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THE ENZYMATIC HALOGENATION OF STEROIDS

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The vast literature related to microbial transformations of steroids is primarily concerned with reactions of an oxidative or reductive nature[1]. Although the production of halometabolites by micro-organisms is not uncommon[2] no biosynthetic reaction yielding halosteroids has been reported previously.

The haloperoxidase of Caldariomyces fumago has been shown[3,4] to chlorinate β-dicarbonyl systems such as 1,3-cyclopentadione to both 2-chloro-1,3-cyclopentadione and 2,2-dichloro-1,3-cyclopentadione and β-ketoadipic acid to δ-chlorolevulinic acid. We have now applied this enzyme system to both chlorinate and brominate steroidal β-diketones and a steroidal β-ketolactone.

In our experiments the cell-free enzyme system was prepared by grinding the acetone-dried mycelial powder of Caldariomyces fumago ATCC 16373 in water and removing the mycelial debris by

centrifugation and filtration. This solution was then added to a suspension containing the steroid in a phosphate buffer (pH 3.0) containing hydrogen peroxide and potassium bromide or potassium chloride and the mixture shaken for one hour at 25° C. Extraction with methyl isobutylketone followed by thin layer chromatography on silica gel gave the halogenated steroid.

The steroid substrates used were 16-ketoprogesterone[5] (I) 16-keto-A-norprogesterone[6] (IV) and 15-keto-1-dehydrotestololactone (IX). The latter compound was prepared by fermentation of 1-dehydrotestolo-lactone[7] (VII) with a *Penicillium* species ATCC 11598 to give 15α-hydroxy-1-dehydrotestololactone (VIII)[8], m.p. 260-262°; $[\alpha]_D^{24}$ -54.9° (chloroform), which was oxidized with Jones reagent[9] to IX[10], m.p. 230-232°; $[\alpha]_D^{24}$ -96.9° (chloroform).

The products obtained in 50% yield from the enzymatic halogenation of I and II have been assigned the 17α-halo structures II, m.p. 124-126°; $[\alpha]_D^{22}$ +18° (chloroform), III, m.p. 155-157°; $[\alpha]_D^{22}$ -41.5° (chloroform), V, m.p. 138-140°, $[\alpha]_D^{22}$ -91° (chloroform), and VI, m.p. 184-186°; $[\alpha]_D^{22}$ -160° (chloroform), on the basis of the following: The ultraviolet spectra no longer showed evidence for the enolic forms exhibited[5,6] by both I and IV but showed only the absorption [$\lambda_{max.}^{alc.}$ 239 mμ (ϵ, 19300) for II, 238 mμ (ϵ, 17700) for III, 232 mμ (ϵ, 19650) for V and 233 mμ (ϵ, 17700) for VI] of the α,β-unsaturated keto system. The carbonyl region of the infrared spectra of each compound was no longer complex as in I and IV but showed three distinct carbonyl stretching frequencies ($\lambda_{max.}^{CCl_4}$ 1754,

1711 and 1652 cm.$^{-1}$ for II; 1756, 1714 and 1679 cm.$^{-1}$ for III; 1752, 1714 and 1625 cm.$^{-1}$ for V; 1760, 1719 and 1628 cm.$^{-1}$ for VI). Further, the NMR data summarized in Table I supports the assignments. There were no hydrogen bonded hydroxyl protons present in II, III, V or VI in the NMR spectra as in I and IV[6] and the chemical shifts for the 21-methyl and 19-methyl groups are consistent with 17α-halo steroids.

TABLE I

Chemical Shifts (τ) for 16,20-Diketo Steroids and 15-Substituted-1-Dehydrotestololactones

Compound	21-CH_3	18-CH_3	19-CH_3
I	7.99	8.93	8.76
II	7.50	8.96	8.78
III	7.61	9.00	8.77
IV	7.98	8.90	8.77
V	7.51	8.98	8.80
VI	7.61	8.99	8.79
VII		8.60	8.78
VIII		8.57	8.77
IX		8.68	8.74
X		8.67	8.74
XI		8.64	8.73

The 17α-assignment for the halogen in these compounds was further supported by the non-enzymatic halogenation of I and

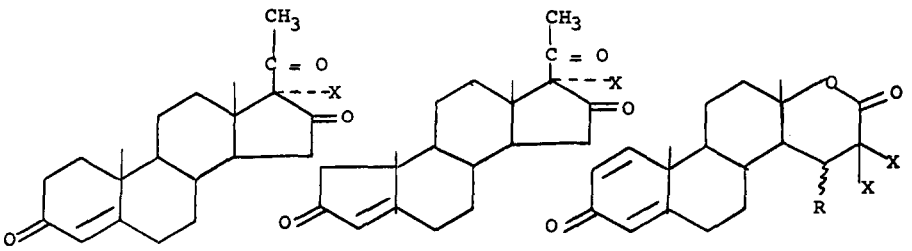
IV with N-chlorosuccinimide to give II and V and N-bromosuccinimide to give III and VI respectively.

Application of the enzymatic halogenating conditions to 15-keto-1-dehydrotestololactone using potassium bromide in the medium gave a dibromo derivative. This compound has been assigned the 16,16-dibromo structure X, m.p. 186-188°, $[\alpha]_D^{26}$ -51° (chloroform), since no band appeared in the NMR spectrum near 4.0τ which would indicate an alpha-proton in an α-bromo-β-ketolactone system and the 14α-proton appeared as a doublet (J = 10 cps) at 6.91τ trans diaxially coupled to the 8β-proton.

Reaction of VII with two moles of N-bromosuccinimide in an acetate buffer (pH 4.4) gave VIII in 70% yield[11] and with N-chlorosuccinimide gave the 16,16-dichloro compound XI, m.p. 245-247°, $[\alpha]_D^{22}$ -66° (chloroform).

That the reactions using the Caldariomyces fumago extract was indeed enzymatic was ascertained by the fact that only starting material could be detected when the enzyme preparation was not added to the reaction mixture.

Progesterone, 16α-hydroxyprogesterone, 16-dehydroprogesterone and 1-dehydrotestololactone were unaffected by the enzyme halogenating conditions.



	X
I	H
II	Br
III	Cl

	X
IV	H
V	Br
VI	Cl

	R	X
VII	H	H
VIII		H
IX	=O	H
X	=O	Br
XI	=O	Cl

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10. Unlike compounds I and IV, 15-keto-1-dehydrotestololactone does not exist as the enol form in neutral solution since in the ultraviolet it had $\lambda_{max.}^{alc.}$ 241 mμ (ε, 16700) and the NMR spectrum had a singlet at 6.52τ equivalent to two protons which could be assigned to position 16. Addition of alkali to an alcoholic solution of VII shifted the ultraviolet absorption to maxima at 244 mμ (ε, 15500) and 277 mμ (ε, 18500) typical of enolizable β-dicarbonyl systems.

11. When one mole of N-bromosuccinimide was used the only compound isolated was VIII indicating that the second bromine atom is introduced at a faster rate than initial bromination.