because of the very rapid single-electron-transfer reaction between $Re(CO)_6^+$ and $Re(CO)_5^-$ in comparison to the single-electron transfer of $Re(CO)_6^+$ and $Mn(CO)_5^-$ ($t_{1/2}$ = 8 h). Reaction 3 yields very little (<10%) of the mixed-metal product. In contrast, reaction between $Re(CO)_6^+$ and $Mn(CO)_5^-$ provides yields of $MnRe(CO)_{10}$ up to 50%.

In this communication, we have reported the first example of two-electron transfer to a kinetic product and the subsequent back transfer of one electron. Studies are continuing to better understand these reactions.

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Trisnorsqualene Alcohol, a Potent Inhibitor of Vertebrate Squalene Epoxidase

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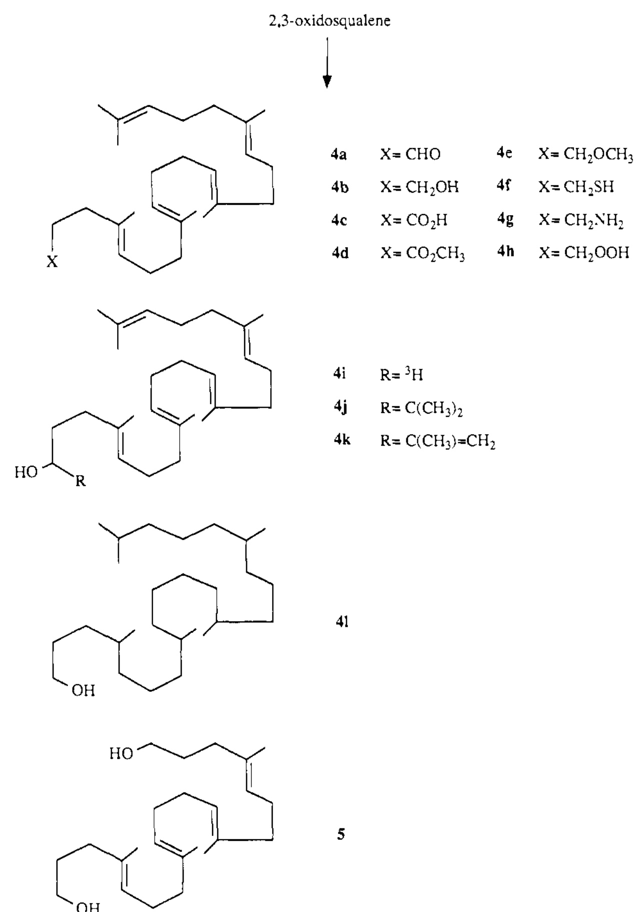
The epoxidation of squalene to (3*S*)-2,3-oxidosqualene and its subsequent cyclization to lanosterol are key steps in cholesterol biogenesis.[1] Despite the clinical importance of lowering serum cholesterol levels in humans, no pharmaceuticals are available which specifically target these committed steps in steroidogenesis.[2] Currently available hypocholesteremic drugs (e.g., mevinolin) inhibit HMG CoA-reductase,[3] thus diminishing the supply of mevalonate available for squalene production. Other potential cholesterol-lowering drugs, the oxysterols,[4] act via a receptor-mediated feedback inhibition of HMG CoA-reductase. Potent in vitro inhibition of oxidosqualene cyclase can be achieved with the aziridyl analogue of oxidosqualene,[5] as well as with several tertiary amine *N*-oxide transition-state analogues.[6] However, few compounds which effectively inhibit squalene epoxidase[7,8] are known. Indeed, very little is understood about the enzymatic mechanism of this apparently non-cytochrome P-450-dependent alkene monooxygenase, even in its purified form.[9] We present

Scheme I[a]



[a] Trisnorsqualene analogues. **4a**: from 2,3-oxidosqualene (**2**), using H_5IO_6, THF/H_2O. **4b**: from **4a**, using $NaBH_4$, EtOH. **4c**: from **4a**, using Ag_2O, THF. **4d**: from **4c**, using CH_2N_2, Et_2O. **4e**: from **4b**, using (1) *n*-BuLi, Et_2O and (2) CH_3I. **4f**: from **4b**, using (1) PPh_3, DIAD, $CH_3C(O)SH$ and (2) $LiAlH_4$, Et_2O. **4g**: from **4b**, using (1) PPh_3, CBr_4, CH_2Cl_2, (2) NaN_3, DMF, and (3) $LiAlH_4$, THF. **4h**: from **4b**, using (1) MsCl, Et_3N, CH_2Cl_2 and (2) H_2O_2, KOH, MeOH. **4i**: from **4a**, using NaB^3H_4, EtOH, **4j**: from **4a**, using $(CH_3)_2CH$-MgBr, THF. **4k**: from **2**, using $LiNEt_2$, THF. **4l**: from **4b**, using H_2, Pd/C, EtOH. **5**: from hexanorsqualene dialdehyde, using $NaBH_4$, EtOH.

Table I. IC_{50} and K_I Values of Squalene Analogues **4a–4l** and **5**[a]

squalene analogue	IC_{50} (μM)	K_I (μM)	squalene analogue	IC_{50} (μM)	K_I (μM)
4a	200		**4h**	4	
4b	4	3.5	**4i**		
4c	>400		**4j**	>400	
4d	>400		**4k**	>>400	
4e	300		**4l**	>>400	
4f	30	13	**5**	400	
4g	200				

[a] Analogues with IC_{50} = >400 showed some inhibition at high [I], while analogues with IC_{50} = >>400 showed essentially no inhibitory effect.

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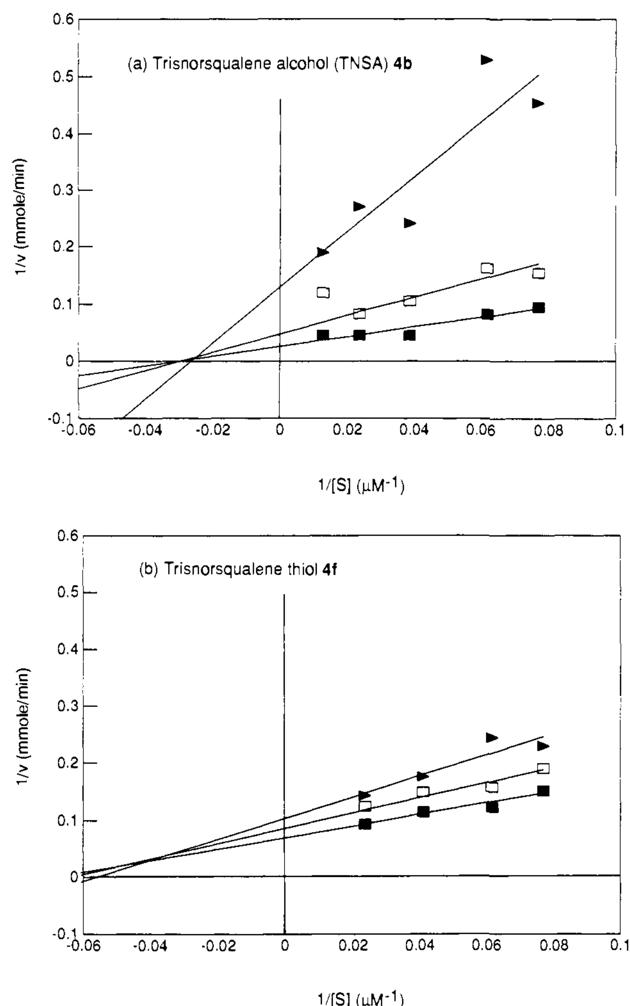
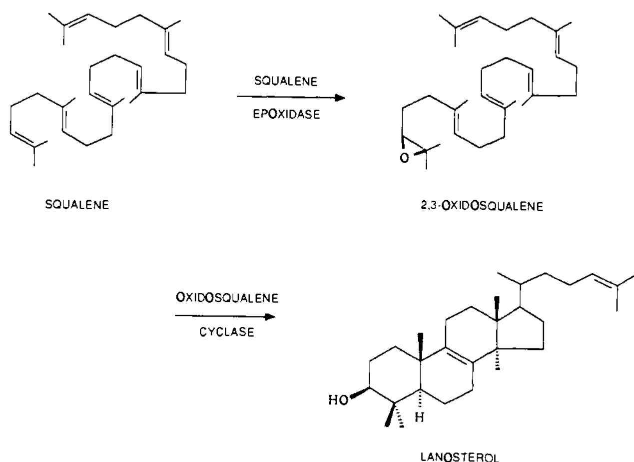


Figure 1. Lineweaver–Burke plot of the inhibition of squalene epoxidase by (a) trisnorsqualene alcohol (TNSA) **4b** and (b) trisnorsqualene thiol **4f**. For each inhibitor, the concentrations used were [I] = 0, [I] = IC_{50}, and [I] = IC_{70}. Velocity was determined by measuring the amount of [^{14}C]-2,3-oxidosqualene produced during a 50-min incubation, after the addition of inhibitor. Results indicate K_I values (inhibitor concentration at which there is a 50% decrease in maximal enzyme velocity) of 4 μM and 13 μM for TNSA and trisnorsqualene thiol, respectively.

herein evidence for potent inhibition of pig liver squalene epoxidase by trisnorsqualene alcohol, and we describe the inhibitory potency of several analogues that define the key structural parameters important in this inhibition.



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Trisnorsqualene alcohol (TNSA) **4b** was prepared by sodium borohydride reduction of the corresponding aldehyde **4a** obtained by periodic acid cleavage of 2,3-oxidosqualene **2**.[10] Trisnorsqualene acid **4c**, methyl ester **4d**, thiol **4f**, amine **4g**, and methyl ether **4e**, as well as squalene isopropyl- and isopropenylcarbinols **4j** and **4k**, were prepared by using standard functional group transformations as shown in the legend of Scheme I. Trisnorsqualane alcohol **4l** was prepared by exhaustive hydrogenation (10 atm, 48 h, 10% Pt/C catalyst) in ethanol. Finally, the symmetrical hexanorsqualene diol **5** was obtained from periodic acid cleavage and reduction of 2,3,22,23-dioxidosqualene.[11]

Enzyme assays for squalene epoxidase and oxidosqualene cyclase were conducted with Tween-80 solubilized enzymes from pig liver microsomes with [^{14}C]squalene (1.37 mCi/mmol) as substrate and NAPDH (1 mM) and FAD (40 μM) as cofactors.[12,13] TNSA **4b** was an unexpectedly potent inhibitor of squalene epoxidase activity, showing an IC_{50} value of 4 μM. Inhibition did not change with time. The requirement for the primary alcohol functionality and the requirement for the entire trisnorsqualenoid skeleton are clearly seen from Table I; only trisnorsqualene thiol **4f** showed comparable inhibitory effect. Furthermore, it appears that the active inhibitor was the alcohol itself and not possible metabolites such as the aldehyde **4a** or acid **4c**.

The importance of the entire trisnorsqualenoid moiety for inhibitory effect suggests that TNSA **4b** and trisnorsqualene thiol function as squalene analogues. However, the absence of classical competitive inhibition (Figure 1) clearly indicates a more complex mode of action. Since squalene epoxidase requires oxygen for enzymatic activity, one possibility is that TNSA functions as a bi-substrate analogue, mimicking a reactive intermediate that incorporates both squalene and an activated form of oxygen. To test this possibility, trisnorsqualene hydroperoxide **4h** was prepared by hydroperoxide anion displacement of the corresponding mesylate. Consistent with our hypothesis, inhibition was essentially identical with that observed for TNSA **4b**.

The metabolic fate of TNSA in vitro was determined with [^{3}H]-TNSA **4i**, synthesized by reduction of trisnoraldehyde **4a** with [^{3}H]-sodium borohydride (sp. act. 2.9 Ci/mmol). The labeled material (0.4 Ci/mmol, 1 000 000 dpm, 5 μM) was incubated for 0, 5 and 10 min with solubilized pig liver microsomes, and products were isolated by extraction from both the saponified and nonsaponified reaction mixtures. [^{3}H]-TNSA was recovered quantitatively, and no radioactive metabolites could be detected by radio-TLC. Thus, activation is not required for inhibition; moreover, covalent attachment to the enzyme does not occur on this time scale. Further studies of the enzyme-inhibitor interaction will be undertaken with purified epoxidase.

Acknowledgment. We thank Dr. Gregory M. Anstead for the preparation of squalene isopropenylcarbinol **4k**. We acknowledge the Center for Biotechnology and the New York State Science and Technology Foundation/Center for Biotechnology for initial

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(13) The final squalene concentration was 40 μM, the apparent K_M for crude pig liver microsomes. Paired experiments were performed, using *N*,-*N'*-dimethyldodecylamine *N*-oxide (100 μM) to inhibit oxidosqualene cyclase in one set of assays. Each inhibitor was added as an isopropyl alcohol stock solution, at inhibitor concentrations from 4 to 400 μM. After a 10-min preincubation, the [^{14}C]-labeled substrate was added and the suspension was agitated at 37 °C for 50 min. The enzyme solutions were then saponified (10% KOH, MeOH), and the labeled substrate and products were extracted (CH_2Cl_2), separated, and quantified with radio-TLC. The IC_{50} values in Table I indicate the inhibitor concentration at which there is a 50% loss of enzymatic activity. Details of the [^{14}C]squalene synthesis and the assay protocols for testing of squalene analogues as inhibitors of squalene epoxidase and oxidosqualene cyclase in our laboratory will be described elsewhere.[8] Relevant assay results for the TNSA analogues are available as supplementary material.

support, and we are grateful to Kirin Breweries, Co., Ltd. for ongoing support of this research.

Supplementary Material Available: Experimental details, elemental analysis data, and ^{1}H NMR, ^{13}C NMR, and IR spectral data, figures showing the inhibition of SE and OSC plus SE by dodecanol, dodecanethiol, **4a,c–g,j–l**, and **5**, and a plot of the time dependency of SE inactivation by **4i** (33 pages). Ordering information is given on any current masthead page.

Hydroxyl and Amido Groups as Long-Range Sensors in Conformational Analysis by Nuclear Overhauser Enhancement: A Source of Experimental Evidence for Conformational Flexibility of Oligosaccharides

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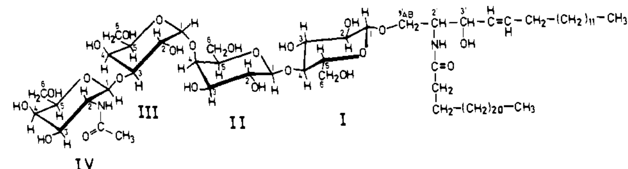
The three-dimensional (3D) structure, or conformation, of biooligomers is known to play a decisive role in their biological activity. One of the most important methods for the determination of the 3D structure of biomolecules is nuclear Overhauser enhancement (NOE) spectroscopy, which enables one to detect proximity in space between protons located in different, yet spatially neighboring, parts of the molecule. Although formally this applies to all classes of molecules, the analysis of oligosaccharide conformation is heavily handicapped as compared to that for proteins,[1] for example, because the number of NOE contacts observed is smaller by almost one order of magnitude. These contacts are usually restricted to interactions between the protons linked to the two carbon atoms at the glycosidic bridge (the anomeric and the aglyconic one), other contacts being rare. An important unfavorable consequence is that, in most cases, the amount of experimental data available for an adequate interactive fit of the theoretically calculated conformation(s) is insufficient to warrant a reliable description of a conformation or a possible conformational equilibrium. The problems arising in this connection have been discussed in detail by several research groups.[2]

We show here a way of supplementing this source of structural information by investigating NOE contacts with unexchanged hydroxyl and amido groups. Protons of these groups protrude farther from the carbon skeleton than the C-linked protons and provide a great number of additional distance constraints that may confirm or disprove hypothetical conformers obtained by energy minimum calculations. We illustrate this approach by analyzing the spectra of native (unexchanged) globoside.



The resonances of the C-linked protons of D_2O-exchanged globoside have been assigned previously[2c,3] and, allowing for their small temperature shifts, confirmed here by one-dimensional (1D) total correlation spectroscopy (TOCSY;[4a] synonymous with homonuclear Hartmann-Hahn spectroscopy—HOHAHA[4b]). Since the deuterium isotope effect on protons separated by three bonds is small, these CH resonances remain practically unchanged in the native globoside, and their scalar coupling connectivities with the OH and NH resonances provide unequivocal assignments of the latter (Figure 1a,b and Table I).

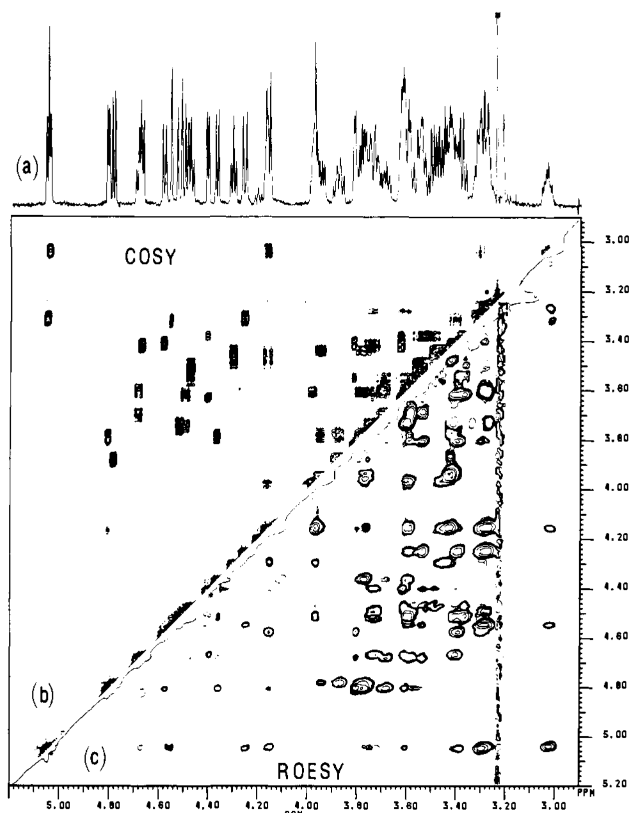


Figure 1. Partial 500 MHz ^{1}H NMR spectra of globoside in Me_2SO-d_6 at 315 K. (a) 1D spectrum; (b) scalar coupling autocorrelated (COSY) spectrum; (c) ROESY spectrum obtained with a mixing time of 200 ms. The diagonal and the exchange cross peaks are drawn with a single contour line; NOE cross peaks are filled.

Table I. Chemical Shifts for Globoside in Me_2SO-d_6 at 315 K

residue		1	2	3	4	5	6a	6b
GalNAcβ-IV	CH	4.53	3.75	3.43	3.63	3.39	3.55	3.51
	OH		7.62[a]	4.67	4.41		4.48	
Galα-III	CH	4.81	3.79	3.61	3.99	4.17	3.49	3.45
	OH		4.37		3.78		4.31	
Galβ-II	CH	4.26	3.32	3.42	3.83	3.56	3.61	3.70
	OH		5.05	4.58			4.69	
Glcα-I	CH	4.16	3.05	3.32	3.32	3.29	3.62	3.75
	OH		5.04	4.55			4.50	

[a] NH.

The dipolar coupling connectivities were obtained by rotating frame NOE experiments (ROESY;[5a-c] synonymous with CAMELSPIN[5d]). A z-filter was added for suppression of scalar coupling cross peaks, and the rf carrier frequency was offset away from the region of sugar proton resonances during the spin lock time and then returned to the middle of the spectrum during acquisition for better digital resolution[5c] (Figure 1c). ROESY

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