Enzymatic Bromination of 16-Dehydroestriadiol 3-Methyl Ether 17-Acetate to 16α-Bromoestrone 3-Methyl Ether

SAUL L. NEIDLEMAN AND MARY A. OBERC

The Squibb Institute for Medical Research, New Brunswick, New Jersey 08903

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A recent communication from these laboratories (S. L. Neidleman et al., Tetrahedron Letters 44:5337, 1966) described the bromination and chlorination of the steroidal β-diketones, 16-ketoprogesterone and 16-keto-A-norpregesterone, and the bromination of the steroidal β-ketolactone, 15-keto-1-dehydrotestolactone, by the chloroperoxidase of Caldarionyeus fumago (F. S. Brown and L. P. Hager, J. Am. Chem. Soc. 89:719, 1967). In a later publication, we detailed the bromination of the α-hydroxymethylene ketosteroids, 2-hydroxymethylene-17β-hydroxy-5α-androstan-3-one and 2-hydroxymethylene-17β-hydroxy-5β-androstan-3-one, by the same enzyme (S. D. Levine and S. L. Neidleman, Tetrahedron, in press). In this note, we wish to extend these findings and to report the ability of a crude preparation of chloroperoxidase to brominate a steroid containing an enolacetate moiety to yield a steroidal α-bromoketone.

Mycelial pads of C. fumago ATCC 16373 were grown as described in an earlier report (S. D. Levine and S. L. Neidleman, Tetrahedron, in press). The crude chloroperoxidase used was prepared by homogenizing six of these pads with 60 g of acid-washed sand and 300 ml of distilled water in a Waring Blendor for 2 min. The homogenate was centrifuged and the supernatant fluid was collected. To 300 ml of the enzyme solution, we added 60 ml of 0.3% hydrogen peroxide, 300 mg of potassium bromide in 60 ml of distilled water, 120 ml of 0.3 M potassium phosphate buffer (pH 3.0), 300 mg of 16-dehydroestriadiol 3-methyl ether 17-acetate in 24 ml of dimethylsulfoxide, and 36 ml of distilled water. The mixture was distributed in 200-ml samples among three 500-ml Erlenmeyer flasks, which were placed on a rotary shaker (280 cycles per min, 2-inch stroke) at 25 C for 30 min. The reaction mixtures were pooled and extracted with 150 ml of chloroform. The chloroform extract was dried with anhydrous sodium sulfate and was concentrated at reduced pressure (1,000 μ) and room temperature (25 C). The concentrate was streaked on an 8 by 16 inch (20.3 by 41 cm) plate of Silica Gel GF24 1 mm in thickness (Brinkmann Instruments, Inc., Westbury, N.Y.) and was chromatographed with benzene-chloroform (3:1). A band of ultraviolet absorbing material, less polar than the substrate, was scraped from the plate and eluted with several 10-ml samples of chloroform-methanol (1:1). The chloroform-methanol extract was dried with anhydrous sodium sulfate, filtered, and concentrated to dryness at reduced pressure (1,000 μ) and room temperature (25 C). The white, crystalline material which was obtained was washed twice with n-hexane. The product was dried under vacuum in a desiccator over phosphorus pentoxide. The yield of crystalline 16α-bromoestrone-3-methyl ether was 30 mg. The melting point of the derivative, determined on a Thomas-Hoover Uni-Melt apparatus,
was 172 to 174 °C (uncorrected), which agrees with the value expressed in the literature (W. S. Johnson and W. F. Johns, J. Am. Chem. Soc. 79:2005, 1957). The nuclear magnetic resonance (NMR) spectrum, determined on a Varian A-60 in deuteriochloroform with tetramethylsilane as internal standard, exhibited signals at 9.05 (s, 18-Me), 6.23 (s, 3-OMe), and 5.46 (m, 16-H). The elementary analysis gave the following results: calculated for C_{18}H_{25}O_{2}Br (363.28): C, 62.81; H, 6.38. Found: C, 63.09; H, 6.35. The transformation involved is shown in Fig. 1.

Small-scale studies indicated that, when either the crude enzyme preparation or the bromide ion was omitted from the reaction mixture, no product was formed.

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