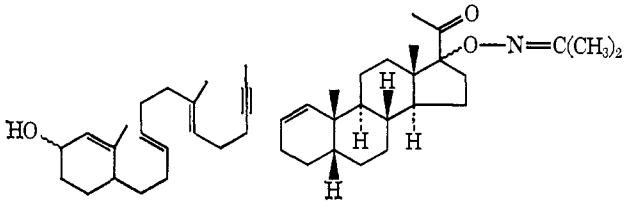
Acetylenic Bond Participation in Biogenetic-Like Olefinic Cyclizations in Nitroalkane Solvents. A Facile Total Synthesis of *dl*-Testosterone Benzoate

Sir:

In an accompanying communication[1] we have described the acid-catalyzed cyclization of trienynol **1** in 2-nitropropane to produce the C-17 epimeric mixture of oxime ethers **2**. We were intrigued by the possibility

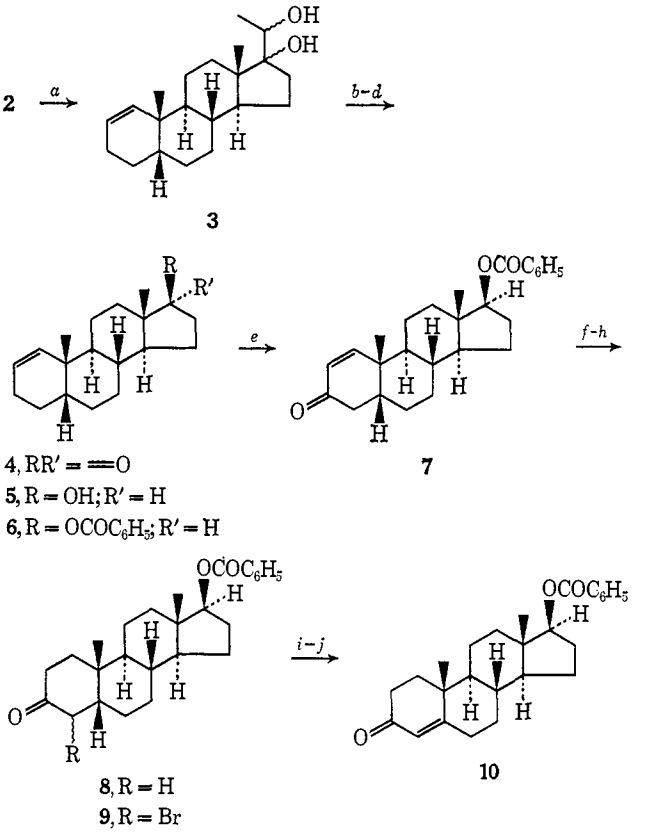


1 **2**

that **2** might serve as an intermediate in the synthesis of 17-oxygenated steroids, and in the present communication we report the realization of this goal with a facile, stereospecific synthesis of *dl*-testosterone benzoate (**10**). The attractiveness of this approach is enhanced by the fact that trienynol **1** has been obtained in its optically active forms which have been shown to undergo cyclization without racemization to give the enantiomeric tetracyclic products.[2] Thus, on the basis of the present study, it is obvious that the *d* form of **1** will lead to the enantiomer **2** of natural configuration, which in turn will give *d*-testosterone benzoate.

The details of the synthesis of racemic material are summarized in the accompanying flow sheet (Scheme I). As previously described,[1] treatment of **2** with lithium aluminum hydride in refluxing tetrahydrofuran gave diol **3** as a mixture of stereoisomers. Treatment of this mixture with periodic acid gave, after preparative tlc on silica gel (3:7 ethyl acetate–hexane), ketone **4**, mp 86–92° (purity >95% by vpc), in 40% yield from **1**. This material was suitable for use in the next step. A sample was recrystallized twice from petroleum ether to give colorless needles: mp 98.5–100.5° (*Anal.* Found: C, 83.7; H, 10.1); $\lambda_{max}^{CHCl_3}$ 5.77 μ. The nmr spectrum[3] included singlets at δ 0.86 (3 H), 1.02 (3 H), and 5.56 (2 H, olefinic protons). Reduction of the ketone **4** with sodium borohydride gave alcohol **5** in quantitative yield. Two recrystallizations from hexane gave colorless needles: mp 137–138° (*Anal.* Found: C, 83.0; H, 11.0); $\lambda_{max}^{CHCl_3}$ 2.76 μ. The nmr spectrum[3] included singlets at δ 0.74 (3 H), 1.02 (3 H), and 5.59 (2 H, olefinic protons). The spectrum also showed a triplet (J = 7 Hz) at 3.63 for the α hydrogen at C-17. Treatment of the unpurified alcohol **5** with benzoyl chloride gave the benzoate **6** which was used, without purification, in the next step. Three recrystallizations of a sample from absolute ethanol gave colorless plates: mp 131–134° (*Anal.* Found: C, 82.5; H, 9.2); $\lambda_{max}^{CHCl_3}$ 5.84, 7.81, and 8.93 μ. The nmr spectrum[3] included singlets at δ 0.93 (3 H), 1.02 (3 H), and

Scheme I



[a] $LiAlH_4$, THF, N_2, reflux, 2 hr. [b] To give **4**: H_5IO_6, CH_3OH, H_2O, N_2, 23°, 14 hr. [c] To give **5**: $NaBH_4$, EtOH, N_2, 23°. [d] To give **6**: C_6H_5COCl, pyridine, 23°. [e] *t*-BuOCrO$_3$H, HOAc, Ac_2O, $Cl_2C{=}CCl_2$, N_2, 85–95°, 0.75 hr. [f] To give **8**: H_2, 10% Pd/C, EtOAc, 24°. [g] Ac_2O, $HClO_4$, EtOAc, N_2, 23°, 10 min. [h] To give **9**: 1.0 mol equiv of Br_2, CCl_4, epichlorohydrin, N_2, 0°. [i] $H_2NNHCONH_2 \cdot HCl$, NaOAc, dioxane, H_2O, N_2, 23°, 14 hr. [j] CH_3COCO_2H, H_2O, N_2, 23°, 14 hr.

5.56 (2 H, olefinic protons) in addition to a triplet (J = 7.5 Hz) at 4.81 (1 H, 17α proton) and characteristic aromatic proton absorption for the benzoate at 7.20–8.20 (5 H). Oxidation of **6** with *tert*-butyl chromate[2b,4] gave enone **7** which was used in the next step without purification. A sample of **7** was recrystallized from hexane–acetone to give colorless needles: mp 190–191° (*Anal.* Found: C, 79.4; H, 8.3); $\lambda_{max}^{CHCl_3}$ 5.84, 5.98, 7.81, and 8.92 μ. The nmr spectrum[3] included singlets at δ 0.96 (3 H) and 1.21 (3 H). The olefinic protons on C-1 and C-2 appeared as an AB quartet at 5.91 and 6.81 (2 H, J_{AB} = 10 Hz, $\Delta\gamma$ = 54 Hz). Catalytic hydrogenation of **7** gave ketone **8** which was partially purified by preparative tlc on silica gel (3:7 ethyl acetate–hexane). A sample was recrystallized twice from absolute ethanol to give colorless plates, mp 112.5–114° (*Anal.* Found: C, 78.9; H, 8.7), identical by nmr, solution ir, and tlc with authentic 17β-hydroxy-5β-androstan-3-one benzoate.[5]

The conversion of **8** into **10** was accomplished in four steps without isolation of intermediates. Thus, reaction of ketone **8** with acetic anhydride in the presence of perchloric acid gave the corresponding Δ^3-enol acetate,[6] λ_{max}^{film} 5.70 and 5.82 μ, which was immediately

(1) D. R. Morton, M. B. Gravestock, R. J. Parry, and W. S. Johnson, *J. Amer. Chem. Soc.*, **95**, 4417 (1973).

(2) (a) R. L. Markezich, W. E. Willy, B. E. McCarry, and W. S. Johnson, *ibid.*, **95**, 4414 (1973); (b) B. E. McCarry, R. L. Markezich, and W. S. Johnson, *ibid.*, **95**, 4416 (1973).

(3) The nmr spectrum at 60 MHz (TMS internal standard, $CDCl_3$ solvent) was entirely consistent with the assigned structure. Details are not recorded here, except for absorptions of particular significance.

(4) K. Heusler and A. Wettstein, *Helv. Chim. Acta*, **35**, 284 (1952).

(5) W. S. Johnson, W. A. Vredenburgh, and J. E. Pike, *J. Amer. Chem. Soc.*, **82**, 3409 (1960).

(6) *Cf.* B. E. Edwards and P. N. Rao, *J. Org. Chem.*, **31**, 324 (1966).

treated with bromine in the presence of epichlorohydrin as an acid scavenger[7] to afford the 4-bromo-3-one **9**: λ_{max}^{film} 5.78 and 5.82 μ. Reaction of **9** with semicarbazide, followed by hydrolysis (aqueous pyruvic acid) of the intermediary α,β-unsaturated semicarbazone[8] and purification by preparative tlc on silica gel (1:9 ethyl acetate–hexane; continuous elution for 4 hr), gave *dl*-testosterone benzoate (**10**), mp 167–178° (homogeneous by tlc), in 45% yield overall from **4** (18% yield overall from trienynol **1**). A sample was recrystallized from absolute ethanol three times to give colorless plates: mp 184–185.5 (*Anal.* Found: C, 79.5; H, 8.2); $\lambda_{max}^{CHCl_3}$ 5.83, 6.01, 7.81, and 8.91 μ. The nmr spectrum[3] included singlets at δ 0.98 (3 H), 1.20 (3 H), and 5.75 (1 H, olefinic proton). This sample of racemic material was identical by nmr, solution ir, and tlc with an authentic, naturally derived specimen of testosterone benzoate.

Acknowledgment. We are indebted to the National Institutes of Health and the National Science Foundation for support of this research.

(7) *Cf.* A. J. Liston and M. Howarth, *J. Org. Chem.*, **32**, 1034 (1967).
(8) *Cf.* B. J. Magerlein, D. A. Lyttle, and R. H. Levin, *ibid.*, **20**, 1709 (1955).

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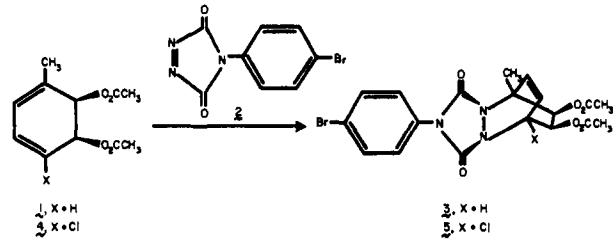
X-Ray Determination of the Absolute Stereochemistry of the Initial Oxidation Product Formed from Toluene by *Pseudomonas putida* 39/D

Sir:

Mammalian enzymes incorporate one atom of molecular oxygen into aromatic hydrocarbons with the resultant formation of arene oxides.[1] Subsequent enzymatic hydration produces trans diols.[2] A different oxidation pathway has been observed in bacteria. Both atoms of molecular oxygen are incorporated into aromatic hydrocarbons and cis diols are the first detectable oxygenated products.[3] However, assignments of cis stereochemistry by infrared spectrometry, by the Karplus equation, or by reaction rates with potassium triacetylosmate are not unambiguous.[4] We now report the absolute stereochemistry of the cis diol that is formed during the oxidation of toluene by *Pseudomonas putida* 39/D. This organism oxidizes several monocyclic aromatic compounds to cis diols.[5]

Toluene was oxidized to (+)-3-methyl-3,5-cyclohexadiene-1,2-diol by *Pseudomonas putida* 39/D and converted to the diacetate **1** as previously described.[5] Freshly sublimed (100° (0.1 mm)) 4-(*p*-bromophenyl)-1,2,4-triazoline-3,5-dione (**2**) in dry acetone (1:10 wt/wt) was added dropwise at 0° to an equimolar amount of **1** in dry acetone (1:10 wt/wt).[6] Throughout the course of addition, the deep red color of **2** was immediately discharged; upon addition of excess reagent, the red color remained.[7] The solvent was removed from the reaction mixture and the crude residue recrystallized two times from hexane–acetone (~1:10, v/v) to give a crystalline adduct in 70% yield: mp 174°; molecular ion mass calcd for $^{12}C_{19}{}^{1}H_{18}{}^{14}N_3{}^{16}O_6{}^{79}Br$, 463.0387; found, 463.0379. On the basis of the ir (5.7, 5.85, 6.9, 7.3, 12.1, 12.3, and 12.7 μ; Nujol mull–NaCl) and 60-MHz pmr (δ 1.9 (s, 1 H), 1.98 (s, 1 H), 5.4 (d of d, J = 8 Hz, 1 H), 6.4 (m, 2 H), 7.35 (d, J = 9 Hz, 2 H), 7.6 (d, J = 9 Hz, 2 H); saturated solution in acetone-d_6) spectral data, the product was assigned the Diels–Alder cycloaddition structure 8,9-diacetoxy-1-methyl-4-(*p*-bromophenyl)-2,4,6-triazatricyclo[5.2.2.0^{4,6}]undec-10-ene-3,5-dione (**3**).



1, X = H
2, X = Cl
3, X = H
5, X = Cl

2 was chosen for characterization of dihydrodiols for the following reasons: (1) the 1,2,4-triazoline-3,5-diones, as a class, are among the most reactive dienophiles known[8] permitting rapid cycloaddition to diacetates, thus minimizing the competitive, labile aromatization of these compounds *via* elimination of acetic acid; *e.g.*, attempted cycloaddition of *p*-chlorotoluenediol diacetate (**4**) and maleic anhydride gave only the *p*-chlorocresyl acetates; in contrast, the cycloaddition of **2** and **4** was complete within 20 min at 0° yielding **5**; (2) Diels–Alder cycloaddition, in the main, occurs cis with retention of configuration of the constituent atoms; thus the stereochemistry of the acetoxy methine carbons should not be affected;[9] and (3) the bromine atom incorporated in **2** permitted the determination *via* X-ray diffraction methods of the absolute stereochemistry of **3** as 8(*S*),9(*R*)-diacetoxy-1(*S*)-methyl-4-(*p*-bromophenyl)-2(*R*),4,6(*S*)-triazatricyclo[5.2.2.0^{4,6}]undec-10-ene-3,5-dione, and thus that of the parent toluene diol as 1*S*,2*R*.

Crystals of **3** are monoclinic, $P2_1$, a = 6.822, b = 24.381, and c = 5.950 Å, β = 94.40°, Z = 2. Using the 2167 observed reflections of 2223 measured with Cu Kα radiation, the structure was solved by the heavy atom method, after location of the bromine atom from a Patterson map. The structure has been refined, including all hydrogen atoms, to a conventional R value of 0.060 and a weighted R value of 0.060, omitting the

(1) D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltzman-Nirenberg, and S. Udenfriend, *Biochemistry*, **9**, 147 (1970).
(2) D. M. Jerina, J. W. Daly, and B. J. Witkop, *J. Amer. Chem. Soc.*, **89**, 5488 (1967).
(3) D. T. Gibson, *Crit. Rev. Microbiol.*, **1**, 199 (1971).
(4) (a) A. R. H. Cole and P. R. Jeffries, *J. Chem. Soc.*, 4391 (1956); (b) M. Karplus, *J. Amer. Chem. Soc.*, 85, 2870 (1963); (c) R. Criegee, R. Marchand, and H. Wannowius, *Justus Liebigs Ann. Chem.*, **550**, 99 (1942); (d) refer to references in B. R. Brown and H. A. H. MacBride, *J. Chem. Soc.*, 3822 (1964).
(5) D. T. Gibson, M. Hensley, H. Yoshioka, and T. J. Mabry, *Biochemistry*, **9**, 1626 (1970).
(6) Prepared by the method of J. C. Stickler and W. H. Pirkle, *J. Org. Chem.*, **31**, 3445 (1966), in 30% yield based on *p*-bromophenyl isocyanate.
(7) Upon exposure to light, solutions of **1** and excess 2 in acetone at ambient temperature soon discharge their color; on the other hand, such solutions kept in the dark retain their color for 2 or more days. I. Stevens, *et al.*, *J. Chem. Soc. C*, 1905 (1967), have observed rapid addition of refluxing acetone to 4-phenyl-1,2,4-triazoline-3,5-dione to yield the nominal 2-hydroxy-2-propene "ene" adduct.
(8) *Cf.* Stevens, *et al.*, cited in ref 7.
(9) For a discussion of Diels–Alder reaction stereochemistry, see C. H. Depuy and O. L. Chapman, "Molecular Reactions and Photochemistry," Prentice-Hall, Englewood Cliffs, N. J., 1972, p 140.