7α-METHYLNORETHINDRONE ENANTHATE 10β-HYDROPEROXIDE: ISOLATION AND CHARACTERIZATION

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ABSTRACT

10β-Hydroperoxy-7α-methylnorethindrone 17-heptanoate (II), a product of allylic autoxidation of 7α-methylnorethindrone enanthate (I), has been isolated and characterized. The synthesis of the hydroperoxide (II) from the 3-ethylene ketal of 7α-methylnorethynodrel (III) was achieved. Esterification of alcohol (III), subsequent deketalization, and photochemical oxygenation resulted in the hydroperoxide (II). Reduction of the hydroperoxide (II) to the 10β-alcohol (VI) and acetylation of (II) to the 10β-acetoxyperoxide (VII) are described. A single subcutaneous injection of the compounds (II), (VI), and (VII) to rats failed to produce long term inhibition of fertility in contrast to the parent compound (I) which is at least five times more effective than norethindrone enanthate as measured by suppression of vaginal cornification and estrous cycles.

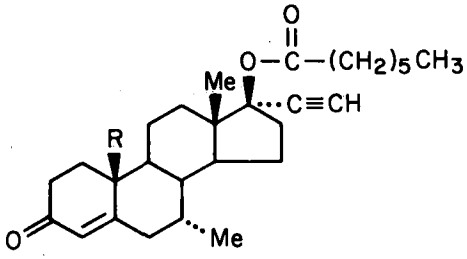
INTRODUCTION

For many years we have sought a compound with a combination of progestational and estrogenic activities which might not only be efficacious in controlling fertility but permit control of uterine bleeding as well. Such a compound would obviate the need for additional estrogen. In combination with ethynylestradiol (17α-ethynyl-1,3,5(10)-estratriene-3,17β-diol) or mestranol (17α-ethynyl-3-methoxy-1,3,5(10)-estratrien-17β-ol), norethindrone (17α-ethynyl-17β-hydroxy-4-estren-3-one) was one of the first synthetic progestational agents to be incorporated into oral contraceptive pills. It has long been known that

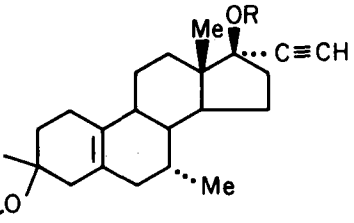
esterification of the 17-hydroxyl group with long chain fatty acids yields compounds with prolonged hormonal activity following intramuscular or subcutaneous injection. The heptanoate ester of norethindrone (norethindrone enanthate) is a long-acting progestational agent currently marketed as an injectable contraceptive but its use has been associated with abnormal bleeding problems characteristic of progestogen-only contraceptive methods. It was known that 7α-methylation of norethindrone conferred substantial estrogenic activity on the parent molecule [1,2]. We have previously described that 7α-methylnorethindrone enanthate (I) exhibited both progestational and inherent estrogenic activity and was found to suppress fertility for prolonged periods following a single subcutaneous injection to rats [3]. Experiments in rats indicate that the 7α-methyl derivative (I) is at least five times more effective than norethindrone enanthate in suppressing fertility as measured by suppression of vaginal cornification and estrous cycles. However, prolonged storage of enanthate (I) at 4°C resulted in a transformation product, the hydroperoxide (II), which is considered to have been the result of autoxidation.

RESULTS AND DISCUSSION

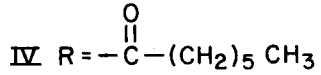
We describe the isolation, characterization, and subsequent synthesis of the autoxidation product of 7α-methylnorethindrone enanthate (I). The structures pertinent to this study are illustrated in Figure I. Storage of a viscous, oily sample of enanthate (I) at 4°C for 7 months afforded a semisolid material in which white crystals had begun to grow on the surface of the viscous oil. Analysis by hplc on a C_{18} μBondapak reverse phase column with acetonitrile and water (85:15) solvent system indicated the presence of a substance more polar than the



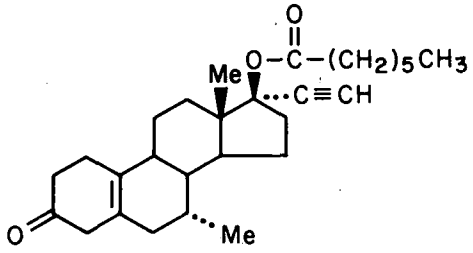
I R = H
II R = —OOH
VI R = —OH
VII R = —OOAc



III R = H



IV



V

FIGURE 1

enanthate (I). The polar material was isolated by hplc, crystallized, and its structure was derived from the following physical data. It melted at 150-153°C (decomp). The uv spectrum exhibited a λ_{max} at 237 nm, a slight hypsochromic shift from the λ_{max} of the enanthate (I) at 240.5 nm. In the nmr spectrum, a peak appeared at δ 6.02 ppm for the C-4 proton of the hydroperoxide (II). The C-4 proton of the enanthate (I) appeared at δ 5.87 ppm. The remainder of the nmr spectrum was virtually identical to that of the enanthate (I). The mass spectrum showed a molecular ion M^+ at m/e = 456 amu, an increase of 32 over the molecular ion of the enanthate (I). Starch-iodide test for peroxides

was positive, and in the light of the above data, we assigned the structure of the white crystals as the hydroperoxide (II)

Steroids possessing the 10-hydroperoxy-4-estren-3-one functionality have been reported previously as oxidative products from a parent β,γ-enone [4,5] and have been found to be less progestational than norethynodrel (17α-ethynyl-17β-hydroxy-5(10)-estren-3-one) but equally potent as contraceptive agents in rats [6]. In the present case, we noted that no migration of the double bond occurred. Apparently, it was simply an instance of slow allylic autoxidation.

In order to prove the structure of the hydroperoxide (II) by an unequivocal method, we synthesized the 10β-hydroperoxide (II), the 10β-hydroxy derivative (VI), and the 10β-acetoxyperoxide (VII) by well established procedures starting from the known [3] intermediate ethylene ketal (III). Alcohol (III) was converted to the ester (IV) in 85% yield by reaction with heptanoic acid, dicyclohexylcarbodiimide, and 4-pyrrolidinopyridine in dichloromethane [7] (an alternative base is 4-dimethylaminopyridine [8]). This procedure is much more effective than the previously employed method involving thallous ethoxide and heptanoyl chloride [9]. Mild deketalization of the ketal (IV) by treatment with acetic acid:tetrahydrofuran:water (3:1:1) gave the nonconjugated enone, 7α-methylnorethynodrel enanthate (V), which upon reaction with oxygen in the presence of fluorescent light [4,5] in carbon tetrachloride-hexane solution afforded the hydroperoxide (II). Reduction of the hydroperoxide with sodium iodide in a solution of ethanol, ether, and acetic acid [4,10] gave the 10β-alcohol (VI), while acetylation of the hydroperoxide (II) with acetic anhydride in pyridine yielded the 10β-acetoxyperoxide (VII). The synthetic hydroperoxide (II) was found to be

identical to the solid compound isolated from enanthate (I) during the storage for biological studies.

It is well known that ascorbic acid is an effective antioxidant. In order to prevent the formation of the undesirable autoxidation product, hydroperoxide (II), we investigated the effect of 1 and 3% concentrations of ascorbic acid on the autoxidation of enanthate (I) to the hydroperoxide (II), and monitored the experimental samples at intervals of 7 to 12 days by means of hplc analysis on a C_{18} μBondapak reverse phase column with acetonitrile and water (85:15) solvent system at a flow rate of 2 ml/min and uv detector at 254 nm. However, the antioxidative effect appears to be inconclusive.

Unlike the parent ester (I), neither the hydroperoxide (II), the alcohol (VI), nor the acetoxyperoxide (VII) exhibited potent estrus-suppressing activity in rats, as measured by suppression of vaginal cornification and estrous cycles, when administered as a single subcutaneous injection of 4 mg in sesame oil. On the other hand, the parent ester (I) [3] showed more potent estrus-suppressing activity at the 4 mg dose level than norethindrone enanthate.

EXPERIMENTAL

Unless otherwise stated, m.p.s were determined with a Thomas-Hoover Model 6406-H apparatus; ir spectra were recorded for potassium bromide pellets with a Perkin-Elmer 467 spectrophotometer; ^{1}H nmr spectra were measured in deuteriochloroform using tetramethylsilane as the internal standard with a Varian EM-390 90 MHz spectrometer; uv spectra were recorded in methanol with a Cary 210 spectrophotometer; elemental microanalyses were obtained by Midwest Microlab, Ltd., Indianapolis, Indiana; mass spectra were recorded on a Finigan quadrupole mass spectrometer at the Southwest Research Institute, San Antonio, Texas, or on a Hewlett-Packard Model 5982 quadrupole mass spectrometer at The University of Texas Health Science Center at San Antonio; optical rotations were determined in chloroform solution with a Rudolph Research Autopol II polarimeter; and analytical hplc was performed with a Water's Associates high-performance liquid chromatograph - Model 202.

Isolation and Characterization of 17α-Ethynyl-10β-hydroperoxy-7α-methyl-3-oxo-4-estren-17-yl heptanoate (II).--Seven months after submission of a sample of 17α-ethynyl-7α-methyl-3-oxo-4-estren-17-yl heptanoate (I) (6.5 g, 98% pure) for biological evaluation, two small semisolid samples, sample A (18.6 mg) and sample B (11.83 mg) as residues remaining from the original sample, were returned to our laboratories for characterization. Analysis by hplc (C_{18} μBondapak; acetonitrile:water, 85:15; 2 ml/min; 0.5 cm/min; uv detector at 254 nm) showed sample (A) to be highly contaminated with a more polar component having a retention time of 3.0 min. Pure (I) had a retention time of 5.8 min. Sample (B) was much less contaminated. The contaminant present in sample (A) was separated by hplc and crystallized from acetonitrile:water (85:15): m.p. 150-153°C (decomp.); $\lambda_{max.}$ 237 nm; δ 0.73-1.00 (9H, overlapping s, d, and t, 13-Me, 7α-Me, and terminal Me), 2.58 (1H, s, 17α-C≡CH), and 6.02 (1H, s, 4-H); MS m/e = 456 (M^+), 440 (M^+-16, loss of 0), 424 (M^+-32, loss of 0_2); positive starch-potassium iodide test for peroxides.

3-Ethylenedioxy-17α-ethynyl-7α-methyl-5(10)-estren-17-yl heptanoate (IV).--Heptanoic acid (3.04 ml) was added to a stirred solution of alcohol (III) [3] (5.10 g), 4-pyrrolidinopyridine (0.424 g), and dicyclohexylcarbodiimide (4.415 g) in dichloromethane (89 ml) and the mixture stirred at ambient temperature for 5 days. The mixture was filtered and the filtrate washed with water, 5% aqueous acetic acid, saturated aqueous sodium bicarbonate solution, water, and saturated sodium chloride solution. Drying (Na_2SO_4), filtration, and concentration in vacuo gave the crude ester (IV) (8.9 g). Dry column chromatography on silica gel (ether:hexane, 1:1) afforded the ester (IV) (5.73 g, 85% yield) as a thick yellow oil. $[\alpha]_D^{22}$ = +15.7° (C = 0.95); $\nu_{max.}$ (Film) 3260 and 1745 cm^{-1}; δ 0.75-1.00 (9H, overlapping s, d, and t, 13-Me, 7α-Me, and terminal Me), 2.60 (1H, s, 17α-C≡CH) and 3.99 (4H, s, 3-OCH_2CH_2O); MS m/e = 468 (M^+). Adequate micro-analysis for C and H could not be performed due to the inherent instability of the compound.

17α-Ethynyl-7α-methyl-3-oxo-5(10)-estren-17-yl heptanoate (V).--A stirred solution of ketal (IV) (2.026 g) in acetic acid (15 ml), tetrahydrofuran (5 ml), and water (5 ml) was heated at 50°C for 1.3 h under an atmosphere of nitrogen. Solvent was removed in vacuo under nitrogen and the residue was diluted with water and extracted with ether. The organic extract was washed with saturated aqueous sodium bicarbonate solution, water, and saturated sodium chloride solution. Drying (Na_2SO_4), filtration, and concentration in vacuo gave an oil (1.818 g) which was purified by dry column chromatography on silica gel (ether:hexane, 1:1) to afford the enone (V) (1.328 g, 72% yield) as an oil. $[\alpha]_D^{22}$ = +40.4° (C = 0.99); $\nu_{max.}$ (Film) 3260, 1745, and 1723 cm^{-1}; δ 0.78-0.98 (9H, overlapping s, d, and t, 13-Me, 7α-Me, and terminal Me) and 2.58 (1H, s, 17α-C≡CH); MS m/e = 424 (M^+). Adequate micro-analysis for C and H could not be performed due to the inherent instability of the compound.

17α-Ethynyl-10β-hydroperoxy-7α-methyl-3-oxo-4-estren-17-yl heptanoate (II).--Oxygen was bubbled into a solution of enone (V) (1.328 g) in carbon tetrachloride (36 ml) and hexane (18 ml) for 21 h while the solution was illuminated with a bank of six fluorescent lights. The

solvent was evaporated under reduced pressure and the residue purified by dry column chromatography on silica gel (ether:hexane, 1:1), to give, after trituration with ether-hexane, the pure hydroperoxide (II) (0.272 g, 19% yield), m.p. 161.5-163.5°C (decomp.); 98% pure by hplc (C_{18} μBondapak; acetonitrile:water, 85:15; 2 ml/min; 0.5 cm/min; uv detector at 254 nm; retention time of 2.6 min); $[\alpha]_D^{23}$ = +2.0° (C = 0.5); $\lambda_{max.}$ 236 nm (ε 16,492); $\nu_{max.}$ 3280, 3200, 1740, 1650, and 1625 cm^{-1}; δ 0.77-1.00 (9H, overlapping s, d, and t, 13-Me, 7α-Me, and terminal Me), 2.58 (1H, s, 17α-C≡CH), 6.03 (1H, s, 4-H), and 8.12 (1H, s, 10β-OOH); MS m/e = 456 (M^+) (Found: C, 73.7; H, 9.0. $C_{28}H_{40}O_5$ requires C, 73.65; H, 8.85%).

17α-Ethynyl-10β-hydroxy-7α-methyl-3-oxo-4-estren-17-yl heptanoate (VI).--Sodium iodide (0.142 g) was added under nitrogen to a solution of hydroperoxide (II) (0.250 g) in absolute ethanol (25 ml), ether (5 ml), and glacial acetic acid (7 drops). The dark solution was stirred at ambient temperature for 18 h, concentrated in vacuo, and the residue diluted with ether. The organic solution was washed with 5% aqueous sodium thiosulfate solution, water, and saturated sodium chloride solution. Drying (Na_2SO_4), filtration, and concentration in vacuo gave a foam (0.256 g) which was purified by dry column chromatography on silica gel (ether:hexane, 8:2) to give crude alcohol (VI) (0.134 g) as an oil. Crystallization from hexane gave fairly pure (VI) (0.087 g) which was combined with alcohol (VI) (0.095 g) from a similar batch. Recrystallization from acetone-hexane (2x) and ether-hexane gave pure alcohol (VI) (0.116 g, 26% yield) as an amorphous white solid, m.p. 128-129°C; 98% pure by hplc (C_{18} μBondapak; acetonitrile:water, 75:25; 2 ml/min; 0.5 cm/min; uv detector at 240 nm; retention time of 4.7 min); $[\alpha]_D^{22}$ = 0.0° (C = 0.62); $\lambda_{max.}$ 235.5 nm (ε 15,106); $\nu_{max.}$ 3460, 3280, 1745, 1665, and 1623 cm^{-1}; δ 0.75-1.03 (9H, overlapping s, d, and t, 13-Me, 7α-Me, and terminal Me), 2.61 (1H, s, 17α-C≡CH), and 5.83 (1H, s, 4-H); MS m/e = 440 (M^+) (Found: C, 76.65; H, 9.25. $C_{28}H_{40}O_4$ requires C, 76.35; H, 9.15%).

10β-Acetoxyperoxy-17α-ethynyl-7α-methyl-3-oxo-4-estren-17-yl heptanoate (VII).--A solution of the hydroperoxide (II) (0.673 g) in acetic anhydride (3.36 ml) and pyridine (3.36 ml) was allowed to stand in the dark overnight. The solvent was removed in vacuo under nitrogen and the residue dissolved in ether. The organic solution was washed with water, ice-cold saturated aqueous sodium bicarbonate solution, water, and saturated sodium chloride solution, dried (Na_2SO_4), filtered, and concentrated in vacuo to the crude acetate (VII) (0.78 g) as an oil, which was combined with crude product from another batch (0.1 g) and purified by dry column chromatography on silica gel (ether:hexane, 8:2) to give pure acetate (VII) (0.467 g, 55% yield) as an oil, 98% pure by hplc (C_{18} μBondapak; acetonitrile:water, 85:15; 2 ml/min; 0.5 cm/min; uv detector at 254 nm; retention time of 3.18 min). A portion (0.231 g) of the sample was dried under vacuum (5 to 1 x 10^{-5} Torr at 50°C for 2 days) to constant weight, 0.224 g. Analysis by hplc showed slight decomposition to 97% purity. $[\alpha]_D^{23}$ = +30.0° (C = 1); $\lambda_{max.}$ 235.8 nm (ε 12,379); $\nu_{max.}$ (Film) 3260, 1775, 1740, 1675, and 1630 cm^{-1}; δ 0.74-1.01 (9H, overlapping s, d, and t, 13-Me, 7α-Me, and terminal Me), 2.02 (3H, s, 10β-OOCOMe), 2.58 (1H, s, 17α-C≡CH), and 6.01 (1H, br.s., 4-H); MS m/e = 326

[M^+-172:loss of heptanoyl (113) and acetate (59)] (Found: C, 72.55; H, 8.8. $C_{30}H_{42}O_6$ requires C, 72.25; H, 8.5%).

BIOLOGICAL ACTIVITY

In an experiment designed to demonstrate suppression of estrus, 4 mg of the test compound was dissolved in sesame oil and administered as a single subcutaneous injection to each of 10 female rats. Ten other animals were treated in the same manner using (I) and 10 control animals were injected with the vehicle alone. Estrus suppression was determined by daily vaginal smears of the test animals, starting on the day of treatment and continuing for 90 days. The duration for which cornification was suppressed equals the number of days between treatment and the first day of the cornification minus 2. A cyclicity index was determined by dividing the total number of cycles observed by the maximum number of 4 day cycles expected after the return of cornification, multiplied by 100.

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