N-Oxide as a Potential Function in the Design of Enzyme Inhibitors. Application to 2,3-Epoxysqualene-sterol Cyclases

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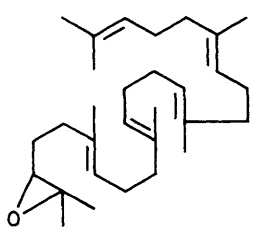
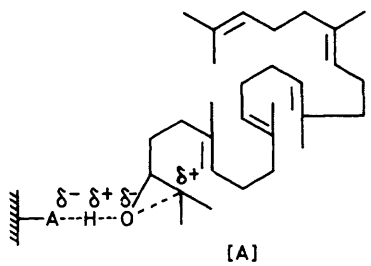
The *N*-oxides of several tertiary amines possessing an aliphatic long chain are more potent inhibitors of 2,3-epoxysqualene-β-amyrin and -lanosterol cyclases than their parent amines.

Non-aromatic tertiary amine *N*-oxides are usually metabolites of natural products or drugs.[1] Apart from an important increase in polarity, no major increase of biological activity has been shown to result from the *N*-oxidation.[1,2] Owing to its strong dipolar moment,[3,4] the *N*-oxide bond is able to mimic dipoles (*e.g.* polarized bonds) involved in enzyme-catalysed reactions and therefore can be used in the design of inhibitors which are transition state (TS) analogues. We have applied this new concept to the case of the enzymic cyclization of 2,3-epoxysqualene (**1**). We have previously shown that 2-azasqualene (**5a**) and derivatives were potent inhibitors of (**1**)-cyclase in both animal and higher plant cell-free systems.[5,6] We have now synthesized the *N*-oxide derivatives (**2**)—(**4**) and studied their ability to inhibit both (**1**)-β-amyrin and -lanosterol cyclases. A molecule such as (**2a**) presents structural and electronic similarities with a possible TS [A] involved in the first step (general acid-catalysed oxirane ring opening) of the enzymic cyclization of (**1**).

The synthesis of the *N*-oxides (**2**)—(**4**) was performed starting from the available parent amines (**5**)—(**7**). The azasqualene *N*-oxides (**2**) were obtained by reaction of H_2O_2



(1)

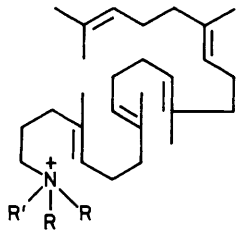


[A]

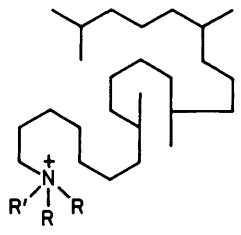
Table 1. Inhibition of 2,3-epoxysqualene cyclases by 2-aza-2,3-dihydrosqualene-*N*-oxide (**2a**), other long chain tertiary amine *N*-oxides (**2b**)—(**4**), and their parent amines (**5**)—(**7**).

Tertiary amines	I_{50}[a]/μM Pea[b]	Rat liver[c]	Tertiary amine *N*-oxides	I_{50}[a]/μM Pea[b]	Rat liver[c]
(**5a**)	1	7.5	(**2a**)	0.3	3.7
(**5b**)	0.55	3.2	(**2b**)	0.15	1.5
(**6a**)	22	21	(**3a**)	1	8
(**6b**)	1.4	18	(**3b**)	0.5	3.5
(**7a**)	50	>100	(**4a**)	0.3	3.0
(**7b**)	0.8	3.2	(**4b**)	0.3	3.6

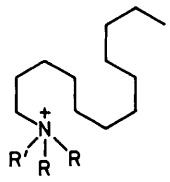
[a] I_{50} value: inhibitor concentration required to reduce reaction velocity by half. [b] (**1**)-β-amyrin cyclase from pea cotyledons.[6] [c] (**1**)-lanosterol cyclase from rat liver.[6]



(**2**) R′ = O⁻
(**5**) R′ = H



(**3**) R′ = O⁻
(**6**) R′ = H



(**4**) R′ = O⁻
(**7**) R′ = H

a; R = Me
b; R = Et

(30%)–anhydrous methanol (30:70, v/v), at room temperature overnight on (**5**).[7] The azasqualane *N*-oxides (**3**) were obtained by reaction of *m*-chloroperbenzoic acid on (**6**) in $CHCl_3$ at room temperature for 1—14 h. Compounds (**6**) were obtained by catalytic hydrogenation of (**5**) in the presence of Pd/C (10% in EtOH) during one week. The *N,N*-diethyldodecylamine *N*-oxide (**4b**) was prepared from the parent amine (**7b**) by treatment with *m*-chloroperbenzoic acid in $CHCl_3$. *N,N*-dimethyldodecylamine *N*-oxide (**4a**) was obtained from Serva Chemicals (Heidelberg). Synthesis of (**5**) and (**7**) has been described previously.[6]

The enzymic systems from pea cotyledons and rat liver, the inhibition assays, and the analytical procedure involved in the purification of the products (β-amyrin and lanosterol) of the enzymic cyclization of (**1**) have been described previously.[6]

Table 1 indicates the comparative results obtained with the amines (**5**)—(**7**) and their corresponding *N*-oxides (**2**)—(**4**). From the data, three structural features are shown to be involved in the inhibition: (i) the *N*-oxide derivatives are systematically more potent than the parent tertiary amines; (ii) the parent *N,N*-diethylamines are more inhibitory than the corresponding *N,N*-dimethyl products; and (iii) in the *N,N*-dimethylamine series, the length of the alkyl chain and the presence of double bonds appear to be important. These three favourable features are gathered in *N,N*-diethylazasqualene *N*-oxide (**2b**), the most potent inhibitor of the series. The inhibition of the (**1**)-β-amyrin and -lanosterol cyclases by (**2a**) was shown to be purely non-competitive with respect to (**1**). The K_i values calculated from Lineweaver–Burk plots (0.3 and 1.8 μM for the two cyclases respectively), when compared with the K_m values (125 ± 25 and 45 ± 14 μM respectively) calculated on the assumption that only one enantiomer of (2,3-*R,S*)-2,3-epoxysqualene is transformed by the cyclases,[6] indicate that both cyclases had an excellent affinity for (**2a**).

Finally the inhibitory power of the *N*-oxide derivatives toward the 2,3-epoxysqualene cyclases is consistent with the potency expected from TS analogues. However the mechanism of inhibition, according to the current views on TS analogues,[8] remains to be firmly established. It should be pointed out in the present context that 2-hydroxynicotinic acid *N*-oxides have been shown to be TS analogue inhibitors of protocatechuate-3,4-dioxygenase.[9] On the other hand, it has been shown in one of our laboratories that 2-azasqualene (**5a**) is transformed in high yields by rat liver microsomes into its corresponding *N*-oxide (**2a**). Thus (**5a**) can be considered as a prodrug of potential pharmacological interest. Studies are now in progress to compare the biological and pharmacological activities of (**2a**) on fungal, higher plant, and animal cells.[10]

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