

requirement for receptor affinity suggests the hypothesis that the C<sup>8</sup> substituent of a xanthine antagonist is binding in the same region as is the N<sup>6</sup> substituent of an adenosine agonist. In our dihydroimidazopyridine, the *R* enantiomer (C-8) preferred A<sub>1</sub> receptor binding. If a C-8 substituent in the dihydroimidazopyridine of 4, 8, and 9 recognizes the same space as does the N<sup>6</sup> substituent of an adenosine agonist or the C-8 substituent of a xanthine antagonist, a new receptor binding mode for this ligand has to be suggested. On the other hand, Quinn's hypothesis<sup>18</sup> suggests that the dihydroimidazole moieties of enantiomers (8 and 9) might recognize a ribose binding domain and produce different activities in adenosine binding. More detailed analysis of the binding mode for this new class of A<sub>1</sub> antagonists will be the subject of a future report.

Water-soluble A<sub>1</sub> antagonists will be widely applicable in biochemical and pharmacological studies.

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**Supplementary Material Available:** Detailed experimental procedures for the preparation of 4-9, the binding assay, and the pharmacological assays (7 pages). Ordering information is given on any current masthead page.

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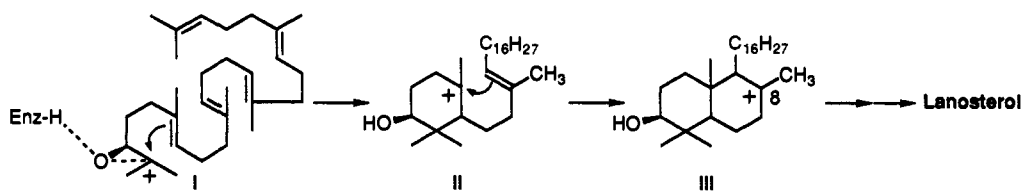
### *N*-(1-Oxododecyl)-4 $\alpha$ ,10-dimethyl-8-aza-*trans*-decal-3 $\beta$ -ol: A Potent Competitive Inhibitor of 2,3-Oxidosqualene Cyclase

Inhibition of the enzyme HMG-CoA reductase is an effective approach for the inhibition of de novo cholesterol biosynthesis and the treatment of hypercholesterolemia.<sup>1</sup> HMG-CoA reductase catalyzes the rate-limiting step in cholesterol biosynthesis; however, its inhibition can lead to an increase in enzyme synthesis.<sup>1b</sup> The enzymes squalene synthase,<sup>2,3</sup> squalene epoxidase,<sup>4</sup> and 2,3-oxidosqualene cyclase (OSC)<sup>5</sup> are attractive targets for drug development since they are positioned in the biosynthetic pathway at a point where sterol synthesis is committed. Therefore, selective inhibition should not interfere with essential non-sterol pathways or result in the accumulation

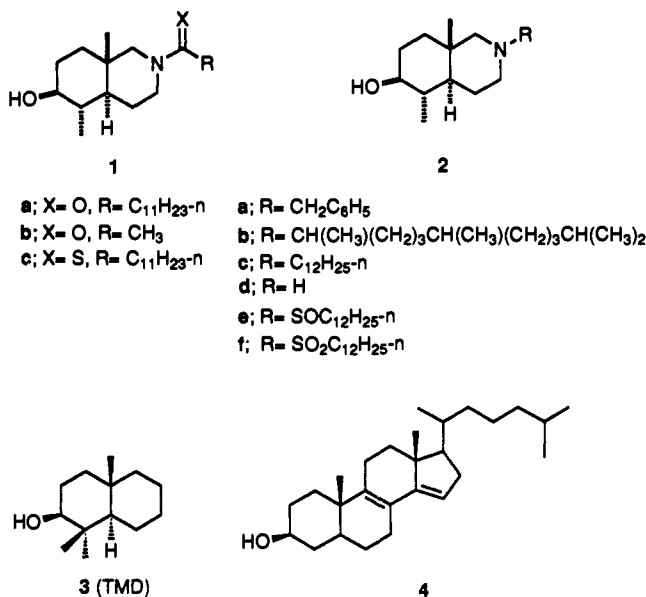
of steroidal precursors.<sup>2,6</sup> Recently, it has been demonstrated that inhibitors of OSC can regulate HMG-CoA reductase via a putative feedback mechanism involving the formation of C25-oxysterols.<sup>7</sup> Our approach to the design of inhibitors of OSC was initially based on the use of amines as mimics (e.g. 2b-d) of high-energy intermediate carbocations involved in the cyclization of 2,3-oxidosqualene (Scheme I) as previously described.<sup>8-11</sup> However, we have determined that the amine functionality is not necessary for potent OSC inhibition. Furthermore, in HepG2 cells, the 8-azadecalins are more potent inhibitors of other enzymes in the cholesterol biosynthesis pathway than OSC. In this communication, we report the synthesis and activity of an amide derivative of 2c, *N*-(1-oxododecyl)-4 $\alpha$ ,10-dimethyl-8-aza-*trans*-decal-3 $\beta$ -ol (1a), which

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## Scheme I



## Chart I



is a potent, competitive inhibitor of 2,3-oxidosqualene cyclase (EC 5.4.99.7) in vitro and inhibits cholesterol biosynthesis in HepG2 cells by blocking this enzyme activity.

**Chemistry, Enzymology, and Cell Culture.** Compounds 2b–d (Chart I) were prepared from 2a as described previously with minor modifications.<sup>10,11</sup> Amide 1a was prepared by acylation of 2a, followed by hydrogenation and

coupling of the resulting amine with lauroyl chloride. Saponification with 1.0 M LiOH in methanol gave 1a as a viscous oil.<sup>12</sup> Thioamide 1b was prepared from the acetate of 1a with Lawesson's reagent followed by saponification.

Rat liver oxidosqualene cyclase was purified and assayed as described.<sup>13</sup> For the determination of IC<sub>50</sub> values, inhibitors (in 5  $\mu$ L of 2-propanol) were added to an enzyme assay mixture (final volume, 200  $\mu$ L) that contained [<sup>3</sup>H]oxidosqualene (10  $\mu$ M), EDTA (0.5  $\mu$ M), dithiothreitol (0.5  $\mu$ M), Tween 80 (0.1%), and phosphate buffer (50  $\mu$ M, pH 7.4). The reaction was initiated by adding enzyme (10  $\mu$ g) solubilized in lauryl maltoside (final assay concentration is 0.25%), and the formation of [<sup>3</sup>H]lanosterol was determined by HPLC after a 20-min incubation. For the determination of K<sub>i</sub> values, Tween 80 was omitted from the assay and substrate (2.5, 5, 10, 15, 25, 33, and 50  $\mu$ M) was dispersed in 0.15% lauryl maltoside (final concentration). Enzyme was assayed in the presence of several different inhibitor concentrations, and the resulting hyperbolic *v* vs [S] curves were evaluated with EZ-Fit Enzyme Kinetic Model Fitting software (Perrella Scientific).

IC<sub>50</sub> values for the inhibition of cholesterol biosynthesis in human hepatoblastoma cells (HepG2) in culture were determined as described.<sup>4b</sup> Inhibitor selectivity for cellular OSC and sterol  $\Delta^{14}$ -reductase was determined by monitoring the accumulation of oxidosqualene (OS) and di-oxidosqualene (DOS) for OSC inhibition and  $\Delta^{8,14}$ -cholestadienol (4) (identified by independent synthesis<sup>14</sup> and spectral analysis) for sterol  $\Delta^{14}$ -reductase inhibition.<sup>15</sup>

**Results and Discussion.** While investigating inhibitors of OSC based on the 8-azadecalin system, we found that the potent inhibition of cholesterol biosynthesis in HepG2 cells from [<sup>14</sup>C]acetate by 2b and 2c resulted from the inhibition of sterol  $\Delta^{14}$ -reductase rather than OSC as evidenced by the accumulation of diene 4 at inhibitor concentrations equal to the IC<sub>50</sub> (Table I). At greater concentrations ( $\sim$ 10  $\mu$ M), 2b and 2c blocked OSC, causing OS and DOS to accumulate. Because the neutral decalin

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**Table I.** IC<sub>50</sub> Values for Cholesterol Biosynthesis Inhibitors

inhibitor	OSC inhibition: IC <sub>50</sub> (μM)	HepG2: IC <sub>50</sub> (μM)
1a	0.11	0.70 <sup>a</sup>
1b	50	>100 <sup>a</sup>
1c	0.21	2.0 <sup>a</sup>
2b	2 <sup>b</sup>	0.07 <sup>c</sup>
2c	0.55	0.01 <sup>c</sup>
2d	d	19 <sup>c</sup>
2e	30	2.8 <sup>a</sup>
2f	>100	52 <sup>a</sup>
3	9 <sup>a</sup>	28 <sup>a</sup>

<sup>a</sup>Diene 4 not detected. <sup>b</sup>Data for inhibition of microsomal OSC taken from ref 11. <sup>c</sup>IC<sub>50</sub> at which diene 4 accumulates. <sup>d</sup>In a microsomal assay, 2d fails to inhibit OSC. See ref 9b. <sup>e</sup>Data taken from ref 9b.

TMD (3) blocks cholesterol biosynthesis in HepG2 cells by the selective inhibition of OSC.<sup>16,17</sup> We hypothesized that the inhibition of the sterol Δ<sup>14</sup>-reductase by 2b–d is a function of the charge<sup>18</sup> or tetrahedral geometry of the basic nitrogen atom and proposed that the neutral and planar amide 1a might inhibit OSC selectively.<sup>19</sup>

Indeed, 1a is a potent inhibitor of purified OSC (Table I, IC<sub>50</sub> = 0.11 μM). In contrast, the less lipophilic *N*-acetyl analogue 1b was a 450-fold less potent inhibitor (IC<sub>50</sub> = 50 μM). An IC value of 0.55 μM was obtained for the amine 2c. Kinetic studies indicated that 1a and 2c are competitive inhibitors of the cyclase having *K*<sub>i</sub> values of 28 and 40 nM, respectively.<sup>20,21</sup> In contrast to 2c, 1a inhibited cholesterol biosynthesis in HepG2 cells (IC<sub>50</sub> = 0.7 μM) without accumulation of the diene 4, indicating that 1a is a more potent inhibitor of OSC than of the sterol Δ<sup>14</sup>-reductase. Thus, 1a is the most potent competitive inhibitor of OSC in HepG2 cells reported to date.

We also prepared and assayed thioamide 1c, sulfoxamide 2e,<sup>22</sup> and sulfonamide 2f.<sup>24</sup> As with amide 1a, compounds 1c, 2e, and 2f contain an electronically neutral nitrogen; however, 2e and 2f lack the planar geometry of 1a.<sup>25</sup> Interestingly, thioamide 1c was nearly equipotent to 1a at inhibiting OSC and cholesterol biosynthesis.<sup>26</sup> Furthermore, cells incubated with 1c did not accumulate the diene 4. The sulfoxamide 2e also blocked cholesterol synthesis (IC<sub>50</sub> = 2.8 μM) in HepG2 cells, but was a 270-fold less potent inhibitor of purified OSC than 1a. The

sulfonamide 2f only weakly inhibited cholesterol biosynthesis and the purified cyclase. Finally, neither 2e nor 2f caused an accumulation of the diene 4.

In summary, we have described the synthesis and activity of a new class of OSC inhibitors exemplified by the amide 1a which inhibits cholesterol biosynthesis in HepG2 cells by selectively blocking 2,3-oxidosqualene cyclase. Kinetic data indicate that 1a binds tightly to the active site of the purified cyclase and has a 460-fold greater affinity for the enzyme than the structurally similar but noncompetitive inhibitor TMD (*K*<sub>i</sub> = 13 μM).<sup>9b</sup> The activity of 1a, while not fully understood at this time, may be a consequence of both lipophilicity and an inhibitor conformation imposed by introduction of the amide functionality. Detailed structure–activity studies are in progress and should aid in the understanding of the activity of 1a and in the development of therapeutic agents which inhibit 2,3-oxidosqualene cyclase.

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**Supplementary Material Available:** Experimental details for syntheses of 1a–c and 2b,c,e,f (7 pages). Ordering information is given on any current masthead page.

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- (18) At physiological pH, 2b–d would be protonated.
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- (20) The discrepancy between *K*<sub>i</sub> and IC<sub>50</sub> values is likely due to differences in the detergent composition and concentration of the two assays.

- (21) To date, the only reported competitive inhibitor of OSC is a C-20 vinyl ether analogue of 22,23-dihydro-2,3-oxidosqualene (*K*<sub>i</sub> = 40 μM). Ceruti, M.; Viola, F.; Dosio, F.; Cattel, L.; Bouvier-Navé, P.; Ugliengo, P. Stereospecific Synthesis of Squalenoid Epoxide Vinyl Ethers as Inhibitors of 2,3-Oxidosqualene Cyclase. *J. Chem. Soc., Perkin Trans. 1* 1988, 461–469.
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- (26) It is possible that the activity of 1c may arise from hydrolysis of the thioamide under the assay conditions (i.e. 1c may be a prodrug to 1a).

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