Inhibition of Higher Plant 2,3-Oxidosqualene Cyclase by 2-Aza-2,3-dihydrosqualene and its Derivatives

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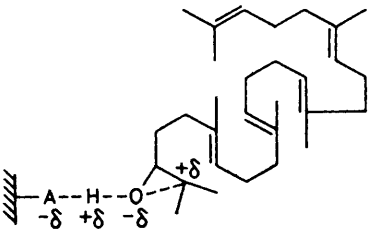
2-Aza-2,3-dihydrosqualene strongly inhibits 2,3-oxidosqualene–β-amyrin and –cycloartenol cyclases.

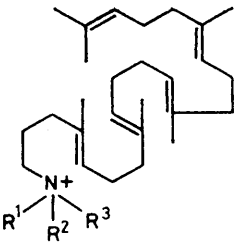
The 2,3-oxidosqualene (SqO) cyclases represent a group of enzymes which convert 2,3-oxidosqualene (**1**)† into polycyclic triterpenoids such as lanosterol, cycloartenol, or α- and β-amyrin.[1] Taking into account the postulated model of the enzymic cyclisation of SqO,[2] we have investigated the possibility of designing compounds which would be selective and potent inhibitors of the SqO cyclases. Accordingly, we have synthesized 2-aza-2,3-dihydrosqualene (**2**) and several derivatives (**3**)—(**9**). These amines, being protonated at physiological pH, could show some electronic and structural similarities with the high energy intermediate C-2 carbonium ion that results from the protonation of the oxiran ring. According to recent theories,[3,4] compounds (**2**)—(**9**) should behave as inhibitors of the SqO cyclase.

The syntheses of (**2**)—(**9**) were performed starting from the readily available 1,1′,2-trisnorsqualene aldehyde. The aldehyde (1 mmol), on treatment in anhydrous methanol with the selected alkylamine (6 mmol) at pH 6, followed by reduction by $NaBH_3CN$ (0.6 mmol), gave the 2-aza-2,3-dihydrosqualene derivatives.[5]

Microsomes (5—10 mg of proteins) from germinated pea cotyledons were incubated in the presence of [3-^{3}H](**1**) (100 μM, 0.1 μCi) and various amounts of inhibitors (0.1—100 μM) for 1 h at 30 °C and pH 7. Controls lacking the inhibitors or consisting of boiled microsomes were incubated in parallel. The reaction was stopped by 6% ethanolic KOH (1 ml) and the radioactive β-amyrin which resulted from the enzymic cyclization of (**1**) was isolated and purified as described previously.[6] From the observed inhibition curves, it was possible to determine an I_{50} value (inhibitor concentration required to reduce reaction velocity by half). As shown in Table 1, the *N*,*N*-dimethyl (**2**) and *N*,*N*-diethyl (**4**) compounds were the most inhibitory, while the *N*,*N*-di-isopropyl (**6**) and the *N*-demethyl (**8**) compounds displayed relatively low activity, and the *N*-monomethyl (**3**) and *N*-monoethyl (**5**) derivatives showed intermediate activity. In order to check whether the neutral or the protonated amine was the inhibitory species, the derivative (**9**) containing a quaternary ammonium function was used as an inhibitor in the assay. The results (Table 1) showed that (**9**) was as strongly inhibitory as (**2**), indicating that the charged species was probably the inhibitory one. The inhibition of the SqO cyclase by (**2**) was shown to be purely non-competitive with respect to SqO. The K_I value



(**1**)



(**2**)-(**9**)

(**2**) $R^1 = R^2 = Me$, $R^3 = H$
(**3**) $R^1 = Me$, $R^2 = R^3 = H$
(**4**) $R^1 = R^2 = Et$, $R^3 = H$
(**5**) $R^1 = Et$, $R^2 = R^3 = H$
(**6**) $R^1 = R^2 = Pr^i$, $R^3 = H$
(**7**) $R^1 = Pr^i$, $R^2 = R^3 = H$
(**8**) $R^1 = R^2 = R^3 = H$
(**9**) $R^1 = R^2 = R^3 = Me$

Table 1. Inhibition of 2,3-oxidosqualene cyclase by 2-aza-2,3-dihydrosqualene (**2**) and derivatives (**3**)—(**9**).

Inhibitor	(**2**)	(**3**)	(**4**)	(**5**)	(**6**)	(**7**)	(**8**)	(**9**)
I_{50} (μM)	1[a]	3	0.5	3	120	50	30	1

[a] A standard deviation of 0.2 has been calculated in the case of the I_{50} value of (**2**).

† 2,3-Epoxy-2,3-dihydrosqualene.

(1 μM), obtained from the Lineweaver–Burk plots, when compared with the K_M value (250 μM) for SqO indicated that the SqO cyclase had a much higher affinity for (**2**) than for SqO. Experiments conducted with an SqO–cycloartenol cyclase from maize seedlings showed that (**2**) inhibited this enzyme as strongly as the SqO–β-amyrin cyclase. However (**2**), at the highest concentration tested (100 μM) failed to inhibit the *S*-adenosylmethionine-cycloartenol-C-24-methyltransferase, another microsomal enzyme involved in higher-plant sterol biosynthesis.[7]

From the results obtained in the present study, it can be concluded that (**2**) strongly inhibited higher plant SqO cyclases, that the inhibition involved the charged amine function, and that the site of the enzyme which is responsible for the binding with the inhibitor was quite sensitive to steric hindrance at the nitrogen atom, as seen with the mono- and di-isopropyl derivatives of (**2**). At first sight the fact that the inhibition of SqO cyclase by (**2**) is not competitive does not seem to favour the hypothesis that (**2**) is a high energy intermediate analogue. However since we are dealing with heterogenous systems, the normal kinetics do not apply.[8] So far the K_I values determined for (**2**) and (**4**) are the lowest reported for an SqO cyclase inhibitor.[9]

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References

1 P. D. G. Dean, *Steroidologia*, 1971, **2**, 143 and references cited therein.
2 E. E. Van Tamelen, *J. Am. Chem. Soc.*, 1977, **99**, 950.
3 W. P. Jencks, *Adv. Enzymol.*, 1975, **43**, 219.
4 R. Wolfenden, *Annu. Rev. Biophys.*, 1976, **5**, 271.
5 R. F. Borch, M. D. Bernstein, and H. Durst Dupont, *J. Am. Chem. Soc.*, 1971, **93**, 2897.
6 A. Dietsch, L. Delprino, P. Benveniste, and L. Cattel, *J. Chem. Res.*, 1980, (*S*) 60; (*M*) 0752.
7 A. S. Narula, A. Rahier, P. Benveniste, and F. Schuber, *J. Am. Chem. Soc.*, 1981, **103**, 2408.
8 W. L. Backes, M. Hogeboom, and W. J. Canady, *J. Biol. Chem.*, 1982, **257**, 4063.
9 2,3-Dihydro-2,3-epiminosqualene, a compound bearing an aziridine ring in place of the oxiran ring has been shown to inhibit SqO–lanosterol cyclase at similar concentrations but the kinetic parameters of the inhibition were not determined: E. J. Corey, P. R. Ortiz de Montellano, K. Lin, and P. D. G. Dean, *J. Am. Chem. Soc.*, 1967, **89**, 2797.