1A and FT-1M in 5 mm o.d. tubes at ambient temperature. Additional spectral parameters are given in conjunction with the figure.
(25) These values follow the convention that positive shifts correspond to resonances at lower field than the standard.
(26) The work of P. L. Timms (*Inorg. Chem.*, **7**, 387 (1968)) demonstrates the ability of $SiCl_2$ to insert into Si–Cl bonds at "ordinary temperatures".

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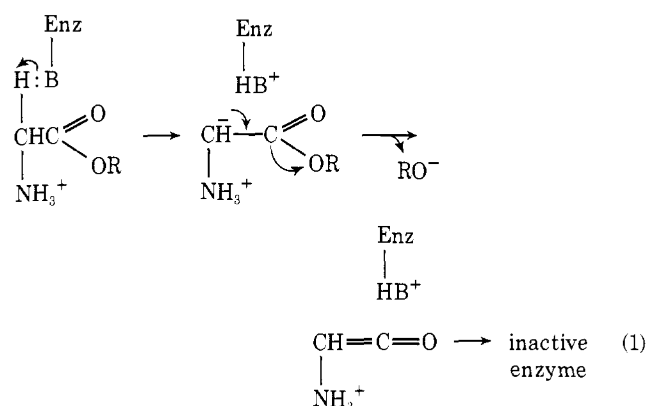
Novel Inactivators of Plasma Amine Oxidase

Sir:

Studies of the mechanism of action of plasma amine oxidase have indicated that at some stage during the oxidation a proton is abstracted from the carbon atom which is oxidized.[1] We have reported that propargylamine and 2-chloroallylamine irreversibly inactivate plasma amine oxidase[2] and proposed a mechanism of inactivation which is based on the ability of the enzyme to abstract a proton from these inactivators. An additional approach to the irreversible inactivation of that enzyme was suggested to us by the report that esters with sufficiently good leaving groups can undergo elimination reactions to form ketenes.[3] Generation of a reactive ketene species at the active site of an enzyme might lead to enzyme inactivation, probably by acylation of a group on the enzyme. Glycine esters appear to be good candidates for this kind of enzyme inactivation. We expected that plasma amine oxidase would catalyze the abstraction of a proton from the α-position of a glycine ester to form a carbanion, which could undergo either the normal oxidative process or an elimination reaction (eq 1) to produce an enzyme bound ketene.



Results obtained with several glycine esters are summarized in Table I. All are good substrates relative to benzylamine. Only the two α-amino esters which have good leaving groups inactivate the enzyme. Ethyl glycinate and the β-amino ester do not inactivate the enzyme. Furthermore, "R" is larger for the phenyl than the p-nitrophenyl ester, which implies that as the leaving group becomes better, partitioning between inactivation and oxidation favors inactivation. When phenyl glycinate is added to plasma amine oxidase, the rate of oxygen consumption decreases to zero

Table I. Kinetic Constants for Substrates and Inactivators

Substrate	V_{max} nm min^{-1} mg^{-1}	K_M, M	$t_{1/2}$ for inactivation	R^a
Benzylamine	140	1×10^{-3}	∞	—
Ethyl glycinate	150	5×10^{-6}	∞	—
Phenyl glycinate[4]	270	1.5×10^{-5}	1.4 min	80
p-Nitrophenyl glycinate[4b]	90	1×10^{-5}	0.3 min	10
Phenyl 3-aminopropionate[4,5]	30	1×10^{-4}	∞	—

^{a} R = (moles O_2 consumed)/(moles enzyme inactivated). Reaction rates were determined by measuring O_2 consumption with a Clark type electrode. Reactions were carried out (at 23°) in 0.4 ml of 50 m*M* potassium phosphate buffer pH 7.0, except for *p*-nitrophenyl glycinate where 100 m*M* potassium phosphate buffer pH 6.0 was used. Specific activity of enzyme 270–400[7] at 23°.

with time, indicating enzyme inactivation. Further addition of substrate at this point results in no additional oxygen consumption. Addition of enzyme, however, leads to resumption of oxygen uptake with the same initial rate as that observed after the first enzyme addition. Inactivation, therefore, is not due to accumulation in solution of a compound enzymically derived from phenyl glycinate. This conclusion was confirmed by measuring the kinetics of phenyl glycinate inactivation in a separate experiment. The inactivation was found to be first order in enzyme for over four half-times. The rate of inactivation by phenyl glycinate is decreased competitively by ethyl glycinate (a noninactivating substrate). "R" (Table I) is independent of phenyl glycinate concentration and is not affected by ^2H substitution in the α-position.[6] These results suggest that oxidation and inactivation occur from the same binding site and may have one or more chemical steps in common.

When the enzyme is inactivated with phenyl [1-^{14}C]glycinate,[7] and then passed through Sephadex G-25 at 2° to remove small molecules, 1.8–2.1 mol of radioactivity are bound per mole of enzyme,[8] and no enzymic activity is observed. In addition, if the enzyme is inactivated with a mixture of phenyl [1-^{14}C]glycinate and phenyl [2-^3H]glycinate,[9] one atom of ^3H is lost per molecule of phenyl glycinate incorporated. These data probably indicate loss of one α-proton upon incorporation rather than a selection against the ^3H speciies, because there is no such selection in the oxidation of ethyl glycinate.[6]

By a process similar to that shown in eq 1, abstraction of a proton adjacent to a cyano group (instead of an ester group) could lead to the formation of a ketenimine, which is also expected to be a reactive species. Therefore, the effect of 2-aminoacetonitrile, NH_2CH_2CN, upon the enzyme was also investigated. The compound showed no substrate activity, but was an effective inhibitor. At 2.5×10^{-5} *M*, $t_{1/2}$ for inactivation was 0.8 min. In other respects, the inactivation resembled that of phenyl glycinate. Experiments with [1-^{14}C]aminoacetonitrile[10] showed that the enzyme became covalently labeled.[11] The rate of inactivation was decreased in the presence of substrates. Aminoacetonitrile inactivates rabbit plasma amine oxidase in vivo. Since concentrations of aminoacetonitrile which completely inactivate the plasma enzyme have no effect on the activity of the mitochondrial amine oxidase (a flavoprotein), the compound can be used in vivo to study the role of these enzymes in the metabolism of amines.[12] It is interesting to note that aminoacetonitrile is one of the most effective lathrogenic agents known.[13] An amine oxidase, believed to be mechanistically similar to plasma amine oxidase, is involved in the crosslinking of collagens.

The results described show that phenyl glycinate and aminoacetonitrile irreversibly inactivate plasma amine oxidase by covalent modification of the enzyme. The results are consistent with α-proton abstraction followed by elimination and reprotonation to form ketene-like intermediates, but until the process is examined in more detail, other mechanisms cannot be ruled out.

Acknowledgments. This work was supported by National Science Foundation Grant No. GB 31952 to R.H.A., National Institutes of Health Training Grant No. GM 212 to R. S., and National Institutes of Health Special Fellowship 5F02 GM 40063-02 to A.M.

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(7) Synthesized from [1-^{14}C]glycine; final specific activity = 2.2×10^{12} dpm/mol. See ref. 4a, 4b, 5.
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Total Biogenetic-Type Synthesis of (±)-Isopimaradienols and (±)-Araucarol

Sir:

Previous nonenzymic di- and triterpenoid terminal epoxide[1] cyclizations have invariably generated tri-, tetra- and pentacyclic systems in which the methyl substituted polyene backbone of the starting material is preserved. In a significant departure from this pattern, we have now observed that the "rearranged", pimaradien-3β-ol/isopimaradien-3-β-ol type (**4**)[2] is produced directly from an all trans 14,15-oxidogeranylgeranyl ester (**1**) by means of a specific sequence involving appearance of the exocyclic methylene bicycle **2**, loss of allylic X, and further cyclization (**3**), an overall process closely related to that by which the same category of tricycles is believed to be formed enzymically from a geranylgeranyl species or terminal epoxide thereof.[3]

Timing of the various chemical events (k_1:k_2:k_3) is crucial—starting material **1** and anticipated, unisolated intermediates **2** and **3** are subject to a number of side reactions, many of which have been observed in similar systems, and all of which must be minimized in order that significant amounts of **4** be produced in one reaction vessel. For exam-