Clearly, reaction 9, which is a key step in the other possible chain mechanism (eq 8 and 9), is not a favorable process in the case of six-coordinate organocobaloximes; indeed, any organsulfonyl radicals which are formed during the reaction with sulfur dioxide may, in appropriate cases, undergo reaction with the organocobaloxime via a reaction analogous to eq 13 which regenerates cobaloxime(II) and thereby initiates reaction 6 of the preferred chain mechanism. It is not surprising therefore that some (≤5%) of the specific sulfone **12**[10] accompanies the insertion product in the reaction of dimethylallylcobaloxime (**11**) with SO_2 (eq 14).

$$Me_2C{=}CHCH_2\cdot \xrightarrow{SO_2} Me_2C{=}CHCH_2SO_2\cdot \xrightarrow[Me_2C{=}CHCH_2Co(dmgH)_2py]{\mathbf{11}} Me_2C{=}CHCH_2SO_2CMe_2CH{=}CH_2 \quad (14)$$

12

(b) There is good precedent for reaction 6 from the direct reactions of SO_2 with cobalt(II) complexes such as $Co(CN)_5^{3-}$ and $Co(dmgH)_2py$. The former gives the well-characterized complex[11] $\{[(NC)_5Co]_2SO_2\}^{6-}$ supposedly via reactions 6 and 10, though the corresponding complex $(py(dmgH)_2Co)_2SO_2$ is less well characterized.[12] It is significant that the latter complex is also formed as a byproduct (≤10%) in the reaction of SO_2 with **1** and with **7**.[13]

The above mechanism is by no means universal in SO_2 insertion reactions, but may also apply to some organoiron complexes under extreme conditions. For example, though the insertion product **5** is also obtained in 5-15% yield[14] from the reaction of SO_2 with mixtures of either **9** with **1**, or **9** with $CH_3Co(dmgH)_2py$,[15] only the two normal insertion products **14** and **15** were formed in the reaction of SO_2 with a mixture of **9** and **13**[16] (eq 15).

$$\mathbf{9} + 4\text{-}FC_6H_4CH_2Mo(CO)_3(\eta\text{-}C_5H_5) \quad \mathbf{13}$$
$$\xrightarrow{SO_2} PhCH_2SO_2Fe(CO)_2(\eta\text{-}C_5H_5) \quad \mathbf{14}$$
$$+ 4\text{-}FC_6H_4CH_2SO_2Mo(CO)_3(\eta\text{-}C_5H_5) \quad \mathbf{15} \quad (15)$$

The implications of the above results are twofold, First, though the free-radical-chain mechanism is dominant in the case of some organocobaloximes and organorhodoximes, it may intrude in other systems only where there is sufficient initiation and where the concentrations are sufficiently high and the stability of the displaced metal such that the propagation step 7 is favored. Second, the sulfur dioxide insertion reactions of organocobaloximes show remarkable similarities of initiation, rates, and regiospecificity to the oxygen insertion reactions of the same complexes.[18] We have endeavored to investigate the intermolecular character of the oxygen insertion in the same manner as above, but have been thwarted by the ready exchange of organic groups between substrates[19] prior to the reaction with oxygen and catalyzed by traces of cobaloxime(II) in solution. However, the analogies are so close for us to propose that some oxygen insertion reactions of the cobaloximes also involve radical-chain reactions, with the same initiation step as for sulfur dioxide insertion, and the corresponding propagation steps of eq 16 and 17.

$$py(dmgH)_2Co\cdot + O_2 \rightarrow py(dmgH)_2CoOO\cdot \quad (16)$$

$$py(dmgH)_2CoOO\cdot + RCo(dmgH)_2py \rightarrow py(dmgH)_2CoOOR + \cdot Co(dmgH)_2py \quad (17)$$

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(3) The benzylrhodoxime **2** is slightly more reactive and hence the appearance of **5** and **6** in preference to **3** indicates that the rhodoxime(II) species is more readily displaced by $MSO_2\cdot$ when M = $Co(dmgH)_2py$ or $Rh(dmgH)_2py$. A necessary consequence is that the yields of **5** and of **6** should be the same, which is what is observed.
(4) The several reagents and insertion products were detected in the reaction mixtures by ^1H NMR and confirmed by isolation after TLC. Only pairs of compounds, e.g., **3** and **5**, could be obtained, but these could be prepared separately by other methods and positively identified in and from the reaction mixtures.
(5) Reaction 2 was allowed to proceed to completion and mixed with the reagents of reaction 3. After 2 h, reaction 3 was complete and only two insertion products, **3** and **4**, were present.
(6) (a) T. Funabiki, B. D. Gupta, and M. D. Johnson, *J. Am. Chem. Soc.*, **98**, 6697 (1976); (b) *J. Chem. Soc., Chem. Commun.*, 653 (1977); (c) K. N. V. Duong, A. Gaudemer, and M. D. Johnson, unpublished work; (d) C. J. Cooksey, D. Dodd, M. D. Johnson, and B. L. Lockman, *J. Chem. Soc., Dalton Trans.*, in press; (e) A. Bury, C. J. Cooksey, T. Funabiki, B. D. Gupta, and M. D. Johnson, *J. Chem. Soc., Perkin Trans. 2*, in press; (f) A. E. Crease, B. D. Gupta, and M. D. Johnson, *J. Chem. Soc., Dalton Trans.*, in press.
(7) Since most organocobaloximes contain traces of cobaloxime(II) species, particularly in solution in the light, there is usually no need for an induced initiation step.
(8) Tungsten lamps (2 × 150-500 W at 10 cm) using water-cooled all-glass apparatus.
(9) A. E. Crease and M. D. Johnson, unpublished observations.
(10) Some *O*-(2-methylbut-3-*en*-2-yl)dimethylglyoxime (≤10%) is also formed.
(11) A. A. Vlcek and F. Basolo, *Inorg. Chem.*, **5**, 156 (1966).
(12) M. D. Johnson and G. J. Lewis, unpublished observations.
(13) During many of the reactions of organocobaloximes with sulfur dioxide, the ^1H NMR spectra quickly become broad owing to the presence of paramagnetic cobaloxime(II) species, but sharpen again toward the end of each reaction. The lack of reaction of methyl(pyridine)cobaloxime with pure dry sulfur dioxide reported earlier[2] may have been a result of the absence of radical initiation rather than, as proposed earlier, the absence of water.
(14) Implying attack of $py(dmgH)_2CoSO_2\cdot$ radicals on the organoiron complex, but not necessarily attack of $(\eta\text{-}C_5H_5)(CO)_2FeSO_2\cdot$ radicals on the organocobaloxime.
(15) These substrates do not insert at the same rates and hence the extent of formation of crossed products would not be expected to be large.
(16) These substrates react with SO_2 at almost identical rates and hence would be expected to give near-statistical yields of crossed and uncrossed products in an intermolecular radical mechanism. Jacobsen and Wojcicki[17] have demonstrated a similar lack of crossed products in the reactions of SO_2 with mixtures of **9** and other organomolybdenum complexes.
(17) S. E. Jacobsen and A. Wojcicki, *J. Am. Chem. Soc.*, **95**, 6962 (1973).
(18) C. Bied-Charreton and A. Gaudemer, *J. Organomet. Chem.*, **124**, 299 (1977), and references quoted therein.
(19) D. Dodd, M. D. Johnson, and B. L. Lockman, *J. Am. Chem. Soc.*, **99**, 3664 (1977).

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Inhibition of 5-Phosphomevalonate Kinase by an Isosteric Analogue of 5-Phosphomevalonate

Sir:

Analogues of the prenyl diphosphate intermediates of sterol biosynthesis proved to be useful probes for testing some enzymic functions[1] and specificities[2] and may point to the design of new substances inhibiting cholesterol biosynthesis.[3] The usefulness of the diphosphate analogues of, e.g., geranyl diphosphate, such as citronellyl, tetrahydrogeranyl, or octyl di-

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phosphate, as inhibitors of cholesterol biosynthesis in vivo—in spite of their effectiveness in vitro[1]—is vitiated by the widespread presence of phosphatases that would hydrolyze such substances before they might reach their target organs. On the other hand, analogues in which the phosphate or diphosphate ester groups were replaced by the methylenephosphonate ($-CH_2PO_3^{2-}$), or phosphonophosphate ($-CH_2P_2O_6^{3-}$) groups might prove more effective because there is no mammalian enzyme known to hydrolyze the C-P bond. Several such compounds have been reported to be inhibitors of enzymes which catalyze the synthesis of farnesyl diphosphate and squalene.[4]

We present evidence that 3-hydroxy-3-methyl-6-phosphonohexanoic acid (**1**), an isosteric analogue of 5-phosphomevalonate (**2**), specifically inhibits phosphomevalonate kinase (ATP: 5-phosphomevalonate phosphotransferase, E.C. 2.7.4.2). The synthesis of **1** has been described elsewhere.[5]

The 10 000*g* supernatant of homogenized rat liver (S_{10})

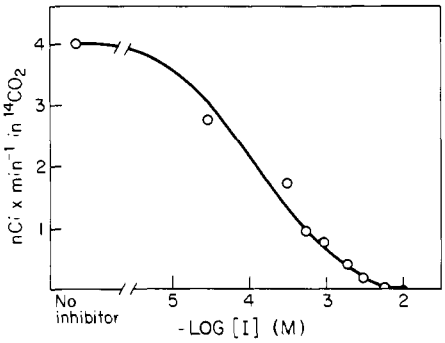
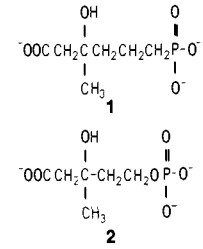


Figure 1. Inhibition of rates of release of $^{14}CO_2$ from [1-^{14}C]mevalonate in incubations of rat liver S_{10} preparations[6,7] by **1**. The rates were calculated from data of 2-, 5- and 10-min incubations during which times linear rates of $^{14}CO_2$ release (and squalene synthesis from [2-^{14}C]mevalonate) were noted without or with varying concentrations of **1**. The reactions were stopped by injecting 1 mL of 10 N citric acid through the rubber cap of the incubation tubes. $^{14}CO_2$ was collected during 1 h in a cup suspended from the rubber cap and containing 0.2 mL of 1 N hyamine hydroxide in MeOH and fluted filter paper. The center wells with their contents were transferred to counting vials containing 10 mL of RPI 3a70B scintillation fluid. Then KOH pellets (to 2 N KOH) and ethanol (to 40%) were added for the hydrolysis of the mixture at 70 °C for 1 h. Squalene and sterols were extracted with light petroleum and separated by chromatography on alumina.[6] The rates for squalene synthesis, which paralleled the rates of $^{14}CO_2$ release, are not shown as these could not be shown on the same scale.



contains all the enzymes needed to convert mevalonate into squalene (or to sterols).[6] When the S_{10} proteins are supplemented with cofactors[7] and incubated under N_2 with [1-^{14}C]- and [2-^{14}C]mevalonate, the former is converted in three steps into unlabeled isopentenyl diphosphate (IPP), $^{14}CO_2$, and unlabeled squalene, whereas the latter gives rise to $^{12}CO_2$, [^{14}C]IPP, and [^{14}C]squalene. The $^{14}CO_2$ and [^{14}C] squalene can be recovered from such incubations with efficiencies of 97 ± 2% (SD) and 91 ± 5% (SD), respectively. The concentration of a test compound that inhibits release of $^{14}CO_2$ from [1-^{14}C] mevalonate by 50% ($I_{50}^{CO_2}$) or the synthesis of squalene by 50% (I_{50}^{Sq}) can be used to evaluate the potency and specificity of a compound as an inhibitor of squalene (or sterol) synthesis.[8] The results of such an experiment with **1** is shown in Figure 1.

The value of $I_{50}^{CO_2}$ and I_{50}^{Sq} was 145 μM at a ratio of inhibitor to substrate concentration of 1.45.[9] There was no noticeable inhibition of squalene synthesis beyond the formation of IPP. Thus, partially purified liver prenyl transferase, assayed according to Holloway and Popják,[10] was not inhibited by **1** at concentrations which inhibited IPP and squalene synthesis. The evidence suggested that the effect on squalene synthesis was accountable by inhibition of one or more of the first three enzymes acting beyond mevalonate in the pathway: mevalonate kinase, phosphomevalonate kinase, or diphosphomevalonate decarboxylase.

The identity of the target enzyme of **1** was deduced by examining which substance derived from mevalonate accumulated in the inhibited incubations. For this purpose 0.1- and 0.4-mL incubations of S_{10} were set up as described[7] except that they contained 50 μM of pure (*R*)-[2-^{14}C]mevalonate (specific activity 16 Ci/mol)[11] with and without 500 μM of **1**. The reactions in the two sets of incubations were stopped after 10 min by the addition of 5 and 20 μL, respectively, of 7.2 M ammonia containing 0.2 M sodium ethylenediamine tetraacetate and ethanol to 33%. The supernatants of the precipitated proteins

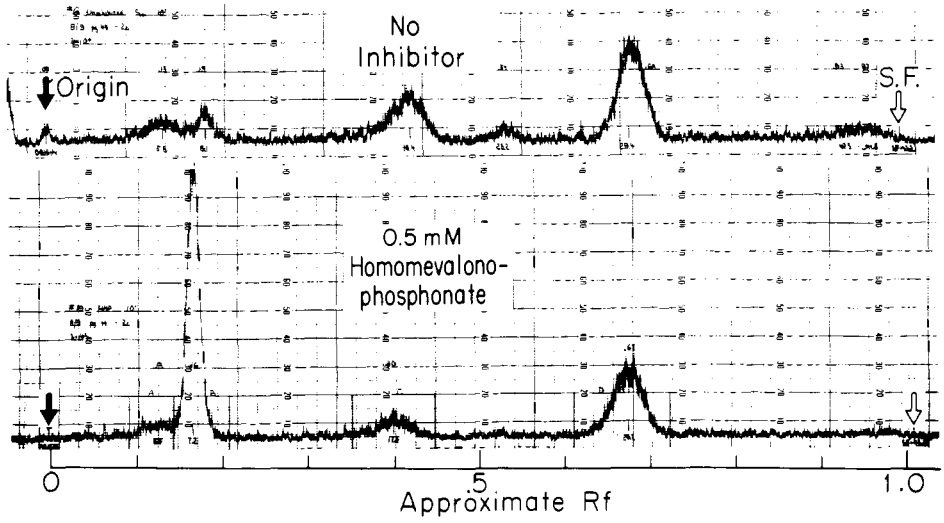


Figure 2. Radiochromatograms illustrating accumulation of 5-phosphomevalonate in incubations of S_{10} preparations with (*R*)-[2-^{14}C]mevalonate and 0.5 mM of **1** (homomevalonophosphonate): top, control incubation at 10 min; bottom, incubation with 0.5 mM inhibitor. The substance at an R_f value of 0.12-0.13 is 5-diphosphomevalonate, the one at R_f 0.16-0.18 is 5-phosphomevalonate, and the one at R_f 0.4-0.42 is IPP. Unused mevalonate is at R_f 0.68. Photographs of original records.

were applied to strips of Whatman 3MM paper and were developed as descending chromatograms with 2-propanol–concentrated ammonia-water (6:3:1, by volume). The radiochromatograms from the 0.1-mL experiments are shown in Figure 2. The chromatograms from the 0.4-mL incubations were used for the quantitation of ^{14}C in each chromatographic peak[12] and for the elution of selected fractions for further identification. In the presence of **1** in the incubations there was a gross accumulation of a product with an R_f value of 0.17 corresponding to **2**. When this substance was eluted from the papers and rechromatographed, it cochromatographed with authentic **2**, and after hydrolysis with alkaline phosphatase it gave mevalonate in 95% yield. Quantitatively, **2** accounted for 40.4% of the total ^{14}C added to the incubations containing **1** compared with 3.4% in the uninhibited reaction mixtures after 10-min incubations. In addition, there was a decrease in the amount of IPP (5.3% compared with 10.0% of the total ^{14}C in the absence of **1**) presumably owing to the decreased availability of 5-diphosphomevalonate.

The observations taken together can only mean that **1** is a specific inhibitor of 5-phosphomevalonate kinase. We examined the possibility that **1** might also be a substrate for phosphomevalonate kinase. However, incubation of S_{10} preparations with **1** and [γ-^{32}P]ATP gave no evidence of the phosphorylation of **1** to a phosphono[^{32}P]phosphate.

The mechanism of inhibition of phosphomevalonate kinase by **1** is unknown at present since we have studied its effects so far only in the multienzyme system of rat liver S_{10} preparations and—in a quantitative way—only at one concentration of mevalonate. Although there is no information about the properties of phosphomevalonate kinase of rat liver, it is worth noting that the K_m value of (*R*)-5-phosphomevalonate for the partially purified enzyme from pig liver was found to be ~300 μM.[13] The inhibition of phosphomevalonate kinase by a racemic mixture of **1** with an apparent K_i ($I_{50}^{CO_2}$) of 145 μM is the more surprising as no substrate inhibition of the pig-liver enzyme could be detected even at a concentration of 1.5 mM of (*R*)-5-phosphomevalonate.[13]

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(5) V. Sarin, B. E. Tropp, and R. Engel, *Tetrahedron Lett.*, **No. 4**, 351 (1977).
(6) G. Popják, *Methods Enzymol.*, **15**, 393 (1969).
(7) The anaerobic incubations contained, in a final volume of 1 mL, 10 mg of S_{10} proteins, 100 μmol of potassium phosphate buffer (pH 7.5), 30 μmol of nicotinamide, 10 μmol of dithiothreitol, 5 μmol $MgCl_2$, 3 μmol of ATP, 3 μmol of glucose 6-phosphates, 1 μmol NADP; 50 nCi of (*R,S*)-[1-^{14}C]- and 50 nCi of (*R,S*)-[2-^{14}C]mevalonate (supplied by Amersham-Searle Corp.), the total amount of potassium (*R,S*)-mevalonate being 100 nmol.
(8) T. S. Parker and G. Popják, *Circulation, Suppl. III*, **56**, 94 (1977).
(9) The substrates and the inhibitor are racemic mixtures; only the (*R*)-mevalonate is used enzymically. It is not known whether the *R* or *S* or both antiomers of **1** are inhibitors.
(10) P. W. Holloway and G. Popják, *Biochem. J.*, **104**, 57 (1967).
(11) The preparation of pure (*R*)- and (*S*)-mevalonates has been described: ref 6; H. L. Ngan and G. Popják, *Bioorg. Chem.*, **4**, 166 (1976); J. W. Bardenheier and G. Popják, *Biochem. Biophys. Res. Commun.*, **74**, 1023 (1977).
(12) ^{14}C compounds can be measured on portions of dried chromatograms with the best efficiency (60 to 65%) by curling up the cut out portions of the paper strips into a roll of ~1-cm diameter and inserting them into the scintillation vials containing 10 mL of RPI 3a70B scintillation fluid. Counting of ^{14}C was done in a Packard Tri-Carb scintillation spectrometer, Model No. 3320.
(13) See figure 5 in H. Hellig and G. Popják, *J. Lipid Res.*, **2**, 235 (1961).
(14) Research Fellow of the American Heart Association Greater Los Angeles Affiliate while this research was carried out.

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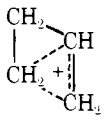
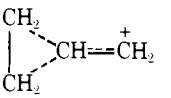
Low-Temperature Carbon-13 Nuclear Magnetic Resonance Spectroscopic Investigation of $C_4H_7^+$. Evidence for an Equilibrium Involving the Nonclassical Bicyclobutonium Ion and the Bisected Cyclopropylcarbinyl Cation[1]

Sir:

Much experimental and theoretical work has been directed toward elucidating the nature of the cationic intermediate(s) involved in cyclopropylcarbinyl, cyclobutyl, and allylcarbinyl interconversions under so-called "stable-ion" as well as solvolytic conditions.[2,3] Whereas all experimental evidence on $C_4H_7^+$ indicates that the species is a nonclassical cation,[2-4] controversy continues regarding the equilibrium geometry of this cation, with some favoring the bicyclobutonium structure **1a**, and others the "bisected" cyclopropylcarbinyl arrangement (**1b**).[2-4] We now report that an investigation of $C_4H_7^+$ under



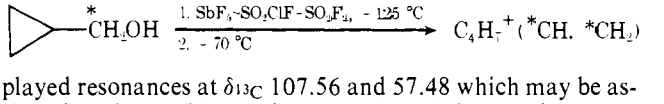
1a



1b

"stable-ion" conditions at low temperatures by ^{13}C NMR spectroscopy indicates the coexistence of at least two structural isomers of $C_4H_7^+$ in rapid equilibrium with one another.

An SbF_5-SO_2ClF-SO_2F_2 solution of $C_4H_7^+$ was prepared according to previously described techniques[3] at ca. −125 °C, employing cyclopropylcarbinol-*1*-^{13}C (43% ^{13}C).[5-7] The 20-MHz ^{13}C NMR spectrum of this solution at −70 °C dis-



played resonances at $\delta_{^{13}C}$ 107.56 and 57.48 which may be assigned to the methine and averaged methylene carbon resonances of $C_4H_7^+$, respectively (Table I).[3d] Under these conditions, the carbon-13 label is distributed nearly randomly between the methylene and methine positions of $C_4H_7^+$, indicating that hydride migrations between methine and methylene centers are occurring at rates which are slow on the NMR time scale.[8]

The ^{13}C NMR chemical shifts obtained on varying the temperature of this SbF_5-SO_2ClF-SO_2F_2 solution of $C_4H_7^+$ between −61 and −132 °C are given in Table I.[9] It is apparent that decreasing temperatures cause substantial movement of the methine and average methylene carbon resonances (downfield and upfield, respectively). The temperature de-

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