Tetrahedron Vol 24, pp 2979 to 2984 Pergamon Press 1968 Printed in Great Britain

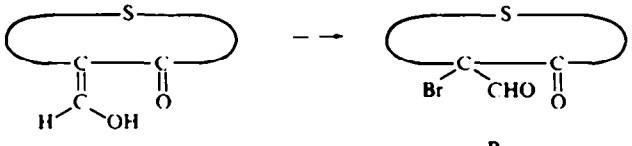
AN ENZYMATIC ROUTE TO α-BROMO STEROIDAL KETONES

S. D. LEVINE, S. L. NEIDLEMAN and M. OBERC
The Squibb Institute for Medical Research, New Brunswick, New Jersey 08903

(*Received in USA* 4 *October* 1967; *accepted for publication* 17 *October* 1967)

Abstract—The enzymatic bromination of α-hydroxymethylene keto steroids using the haloperoxidase of *Caldariomyces fumago* has been investigated. The isolated products are not the anticipated brominated keto aldehydes, but α-bromo-ketones. The mechanism of this reaction is discussed. This method affords a practical route to 2β-bromo-3-keto-5β-androstanes.

A RECENT report from these laboratories[1] described the chlorination and bromination of 16-keto-progesterone, 16-keto-A-norprogesterone and 15-keto-1-dehydrotestololactone using the haloperoxidase of *Caldariomyces fumago*.[2] In this paper, we describe our results when another type of enolizable β-dicarbonyl system was used as a substrate for the halogenation. We set out to explore the enzymatic halogenation of α-hydroxymethylene keto steroids (A) and envisioned obtaining products of structural type B.

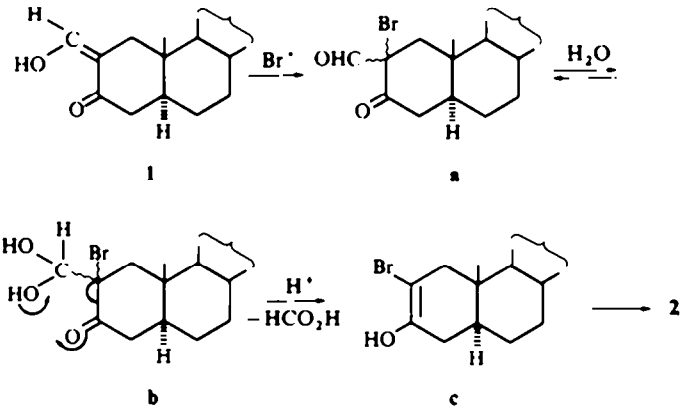


A B

S = Steroid residue

When 2-hydroxymethylene-17β-hydroxy-5α-androstan-3-one[3] (1) was incubated for 0·5 hr at room temperature with the enzyme system containing hydrogen peroxide perxide and potassium bromide and the crude product chromatographed on neutral alumina, a halogenated (+ Beilstein) steroid was obtained in 20–25% yield. Since the IR spectrum exhibited only one CO peak at 5·79 μ, it was obvious that the product was not a brominated keto aldehyde. This compound was formulated as 2α-bromo-17β-hydroxy-5α-androstan-3-one (2) on the basis of its microanalysis, infrared and NMR spectra. The C-2 proton appeared as a quartet centered at τ 5·27 (J = 6·5, 12·8 c/s). This is typical of an axial proton[4] and provided further evidence for the equatorial configuration of the Br atom. The m.p. and optical rotation of 2 were not in agreement with those reported in the literature.[5,6] However, acetylation gave the 17β-acetate[7] (3) and Jones oxidation[8] gave the 17-ketone[9] (4) both of whose physical properties were in agreement with previously prepared samples. The bromo ketone 2 could also be prepared non-enzymatically by bromination of 1 with N-bromosuccinimide in a pH 4·3 buffer. As in the enzymatic case, 2 is obtained directly without base-catalyzed cleavage of the expected bromo keto aldehyde. During the preparation of

2α-fluoro steroids[10] by reaction of the sodio salt of 2-hydroxymethylene-3-keto steroids with perchloryl fluoride, the resultant 2-aldehydo-2-fluoro compounds (no physical data was given to support the presence of this grouping) were treated with alkali to eliminate the aldehyde. Although we cannot exclude the possibility that the loss of the C-2 formyl group during the enzymatic bromination occurs *via* an enzymatic process, we wish to propose the following mechanism for both the chemical and enzymatic bromination. The 2-hydroxymethylene steroid (**1**) is brominated[2] by Br^+



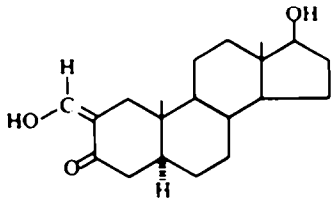
to afford the anticipated bromo keto aldehyde (**a**). The hydration of **a** is facilitated by the presence of the electron withdrawing Br atom adjacent to the aldehyde. The resulting hydrated aldehyde (**b**) can either lose water to reform **a** or expel a molecule of formic acid to give the enol (**c**). Ketonization of **c** then gives rise to the more stable (equatorial) bromo ketone (**2**).

An attempt to prepare 2-formyl-17β-hydroxy-androst-1-ene-3-one (**5**) by the bromination of **1** followed by dehydrobromination was reported to be unsuccessful,[11] but the nature of the brominating agent, reaction conditions, and products obtained was not presented. From the results presented herein, it appears likely that the product obtained after the bromination step did not contain any aldehyde group, and that the compound which was actually dehydrobrominated was **2**.

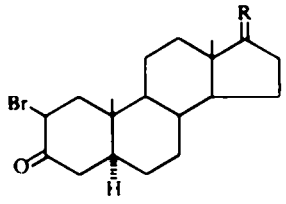
In a similar fashion, enzymatic bromination of 2-hydroxymethylenetestosterone[12] (**6**) gave non-crystalline 2α-bromo testosterone (**7**), which was characterized by its NMR spectrum and conversion by acetylation to **8**[13] and by oxidation to **9**.[6]

These results suggested that a convenient and practical route to 2β-bromo-3-keto-5β-androstanes was feasible since A/B *cis*-3-ketones are preferentially formylated at C-2.[14] Recently, Liston[15] has investigated the enol acetylation of 17β-acetoxy-5β-androstane-3-one and demonstrated that under kinetic control a 7:3 mixture of Δ^3 and Δ^2-enol acetates was formed. These were separable by preparative GLPC and the Δ^2-enol acetate could be isolated in ca. 5% yield and brominated to **12** which was characterized by its microanalysis and infrared spectrum. Incubation of 2-hydroxymethylene-17β-hydroxy-5β-androstan-3-one[14] (**10**) with the brominating enzyme system gave the oily 2β-bromo-17β-hydroxy-5β-androstan-3-one (**11**) in ca. 50% yield. In accord with this structure, the NMR spectrum exhibited signals at τ 9·24 (s, 18-Me), 8·94 (s, 19-Me), 6·33 (m, 17α-H) and 5·29 (q, J = 5·5, 14 c/s, 2α-H).

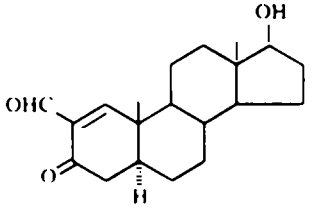
Acetylation of **11** gave the previously mentioned acetate[15] (**12**) and oxidation gave the 17-keto compound (**13**).



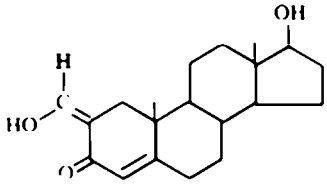
1



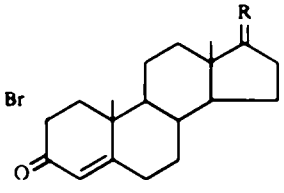
2: R = OH (α)
3: R = OAc (α)
4: R = O



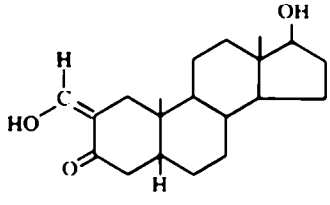
5



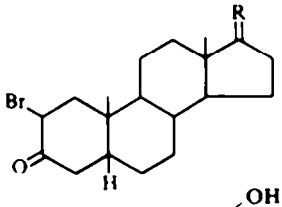
6



7: R = OH (α)
8: R = OAc (α)
9: R = O



10



11: R = OH (α)
12: R = OAc (α)
13: R = O

That the brominated derivatives described herein were enzymatically produced was demonstrated by the fact that omission of any of the components of the complete system (enzyme preparation, potassium bromide or hydrogen peroxide) prevented formation of these products and only starting materials could be detected.

EXPERIMENTAL

M.ps were taken on a Fisher–Johns m.p. apparatus and are uncorrected. Optical rotations were determined in $CHCl_3$ on a Perkin–Elmer 141 polarimeter and the values of $[\alpha]_D$ have been approximated to the nearest degree. IR spectra were determined on a Perkin–Elmer 21 spectrometer in pressed KBr pellets, and NMR spectra on a Varian A-60 in $CDCl_3$ with TMS as internal standard. The alumina used for chromatography refers to Merck, A.G. neutral alumina (activity V). Solns were dried over Na_2SO_4 and all evaporations were carried out *in vacuo*.

Growth of mycelial pads of Caldariomyces fumago ATCC 16373, *American type culture collection, Rockville, Maryland.* The organism was maintained on potato agar slants prepared as follows: Sliced potato (200 g) was cooked in distilled water (500 ml) for 40 min and then strained. A soln of glucose (21 g) and agar (20 g) in distilled water (500 ml) was added to the strained soln. The pH was adjusted to 6·8 and the volume brought to 1 l. with distilled water. The medium was sterilized at 121° for 15 min.

The organism was grown for about one week on these slants at room temp and was then used to inoculate 250 ml. Erlenmeyer flasks containing a soybean-glucose medium (50 ml) prepared as follows: To 1 l. of distilled water were added extraction process soybean meal (30 g), glucose (30 g) and $CaCO_3$ (7 g). The medium was sterilized at 121° for 30 min.

The organism was grown for 4–5 days on a rotary shaker at 25°. Five ml of this material was used to inoculate each 500 ml Erlenmeyer flask containing 100 ml of a modified Czapek-Dex medium prepared by adding the following to 1 l. of distilled water: $NaNO_3$ (3 g), KH_2PO_4 (1 g), KCl (0·5 g), $MgSO_4 \cdot 7H_2O$ (0·5 g), $FeSO_4 \cdot 7H_2O$ (10 mg) and glucose (40 g). The medium was sterilized at 121° for 20 min.

The organism was grown under static conditions at room temp for 5–7 days. The black mycelial pads which formed were collected, rinsed with distilled water and stored in plastic bags in a deep-freeze for subsequent use.

Enzymatic bromination of 2-hydroxymethylene-17β-hydroxy-5α-androstan-3-one (1). The halogenating enzyme soln was prepared by grinding 6 mycelial pads of the organism (prepared as described above) with acid-washed sand (60 g) and distilled water (300 ml) in a Waring Blendor for 2 min and then centrifuging. The supernatant was treated with 0·3% H_2O_2 (60 ml), KBr (300 mg) in water (60 ml), 0·3M potassium phosphate buffer (pH 3·0, 120 ml), 1 (300 mg) in DMSO (24 ml) and distilled water (36 ml). The mixture was distributed in 200 ml aliquots among three 500 ml Erlenmeyer flasks and placed on a rotary shaker at 25° for 30 min. $CHCl_3$ (120 ml) was added to the mixture and filtered through Hy-flo. The $CHCl_3$ layer of the filtrate was separated and the aqueous phase extracted with $CHCl_3$ (3 × 250 ml), dried and evaporated. The residue was plate-chromatographed on alumina using $CHCl_3$–hexane (3:2) as the developing solvent and gave a major band detectable with I_2 vapor. Elution with EtOAc, evaporation, and crystallization of the residue from EtOAc–ether gave 2 (82 mg, m.p. 165–166° (d). Recrystallization from EtOAc–ether gave the analytical sample: m.p. 167–168° (d) [lit. 180–181°[5]]; $[\alpha]_D^{26}$ +55° (*c* 0·265) [lit. +29°[6]]; λ 5·79 μ; τ 9·24 (3H s,* 18-Me), 8·91 (3H s, 19-Me), 6·37 (1H m, 17α-H) and 5·27 (1H qu, J = 6·5, 12·8 c/s, 2β-H). (Found: C, 61·40; H, 8·00; Br, 21·51. Calc. for $C_{19}H_{29}BrO_2$: C, 61·77; H, 7·91; Br, 21·64%).

A soln of 2 (20 mg) in Ac_2O (0·05 ml) and pyridine (0·1 ml) was left at room temp overnight. The reaction mixture was diluted with water and the ppt collected by filtration. Crystallization from MeOH-isopropyl ether gave 3: m.p. 175–176°; $[\alpha]_D^{26}$ +29° (*c* 0·569) [lit. 175–177°; $[\alpha]_D$ +32°[7]].

A soln of 2 (20 mg) in acetone (5 ml) was treated with a slight excess of Jones reagent. MeOH was added to decompose excess reagent and the suspension filtered. The filtrate was concentrated, diluted with water and extracted with $CHCl_3$. The $CHCl_3$ extracts were washed with 8% salt soln, dried and evaporated. Two recrystallizations from $CHCl_3$–MeOH gave 4: m.p. 211·5–212·5°; $[\alpha]_D^{25}$ +99° (*c* 0·244) [lit. 215–216°; $[\alpha]_D^{20}$ +102°[6]].

Chemical bromination of 2-hydroxymethylene-17β-hydroxy-5α-androstan-3-one (1). A soln of 1 (300 mg) in dioxan (15 ml) was treated with N-bromosuccinimide (175 mg) and a buffer soln (6 ml) prepared by

* s = singlet, m = multiplet, qu = quartet.

dissolving NaOAc (6·6 g) and AcOH (6·6 ml) in enough water to make a final volume of 100 ml. After 20 min at room temp, water was added and the reaction mixture extracted with $CHCl_3$. The $CHCl_3$ extracts were washed with 8% salt soln, dried and evaporated. Chromatography of the residue as described in the previous example and crystallization from EtOAc-isopropyl ether gave **2**, (91 mg, m.p. 166·5–167·5°).

Enzymatic bromination of 2-hydroxymethylene testosterone (**6**). The incubation (5 min) of **6** (300 mg) and workup was carried out as described for **1**. The residue was plate chromatographed on alumina using $CHCl_3$ hexane (3:1) as the developing solvent and gave a major band detectable by UV. Elution with EtOAc and evaporation gave oily **7** (87 mg). The NMR spectrum was in accord with this structure: τ 9·39 (3H s, 18-Me), 8·72 (3H s, 19-Me), 6·34 (1H m, 17α-H), 5·18 (1H qu, J = 6,14 c/s, 2β-H) and 4·17 (1H s, 4-H).

Acetylation of **7** with Ac_2O-pyridine overnight at room temp gave after crystallization from acetone isopropyl ether **8**: m.p. 169·5 170·5° [lit. 169°[13]]; τ 9·16 (3H s, 18-Me), 8·71 (3H s, 19-Me), 7·95 (3H s, 17-O—C(=O)—CH_3), 5·19 (1H qu, J = 5·5, 14·5 c/s, 2β-H) and 4·18 (1H s, 4-H). Oxidation of **7** with Jones reagent gave after crystallization from acetone-isopropyl ether, **9**: m.p. 169·5 170·5° (d) [lit. 167 168° (d)[6]]; $\lambda_{max}^{CHCl_3}$ 5·77, 5·96 and 6·18 μ.

Enzymatic bromination of 2-hydroxymethylene-17β-hydroxy-5β-androstan-3-one (**10**). The incubation (30 min) of **10** (300 mg) and workup was carried out as described for **1**. The residue was plate chromatographed on alumina using $CHCl_3$–hexane (7:3) as the developing solvent and gave a major band detectable with I_2 vapor. Elution with EtOAc and evaporation gave oily **11** (180 mg). The NMR spectrum was in agreement with this structure: τ 9·24 (3H s, 18-Me), 8·94 (3H s, 19-Me), 6·33 (1H m, 17α-H) and 5·29 (1H qu, J = 5·5, 14 c/s, 2α-H).

Acetylation of **11** with Ac_2O-pyridine on a steam bath for 2 hr gave after 2 recrystallizations from $CHCl_3$ isopropyl ether, **12**: m.p. 201·5 203° (d) [lit. 201 202°[15]]; $[\alpha]_D^{23}$ −26° (*c* 0·424); τ 9·19 (3H s, 18-Me), 8·94 (3H s, 19-Me), 7·96 (3H s, 17-O—C(=O)—CH_3), 5·43 (1H m, 17α-H) and 5·27 (1H qu, J = 5·5, 14 c/s, 2α-H).

Oxidation of **11** with Jones reagent gave after crystallization from EtOAc-isopropyl ether, **13**: m.p. 201 202° (d); $[\alpha]_D^{23}$ +38° (*c* 0·450); λ 5·79 μ; τ 9·11 (3H s, 18-Me), 8·92 (3H s, 19-Me) and 5·31 (1H qu, J = 5·3, 13·5 c/s, 2α-H). (Found: C, 62·26; H, 7·66; Br, 21·95. Calc. for $C_{19}H_{27}BrO_2$: C, 62·10; H, 7·41; Br, 21·75%).

Chemical bromination of 2-hydroxymethylene-17β-hydroxy-5β-androstane-3-one (**10**). A soln of **10** (380 mg) in dioxan (20 ml) was treated for 20 min with N-bromosuccinimide (230 mg) and buffer soln (8 ml) as described for the preparation of **2**. After the workup, the residue was plate chromatographed as in the previous example and gave **11** (300 mg). Acetylation of **11** with Ac_2O-pyridine overnight at room temp gave after crystallization from acetone-isopropyl ether **12**: 150 mg m.p., 201–202° (d).

REFERENCES

1. S. L. Neidleman, P. A. Diassi, B. Junta, R. M. Palmere and S. C. Pan, *Tetrahedron Letters* No. 44, 5337 (1966).
2. F. S. Brown and L. P. Hager, *J. Am. Chem. Soc.* **89**, 719 (1967) and Refs therein.
3. R. O. Clinton, A. J. Manson, F. W. Stonner, H. C. Neumann, R. G. Christiansen, R. L. Clarke, J. H. Ackerman, D. F. Page, J. W. Dean, W. B. Dickinson and C. Carabateas, *Ibid.* **83**, 1478 (1961).
4. N. S. Bhacca and D. H. Williams, *Application of NMR Spectroscopy in Organic Chemistry*, pp. 47–51. Holden-Day, San Francisco (1964).
5. A. Butenandt and H. Dannenberg, *Chem. Ber.* **69**, 1158 (1936).
6. C. Djerassi and C. R. Scholz, *J. Org. Chem.* **13**, 697 (1948).
7. J. Fajkos, *Chem. listy* **51**, 1885 (1957).
8. K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.* 39 (1946).
9. J. Fajkos and F. Šorm, *Chem. listy* **52**, 2115 (1958).
10. J. Edwards and H. J. Ringold, *J. Am. Chem. Soc.* **81**, 5262 (1959).
11. J. Edwards, J. C. Orr and A. Bowers, *J. Org. Chem.* **27**, 3378 (1962).
12. F. L. Weisenborn, D. C. Remy and T. L. Jacobs, *J. Am. Chem. Soc.* **76**, 552 (1954).

[13] H. H. Inhoffen and G. Zuhladorff, Ger. Patent 864, 255 (1953).
[14] R. O. Clinton, R. L. Clarke, F. W. Stonner, A. J. Manson, K. F. Jennings and D. K. Phillips, *J. Org. Chem.* **27**, 2800 (1962).
[15] A. J. Liston, *Ibid.* **31**, 2105 (1966).