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Note

Reconsideration of Mechanism of Enzymatic Decarboxylation of 3β-hydroxycholest-7-ene-4α-carboxylic Acid

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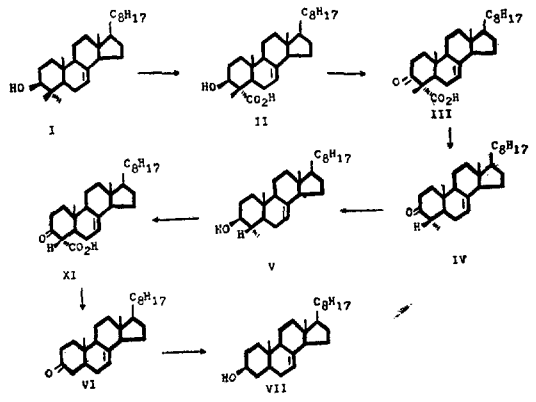
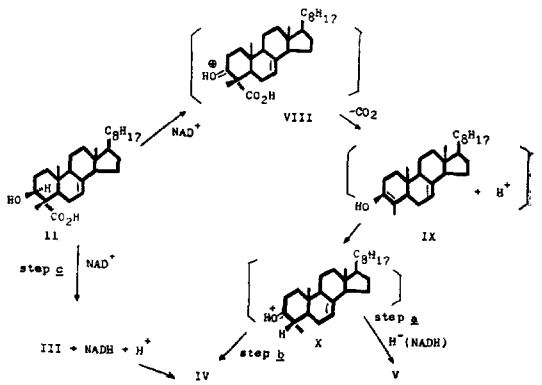
The participation of 3-oxosteroids (III, IV, XI and VI) in the biogenesis of cholesterol from lanosterol was first suggested by Bloch and his collaborators [1] based mainly on the observation that the 3α-^{3}H was lost during the demethylation at C-4 of lanosterol. Recently Gaylor and his collaborators have isolated the 3-oxo intermediate IV either during the demethhyphen ylation of I in the liver microsome or during the decarboxylation of II in partially purified enzyme(s) [2,3]. Their results appear to firmly establish the 3-oxosteroids as obligatory intermediates (*Scheme* 1).[4]



Scheme I



Scheme II

However, there is some question whether 3-oxosteroids (III, IV, XI, and VI) function as intermediates during demethylation, since IV and VI were isolated [2,3] under conditions which did not promote demethylation in the liver homogenates[8]. In addition, one of the arguments against the intermediate role of 3-oxosteroids is the finding by Gaylor and his collaborators [2] that the 3-oxosteroid (IV) is not demethylated, while its precursor I is demethylated in the washed liver microsome. However, when supernatant was added to the washed microsome (or an NADPH generating system added), IV eventually undergoes demethylation with no significant increase in the demethylation of I.

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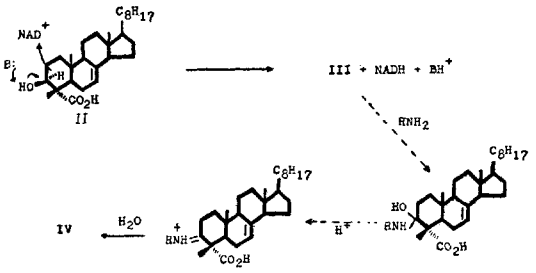
This may indicate that demethylation of IV in the homogenates (or microsome+supernatant) is not related to the over-all process of the demethylation of I.

The main purpose of this paper is to propose a modified Bloch-Gaylor mechanism (*Scheme* II*a*) which is chemically more feasible and does not require the 3-oxosteroids as intermediates. Perhaps, the proposed mechanism will better explain all the known facts than the Bloch-Gaylor mechanism does (*Scheme* I).

Discussion

The decarboxylation of II has shown an absolute requirement of NAD^+ under anaerobic condition[2,4b]. Enzyme-mediated one-hydrogen transfer(as H^-) from II to the NAD^+ [4a] will give transient species (non-isolable) VIII which will spontaneously decarboxylate to give X (*Scheme* II). The fate of oxonium ion X is either to lose H^+ (Step *b*) or accept H^- (Step *a*). If a hydrogen acceptor (possibly basic conditions or molecular oxygen) is present H^+ loss may be the main pathway (Step *b*). However, if there is no acceptor available, as in the conversion of II in the liver microsome which occurs under anaerobic condition, and possibly neutral conditions (pH$\simeq$7), the alternative Step *a*, will be the main pathway which leads to V. In addition, it is more than likely that the conversion of IV to V, if any IV is formed, goes through the protonated species, X, followed by H^- transfer (Step *a*) (theory of micro-reversibility). Thus, Step *b* is not sensible.

Another possible pathway for the decarboxylation of II is the synchronous H^- transfer with the loss of H^+(Step *c* in Scheme II) to form III. It has been well established that the enzymatic decarboxylation of β-keto acids goes through the Schiff base under acid catalysis[6]. It is likely that IV, if present, can be protonated



Scheme III

first under the conditions (H^+ was formed) and then form the Schiff base. Thus, Step *c* is not sensible, if III undergoes decarboxylation through the Shiff base. One may argue that III is not basic enough to be protonated under physiological conditions, thus protonation occurs only after the formation of the carbinol amine (dotted pathway in Scheme III). This is not likely, as H^+ was released during the formation of III and this can produce enough local acidity to cause protonation of III. Anyhow, the unsuccessful demethylation of IV in the liver microsomes speaks against the intermediate role of IV and thus Steps *b* and *c*.

The proposed mechanism (Step *a* in *Scheme* II) appears to be more plausible than the Bloch-Gaylor mechanism(Step *b* or *c*) under physiological conditions (neutral and anaerobic). The so-formed oxonium ion (VIII) can facilitate decarboxylation more than does the free 3-oxo species (III) by providing an active electron sink and the free enol as the product. It can explain the isomerization of the β-Me group at C-4 of II after decarboxylation, (see IX in *Scheme* II) and also the incorporation of the 3α-H of II to the NAD^+ [4b]. It further suggests that formation of 3-oxosteroids under basic and/or aerobic conditions is possible but may not occur under physiological conditions (neutral and anaerobic);thus it can explain the unsuccessful demethylation of 3-oxosteroids in the liver microsome[2] because 3-oxosteroids are not intermediates in the proposed mechanism.

Indirect evidence for supporting the proposed mechanism may be the unsuccessful isolation of labelled NADH from the incubation of 3α-3H II which is supposed to give IV and labelled NADH, according to the Bloch-Gaylor mechanism in the liver microsome [4b]. Most of the label was found at nicotinamide which was suggested as the degradation product of NAD^+ [4b]. Thus, this finding may support the formation of NAD^+ during the demethylation which coincides with the proposed mechanism.

Another line of indirect evidence for supporting the proposed mechanism may be the findings that the oxygen atom of the 3-hydroxy in cholesterol originates from the lanosterol and no exchange has been observed.[7] If IV is an obligatory intermediate during the process, it is likely that decarboxylation of III occurs through the Schiff base. Thus the oxygen atom at C-3 in lanosterol should exchange with the medium during the conversion to cholesterol.[6] The proposed intermediate, VIII, is a protonated ketone and can decardoxylate without forming the Schiff base intermediate. Therefore, there will be no exygen atom exchange during the conversion.[7]

The only observation which is against the proposed mechanism is the isolation of 3-oxosteroids (IV and VI) during the demethylation. However, the formation of IV as a major product from the decarboxylation of II[3] in a partially purified enzyme system has different conditions than the over-all process of demethylation of I.[8] Bloch and his collaborators[8] have shown that the demethylation of I (determined by CO_2 production) was optimum at pH 7.0 with no significant demethylation over pH 7.5(extrapolated from Bloch's diagram) [8], while the formation of IV from II by Gaylor and his collaborators [3] was optium at pH 9.0. In addition, the formation of CO_2 from II was an anaerobic process, but that of IV from II was undre aerobic conditions. It is difficult to believe that the formation of one product (CO_2) is maximum under anaerobic, and the other product (3-oxosteroids) is maximum under aerobic conditions. This may suggest that the formation of CO_2 and 3-oxosteroids occur by different pathways. One possible explanation for the formation of 3-oxosteroids in the partially purified enzyme at pH 9 under aerobic conditions is that, under basic conditions, such as pH 9, Step *b* or *c* (*Scheme* II) is favred over Step *a*, as the base acts as a hydronium ion acceptor and molecular oxygen also can oxidize NADH to NAD^+ to suppress Step *a*.

Conclusion

The 3-oxosteroids (III, IV, XI, and VI) in the biogenesis of cholestrol from lanosterol may not be true intermediates. The oxonium ion (VIII) resulting from hydride loss from the alcohol is postulated as the actual intermediate during the demethylation.

Many of the reactions of ketones are catalyzed by acid in the test tube. In living cells, however, it is very difficult to achieve significant protonation of ketones as it usually requires moderately strong acid. The hydride shift from an alcohol to form a protonated ketone may be the way enzymes mimic the acid catalyst.

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