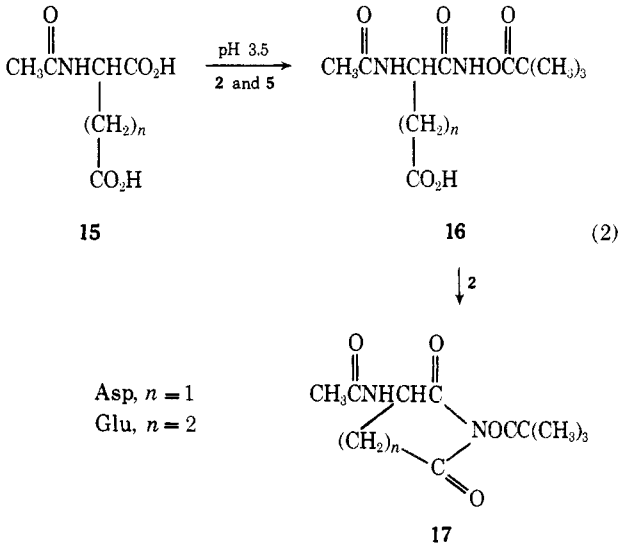
The advantages of our procedure are (1) the use of aqueous solutions (or mixed solvents; note that the insulin degradation was successful in 8 *M* urea); (2) the high yields of the procedure; (3) the fact that peptides need not (but may) be protected at the amino terminus[17] or at side-chain functionality; (4) the successful removal of proline,[7] asparagine, and presumably glutamine[18] in high yield; (5) successful analyses of peptides as large as the insulin chains; and (6) the overall simplicity of the method.

Our procedure currently has the following limitations. (1) Carboxyl-terminal Glu and Asp suffer low degradation yield (ca. 40%) under the conditions described above because they form a cyclized intermediate in the coupling step (**17**, eq 2) which regenerates Glu or Asp after hydrolysis.[19]



(The structure of **17** is based on actual isolation and identification.) However, carrying out the coupling procedure at pH 0.75 increases the yield somewhat (Table I). (2) Peptides currently must be small enough to analyze by difference amino acid analysis, although we are developing methods to identify the aldehyde on a submicro scale; however, no other size limitation appears to exist. (3) That the Lossen rearrangement step takes more time than we consider optimum, although an object of further development, is not a serious problem, since the rearrangement can be monitored by a pH-stat and left essentially unattended.

Variations of this method which will permit extension of this chemistry to a sequential C-terminal degradation are currently under study.

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(15) No other non-C-terminal residues were affected by the method, except for Tyr (ca. 30% loss), the amino-terminal residue (ca. 25% loss), and Trp (ca. 50% loss). These losses were also incurred in the presence of OPHA, but *absence* of carbodilimide (conditions under which the C-terminal degradation does not take place); this observation suggests that the N-terminal amino group and the activated aromatic rings were undergoing electrophilic amination by OPHA. Consistent with this suggestion is the complete protection of the amino terminal residue from loss by prior acetylation. The amination of the amino terminus suggests the possibility of a simultaneous C- and N-terminal degradation.
(16) R. G. Dickinson and N. W. Jacobsen, *Chem. Commun.*, 1719 (1970).
(17) The large excess of OPHA over free amino terminus assures the virtual absence of peptide–peptide coupling; see also note 15.
(18) A full kinetic study was carried out for degradation of *N*-acetylglutamine. This amino acid was degraded in 99.5% yield. Similarly, the γ-Glu residue can be thought of as a "substituted Gln"; it degrades normally (Table I); this observation is the basis for an analysis for ω-linked dicarboxylic amino acids.
(19) Obviously, either the side-chain or α-carboxyl may be activated first, and **16** represents only the latter possibility; the derivative **17** is of course obtained in either case.
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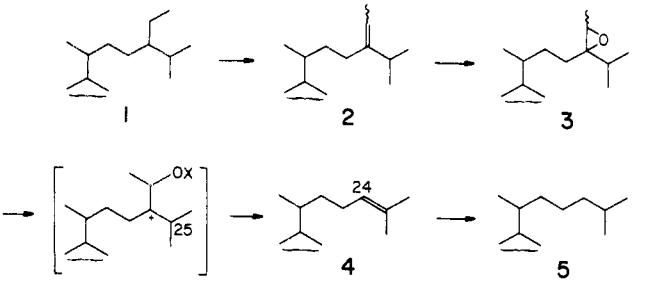
Stereospecificity in the Conversion of Fucosterol 24,28-Epoxide to Desmosterol in the Silkworm, *Bombyx mori*

Sir:

Insects depend on phytosterols as a steroid source since they lack the ability to synthesize cholesterol.[1] The general scheme of sitosterol (**1**) dealkylation is summarized in Scheme I.[2–4] Intermediacy of the epoxide **3** has been demonstrated by isotope incorporation techniques[3] and is also supported by the recent findings showing that ^3H in [25-^3H]-24-ethylcholesterol migrates to C-24 during conversion to desmosterol **4** in *Bombyx mori*[4] and *Tenebrio molitor*.[5]

Scheme I



The absolute stereochemistry of the intermediate epoxide involved in the transformation of sitosterol **1** to cholesterol **5** in *B. mori* has been determined to be (24*S*,28*S*)-**7a** as follows. Selective epoxidation of fucosteryl acetate **6** with *m*-chloroperbenzoic acid (in chloroform, 0°, 15 min) gave a nonseparable 1:1 mixture of the epoxides **7a**/**7b** (with 3-OAc). Presence of a mixture was clear from the ^{13}C NMR which gave paired peaks for the asterisked carbons in **7**; the two C-29 methyl signals, however, overlapped at 14.3 ppm, a point which proved to be of diagnositc value (see below).

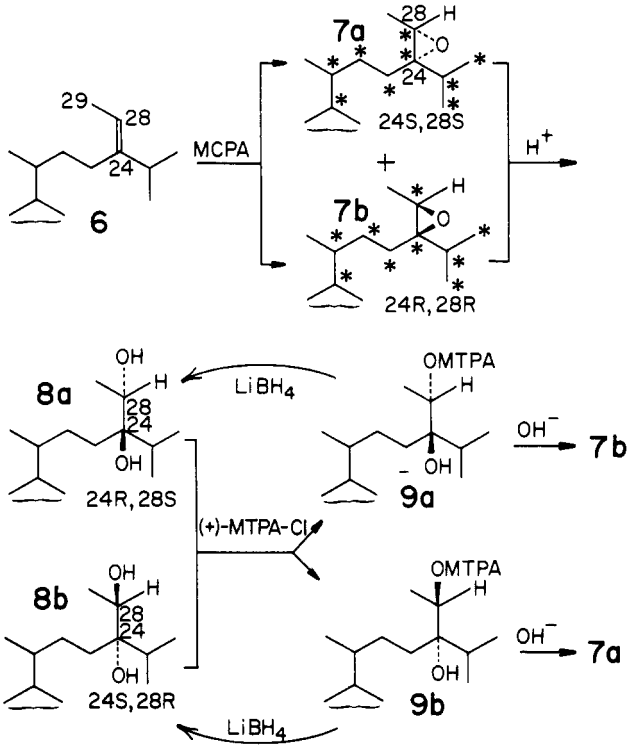
Acid catalyzed (H_2SO_4 in aqueous THF) cleavage of epoxides **7**(3-OAc) gave the glycol mixture **8**(3-OAc), still nonseparable. However, treatment of mixture **8** with (+)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) chloride[6] yielded the MTPA ester which could now be separated

Table I. Relative Radioactivity Incorporation of [3-^{3}H]Epoxides

Substrate	Specific activity (Ci/mol)	% cholesterol benzoate (1)	% desmosterol benzoate (4)[a,b]	% epoxide benzoate (recovered)	% polar fraction
Epoxide mixture **7**	101	0.1	21	28	49
(24*S*, 28*S*)-Epoxide **7a**	47	0.3	37	25	36
(24*R*, 28*R*)-Epoxide **7b**	39		0.9	31	67

[a] The cell-free system employed here seems to lack the enzyme system to reduce desmosterol to cholesterol.[8] [b] The desmosterol benzoates produced from **7** and **7a** were recrystallized with carrier until constant specific activity was attained.

Scheme II



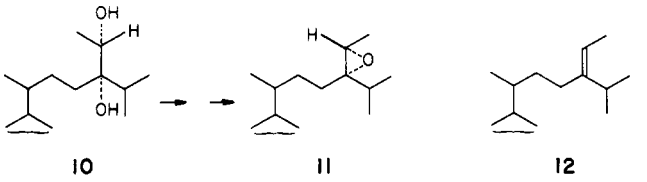
by high-pressure liquid chromatography into two major components **9a** and **9b**(3-OAc) after five recycles, Corasil II, 9 ft × ⅜ in., 19% ether–*n*-hexane.

Mass spectroscopic studies of the glycol mixture **8**(3-OAc) obtained by acid cleavage of epoxide acetates **7** with $H_2{}^{18}O$ showed that ^{18}O was distributed between C-28 and C-24 in a ratio of 13:87. The stereochemical course of the epoxide cleavage and formation reactions are thus well defined. Namely, epoxide cleavage of **7a**(3-OAc) gives **8a** and **8b**(3-OAc) (ratio ca. 87:13), most likely with 24-inversion/28-retention and 24-retention/28-inversion, respectively (this result is corroborated below); likewise, **7b** gives **8a** and **8b** in a ratio of ca. 13:87. A similar preferred opening at the more hindered carbon has been noted previously.[7a]

Treatment of the separated MTPA esters with $LiBH_4$ afforded pure triols **8a** and **8b**(3-OH), the C-24/C-28 absolute configurations of which were then determined by the $Pr(dpm)_3$ method.[7] CD measurements were carried out with 10^{-4} *M* solutions of a 1:1 mixture of glycol and $Pr(dpm)_3$ in carbon tetrachloride, 10 min after preparation of solution: **8a**(3-OH) 24*R*:28*S*, $\Delta\epsilon_{317}$ +2.7, $\Delta\epsilon_{297}$ −3.0; **8b**(3-OH) 24*S*:28*R*, $\Delta\epsilon_{317}$ −3.5, $\Delta\epsilon_{297}$ +2.5.

Ring closures of the separated MTPA esters **9a** and **9b**(3-OAc) with 5% methanolic KOH each gave epoxide **7**(3-OH), the C-29 ^{13}C NMR peaks of which were *both* at 14.3 ppm, i.e., at a shift identical with that present in the original epoxide acetate mixture. It is clear that re-formation of the epoxide involves SN2 displacement at C-28, and hence the epoxides obtained from **9a** and **9b** should be represented as (24*R*,28*R*)-**7b** and (24*S*,28*S*)-**7a**, respectively.

The following experiments were carried out in order to secure further evidence for the route of epoxide cleavage. If (24*S*,28*S*)-**7a** had given the (24*S*,28*S*)-glycol **10** with configurational retention at both centers (instead of **8**), the epoxide derived from **10**-MTPA ester would have given the (24*S*,28*R*)-epoxide **11**, i.e., isofucosterol epoxide. Isofucosteryl acetate **12** was therefore epoxidized. As in the case of fucosteryl acetate **6**, it gave a mixture of epoxides, i.e., (24*S*,28*R*)-**11** and (24*R*,28*S*)-**11** which again had different ^{13}C NMR shifts for most side-chain carbons. Fortunately, however, the C-29 peaks now overlapped[8] at 13.4 ppm, a chemical shift which was distinctly different from the 14.3 ppm value of fucosterol epoxide 3-acetates (and 3-OH) **7**. Thus the unlikely mechanism of acid-catalyzed cleavage involving configurational retention at both C-24 and C-28 can be discarded.



Tritium was introduced at C-3 of the epoxide mixture **7**(3-OH), (24*S*,28*S*)-epoxide **7a**, and (24*R*,28*R*)-epoxide **7b**, by Collins oxidation and reduction with [^{3}H]-$NaBH_4$. The supernatant obtained from homogenates[9] of five guts of *B. mori* fifth instar larvae were incubated with ^{3}H-labeled epoxides, respectively, at 30°, 2 hr in air. An aliquot of the nonsaponifiable material was benzoylated and separation of the four fractions (Table I), i.e., cholesterol benzoate, desmosterol benzoate, epoxide benzoate, and polar fraction, was effected by silica gel chromatography and subsequent $AgNO_3$-impregnated TLC or HPLC (Zorbax SIL, 4% CH_2Cl_2 in hexane).

Results shown in Table I indicate that the (24*S*,28*S*)-epoxide **7a**(3-OH) is effectively converted into desmosterol in contrast to the 24*R*,28*R* isomer **7b**(3-OH), and hence strongly suggest that **7a**(3-OH) is the actual precursor.[10]

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(10) Supported by NIH, AI 10187 (to K.N.) and Sea Grant 175-025 (to Y.S.).

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Metal Clusters in Catalysis. II. An Electron Spin Resonance Study of Dinuclear Metal Complex Fragments and Their Interaction with Organic Substrates

Sir:

Metal clusters are attractive catalytic species especially for template syntheses as illustrated[1] for $Ni_4[CNC(CH_3)_3]_7$. In addition, the weakest bonds in clusters may often prove to be the framework cluster bonds;[2] hence there is the further potential in cluster complexes of reversibly generating reactive fragments. In this context, the simplest, most readily available class comprises dinuclear metal complexes. Herein we describe for a group of readily dissociable iron complexes, $[(allyl)Fe(CO)_2L]_2$, catalytic chemistry and an ESR study that provides, (1) an accurate measure of Fe–Fe bond energies, (2) a kinetic, thermodynamic, and electronic view of the interaction of $(allyl)Fe(CO)_3$ with unsaturated organic substrates, and (3) a demonstration that two mononuclear isomers are usually present in each system and that these are highly fluxional.

The binuclear $[C_3H_5Fe(CO)_3]_2$ complex, A, dissociates in solution to give a paramagnetic species. Equilibria, presumed to be complex,[3a] are now shown to be singular. Analysis of ESR signal integral intensities over a temperature range of +40 to −90° establish that the solution state of A is fully[4] represented by dissociation 1.

$$\underset{\mathbf{A}}{[C_3H_5Fe(CO)_3]_2} \rightleftharpoons \underset{\mathbf{B}}{2C_3H_5Fe(CO)_3} \qquad (1)$$

No gas phase equilibria data are available but mass spectral studies show dimer to be present. Solvent effects upon equilibrium 1 were small and most significant with toluene (see Table I). The most informative solvent interaction, vis a vis catalytic reactions, is with hexenes. When A was dissolved in cold 1-hexene, the equilibrium was similar to that of A in toluene. However, a reaction of the complex, probably B, with 1-hexene occurred with *no* CO loss and an activation energy of ~10 kcal/mol[5,6] to give η^1-$C_3H_5Fe(CO)_3$(1-hexene) which in dimeric form exhibits a substantially reduced Fe–Fe bond energy. This type of olefin complex must be an important intermediate[7a] in the catalytic chemistry of A. We found that A rapidly[7b] isomerized 1-hexene to *trans*-2-hexene at 25°, initiated vinyl polymerization, e.g., ethyl vinyl ether and styrene, and rapidly polymerized allene at 22° to a solid $-(-C(=CH_2)CH_2-)_x-$ polymer. In the isomerization itinerary that follows olefin adduct formation, conventional isomerization pathways of hydride insertion–elimination[7c] or olefin adduct → η^3-allylmetal hydride → internal olefin adduct formation cannot be followed precisely. A possible intermediate is (η^1-allyl)(η^1-alkylallyl)-$FeH(CO)_3$.[7d] Interestingly, there was no extensive hydride insertion into the C_3H_5 group because the original complex A was recovered unchanged from isomerization reactions.[3b] For the analogous reaction system of A with 2-hexene, rate of adduct formation was lower than with 1-hexene.[7e] Entropy data (Table I) and the relatively large activation energy for solvation suggest that hexene loss does not occur in the dimerization of the olefin adducts, σ-$C_3H_5Fe(CO)_3$(hexene).[8]

In $C_3H_5Fe(CO)_2L$ derivatives, the iron–iron bond energy for the dimeric form is close to that of A while the entropy loss on dimerization is invariably larger than for the sterically less encumbered parent. Steric factors are evident also in the bond energy data for the phosphine series (Table I, enthalpies may be read as iron–iron bond energies). Nonetheless, barring extreme ligand bulkiness, there was a small perturbation of the iron–iron bond energy as ligands were varied in the phosphine and phosphite series; electronic ligand effects seem to be well buffered by the remaining allyl and carbonyl ligands which have donor–acceptor bonding

Table I. Thermodynamic Data for Dimer–Monomer Equilibria (1) in $C_3H_5Fe(CO)_2L$·S and ESR Parameters for Monomer

L[a]	Medium[a]	ΔH^b	ΔS^c	$(g_0 - 2)^d$	$(g_\parallel - 2)^e$	$(g_\perp - 2)^e$	$a_H{}^f$ $(a_P)^f$
CO	crys			0.0446	0.0068	0.0667 (0.0232)	
CO	nuj			0.0455	0.0055	0.0668	
CO	pe	13.5	37	0.0467			
CO	thf	13	41	0.0455			5.7
CO	mthf	12.5	39	0.0458	0.0051 (0.0756)	0.0646 (0.0257)	6.0
CO	tol	11	30	0.0459	0.0055	0.0668 (0.0239)	6.0
CO	1-hex	12	32	0.0449			~5.6
(CO)(1-hex)	1-hex	9	41	0.0448	0.0159 (0.0229)	0.0568 (0.0635)	5.4
(CO)(2-hex)	2-hex	13	61.5	0.0447	0.0056	0.0624	5.4
CO·(2-but)	2-but	11.5	31	0.0449	−0.0158	0.0755 (0.0242)	
$P(CH_3)_3$	tol	12	39	0.0449			6.0 (11.2)
$P(CH_3)_2C_6H_5$	tol	13	42	0.0478			6.0
$P(CH_3)_2C_6H_5$	pe	13.5	46	0.0473			6.0
$PCH_3(C_6H_5)_2$	tol	10	40	0.0482			7.4 (7.2)
$P(C_6H_5)_3$	tol	No dimerization		0.0504	−0.0046	0.0775 (0.0251)	5.7,[g] (16.7)[g]
$P(C_2H_5)_3$	tol	10.5	44	0.0463			5.7 (11.4)
$P(OCH_3)_3$	pe	14	46.5	0.0462			7.6 (7.7)

[a] Key: crys, monomer defects in dimeric crystals; pe, pentane; nuj, nujol; thf, tetrahydrofuran; mthf, 2-methyltetrahydrofuran; tol, toluene; 2-but, 2-butyne; 1-hex, 1-hexene; 2-hex, 2-hexene. [b] In kcal/mol, precision ~7%. [c] In eu, precision ~10%. [d] At 25°C, ±0.0005. [e] At −160°C, ±0.0010, less intense (isomer) signal in brackets. [f] Absolute value of isotropic hyperfine coupling constant × 10^4 $cm.^{-1}$, ±<0.2. [g] $A_{H(\parallel)} = 5.3$, $A_{P(\parallel)} = 43$, $A_{P(\perp)} = 51$ for more abundant isomer, $A_{H(\parallel)} = 6.2$ for the other isomer.