Microbial Oxidation of 17α-Methyl-B-nortestosterone. I[1]

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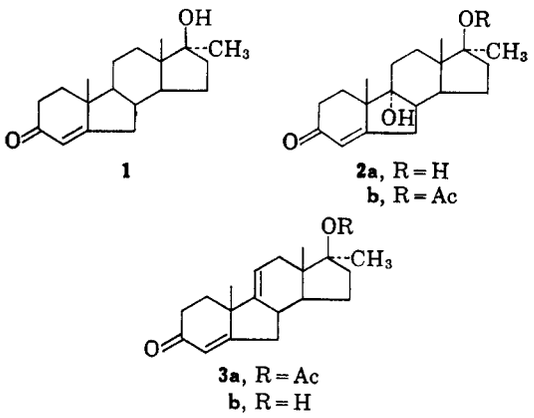
Fermentation of 17α-methyl-B-nortestosterone (**1**) with *Protaminobacter ruber* produced three metabolites. One was identified as 9α-hydroxy-17α-methyl-B-nortestosterone (**2a**). The others proved to be the 9,10-secophenols (**6a** and **8a**). One of the phenols (**8a**), hydroxylated at C-4, is of particular interest, because, although 4 hydroxylation has been shown to be the most probable route to further microbial degradation of steroidal 9,10-secophenols, a 4-hydroxylated intermediate has not been isolated until now.

As an extension of our work[2] with the antiandrogen 17α-methyl-B-nortestosterone (**1**), we investigated its microbial oxidation. Fermentation[3] of this compound with *Protaminobacter ruber* produced three metabolites. One was separated from the nonacidic portion of the crude fermentation product by chromatography and recrystallization. Elemental analysis of the metabolite showed that it contains an additional oxygen atom with respect to **1**, and its infrared and ultraviolet spectra indicated that the 3-keto-Δ^4 function was still intact. The metabolite was not affected by mild acetylation or oxidation conditions; however, its infrared spectrum showed a hydroxyl peak of approximately twice the intensity of that of **1**. The additional oxygen atom in the metabolite therefore seemed to be present as a tertiary hydroxyl group. In confirmation of the tertiary nature of the hydroxyl function the nmr spectrum of the metabolite showed no signal in the range δ 3–5.

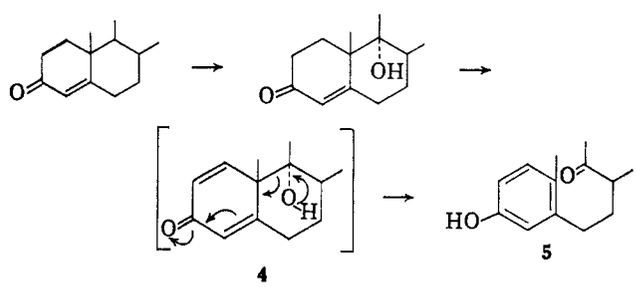
Enzymatic hydroxylations of steroids at tertiary carbons have been shown to proceed with retention of configuration.[4] Therefore, the only positions that need to be considered for the tertiary hydroxyl group are 8β, 9α, and 14α. Of these, the 9α formulation (**2a**) best fits the observed shifts of the C-18 and C-19 methyl resonances in the nmr spectrum of the metabolite relative to those of **1** (δ 0.02 and 0.10, respectively). The relatively large shift of the C-19 methyl resonance reflects its proximity to the hydroxyl function. If the hydroxyl were located at 8β (1,3-diaxial to C-18 and C-19) large shifts of both angular methyl resonances should occur, and if it were at 14α the C-18 methyl resonance should be shifted more than that of the C-19 methyl.[5]

Further proof that the metabolite has structure **2a** is offered by the following reactions. Refluxing acetic anhydride readily converted **2a** to a monoacetate (**2b**), which on treatment with thionyl chloride in pyridine gave **3a**, identical with an authentic sample prepared by acetylation of 17β-hydroxy-17α-methyl-B-norandrosta-4,9(11)-dien-3-one (**3b**).[6]

The remaining metabolites are both phenols, as



evidenced by their extraction from organic solvents with dilute sodium hydroxide but not with sodium bicarbonate, and by their ultraviolet, infrared, and nmr spectra. The isolation of phenols accompanying 9α-hydroxylated metabolites of steroids has been reported[7] in several instances. In all cases the phenols proved to be 9,10-seco compounds of type **5**. They are formed by 9 hydroxylation followed by 1,2 dehydrogenation, or the reverse, to give the intermediate **4** which aromatizes by a reverse aldol reaction.



In the present work one of the phenolic metabolites, produced in low yield, was easily identified as the B-nor analog of **5**, namely **6a**. The infrared spectrum of **6a** showed a peak for a ketone in a six-membered ring. Acetylation under mild conditions gave a monacetate (**6b**), whose nmr spectrum showed signals for a methyl group on an aromatic ring and three aromatic protons.

The remaining phenolic metabolite, the major product of the fermentation, contains an additional oxygen atom with respect to **6a** and was formed on fermentation of **6a** with *P. ruber*. Therefore, a hy-

(1) Presented in part at the First Middle Atlantic Regional Meeting of the American Chemical Society, Philadelphia, Pa., Feb 3, 4, 1966.

(2) H. L. Saunders, K. Holden, and J. F. Kerwin, *Steroids*, **3**, 687 (1964).

(3) For fermentation of another B-nor steroid, see Z. Prochazka, J. Fajkos, J. Joska, and F. Šorm, *Collection Czech. Chem. Commun.*, **26**, 2068 (1961).

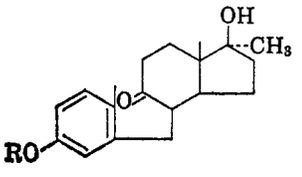
(4) M. Hayano, M. Gut, R. I. Dorfman, O. K. Sebek, and D. H. Peterson, *J. Am. Chem. Soc.*, **80**, 2336 (1958).

(5) See, for example, N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964, pp 13–32.

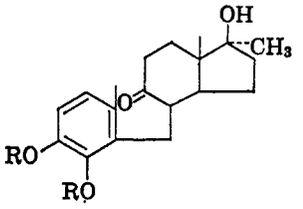
(6) This compound was prepared by elimination of toluenesulfonic acid from the 11-tosylate of 11α-hydroxy-17α-methyl-B-nortestosterone, whose preparation and structure proof will be presented in a subsequent publication. The nmr spectrum of **3b** showed the presence of one vinyl hydrogen, in addition to the C-4 hydrogen, with the expected splitting pattern.

(7) (a) R. M. Dodson and R. D. Muir, *J. Am. Chem. Soc.*, **80**, 5004 (1958); (b) R. M. Dodson and R. D. Muir, *ibid.*, **80**, 6148 (1958); (c) K. Schubert, R. H. Bohme, and C. Horhold, *Z. Naturforsch.*, **15b**, 584 (1960); (d) R. M. Dodson and R. D. Muir, *J. Am. Chem. Soc.*, **83**, 4627 (1961); (e) R. M. Dodson and R. D. Muir, *ibid.*, **83**, 4631 (1961); (f) C. J. Sih, *Biochim. Biophys. Acta*, **62**, 541 (1962); (g) C. J. Sih and A. M. Rahim, *J. Pharm. Sci.*, **52**, 1075 (1963); (h) K. C. Wang and C. J. Sih, *Biochemistry*, **2**, 1238 (1963); (i) E. Kondo and K. Tori, *J. Am. Chem. Soc.*, **86**, 736 (1964).

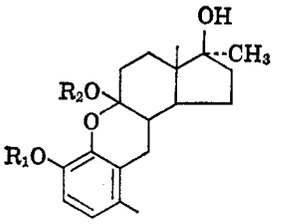
droxylated derivative of **6a** was strongly suggested for the structure of the major metabolite. However, it was difficult at first to reconcile the spectral data with this assumption. The infrared spectrum of the compound showed no carbonyl absorption, and its nmr spectrum indicated that no primary or secondary hydroxyl groups were present. In addition, signals for only two aromatic protons, plus an aromatic methyl group, showed that the newly introduced oxygen was most probably attached to the benzene ring. These data led us to consider structure **8a** for the major phenolic metabolite. Structure **8a** is the result of 4 hydroxylation of **6a** to give **7a** followed by hemiketalization of the C-9 ketone by the newly introduced hydroxyl.



6a, R = H
b, R = Ac



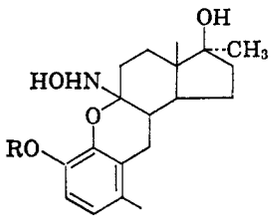
7a, R = H
b, R = Ac
c, R = CH_3



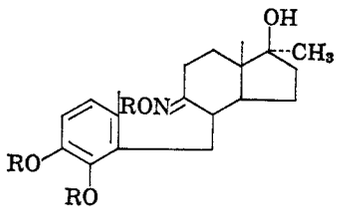
8a, $R_1 = R_2 = H$
b, $R_1 = Ac$; $R_2 = H$
c, $R_1 = R_2 = Ac$
d, $R_1 = CH_3$; $R_2 = H$
e, $R_1 = SO_2CH_3$; $R_2 = H$

Mild acetylation of **8a** produced a monoacetate (**8b**). Similarly, reaction with excess diazomethane for an extended period gave only a monomethyl ether (**8d**). These reactions show that only one phenolic hydroxyl is readily available for reaction. More prolonged acetylation of either **8a** or **8b** produced a mixture of diacetates **7b** and **8c**. The structure of the ring-opened diacetate (**7b**) follows from its infrared spectrum which showed absorption at 5.65 (aromatic acetates) of approximately twice the intensity of a band at 5.87 μ (six-membered ring ketone), and its nmr spectrum which showed signals for two aromatic protons and a methyl group attached to an aromatic ring. The second diacetate (**8c**) showed infrared peaks of approximately equal intensity at 5.66 (aromatic acetate) and 5.83 μ (hemiketal acetate). The nmr spectrum of **8c** showed a signal for an aromatic acetate at δ 2.28 and one at unusually high field (1.95) which must be due to the hemiketal acetate.

In order to further establish the presence of a masked ketone function in **8a**, both **8a** and **7b** were treated with excess hydroxylamine hydrochloride in pyridine. The same product (**9a** or **10a**) was isolated in each case, these conditions being sufficient to hydrolyze the acetate functions of **7b**. The cyclic structure (**9a**) for the oxime is preferred, because, although acetylation of the oxime gave the ring-opened triacetate **10b**, treatment of the oxime with excess diazomethane gave only a monomethyl ether (**9b**). Failure to form a polymethylated product favors cyclic structures **9a** and **b**.

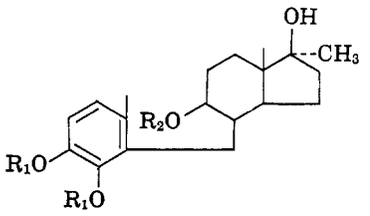


9a, R = H
b, R = CH_3



10a, R = H
b, R = Ac

Reduction of **8a** with sodium borohydride gave tetrol **11a**, in which the 9-hydroxy group is assigned the β (equatorial) configuration.[8] The nmr spectrum of its acetate (**11b**) showed a broad peak for the 9α proton at δ 4.50 with a half-band width of approximately 18 cps. Such a broad peak must be due to large diaxial, as well as smaller axial–equatorial, couplings.[9] Methylation of **11a** with diazomethane gave the dimethyl ether **11c**, which was oxidized to dimethoxy ketone **7c**.



11a, $R_1 = R_2 = H$
b, $R_1 = R_2 = Ac$
c, $R_1 = CH_3$; $R_2 = H$

The above series of reactions serves to show the mutual masking of the 4-hydroxyl and 9-keto functions of **8a**. Thus, prior to reduction of the 9-keto function of **8a** only one phenolic hydroxyl was readily available for acetylation or methylation, and the infrared spectrum of **8a** showed no carbonyl absorption. After reduction, two phenolic hydroxyls were readily available for acetylation or methylation. Back-oxidation of the 9-hydroxyl function of **11c** gave **7c**. Since methylation of the C-4 hydroxyl now blocked its hemiketalization of the C-9 ketone, the infrared spectrum of **7c** showed the expected carbonyl absorption.

The nmr spectrum of **8a** presented a rather interesting problem in that the C-1 and C-2 protons did not produce the expected AB quartet, but rather a single sharp peak at δ 6.67. Examination of the nmr spectra of several derivatives of **8a**, *viz.* **7b**, **9a**, and **11b**, revealed weak satellites located at approximately 8 cps from the main peak. In one derivative (**7c**) an AB quartet was clearly visible at δ 6.77 (J = 8 cps) with its center peaks separated by 4 cps. Therefore, it is probable that the C-1 and C-2 protons of **8a** are magnetically equivalent, whereas those of some of its derivatives are only nearly equivalent. In order to more clearly define this point, compound **8c**, the 3-methanesulfonyl ester of **8a**, was prepared. The proximity of the meth-

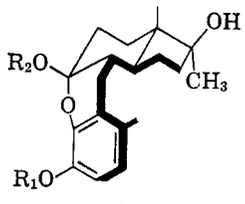
(8) This assignment is in agreement with that of S. Mahapatra and R. M. Dodson [*J. Med. Chem.*, **9**, 147 (1966)] who reduced a similar 9-keto function.

(9) Reference 5, pp 77–85.

anesulfonyl function to the C-2 proton caused its signal to be shifted away from that of the more distant C-1 proton so that a very clearly defined AB quartet with its center peaks separated by 10 cps (δ 6.92, J = 8 cps) resulted. Therefore, **8a** must contain two adjacent hydrogens, and the newly introduced hydroxyl function must be located at C-4 rather than at C-1 or C-2.

The only point remaining to be explained in the structure of **8a** is the configuration at C-9. Examination of the nmr spectra of **8a** and its derivatives resolved this problem. The ring-opened derivatives of **8a** (**7b,c**, **10b**, and **11a,b,c**) all showed the expected sharp singlets for the C-17 and C-18 methyls. However, **8a** and all of its ring-closed derivatives, except one (**8c**) showed four peaks of approximately equal intensity for their tertiary methyls.

The presence of four tertiary methyl peaks in the nmr spectra of the ring-closed compounds but not in those of the ring-opened derivatives is most easily explained by assuming that the ring-closed compounds are mixtures (approximately 1:1) of C-9 epimers. Examination of molecular models indicated that, in the case of the epimer with a 9α-ether function (**12**), the aromatic ring is oriented so that long-range shielding of the C-17 methyl is possible.[10] It is suggested, therefore, that **12** is responsible for the higher field C-17 methyl signal (δ 1.12–1.17), whereas **13** shows its C-17 methyl signal at relatively lower field (δ 1.28–1.30). As expected, there was only a slight separation of the C-18 methyl signals for the epimeric pairs (0.03 ppm). The only ring-closed derivative which is apparently a single epimer is **8c**, one of the two diacetates obtained from **8a**. The nmr spectrum of **8c** showed only two peaks for its tertiary methyl groups. The C-17 methyl signal appeared at δ 1.17, indicating that **8c** has its ring oxygen in the α configuration (**12**). This assignment seems reasonable, since acetylation of the hemiketal hydroxyl of **8a** could occur most easily if the hydroxyl were equatorial, as in **12**. The other epimer of **8a** (**13**) is apparently the source of ring-opened diacetate **7b**.



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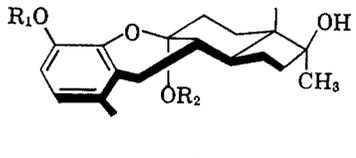

13

where R_1 and R_2 are the same as those for **8**

The pathway for the formation of **6a** from **1** by *P. ruber* parallels the formation of similar 9,10-secophenols from steroids with the usual six-membered B ring.[7] In this case the first step is 9α hydroxylation to yield **2a**, followed by 1,2 dehydrogenation and rupture of ring B by a reverse aldol reaction. The reverse order of events, 1,2 dehydrogenation followed by 9α hydroxylation, appears unlikely, since the 9α-hydroxylated derivative (**2a**) was readily converted to **6a** by *P. ruber*, whereas 17β-hydroxy-17α-methyl-B-norandrosta-1,4-dien-3-one[11] was not an efficient substrate. Incubation of **6a** with *P. ruber* produced the major product of the fermentation (**8a**), showing that the over-all pathway must be as shown.

$$\mathbf{1} \xrightarrow{9\alpha\text{ hydroxylation}} \mathbf{2a} \xrightarrow[2.\text{ reverse aldol reaction}]{1.\text{ 1,2 dehydrogenation}}$$

$$\mathbf{6a} \xrightarrow[2.\text{ hemiketal formation}]{1.\text{ 4 hydroxylation}} \mathbf{8a}$$

The isolation of **8a** is of particular interest because its structure is relevant to the mechanism of microbial degradation of ring A of steroids. Sih and co-workers[12] have presented convincing evidence that 9,10-seco steroids of type **5** are degraded by microorganisms *via* 4 hydroxylation. However, they were not able to isolate the 3,4-dihydroxy compound as a metabolic product owing to its rapid further oxidation leading to ring-A fission. In the B-nor steroid example reported here, formation of a stable, six-membered hemiketal ring protects the sensitive catechol moiety from further microbial oxidation, allowing **8a** to accumulate. Thus the transformation of **1** to **8a** represents a special case of the degradation studied in detail by Sih and coworkers.

Experimental Section[13]

Fermentation of 17α-Methyl-B-nortestosterone (1) with *P. Ruber* (ATCC 8547).—To 9 l. of sterile trypticase soy broth in a 10-l. New Brunswick stir jar unit was added 1 l. of a standard inoculum of *P. ruber*. After incubation for 24 hr at 30° with an impeller speed of 200 rpm and an aeration rate of 0.3 l. of air per minute per liter of medium, a solution of 10 g (34.8 mmoles) of 17α-methyl-B-nortestosterone[14] in 100 ml of ethanol was added. The impeller speed and rate of aeration were increased to 300 and 1, respectively. After 8 hr the fermentation medium was adjusted to pH 3 with phosphoric acid and the cells were removed by centrifugation. The broth was extracted twice with equal volumes of methylene chloride. The cells were extracted with 250 ml of methylene chloride–ethanol (1:1). The combined extracts were concentrated to 1 l., washed with 5% sodium bicarbonate solution (three 200-ml portions) followed by 2% sodium hydroxide solution (four 200-ml portions), dried, and evaporated to give a 12.2 g of neutral fraction. Acidification of the sodium bicarbonate solution followed by extraction with methylene chloride gave 0.6 g of oily acids which was discarded. The sodium hydroxide solution was acidified and extracted with methylene chloride (three 150-ml portions). Drying and evaporation of the methylene chloride extracts gave a 7.3 g of phenolic fraction.

9α-Hydroxy-17α-methyl-B-nortestosterone (2a).—The neutral fraction (12.2 g) was dissolved in 100 ml of benzene and chromatographed on 100 g of activity III Woelm alumina. Elution with benzene (400 ml) gave 9.6 g of nonsteroidal material, which was discarded. Elution with methylene chloride (500 ml) gave 1.8 g, which was recrystallized from acetone–hexane to give 1.24 g (4.08 mmoles, 12%) of 9α-hydroxy-17α-methyl-B-nortestoster-

(10) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press Inc., New York, N. Y., 1959, pp 125–129. See also ref 5, p 152, for a similar case in the steroid series.

(11) This compound will be fully described in a subsequent publication.

(12) (a) C. J. Sih, S. S. Lee, Y. Y. Tsong, and K. C. Wang, *J. Am. Chem. Soc.*, **87**, 1385 (1965); (b) C. J. Sih, K. C. Wang, D. T. Gibson, and H. W. Whitlock, *ibid.*, **87**, 1386 (1965); (c) C. J. Sih, S. S. Lee, Y. Y. Tsong, and K. C. Wang, *J. Biol. Chem.*, **241**, 540 (1966); (d) D. T. Gibson, K. C. Wang, C. J. Sih, and H. Whitlock, *ibid.*, **241**, 551 (1966).

(13) Melting points were determined using a Thomas–Hoover apparatus and are corrected. Infrared spectra were determined as Nujol mulls on a Perkin-Elmer Infracord, and ultraviolet spectra were recorded in ethanol solutions on a Cary Model 14 instrument. Nmr spectra were recorded on a Varian Associates A-60 spectrometer in $CDCl_3$ solutions with internal tetramethylsilane (δ = 0 ppm). Thin layer chromatograms (tlc) were developed on silica gel G, 5 × 20 cm plates (Analtech Inc.) using a mixture of ethyl acetate and cyclohexane unless otherwise stated. The ratio of ethyl acetate to cyclohexane employed is reported in brackets following the R_f value. Visualization of the chromatograms was accomplished by spraying with 40% aqueous sulfuric acid followed by heating.

(14) J. Joska, J. Fajkos, and F. Šorm, *Chem. Ind.* (London), 1665 (1958).

one, mp 181–183°. The analytical sample, obtained by a second recrystallization from the same solvents, had mp 183–184°; $[\alpha]^{25}D$ −52.7° ($CHCl_3$); λ_{max} 242 mμ (ε 14,300); λ_{max} 2.88 (m), 6.05 μ (s); nmr absorption at δ 0.88 (C-18 H_3, s), 1.17 (C-19 H_3, s), 1.23 (C-17 CH_3, s), 5.59 (C-4 H, s), tlc, R_f 0.40 (EtOAc).

Anal. Calcd for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 75.13; H, 9.14.

3,17β-Dihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one (6a).—The phenolic fraction (7.3 g) was crystallized from ethyl acetate to give 0.70 g (2.32 mmoles, 7%) of 3,17β-dihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one: mp 208–211°; $[\alpha]^{25}D$ +50.3° (CH_3OH); λ_{max} 281 mμ (ε 2,400); λ_{max} 2.85 (m), 2.95 (m), 5.95 (s), 6.24 μ (m); tlc, R_f 0.28 (1:1).

Anal. Calcd for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.39; H, 8.57.

3,4,17β-Trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-Hemiketal (8a).—The mother liquor residue (6.6 g) from the isolation of **6a** was dissolved in 200 ml of benzene–ethyl acetate (3:1) and chromatographed on 125 g of silica gel. Three 250-ml fractions were collected using benzene–ethyl acetate (1:1). Fractions 2 through 6 were combined on the basis of their thin layer chromatograms and crystallized from benzene to give 2.48 g (7.80 mmoles, 22%) of 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-hemiketal: mp 158–162° with prior softening beginning at 128°; $[\alpha]^{25}D$ −27.0° ($CHCl_3$); λ_{max} 280 mμ (ε 1,970); λ_{max} 2.80 (m), 3.00 (m), 6.72 μ (m); nmr absorption at δ 0.97 (C-18 H_3, s), 1.00 (C-18 H_3, s), 1.12 (C-17 CH_3, s), 1.28 (C-17 CH_3, s), 2.14 (C-19 H_3, s), 6.67 (C-1 H, C-2 H, s); tlc, R_f 0.24 (1:1).

Anal. Calcd for $C_{19}H_{26}O_4$: C, 71.67; H, 8.23. Found: C, 71.83; H, 8.15.

9α-Hydroxy-17α-methyl-B-nortestosterone 17-Acetate (2b).—A solution of 0.50 g (1.64 mmoles) of 9α-hydroxy-17α-methyl-B-nortestosterone (**2a**) in 10 ml of acetic anhydride was heated at reflux for 2 hr. Most of the acetic anhydride was evaporated, and the residue was diluted with aqueous sodium carbonate. The resulting precipitate was filtered, washed with water and aqueous alcohol, dried, and recrystallized from acetone–hexane to give 0.50 g (1.44 mmoles, 88%) of 9α-hydroxy-17α-methyl-B-nortestosterone 17-acetate: mp 205–209°; λ_{max} 2.90 (m), 5.76 (s), 6.09 (s), 8.00 μ (s).

Anal. Calcd for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.52; H, 8.77.

17β-Hydroxy-17α-methyl-B-norandrosta-4,9(11)-dien-3-one 17-Acetate (3a). A. From 9α-Hydroxy-17α-methyl-B-nortestosterone 17-Acetate (2b).—A solution of 0.46 g (1.33 mmoles) of 9α-hydroxy-17α-methyl-B-nortestosterone 17-acetate (**2b**) in 10 ml of pyridine was treated with 0.15 ml (2.1 mmoles) of thionyl chloride. After 5 min the reaction mixture was poured into dilute sodium carbonate solution and extracted with methylene chloride. The methylene chloride extracts were washed with dilute phosphoric acid, dried, and evaporated to a residue. The residue was dissolved in 20 ml of benzene and filtered through a column of 10 g of Woelm activity III alumina. The column was washed with an additional 100 ml of benzene and the combined benzene filtrates were evaporated. The residue was recrystallized from acetone–hexane to give 0.20 g (0.61 mmole, 46%) of 17β-hydroxy-17α-methyl-B-norandrosta-4,9(11)-dien-3-one 17-acetate: mp 183–185°; λ_{max} 5.78 (s), 6.00 (s), 8.02 μ (s).

Anal. Calcd for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: C, 76.66; H, 8.63.

B. From 17α-Hydroxy-17α-methyl-B-norandrosta-4,9(11)-dien-3-one (3b).—A solution of 0.05 g of 17β-hydroxy-17α-methyl-B-norandrosta-4,9(11)-dien-3-one (**3b**) in 5 ml of acetic anhydride was refluxed for 2 hr. The cooled reaction mixture was poured into excess sodium carbonate solution and extracted with methylene chloride. The methylene chloride extracts were dried and evaporated to a residue which was recrystallized from acetone–hexane to give 17β-hydroxy-17α-methyl-B-norandrosta-4,9(11)-dien-3-one 17-acetate, mp 183–186°, identical in all respects with that prepared above, mmp 183–186°.

3,17β-Dihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 3-Acetate (6b).—A solution of 0.18 g (0.59 mmole) of 3,17β-dihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one (**6a**) in 1 ml of pyridine and 1 ml of acetic anhydride was allowed to stand at room temperature for 16 hr. The reaction mixture was poured into sodium carbonate solution and extracted with methylene chloride. The organic extracts were washed with dilute phosphoric acid, dried, and evaporated to a residue which was recrystallized from acetone–hexane to give 0.12 g (0.35 mmole, 59%) of 3,17β-dihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 3-acetate. The analytical sample was sublimed at 100° (high vacuum) and had mp 129–132°; λ_{max} 2.77 (m), 5.68 (s), 5.90 (s); 8.27 μ (s); nmr absorption at δ 1.20 (C-18 H_3 or C-17 CH_3, s), 1.25 (C-18 H_3 or C-17 CH_3, s), 2.28 (C-3 CH_3CO_2, C-19 H_3, s), 6.97 (C-1 H, C-2 H, C-4H, m); tlc R_f 0.49 (1:1).

Anal. Calcd for $C_{21}H_{28}O_4$: C, 73.23; H, 8.19. Found: C, 73.23; H, 8.16.

Acetylation of 3,4,17β-Trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-Hemiketal (8a).—A solution of 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-hemiketal (0.1 g, 0.31 mmole) in 1 ml of pyridine and 0.5 ml of acetic anhydride was allowed to stand at room temperature for 2 hr. The solution was diluted with methylene chloride, washed with cold, dilute sodium hydroxide and phosphoric acid solutions dried, and evaporated to a residue. Although thin layer chromatography indicated that the residue was homogenous it could not be crystallized. Dissolving the residue in methylene chloride followed by rapid evaporation of the solvent produced a foam which was pulverized to a white, amorphous powder of **3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-hemiketal 3-acetate (8b,** 0.09 g, 0.25 mmole, 81%): λ_{max} 270 mμ (ε 1200), 274 (1200), 278 (1200); λ_{max} 2.83 (s), 5.69 (s), 8.32 μ (s); nmr absorption at δ 0.97 (C-18 H_3, s), 1.00 (C-18 H_3, s), 1.17 (C-17 CH_3, s), 1.30 (C-17 CH_3, s); 2.20 (C-19 H_3, s), 2.27 (C-3 CH_3COO, s), 6.79 (C-1 H, C-2 H, s); tlc, R_f 0.37 (1:1).

Anal. Calcd for $C_{21}H_{28}O_5$: C, 69.98; H, 7.83. Found: C, 69.72; H, 7.87.

A solution of 1.08 g (3.40 mmoles) of 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-hemiketal (**8a**) in 10 ml of pyridine and 5 ml of acetic anhydride was allowed to stand at room temperature for 24 hr. The solution was poured into cold, dilute sodium carbonate solution and extracted with methylene chloride. The methylene chloride extracts were washed with dilute phosphoric acid, dried, and evaporated to a residue. The residue was dissolved in 20 ml of benzene–petroleum ether (bp 30–60°) (1:1) and chromatographed on 30 g of activity III Woelm alumina. Elution with benzene–petroleum ether (1:1) and (3:1) gave **3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-hemiketal 3,9-diacetate (8c,** 0.21 g, 0.52 mmole, 15%): mp 170–173° after recrystallization from benzene–hexane; λ_{max} 2.76 (m), 5.66 (s), 8.43 μ (s); λ_{max} 268 mμ (ε 1440), 272 (1470), 277 (1550); nmr absorption at δ 1.08 (C-18 H_3, s), 1.17 (C-17 CH_3, s), 1.95 (C-9 CH_3CO_2, s), 2.20 (C-19 H_3, s), 2.28 (C-3 CH_3CO_2, s), 6.71 (C-1 H, C-2 H, s); tlc, R_f 0.57 (1:1).

Anal. Calcd for $C_{23}H_{30}O_6$: C, 68.64; H, 7.51. Found: C, 68.67; H, 7.58.

Further elution with benzene and benzene–methylene chloride (1:1 gave **3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 3,4-diacetate (7b)** which could not be crystallized[15] (0.25 g, 0.62 mmole, 18%). Thin layer chromatography showed the sample to be homogenous: R_f 0.20 (1:1); λ_{max} 265 mμ sh (ε 1080), 267 (1090), 272 sh (1050); λ_{max} 2.80 (m), 5.65 (vs), 5.87 (s), 8.30 μ (s); nmr absorption at δ 1.13 (C-18 H_3 or C-17 CH_3, s), 1.20 (C-18 H_3 or C-17 CH_3, s), 2.22 (C-19 H_3, s), 2.28 (C-3 CH_3COO, s); 2.38 (C-4 CH_3COO, s), 7.02 (C-1 H, C-2 H, s).

Elution with benzene–methylene chloride (1:1) and methylene chloride gave **3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-hemiketal 3-acetate (8b,** 0.16 g, 0.44 mmole, 13%), identical with that prepared above. When this material was reacetylated for 48 hr it was converted to a mixture of 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-hemiketal 3,9-diacetate (**8c**) and 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 3,4-diacetate (**7b**).

3,4,17β-Trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-Hemiketal 3-Methyl Ether (8d).—To a solution of 0.50 g (1.57 mmoles) of 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-hemiketal (**8a**) in 50 ml of ether was added approximately 0.5 g (12 mmoles) of

(15) Many of the compounds in this series (**7b,c, 8b,d,e, 10b,** and **11b,c**) were noncrystalline, and elemental analyses usually were not obtained. These compounds were characterized as far as possible by spectrographic means, and their purity was checked by thin layer chromatography (tlc).

diazomethane in 30 ml of ether. After 72 hr at 0° the reaction appeared to be nearly complete as judged by tlc. The ether solution was washed with sodium bicarbonate solution to destroy excess diazomethane and then with sodium hydroxide solution to remove unreacted starting material. Evaporation of the dried ether phase gave 0.42 g of a residue which was dissolved in benzene and chromatographed on 15 g of activity III Woelm alumina. Elution with benzene–methylene chloride (1:1) and methylene chloride gave 0.34 g (1.02 mmoles, 65%) of 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-hemiketal 3-methyl ether which could not be crystallized. An amorphous solid was produced by precipitation from hexane which showed a single spot on tlc, R_f 0.43 on alumina (3:2); λ_{max} 275 mμ sh (ε 1700), 279 (1720), 281 sh (1620); λ_{max} 2.83 (m), 6.71 μ (m); nmr absorption at δ 0.97 (C-18 H_3, s), 1.00 (C-18 H_3, s); 1.13 (C-17 CH_3, s), 1.30 (C-17 CH_3, s), 2.20 (C-19 H_3, s), 3.88 (C-3 CH_3O, s), 6.77 (C-1 H, C-2 H, s).

3,4,17β-Trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-Hemiketal 3-Methanesulfonyl Ester (8e).—To a solution of 0.25 g (0.79 mmole) of 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-hemiketal (**8a**) in 10 ml of pyridine at 0° was added 0.25 ml of methanesulfonyl chloride. After 20 min at 0° the reaction mixture was poured into cold sodium carbonate solution and extracted with methylene chloride. The methylene chloride extracts were washed with dilute phosphoric acid and sodium hydroxide solutions, dried, and evaporated to a residue which could not be crystallized, but which was homogenous on tlc, R_f 0.85 on alumina (ethyl acetate); nmr absorption at δ 0.97 (C-18 H_3, s), 1.00 (C-18 H_3, s), 1.12 (C-17 CH_3, s), 1.28 (C-17 CH_3, s), 2.22 (C-19 H_3, s), 3.18 (C-3 CH_3SO_3, s), 6.92 (C-1 H, C-2 H, q, $J_{1,2}$ = 8 cps, center peaks separated by 10 cps).

3,4,17β-Trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one Oxime (9a or 10a). A. From 3,4,17β-Trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-Hemiketal (8a).—A solution of 0.20 g (0.63 mmole) of 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-hemiketal in 2.5 ml of pyridine was treated with 0.2 g of hydroxylamine hydrochloride and allowed to stand for 20 hr at room temperature. The reaction mixture was diluted with water and extracted with methylene chloride. The methylene chloride extracts were washed with dilute phosphoric acid, dried and evaporated to a crystalline residue. Recrystallization from methanol–methylene chloride gave 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one oxime (**9a** or **10a**): mp 232° dec; λ_{max} 2.71 (m), 2.91 (m), 6.09 μ (m); nmr absorption at δ 1.08 (C-18 H_3, s), 1.23 (C-17 CH_3, s), 2.22 (C-19 H_3, s), 6.57 (C-1 H, C-2 H, s); tlc, R_f 0.25 (1:1).

Anal. Calcd for $C_{19}H_{27}NO_4$: C, 68.44; H, 8.16; N, 4.20. Found: C, 68.30; H, 8.19; N, 4.06.

B. **From 3,4,17β-Trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 3,4-Diacetate (7b).**—A solution of 0.20 g (0.50 mmole) of 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 3,4-diacetate (**7b**) in 2.5 ml of pyridine was treated with 0.2 g of hydroxylamine hydrochloride as described above. The product, mp 232° dec, was identical in all respects with 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one oxime (**9a** or **10a**) prepared as described above.

3,4,17β-Trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one Oxime Triacetate (10b).—A solution of 0.40 g (1.20 mmoles) of 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one oxime (**9a** or **10a**) in 5 ml of pyridine and 2.5 ml of acetic anhydride was allowed to stand at room temperature for 2 hr. The reaction mixture was poured into cold, dilute sodium carbonate solution and extracted with methylene chloride. The methylene chloride extracts were washed with dilute phosphoric acid, dried, and evaporated to a residue. Chromatography on activity III Woelm alumina resulted in a poor recovery of product owing to decomposition on the column. The crude product was purified by dissolving it in methylene chloride and filtering it rapidly through 3 g of activity III Woelm alumina. Evaporation of the filtrate gave 0.05 g (0.11 mmole, 9%) of 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one oxime triacetate as an oil which appeared homogenous on tlc, R_f 0.43 (ethyl acetate); λ_{max} 2.78 (m), 5.67 (vs), 6.16 (w), 8.28 μ (vs); nmr absorption at δ 1.00 (C-18 H_3, s), 1.23 (C-17 CH_3, s), 2.12 (C-19 H_3 or C-9 CH_3COON= or C-3 CH_3COO or C-4 CH_3COO, s), 2.25 (C-19 H_3 or C-9 CH_3COON= or C-3 CH_3COO or C-4 CH_3COO, s), 2.32 (C-19 H_3 or C-9 CH_3COON= or C-3 CH_3COO or C-4 CH_3COO, s), 2.42 (C-19 H_3 or C-9 CH_3COON= or C-3 CH_3COO or C-4 CH_3COO, s); 7.00 (C-1, C-2 H, s).

3,4,17β-Trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one Oxime 3-Methyl Ether (9b).—To a solution of 0.03 g (0.09 mmole) of 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,4-trien-9-one oxime (**9a** or **10a**) in 5 ml of methanol and 10 ml of ether was added approximately 0.1 g (2.5 mmoles) of diazomethane in 20 ml of ether. After 18 hr at 0° the reaction mixture was washed with sodium bicarbonate and dilute sodium hydroxide solutions, dried, and evaporated to a residue. Recrystallization from methanol–methylene chloride gave 0.02 g (0.06 mmole, 64%) of 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one oxime 3-methyl ether: mp 225–228°; λ_{max} 2.82 (m), 2.98 (m), 6.02 (w), 6.34 μ (m); nmr absorption at δ 1.10 (C-18 H_3, s), 1.23 (C-17 CH_3, s), 2.25 (C-19 H_3, s), 3.82 (C-3 CH_3O, s), 6.62 (C-1 H, C-2 H, s); tlc, R_f 0.60 (ethyl acetate).[16]

17α-Methyl-9,10-seco-B-norandrosta-1,3,5-triene-3,4,9β,17β-tetrol (11a).—To a cold, stirred solution of 0.85 g (2.67 mmoles) of 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 4,9-hemiketal (**8a**) in 20 ml of water containing 0.5 g of sodium hydroxide was added 0.79 g (21.4 mmoles) of sodium borohydride in portions. After 20 min at 0° the reaction mixture was slowly poured into ice water containing 10 ml of 85% phosphoric acid and extracted with methylene chloride. Evaporation of the dried methylene chloride extracts gave 0.66 g of crystalline material. Filtration of the suspended solid in the aqueous phase gave an additional 0.12 g of the same material. The crude 17α-methyl-9,10-seco-B-norandrosta-1,3,5-triene-3,4,9β,17β-tetrol (0.78 g, 2.44 mmoles, 91%) was recrystallized from ethyl acetate: mp 237–244°; λ_{max} 2.73 (m), 2.85 μ (m); nmr absorption at δ 0.93 (C-18 H_3, s), 1.22 (C-17 CH_3, s), 2.24 (C-19 H_3 s), 6.58 (C-1 H, C-2 H, s); tlc, R_f 0.50 (ethyl acetate).

Anal. Calcd for $C_{19}H_{28}O_4$: C, 71.22; H, 8.81. Found: C, 71.26; H, 8.77.

17α-Methyl-9,10-seco-B-norandrosta-1,3,5-triene-3,4,9β,17β-tetrol 3,4,9-Triacetate (11b).—A solution of 0.20 g (0.63 mmole) of 17α-methyl-9,10-seco-B-norandrosta-1,3,5-triene-3,4,9β,17β-tetrol (**11a**) in 10 ml of pyridine and 5 ml of acetic anhydride was allowed to stand at room temperature for 16 hr. The reaction mixture was poured into cold sodium carbonate solution and extracted with methylene chloride. The methylene chloride extracts were washed with dilute phosphoric acid, dried, and evaporated. The residue was dissolved in benzene–petroleum ether (1:2) and chromatographed on 4 g of activity III Woelm alumina. The product, eluted with benzene, was homogenous on tlc, R_f 0.55 (ethyl acetate); λ_{max} 2.77 (m), 5.63 (vs), 5.78 (s), 8.30 μ (vs); nmr absorption at δ 0.95 (C-18 H_3, s), 1.23 (C-17 CH_3, s), 1.65 (C-9 CH_3COO, s), 2.23 (C-19 H_3, s), 2.30 (C-3 CH_3COO, C-4 CH_3COO, s), 4.50 (C-9 H, m), 6.99 (C-1 H, C-2 H, s).

3,4,17β-Trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 3,4-Dimethyl Ether (7c).—A solution of 0.32 g (1.00 mmole) of 17α-methyl-9,10-seco-B-norandrosta-1,3,5-triene-3,4,9β,17β-tetrol (**11a**) in 15 ml of methanol–ether (1:4) was treated with a solution of approximately 0.17 g (4 mmoles) of diazomethane in 20 ml of ether. After 16 hr at 0° the reaction mixture was washed with sodium bicarbonate solution to destroy excess diazomethane, dried, and evaporated to a residue of crude **17α-methyl-9,10-seco-B-norandrosta-1,3,5-triene-3,4,-9β,17β-tetrol 3,4-dimethyl ether (11c).**

The total crude product was dissolved in 20 ml of acetone and treated with excess Jones reagent[17] at 0° with stirring. After 5 min the reaction mixture was poured into water and extracted with methylene chloride. The methylene chloride extracts were washed with dilute sodium hydroxide, dried, and evaporated. The residue was dissolved in benzene–petroleum ether (1:3) and chromatographed on 8 g of activity III Woelm alumina. The product, 3,4,17β-trihydroxy-17α-methyl-9,10-seco-B-norandrosta-1,3,5-trien-9-one 3,4-dimethyl ether (0.11 g, 0.32 mmole, 32%), was eluted with benzene and showed a single spot on tlc, R_f 0.55 (ethyl acetate); λ_{max} 2,80 (m), 5.88 μ (s); nmr absorption at δ 1.15 (C-18 H_3, s), 1.23 (C-17 CH_3, s), 2.33 (C-19

(16) Because of the small amount of this sample its nmr spectrum was determined in preference to elemental analysis, because the former would better define the number of methoxyl groups present.

(17) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

H_3, s), 3.70 (C-3 CH_3O or C-4 CH_3O, s), 3.83 (C-3 CH_3O or C-4 CH_3O, s), 6.77 (C-1 H, C-2 H, q, $J_{1,2}$ = 8 cps, center peaks separated by 4 cps).

Registry No.—**1**, 3570-10-3; **2a**, 7635-63-4; **6a**, 7635-64-5; **8a**, 7635-65-6; **2b**, 7635-66-7; **3a**, 7635-67-8; **6b**, 7756-52-7; **8b**, 7635-68-9; **8c**, 7635-69-0; **7b**, 7635-70-3; **8d**, 7635-71-4; **8e**, 7635-72-5; **9a**, 7635-73-6; **10a**, 7635-74-7; **10b**, 7635-75-8; **9b**, 7635-76-9; **11b**, 7635-77-0; **7c**, 7635-78-1; **11c**, 7635-79-2; **11a**, 7635-80-5.

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Stereochemical Studies of Monoterpene Compounds. II.[1] The Conformation of 4-Hydroxymenthones[2]

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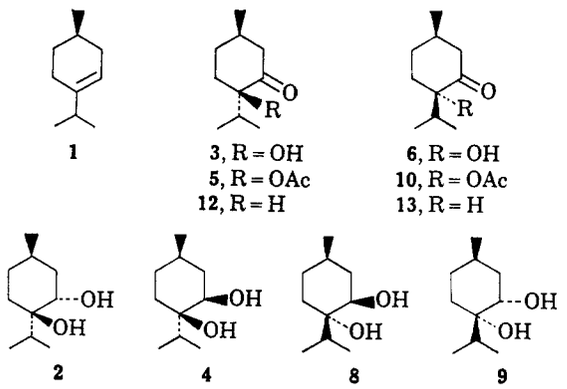
A diastereoisomeric pair of 4-hydroxymenthones was prepared from (+)-*p*-menth-3-ene by oxidation. The configuration of these isomers was assigned as (1*R*:4*R*)-(−)-4-hydroxymenthone (**3**) and (1*R*:4*S*)-(+)-4-hydroxyisomenthone (**6**) by chemical evidences. The conformation of hydroxy ketones **3** and **6** was examined by a combination of ultraviolet, infrared, and nuclear magnetic resonance spectra, optical rotatory dispersion, and circular dichroism measurements. α-Hydroxymenthone (**3**) exhibited the inversion in the sign of the Cotton effect upon changing solvent; this phenomenon was interpreted as indicating the existence of the conformational equilibrium **3a** ⇄ **3b**. On the other hand, another isomer (**6**) did not show such an inversion of the sign of the Cotton effect, taking preferentially conformation **6a**.

Optical rotatory dispersion[3,4] (ORD) and circular dichroism[4,5] (CD) have been found useful in studying the conformation of optically active, flexible substituted cyclohexanones, because of the peculiar sensitivity of this method to subtle conformational alteration. α-Hydroxycyclohexanones represent an interesting example of such mobility, since intramolecular hydrogen bonding can be expected together with a possible chair–chair conformer equilibrium. Djerassi, *et al.*, have recently demonstrated the conformational mobility in (+)-1-hydroxycarvomenthone on the basis of the optical rotatory dispersion[6] and the variable-temperature circular dichroism.[7] We wish to report here on the preparation of and the assignment of the conformation to a diastereoisomeric pair of 4-hydroxymenthones, (1*R*:4*R*)-(−)-4-hydroxymenthone (**3**) and (1*R*:4*S*)-(+)-4-hydroxyisomenthone (**6**).

Preparation and Configuration.—(−)-α-Hydroxy ketone **3** was synthesized by the *t*-butyl chromate oxidation of (+)-*trans*-4-hydroxyneomenthol (**2**), which was derived from (+)-*p*-menth-3-ene (**1**) by the performic acid oxidation. On the other hand, (+)-α-hydroxy ketone **6** was prepared from **1** by the potassium permanganate oxidation. Both **3** and **6** yielded the same derivative, (−)-*p*-menth-3-en-5-one 2,4-dinitrophenylhydrazone, when treated with a hot ethanol–sulfuric acid solution of 2,4-dinitrophenylhydrazine. However, on treatment with a cold, dilute hydrochloric acid



solution of the reagent, each of **3** and **6** yielded the corresponding 2,4-dinitrophenylhydrazone derivatives. These facts and some of physical properties shown in Table I indicate **3** and **6** to be a diastereoisomeric pair.

TABLE I

PHYSICAL PROPERTIES OF 4-HYDROXYMENTHONES

Compd	$\lambda_{max}^{isooctane}$	ε	$\nu_{C=O}^{CCl_4}$,[a] cm^{-1}	$\delta_{CH_3\ to\ C\text{-}1}^{CCl_4}$, ppm	$[\alpha]^{25}D$ (neat), deg	Mp of 2,4-DNPH,[b] °C
3	279	35.3	1711	0.96	−32.50	102.5–103
6	276	75.3	1712	1.04	+72.46	123–124

[a] Ketols **3** and **6** showed distinctive differences in the fingerprint region of the infrared spectra. [b] 2,4-DNPH = 2,4-dinitrophenylhydrazone.

On reduction with lithium aluminum hydride, **3** gave (+)-*trans*-4-hydroxyneomenthol (**2**)[1] and (+)-*cis*-4-hydroxymenthol (**4**),[8] and **6** yielded a glycol (**7**) (mp 93–94°) and (−)-*trans*-4-hydroxyneoisomenthol (**8**).[8] Because the absolute configuration of the diols **2**, **4**, and **8** has already been established, hydroxy ketones **3** and **6** were proved to be (1*R*:4*R*)-(−)-4-hydroxymenthone and (1*R*:4*S*)-(+)-hydroxyisomen-

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