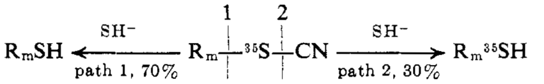
reaction of $\mathrm{R_mSCN}$ with $\mathrm{SH^-}$. For this purpose, $\mathrm{R_m{}^{35}SCN}$ was prepared by the following reaction sequence

$$\mathrm{R_mSH} \xrightarrow{\mathrm{CNBr}} \mathrm{R_mSCN} \xrightarrow{\mathrm{^{35}SH^-}} \mathrm{R_m{}^{35}SH} \xrightarrow{\mathrm{CNBr}} \mathrm{R_m{}^{35}SCN}$$

The separation of $\mathrm{R_m{}^{35}SH}$ and $\mathrm{^{35}SH^-}$ was achieved by chromatography on DEAE-Sephadex, taking advantage of the difference in the pK_a's of the two compounds ($\mathrm{R_mSH}$, pK 8.2; $\mathrm{H_2S}$, pK 7). The $\mathrm{R_m{}^{35}SCN}$ thus prepared showed a specific activity of 160 cpm/nmol. It was treated with $\mathrm{SH^-}$ and the specific activity of the product $\mathrm{R_mSH}$ was found to be 49 cpm/nmol. This indicates that 70% of the reaction proceeds with ring C–S bond fission as depicted below. There was no exchange reaction between $\mathrm{R_mSH}$ and $\mathrm{^{35}SH^-}$ under the experimental conditions used.



$$\mathrm{R_mSH} \xleftarrow[\text{path 1, 70\%}]{\mathrm{SH^-}} \mathrm{R_m}\overset{1}{\vdots}\mathrm{{}^{35}S}\overset{2}{\vdots}\mathrm{CN} \xrightarrow[\text{path 2, 30\%}]{\mathrm{SH^-}} \mathrm{R_m{}^{35}SH}$$

These results encouraged us to try to label mixed *E. coli* tRNAs *in vitro* with $\mathrm{^{35}SH^-}$ for biological studies. 4-Thiouridine present in mixed *E. coli* tRNAs was converted to 4-thiocyanatouridine by CNBr following a modified method.[6] One milliliter of aqueous tRNA ($A_{260} = 61$, $A_{340} = 1.15$) was treated with 25 μl of 0.5 M phosphate buffer, pH 8, followed by 10 μl of 1 M ethanolic CNBr. The reaction mixture was allowed to stand at 27° for 15 min and then evaporated at 27° under high vacuum. The residue was made up to 1 ml with oxygen-free water and treated with 25 μl of 0.4 M $\mathrm{NaH^{35}S}$ (Amersham, 5.4 Ci/mol) in nitrogen atmosphere and allowed to stand 1 hr at 27° and overnight at 4°. It was then freed from excess reagent by chromatography on a 1-ml column of hydroxylapatite. The unreacted $\mathrm{NaH^{35}S}$ was eluted with 0.05 M phosphate buffer, pH 6.8. The tRNA was eluted with 0.2 M phosphate buffer, pH 6.8, and it was dialyzed against distilled water at 4° to remove the phosphate. The radioactivity incorporated in the tRNA was found to be 9550 cpm/A_{260}. By comparison, a very small amount of radioactivity, 290 cpm/A_{260}, was incorporated by direct exchange between tRNA and $\mathrm{NaH^{35}S}$.

In order to demonstrate that the radioactivity was located in the 4-thiouridine moiety of the tRNA, we hydrolyzed the tRNA to nucleoside level in two steps, by incubating the tRNA with RNase $\mathrm{T_1}$ at 37° at pH 7.2 for 30 min followed by incubation at 45° for 3 hr with phosphatase and venom phosphodiesterase at pH 8.8. The hydrolyzed tRNA was then analyzed on a Bio-Rad A6 column (20 × 0.63 cm) by both the anion-exclusion method of Singhal[7] using ammonium carbonate, pH 9.8, and the cation-exchange method using 0.05 M $\mathrm{NH_4Cl}$, pH 5.3. In the former system, the radioactivity was found to be associated with the 4-thiouridine peak and no other nucleoside was found to be associated with radioactivity. Unfortunately, this system does not separate $\mathrm{SH^-}$ from 4-thiouridine; in addition, there is an overlapping of the enzyme peak with the 4-thiouridine peak. In order to rule out the possibility of the presence of $\mathrm{^{35}SH^-}$ as a contaminant, we used the second system, which does separate 4-thiouridine, $\mathrm{^{35}SH^-}$, and the enzyme mixture. In this system, the radioactivity was found to be associated with the enzyme peak (breakthrough) and the 4-thiouridine peak. No radioactivity was found in the fractions eluting at the $\mathrm{SH^-}$ peak position. About 17% of the total radioactivity was associated with the enzyme peak and 83% with the 4-thiouridine peak. There could be several explanations for the radioactivity in the enzyme peak: (1) some minor nucleoside may incorporate $\mathrm{^{35}S}$ by this process and elute at this position; (2) oligonucleotides containing labeled 4-thiouridine due to incomplete hydrolysis may elute at this position; (3) enzymes may incorporate radioactivity from labeled 4-thiouridine. We have checked the last possibility by incubating a fresh enzyme mixture with radioactive 4-thiouridine and found that the enzyme did incorporate radioactivity. This activity could not be dialyzed against distilled water but could be dialyzed against β-mercaptoethanol. Although the first possibility cannot be ruled out, the second one is remote since the reaction conditions were carefully selected to ensure complete hydrolysis of the tRNA. We have also subjected brewer's yeast tRNA (Schwartz) to this labeling procedure. It was found to incorporate a small amount of radioactivity, 180 cpm/A_{260}, which was associated with the enzyme peak in the chromatogram of the tRNA hydrolyzate (Bio-Rad A6, 0.05 M $\mathrm{NH_4Cl}$, pH 5.3).

The exchange method of labeling compounds by heating with $\mathrm{^{35}S}$ in benzene–pyridine has been used successfully;[8] obviously such harsh treatment cannot be used with sensitive macromolecules like tRNA. The present method accomplishes the labeling of 4-thiouridine containing tRNA's *in vitro* with $\mathrm{^{35}S}$ in a simple and mild way.

Acknowledgment. The authors are grateful to Dr. Waldo E. Cohn for his interest and encouragement.

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(6) M. Saneyoshi and S. Nishimura, *Biochim. Biophys. Acta*, **204**, 389 (1970).

(7) R. P. Singhal, *Arch. Biochem. Biophys.*, **152**, 800 (1972).

(8) J. L. Darlix, P. Fromageot, and E. Reich, *Biochemistry*, **12**, 914 (1973).

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A Method for Direct Hydroxylation of Enolates. Transition Metal Peroxide Reactions

Sir:

Base catalyzed oxygenation can be used to prepare α-hydroxy derivatives from occasional ketones and esters having a tertiary α-carbon.[1] The most practical procedure employs triethyl phosphite for *in situ* reduction of labile hydroperoxide intermediates,[2] a modification

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(1) (a) E. J.Bailey, D. H. R. Barton, J. Elks, and J. F. Templeton, *J. Chem. Soc.*, 1578 (1962); (b) R. Hanna and G. Ourisson, *Bull. Soc. Chim. Fr.*, 3742 (1967); 1945 (1961); (c) H. Muxfeldt, G. Hardtmann, F. Kathawala, E. Vedejs, and J. B. Mooberry, *J. Amer. Chem. Soc.*, **90**, 6534 (1968); (d) G. Buchi, P. Kulsa, and R. L. Rosati, *ibid.*, **90**, 2448 (1968); (e) G. Buchi, W. Pickenhagen, and H. Wüest, *J. Org. Chem.*, **37**, 4192 (1972); (f) M. Avramoff and Y. Sprinzak, *J. Amer. Chem. Soc.*, **85**, 1655 (1963); (g) H. R. Gersmann, H. J. W. Nieuwenhuis, and A. F. Bickel, *Proc. Chem. Soc., London*, 279 (1962).

(2) J. N. Gardner, F. E. Carlon, and O. Gnoj, *J. Org. Chem.*, **33**, 3294 (1968); J. N. Gardner, T. L. Poppen, F. E. Carlon, O. Gnoj, and H. L. Herzog, *ibid.*, **33**, 3695 (1968); G. Buchi, P. Kulsa, K. Ogasawara, and R. L. Rosati, *J. Amer. Chem. Soc.*, **92**, 999 (1970); P. R. Enslin, *Tetrahedron*, **27**, 1909 (1971); J. J. Plattner, R. D. Gless, and H. Rapoport, *J. Amer. Chem. Soc.*, **94**, 8613 (1972).

which minimizes the serious side reaction of oxidative α-carbon cleavage.[1a,b,f,3] In systems having an enolizable methylene group, hydroperoxide fragmentation to an α-dicarbonyl compound is a further complicating factor.[1a,b,4] There appears to be no successful example of conversion of a ketone α-methylene group to the acyloin by enolate oxygenation described in the literature.

This communication reports a new method for enolate hydroxylation using the readily available molybdenum peroxide $\mathrm{MoO_5 \cdot Py \cdot HMPA}$ (MoOPH).[5–8] A variety of enolates react with this reagent at temperatures between −70 and −40°, presumably by nucleophilic attack at peroxide oxygen to form a $\mathrm{Mo^{VI}}$ ester.[9,10] After aqueous work-up, α-hydroxy esters and ketones are obtained in good yield and without contamination by oxidative cleavage products.

The reaction conditions have been optimized for hydroxylation of ethyl bicyclo[2.2.2]octene-5-carboxylate. A solution of the ester (1 mmol) in dry THF (5 ml) is added dropwise to 2 ml of 0.65 *M* lithium diisopropylamide (LDA) in 2:1 THF–hexane at −70° under nitrogen. After 30 min at −70°, powdered MoOPH (1.3 mmol) is added to the enolate in one portion with vigorous stirring. An orange-red color develops as the reagent slowly dissolves. After 1 hr at −70° the cooling bath is removed, resulting in a gradual color change to green. After the reaction mixture reaches 0° (or, in some examples, as soon as the reaction becomes homogenous) water is added and the product is extracted with ether. The organic phase is washed with 5% carbonate and 5% HCl to remove Mo salts and pyridine, dried, evaporated, and chromatographed to separate starting material (3–5%) from the α-hydroxy ester (80–85%). No other products are present according to glpc analysis.

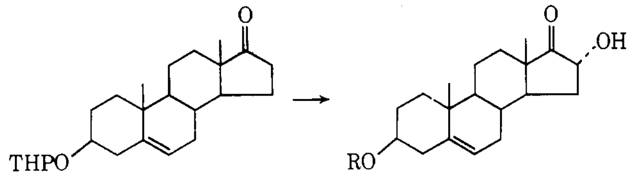
The conditions detailed above have been used with other carbonyl compounds (Table I) without optimizing individual examples. Esters, lactones, and ketones having an enolizable methine or methylene group are hydroxylated successfully. Among the several examples, only deoxybenzoin suffers overoxidation. Benzil is a major product unless <1 mol of base and 0.5 mol of MoOPH/(mole of ketone) are employed. However, conversion is inefficient under the latter conditions and the product consists of benzoin (15–20%) and starting material.

Table I. MoOPH Hydroxylations

Starting carbonyl compound	Product (yield)
Ethyl phenylacetate	Ethyl mandelate (58%)[a]
Ethyl dihydrocinnamate	Ethyl α-hydroxydihydrocinnamate (60%)[a]
Ethyl bicyclo[2.2.2]-octene-5-carboxylate	Ethyl 5-hydroxybicyclo[2.2.2]octene-5-carboxylate (85%)[a,b]
α-Butylbutyrolactone	α-Hydroxy-α-butylbutyrolactone (73%)[b]
γ-Phenyl-γ-methyl-butyrolactone	α-Hydroxy-γ-phenyl-γ-methylbutyrolactone (56%)[a]
Isobutyrophenone	α-Hydroxyisobutyrophenone (65%)[a]
2-Phenylcyclohexanone	*trans*-2-Hydroxy-6-phenylcyclohexanone (70%)[a]
3-β-Tetrahydropyranyl-oxyandrost-5-en-17-one	3β,16α-Dihydroxyandrost-5-en-20-one-3-tetrahydropyranyl ether (75%)[c]
Deoxybenzoin	Benzoin (34%),[c] benzil (26%)[c]

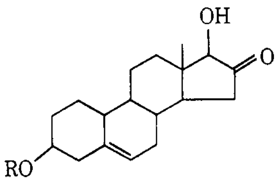
[a] Isolated yield of liquid homogenous by glpc, tlc, and nmr. [b] Yield by glpc. [c] Isolated yield of crystalline product.

Unsymmetrical acyloins are formed with high regioselectivity. Treatment of 3-β-tetrahydropyranyloxyandrost-5-ene-17-one[11] with LDA and MoOPH (1:1.5:1.5 mmol) gives a 75% yield of **2a**, characterized by acid hydrolysis to **2b**.[12] The well-known base catalyzed conversion of 16-hydroxy 17-keto steroids into the more stable 17-hydroxy 16-keto isomers (such as **3**) is not observed[12b,13] under the conditions of hydroxylation.



1

2a, R = THP
b, R = H



3

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(3) W. v. E. Doering and R. M. Haines, *J. Amer. Chem. Soc.*, **76**, 482 (1954); F. G. Bordwell and A. C. Knipe, *ibid.*, **93**, 3416 (1971); D. H. R. Barton and N. H. Werstiuk, *J. Chem. Soc. C*, 148 (1968); W. Cocker, K. J. Crowley, and K. Srinivasan, *J. Chem. Soc., Perkin Trans. 1*, 1971 (1972); W. H. Richardson and R. S. Smith, *J. Amer. Chem. Soc.*, **89**, 2230 (1967); W. Adam and J-C. Liu, *ibid.*, **94**, 2894 (1972).

(4) E. P. Kohler and R. B. Thompson, *J. Amer. Chem. Soc.*, **59**, 887 (1937); D. H. R. Barton, S. K. Pradha, S. Sternhell, and J. F. Templeton, *J. Chem. Soc.*, 255 (1961); J. F. Biellman and M. Raji, *Bull. Soc. Chim. Fr.*, 441 (1962); B. Laundon and G. A. Morrison, *J. Chem. Soc. C*, 1694 (1971); R. E. Lack and A. B. Ridley, *ibid.*, 3017 (1968).

(5) H. Mimoun, L. Seree de Roch, and L. Sajus, *Bull. Soc. Chim. Fr.*, 1481 (1969); a modified procedure was used to prepare MoOPH free of hydrated impurities. One equivalent of pyridine was added slowly to a saturated THF solution of anhydrous $\mathrm{MoO_5 \cdot HMPA}$. The precipitated crystals were air-dried and stored in the refrigerator. Prolonged storage at room temperature or exposure to daylight for several hours causes gradual decomposition.

(6) The related reagent $\mathrm{MoO_5 \cdot HMPA}$ is reported to react with excess *n*-butyllithium to form lithium butoxide: S. L. Regen and G. M. Whitesides, *J. Organometal. Chem.*, **59**, 293 (1973). Although $\mathrm{MoO_5 \cdot HMPA}$ is more conveniently soluble in THF, MoOPH is nonhygroscopic, is more easily stored, and generally gives higher yields in the enolate hydroxylation process.

(7) To pronounce this abbreviation correctly, one must recall that there are five O's in MoOPH.

(8) We have observed no indication that MoOPH is explosive. The substance has been ground in a mortar (behind a safety shield) without incident, and decomposes with only modest violence on a hot plate. However, a referee has noted that $\mathrm{MoO_5 \cdot HMPA \cdot H_2O}$ may explode on storage at room temperature. Precautions should be taken when crystalline MoOPH is in contact with oxidizable materials.

(9) For other reactions of isolated $\mathrm{MoO_5}$ derivatives, see H. Mimoun, I. Seree de Roch, and L. Sajus, *Tetrahedron*, **26**, 37 (1970); G. A. Tolstikov, U. M. Dzhemilev, and V. P. Yur'ev, *Zh. Org. Khim*, **8**, 2204 (1972); G. A. Tolstikov, U. M. Dzhemilev, V. P. Yur'ev, and S. R. Rafikov, *Dokl. Akad. Nauk SSSR*, **208**, 376 (1973); S. A. Matlin and P. G. Sammes, *J. Chem. Soc., Chem. Commun.*, 1222 (1972). K. B. Sharpless, J. M. Townsend, and D. R. Williams, *J. Amer. Chem. Soc.*, **94**, 1296 (1972).

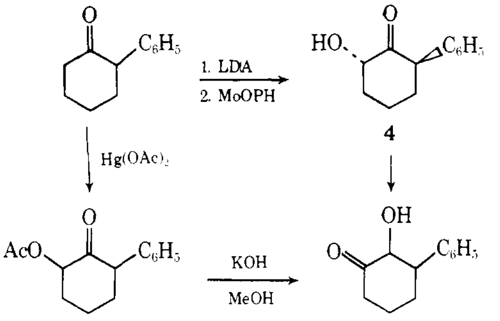
(10) MoOPH does not epoxidize olefins[9] under the enolate hydroxylation conditions.

(11) A. C. Ott, M. F. Murray, and R. F. Pederson, *J. Amer. Chem. Soc.*, **74**, 1239 (1952).

(12) (a) K. Fotherby, A. Colas, S. Atherden, and G. Marrian, *Biochem. J.*, **66**, 664 (1957); (b) A. Hassner and P. Catsoulacos, *J. Org. Chem.*, **31**, 3149 (1966).

(13) C. Fenselau in "Steroid Reactions," C. Djerassi, Ed., Holden Day, San Francisco, Calif., 1963, Chapter 13, p 565–566.

A labile acyloin **4**[14] is formed from 2-phenylcyclohexanone in 70% yield. Structure **4** has previously been assigned to a compound obtained by reaction of 2-phenylcyclohexanone with mercuric acetate followed by saponification of the initial product, 2-acetoxy-6-phenylcyclohexanone.[15] The structure of the acetate is supported by spectral data, but the nmr spectrum of the saponification product can only be reconciled with the isomeric acyloin **5**.[16] Methanolic KOH converts **4** into



4

5

5 within minutes at 25°, but **5** is not present in the crude MoOPH product by tlc analysis. Thus, it is possible to prepare the less stable acyloin by hydroxylation of the kinetically favored enolate[17] without interconversion of acyloin isomers.

Numerous synthetic applications are anticipated for transition metal peroxide hydroxylations, including oxidative degradation of esters and ketones and ring expansions of cycloalkane acyloins[19] or of pinacol rearrangement systems[20] available from cycloalkane α-hydroxy esters. We are also investigating hydroxylation of other carbanions and the behavior of related metal peroxide reagents.

Acknowledgment. The author wishes to thank the Upjohn Company for steroid samples used in this work.

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[14] Noncrystalline, purified by preparative layer chromatography: nmr ($CDCl_3$, δ) 7.1–7.6 (5 H, m), 4.18 (1 H, dd, J = 11, 6 Hz), 4.03 (1 H, br s), 3.7 (1 H, br s, exchanged by D_2O), 1.4–2.8 (m, 6 H); ir (cm^{-1} neat) 3340 (br), 1720 (s).

[15] W. Treibs and M. Weissenfels, *Chem. Ber.*, **93**, 1374 (1960).

[16] A sample of **5** was prepared by the mercuric acetate–saponification procedure[15]: mp 118–119°; nmr ($CDCl_3$, δ) 7.24 (5 H, br s) 4.28 (1 H, dd, J = 12, 1.5 Hz; 1.5 Hz coupling disappears after shaking with D_2O), 3.6 (1 H, d, J = 1.5 Hz; D_2O exchangeable), 1.5–2.9 (7 H, m).

[17] Exclusive (>99%) formation of the less substituted enolate from 2-phenylcyclohexanone and LDA has been verified by Professor H. J. Reich (personal communication). Similar behavior is apparent in the case of acid-catalyzed bromination,[18] as well as the mercuric acetate oxidation.[15]

[18] B. Miller and H-S. Wong, *Tetrahedron*, **28**, 2369 (1972).

[19] I. Elphimoff-Felkin, G. LeNy, and B. Tchoubar, *Bull. Soc. Chim. Fr.*, 522 (1958).

[20] C. D. Gutsche and D. Redmore, *Advan. Alicyclic Chem., Suppl. 1*, 61, 99 (1968).

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Reversible Oxygen Binding by Divalent Chromium(II) Ion Exchanged Molecular Sieve

Sir:

Reversible binding of molecular oxygen by transition metal complexes continues to be of interest and the subject has recently been reviewed by Valentine.[1] To date iridium,[2] rhodium,[3] palladium, platinum, nickel,[4] and iron[5] reversibly formed dioxygen complexes have been reported. In addition there are the numerous reversibly formed dioxygen complexes of cobalt.[1] Though simpler than the hemoglobin molecule whose reversible oxygenation is of prime interest, most, if not all, of these complexes involve extensive covalent binding of the ligands with the central ion, raising the question of the extent to which ligands other than oxygen participate in the binding scheme.

We report on a heterogeneous oxygen carrier based on chromium(II) introduced by ion exchange into a zeolite cavity and coordinated by no ligands other than the zeolite framework.

Exchange of transition metal ions into A type zeolites and subsequent dehydration leaves the exchanged ion in trigonal coordination in a distorted oxygen six ring which links the α and β cages of the zeolite.[6–8] The ions are coordinatively unsaturated to a high degree, allowing them to bind "guest molecules" which are small enough to enter the zeolite cavities. Examples of zeolitic complexes in which a transition metal ion is bound partly to the zeolitic skeleton and partly to a guest molecule are the olefin and acetylene ($(Na_{0.834}Co(II)_{0.083})$-A,[9] acetylene $(Na_{0.25}Mn(II)_{0.375})$-A,[10] and the water, cyclopropane, and ethylene complexes of $(Na_{0.715}Ni(II)_{0.143})$-A.[6]

Ion exchange of divalent chromium into zeolite A under oxygen-free conditions yielded a pale blue air-stable material containing 1.5 Cr(II) ions per unit cell, $(Na_{0.75}Cr(II)_{0.125})$-A, having a diffuse reflectance electronic spectrum characteristic of the hexaaquo Cr(II) ion. Similar behavior was observed for the nickel[6] and cobalt[9] exchanged zeolites. Dehydration at 350° and 10^{-6} Torr induced a pale blue-lilac color in the zeolite and an electronic diffuse reflectance spectrum having two peaks (Table I).

Table I. The Diffuse Reflectance Electronic Spectra (cm^{-1}) of Anhydrous and of Oxygenated Anhydrous Chromium(II) Ion Exchanged A Type Zeolite[a]

$(Na_{0.75}Cr(II)_{0.125})$-A	$(Na_{0.75}Cr(II)_{0.125})$-A + O_2
12,300 m	4,000 w
17,000 m	10,000 s, sh
	14,200 s
	18,000 m
	20,600 m
	26,200 w

[a] Key: m = medium, w = weak, s = strong, sh = shoulder.

Magnetic susceptibility measurements showed that the chromium ion was in a high spin state ($2S + 1$ =

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[1] J. Valentine, *Chem. Rev.*, **73**, 235 (1973).

[2] L. Vaska, *Science*, **140**, 809 (1963).

[3] L. Vaska, L. S. Chen, and W. V. Miller, *J. Amer. Chem. Soc.*, **93**, 6671 (1971).

[4] G. Wilke, H. Schott, and P. Heimbach, *Angew. Chem., Int. Ed. Engl.*, **6**, 92 (1967).

[5] J. P. Collman, R. R. Gagne, and C. A. Reed, *J. Amer. Chem. Soc.*, **96**, 2629 (1974).

[6] K. Klier and M. Ralek, *J. Phys. Chem. Solids*, **29**, 951 (1968).

[7] R. Polak and V. Cerny, *J. Phys. Chem. Solids*, **29**, 945 (1968).

[8] P. E. Riley and K. Seff, *J. Chem. Soc., Chem. Commun.*, 1287 (1972).

[9] K. Klier, R. Kellerman, and P. J. Hutta, submitted to *J. Chem. Phys.*

[10] P. E. Riley and K. Seff, *J. Amer. Chem. Soc.*, **95**, 8180 (1973).