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ENZYMATIC BROMOHYDRIN FORMATION

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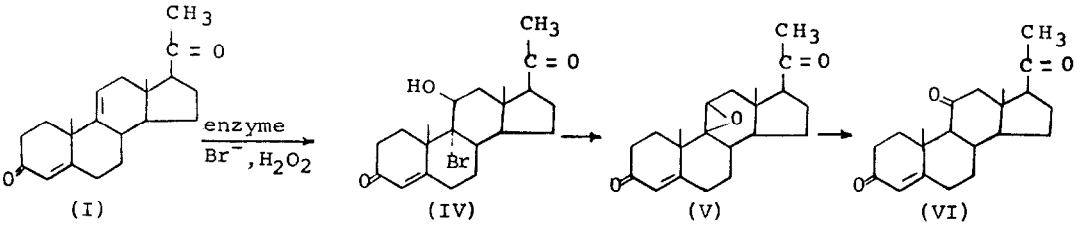
We have previously described the enzymatic bromination of steroidal β-diketones (1), α-hydroxymethylene keto-steroids (2), and a steroidal β-keto-lactone (1) by the chloroperoxidase of Caldariomyces fumago (3). In this communication, we report the reaction of the enzyme with the isolated double bonds of 9(11)-dehydroprogesterone (I), pregnenolone (II), and pregnenolone acetate (III). This represents a novel route to steroidal epoxides via enzymatically produced bromohydrins, and is the first example of enzymatic bromohydrin formation.

The procedures for growing the microorganism Caldariomyces fumago ATCC 16373, and preparing the crude chloroperoxidase have been described earlier (2). The substrates were added to a solution of the crude enzyme in phosphate buffer (0.3M, pH 3.0) in the presence of potassium bromide and hydrogen peroxide and shaken at 25° C. Extraction with chloroform, followed by thin layer chromatography on alumina, yielded the products described below. In the absence of added chloroperoxidase, potassium bromide, or hydrogen peroxide, no bromohydrin formation was observed.

With I as substrate and a reaction time of 15 minutes, we obtained 9α-bromo-11β-hydroxyprogesterone (IV)* (4) (48% yield). When the reaction time

* The physical constants of the isolated products were in agreement with previously prepared samples.

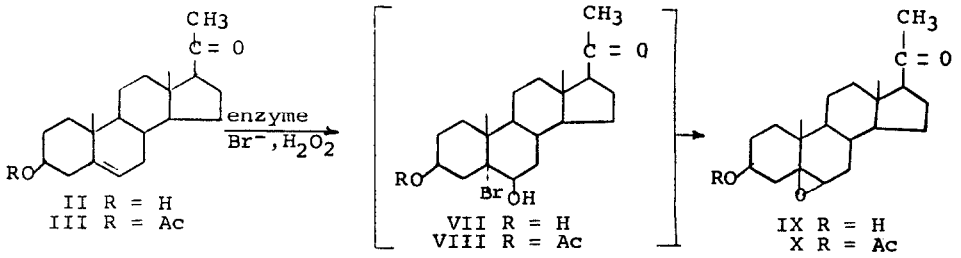
was extended to four hours, in addition to IV, we obtained 9β,11β-epoxy-progesterone (V) (4) (2% yield), and 11-ketoprogesterone (VI) (5) (8% yield).



Non-enzymatic formation of V and VI from IV was demonstrated to occur in phosphate buffer (0.3M, pH 3.0) shaken for 30 minutes.

With II as substrate and a reaction time of 5 minutes, we isolated 3β-hydroxy-5,6β-epoxy-5β-pregnan-20-one (IX) (6) (22% yield).

Utilizing III as substrate and a reaction time of 10 minutes, we isolated 3β-acetoxy-5,6β-epoxy-5β-pregnan-20-one (X) (6) (8% yield).



With II or III as substrates, we did not isolate any bromohydrin. However, with III as substrate and a reaction time of 10 minutes, we were able to detect a phosphomolybdic acid positive material on silica gel with an R_f value identical to that of chemically prepared VIII (7). Chromatography of VIII on silica gel led to a poor recovery of this material and thus its isolation from an enzymatic reaction was deemed impractical. When VIII was chromatographed on alumina, the only isolated product was X. Since bromide was required for the production of the epoxides, we believe that the transformation must pass through VII and VIII. Therefore, the isolated epoxides IX and X probably represent non-enzymatic products formed from enzymatically produced

VII and VIII.

The possible biosynthetic significance of enzymatic bromohydrin formation and other enzymatic halogenations will be discussed in a subsequent publication.

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