The Mechanism of Introduction of Alkyl Groups at C-24 of Sterols

IV. INHIBITION BY TRIPARANOL*

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SUMMARY

C1 transfer to the Δ24 bond of 8,24-lanostadien-3β-ol (lanosterol) or of 9,19-cyclo-24-lanosten-3β-ol (cycloartenol) was inhibited by triparanol when either of the two steroids was incubated with methionine as the C1 source in a cell-free system from germinating peas. Specificity was demonstrated by the failure of triparanol to inhibit any of the steps between mevalonate and squalene. The mechanism of inhibition is probably through coordination of the lone electron pair of the nitrogen atom of triparanol with the electrophilic center of the enzyme which then interferes with binding of the Δ24 substrate to the enzyme.

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EXPERIMENTAL PROCEDURE

Materials and Methods—Lanosterol and cycloartenol were obtained and separated from their 24,25-dihydro derivatives and other contaminants as previously described (17). Triparanol was the gift of Dr. Thomas R. Blohm of the Wm. S. Merrell Company, Cincinnati, Ohio.

Incubations of the unlabeled steroids were performed for 6.0 hours at 36° with 0.50 mg of steroid and various amounts of triparanol (suspended in 80 mg of Tween 20) and 0.025 mg of [methyl-14C]methionine (5.0 X 10^6 dpm) in 23 ml of the supernatant obtained from an homogenate of 27 peas (14). Solid ATP-disodium salt (8 mg) and solid NADPH (3.0 mg) were added to each 23 ml of supernatant. The supernatant was obtained from the homogenate by centrifugation at 8720 X g for 30 min at 0°. The homogenate was prepared from peas (Burpee's Blue Bantam) which were placed in water for 16 hours and then germinated for an additional 5 days at room temperature on moist filter paper, when [14C]methionine was the labeled substrate. When [14C]mevalonate was the substrate, the supernatant was prepared in the latter way except that after 16 hours of immersion in water the peas were allowed to germinate on filter paper for only 24 hours more. The incubations were terminated by the addition of 25 ml of 10% ethanolic KOH. Saponification was achieved by allowing

Sterol nomenclature used is: lanosterol, 8,24-lanostadien-3β-ol; cycloartenol, 9,19-cyclo-24-lanosten-3β-ol.
the mixture to remain at room temperature under nitrogen for 12 to 14 hours followed by heating at the reflux temperature for 1 hour. The neutral lipids (ether-soluble fraction after addition of water) were chromatographed on a thin layer of Silica Gel G at C-24. \( \text{CH}_3^+ \) is derived from \( S \)-adenosylmethionine, \( \text{H}^+ \) from reduced pyridine nucleotide, and \( \text{H}^+ \) from water, presumably via the enzyme.

[Scheme 1. Similarity of mechanism of reduction and alkylation at C-24. \( \text{CH}_3^+ \) is derived from \( S \)-adenosylmethionine, \( \text{H}^+ \) from reduced pyridine nucleotide, and \( \text{H}^+ \) from water, presumably via the enzyme.]
50% inhibition was achieved at 0.33 mg of inhibitor per ml of incubate (0.75 mM). However, triparanol did not inhibit the formation of squalene (Table II) at concentrations which led to essentially complete inhibition of C\textsubscript{1} transfer. The incorporation of \textsuperscript{14}C from [2-\textsuperscript{14}C]mevalonate into neutral lipids was unchanged by the presence of the inhibitor. Chromatography revealed the presence of labeled squalene also in undiminished quantity. In fact, triparanol actually increased the amount of squalene and decreased the amount of label in the lipids which remained at the origin of a thin layer chromatoplate. This is provisionally interpreted as meaning that triparanol acted as an antioxidant preventing artifact formation. When a similar incubation was performed in the presence of glutathione and in the absence of triparanol, the distribution of \textsuperscript{14}C in the lipid fraction was similar to that obtained in the absence of glutathione and the presence of triparanol (Table II).

**DISCUSSION**

The conversion of mevalonate to squalene involves two phosphorylations (21-23), phosphorylative decarboxylation and elimination of phosphate ion (24, 25), trimerization of the Cs unit (26-28), and multistep reductive coupling (29-33). The failure of triparanol to inhibit any of these very different reactions constitutes good evidence that it is not a general inhibitor of plant enzymes. Similar evidence is available in the animal kingdom (3). Rats which have been fed triparanol, accumulate desmosterol, indicating that the ∆\textsuperscript{A\textsubscript{24}} reductase is the only step beyond cyclization other than alkylation or reduction. However, our enzyme preparation did not contain a sufficient concentration of the cyclase, etc. to allow definition of this point.

Since triparanol does inhibit both alkylation and reduction (2-10) at C-24, a rather specific mechanism of action dependent on electrons in the substrate must be involved. We would like to extend the hypothesis of Blomh et al. (10) by suggesting that triparanol acts as an inhibitor in both cases through binding at the electrophilic sites of the enzymes which are normally associated with reaction of the π electrons of the ∆\textsuperscript{A\textsubscript{24}} bond. As shown in Scheme 2, the diethylaminoethoxy side chain of triparanol corresponds in length and general conformation to the side chain of steroids (10), and one can imagine the lone electron pair on the nitrogen atom, which is situated at a position analogous to C-24 of the steroid, playing the same binding role as the π electrons of the ∆\textsuperscript{A\textsubscript{24}} substrate. The bulk of the triparanol molecule simply would play a rough space-filling role corresponding to the steroid nucleus. As pointed out by Blomh et al. (10), evidence for the latter is that cis- and trans-clomiphene, which bear the same diethylaminoethoxy side chain but differ in the rest of the molecule, also are ∆\textsuperscript{A\textsubscript{24}} reductase inhibitors (10) as are 3(2)-diethylaminoethoxyestradiol (34), 25-azasterols (35, 36), and a diethylaminoethoxy derivative of stilbestrol (34).

It is interesting to note that triparanol and these various other nitrogen containing inhibitors could conceivably be converted to their protonated (in the case of the reductase) or methylated (in the case of the alkylase) derivatives (cf. Scheme 2). However, even if such a reaction occurred, the products, unlike the ∆\textsuperscript{A\textsubscript{24}} steroid metabolites, would be positively charged and thus could remain attached to the enzyme through interaction with whatever forces there are which stabilize the electrophilic center of the enzyme. In the normal course of events (reduction or alkylation) the charge status of the enzyme would not change. In reduction H\textsuperscript{+} and H\textsuperscript{+} are lost yielding a net change in charge of 0, and in alkylation the loss of CH\textsubscript{3}\textsuperscript{+} is balanced by a gain of H\textsuperscript{+}. With the nitrogen atom of the inhibitor it is not possible to regenerate the original charge status unless the now positively charged product remains bound to the enzyme.

**REFERENCES**

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