LONG-ACTING CONTRACEPTIVE AGENTS: NORETHISTERONE ESTERS OF POLYUNSATURATED ACIDS

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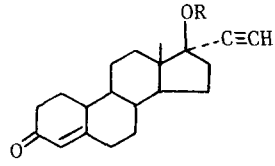
ABSTRACT

Some new derivatives of norethisterone (17α-ethynyl-17β-hydroxyestr-4-en-3-one) are described in which the 17β-hydroxyl group of the steroid is esterified with polyunsaturated aliphatic acids. The potential of these compounds as long-acting contraceptive agents has been evaluated.

INTRODUCTION

Despite the continued demand for long lasting injectable contraceptive agents in many of the less developed countries, research in this field by the pharmaceutical industry has decreased in the last decade. Consequently, the Special Programme of Research, Development and Research Training in Human Reproduction of the World Health Organization has



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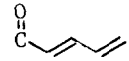
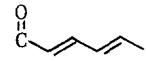
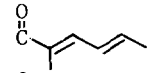
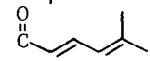
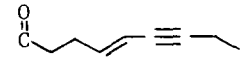
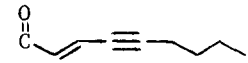
I	
II	
III	
IV	
V	
VI	
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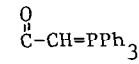
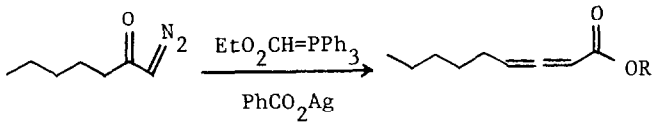
VIII	



IXa R = Et

IXb R = H

Scheme

established a task force to develop an international chemical synthesis programme aimed to find new, long-acting contraceptive steroids (1).

Some injectable, long-acting contraceptive steroids are known, such as norethisterone enanthate and medroxyprogesterone acetate (17α-acetoxy-6α-methylpreg-4-ene-3,20-dione), but some major side effects have been detected in the clinical evaluation of these substances (2,3). The objective of the chemical synthesis programme was to prepare some new 17-OH esters of the active oral progestogens, such as norethisterone. We describe here the synthesis and physical properties of some esters of norethisterone with polyunsaturated acids (I) - (VII). The biological activities of these esters in an estrus suppression assay in rats are reported in an accompanying paper in this Journal by Bialy et al.

CHEMICAL SYNTHESIS

All of the acids except for the E,E-hexa-2,4-dienoic acid, which is commercially available, were synthesized for this work. The syntheses of E-penta-2,4-dienoic acid, E,E-2-methylhexa-2,4-dienoic acid and E-non-2-en-4-ynoic acid are described in an accompanying paper in this Journal.

E-5-Methylhexa-2,4-dienoic acid was prepared by Wittig reaction of isopropylidene triphenylphosphorane with ethyl 4-oxobut-2-enoate.

Alkylation with 1,3-dibromopropene of the sodium salt of diethyl malonate, followed by saponification and decarboxylation, gave E-5-bromo-pent-4-enoic acid (4), which by reaction with the lithium salt of propyne afforded E-non-4-en-6-ynoic acid.

Reaction of the stabilized ylide, carboxymethylene triphenylphosphorane, with 1-diazoheptan-2-one in the presence of silver benzoate

(Scheme) produced the α-allenic ester (IXa). Unfortunately, however, this could not be hydrolysed to the corresponding acid (IXb) without isomerization of the allenic acid to the β-acetylenic acid. Alternatively we have found that the allenic ester (VII) can be obtained directly from the steroidal phosphorane ester (VIII), via the Wittig reaction with the ketene formed in situ from 1-diazoheptan-2-one.

The esterifications were performed by reaction of the corresponding acid chlorides with the thallium derivative of norethisterone, prepared either with thallous ethoxide (5,6) or with thallous carbonate.

EXPERIMENTAL

E-5-Methylhexa-2,4-dienoic acid:

A solution of ethyl E,E-hexa-2,4-dienoate (180 g) in CH_2Cl_2 (1.8 l) and MeOH (69.4 ml) was ozonized at -30°C. Then, Me_2S (198 ml) was added and the reaction mixture stirred for 16 h. at room temperature. After washing the solution with aqueous $NaHCO_3$ and elimination of the solvent, ethyl 4-oxobut-2-enoate was obtained by vacuum distillation (70-2°C, 11 mm Hg), 124 g. NMR ($CDCl_3$): 9.83 (1H, dd), 6.9 (2H, m), 4.32 (2H, q), 1.34 (3H, t) δ.

n-BuLi in n-hexane (0.35 mole) was added at 0°C under argon to a THF solution (1.2 l) of isopropyl triphenylphosphonium bromide (0.6 mole) and the reaction was kept at room temperature for 2 h. A solution of ethyl 4-oxobut-2-enoate (0.75 mole) in THF (150 ml) was added dropwise after cooling the reaction mixture to -20°C. The mixture was then kept for 2 h. at room temperature, filtered and worked up as usual. The crude material was distilled under vacuum (78-80°C, 5.5 mm Hg) to afford ethyl E-5-methylhexa-2,4-dienoate, 33 g. NMR ($CDCl_3$): 7.83, 7.63, 7.58, 7.39 (1H), 6.10, 5.90, 5.65 (2H), 4.22 (2H, q), 1.88 (6H, s), 1.29 (3H, t) δ. MS: m/e 154 (M^+), 139, 111.

A solution of this ester (30 g) in KOH/MeOH (600 ml, 4%) was refluxed for 2 h., the mixture poured into water, extracted with ether and acidified with dil. HCl. The liberated acid was extracted with ethyl acetate and after concentration in vacuum, E-5-methylhexa-2,4-dienoic acid was obtained, 20.5 g. NMR ($CDCl_3$): 11.10 (1H, s), 7.93, 7.72, 7.68, 7.48 (1H), 6.15, 5.90, 5.65 (2H), 1.90 (6H, s) δ. MS: m/e 126 (M^+).

E-Non-4-en-6-ynoic acid:

A mixture of Z and E-5-bromopent-4-enoic acids was obtained by alky-

lation of diethyl malonate with the isomeric mixture of 1,3-dibromopropenes followed by hydrolysis and decarboxylation of the intermediate products. The desired isomer, E-5-bromopent-4-enoic acid, was isolated from the mixture by the following procedure: the mixture (0.163 mole) in methanol (25 ml) was treated with an aqueous solution of KOH (0.356 mole). The reaction mixture was heated at 50°C for 30 min., cooled, acidified with conc. HCl and extracted with ether. The ethereal phase was washed with water, dried over sodium sulphate and evaporated to give a crystalline residue containing pent-4-ynoic acid and E-5-bromopent-4-enoic acid as major products. Pent-4-ynoic acid was distilled off under reduced pressure (2 mm Hg, with the bath temperature below 100°C) and the residue was crystallized from n-hexane to give E-5-bromopent-4-enoic acid, 12.1 g (45%), m.p. 56-8°. This was converted to the methyl ester with CH_2N_2.

But-1-yne (0.24 mole) was condensed at -78°C under argon, diluted with THF (80 ml) and treated dropwise with n-BuLi (0.23 mole) in hexane. A solution of $ZnCl_2$ (0.24 mole) in THF was added and the reaction mixture was allowed to warm to room temperature. After 1 h., a solution of E-5-bromopent-4-enoic ester (0.12 mole) in THF (25 ml) was added, followed by a solution of catalyst which was prepared by adding diiso-butyl aluminium hydride (4.4 ml) to a suspension of $Pd(PPh_3)_2Cl_2$ (6.2 g) in THF (160 ml). The reaction mixture was left at room temperature overnight, the solvent evaporated and the residue washed with 2N HCl and extracted with petroleum ether. The organic layer was dried over $MgSO_4$ and filtered through a short column of alumina (act. III) and after removal of the solvent the crude ester (19.7 g) was obtained. Bulb-to-bulb distillation (55°C, 0.5 mm Hg) afforded pure E-non-4-en-6-ynoic acid methyl ester, 18.5 g (93%). NMR ($CDCl_3$): 1.14 (3H, t, J = 7Hz), 2.0-2.6 (6H, m), 3.61 (3H, s), 5.41 (1H, d, J ≅ 16Hz), 6.0 (1H, m) δ.

The methyl ester (18 g) was dissolved in methanol and stirred at room temperature for 3 h. with a solution of KOH (9 g) in water (12 ml). The reaction mixture was diluted with water and extracted with ether, then the aqueous solution acidified and the product taken up in ether. After drying over $MgSO_4$ and removal of the solvent, the crystalline E-non-4-en-6-ynoic acid was recrystallized from n-pentane, 13.6 g (82%), m.p. 76.5-78°C. IR (KBr): 3300-2700, 2215, 1700, 1630, 1320, 965 cm^{-1}. NMR (CCl_4): 1.08 (3H, t, J = 7Hz), 2.0-2.6 (6H, m), 5.32 (1H, d, J = 16 Hz), 5.87 (1H, m), 11.0 (1H, s) δ.

Anal: $C_9H_{12}O_2$ requires C: 71.03 H: 7.95
Found C: 70.98 H: 7.89

Norethisterone nona-2,3-dienoate (VII):

To a solution of norethisterone (10 g) in dry dioxane (45 ml) and symcollidine (25 ml) at 70°C a hot solution of chloroacetic anhydride (20 g) in dry dioxane (5 ml) was added. The mixture was stirred at 70-5°C for 1 h. and left at room temperature overnight. The mixture was extracted with dichloromethane-ether, washed with water and 2N HCl, and the extract diluted with pentane to remove tars. After filtration through silica gel and removal of the solvents, crude norethisterone

chloroacetate was obtained, 13 g. A solution of triphenylphosphine (9 g) and the chloroacetate (13 g) in benzene (80 ml) was refluxed until the insoluble phosphonium salt was formed. The precipitate was dissolved in water and washed with ether to remove non-polar impurities. The water extract was made alkaline with 1N NaOH. The oily phosphorane (VIII) was recovered by extraction with dichloromethane, 11.6 g (58%). NMR (CCl_4): 2.55 (1H, s), 5.82 (1H, s), 7.3-8.0 (15H, m) δ.

A solution of the phosphorane (VIII) (0.15 mole) in THF (500 ml) was added at -20°C to a solution of 1-diazoheptan-2-one (0.18 mole), prepared from pentanoyl chloride and diazomethane, in THF (100 ml). This mixture was further treated with silver benzoate (0.037 mole) in dry dimethyl sulphoxide (70 ml). The reaction was allowed to warm up to room temperature overnight and after concentration under reduced pressure the mixture was extracted with ether. The organic phase was washed, dried and evaporated and the crude product purified by column chromatography on silica gel, eluted with benzene/ethyl acetate (50:1), yielding the ester (VII) (39%) as an oil, $[\alpha]_D$ + 56° ($CHCl_3$)

UV: 239 nm (MeOH), ε 17600.
IR: (KBr): 3250, 1960, 1720, 1670, 1620, 1262, 1155, 910, 733 cm^{-1}
NMR: (CCl_4): 0.88 (3H, s), 2.55 (1H, s), 5.4-5.7 (2H, m), 5.81 (1H, s) δ
MS: m/e 434 (M^+).

General procedures for the preparation of acid chlorides:

a) Oxalyl chloride (0.19 mole) was added dropwise at 0°C to a solution of the acid (0.15 mole) in dry benzene (40 ml) and pyridine (7 drops). The mixture was allowed to warm to room temperature and was then heated to 35-40°C for 1 h. In order to eliminate the excess of oxalyl chloride, benzene was distilled off (vacuum, 30-40°C) and further benzene (40 ml) added and distilled off twice more. The acid chloride was purified by vacuum distillation.

b) The acid (0.0724 mole) in dry benzene (50 ml) was cooled in an ice bath and thionyl chloride (0.212 mole) added dropwise. The mixture was stirred overnight at room temperature, the benzene removed under reduced pressure and three subsequent portions of benzene (80 ml) added and distilled out to remove excess thionyl chloride. The residue was then vacuum distilled.

General procedures for the esterification of norethisterone:

a) Thallous ethoxide (43 mmole) was added at room temperature under nitrogen to a solution of norethisterone (29 mmole) in dry benzene (800 ml). Benzene (500 ml) was distilled off whilst being continuously replaced with the same volume of fresh benzene (approx. 2.5 h). The mixture was cooled to room temperature and after addition, with stirring, of the acid chloride (43 mmole) in dry benzene (100 ml), was refluxed for 2 h. The precipitated thallium chloride was filtered off and the benzene solution thoroughly washed with water. After evaporation of the solvent

under reduced pressure, the crude mixture was purified by preparative thin layer chromatography or column chromatography.

b) Norethisterone (40 mmole) was dissolved under argon in dry toluene (600 ml) together with thallium carbonate (34 mmole) and dibenzo-18-crown-6 (1.5 g). The suspension was heated to reflux and toluene (250 ml) distilled off. After cooling to room temperature, another 200 ml of dry toluene was added, and the acid chloride (45 mmole) in dry toluene (50 ml) was then added dropwise. The reaction flask was fitted with a Soxhlet filled with 3A molecular sieves as a water trap and the suspension was refluxed for 8 h. with vigorous stirring. After cooling, the mixture was filtered over silica, which was washed with dichloromethane. The combined organic solutions were evaporated under reduced pressure and the residue chromatographed on silica.

Norethisterone E-penta-2,4-dienoate (I): m.p. 250°C(dec), $[\alpha]_D$ + 18°

UV: 245 nm (EtOH), ε 40300.
IR: (KBr): 3260, 1720, 1660, 1635, 1620, 1600 cm^{-1}
NMR: ($CDCl_3$): 7.5-5.4 (4H, m), 5.83 (1H, s) 2.62 (1H, s), 0.96 (3H, s) δ.
MS: m/e 378(M^+), 363, 297.
Anal: $C_{25}H_{30}O_3$ requires C: 79.33 H: 7.99
Found C: 79.30 H: 7.91

Norethisterone E,E-hexa-2,4-dienoates (II): m.p. 164-6°C, $[\alpha]_D$ + 40°.

UV: 254 nm (EtOH), ε 48,000.
IR: (KBr): 3240, 1722, 1670, 1645, 1620 cm^{-1}
NMR: ($CDCl_3$): 7.38, 7.13 (1H, m), 6.16 (2H, m), 5.77 (1H, d), 5.84 (1H, s), 2.60 (1H, s), 1.84 (3H, d), 0.95 (3H, s) δ.
MS: m/e 392(M^+), 377, 297, 283.
Anal: $C_{26}H_{32}O_3$ requires C: 79.56 H: 8.22
Found C: 79.44 H: 8.18

Norethisterone E, E-2-methylhexa-2,4-dienoate (III): m.p. 177-180°C, $[\alpha]_D$ +60°.

UV: 255 nm (EtOH), ε 30700.
IR: (KBr): 3290, 2100, 1710, 1675, 1640, 1615 cm^{-1}
NMR: ($CDCl_3$): 7.15 (1H, d), 6.26 (2H, m), 5.87 (1H, s), 2.60 (1H, s), 1.92 (3H, s), 1.89 (3H, d), 0.99 (3H, s) δ.
MS: m/e 406(M^+), 391, 362, 297, 283.
Anal: $C_{27}H_{34}O_3$ requires C: 79.77 H: 8.43
Found C: 79.70 H: 8.39

Norethisterone E-5-methylhexa-2,4-dienoate (IV): m.p. 193-5°C, $[\alpha]_D$ +32°.

UV: 246 nm (EtOH), ε 20300.
IR: (KBr): 3245, 1725, 1670, 1640, 1620 cm^{-1}
NMR: ($CDCl_3$): 7.82, 7.63, 7.58, 7.38 (1H), 6.11, 5.88, 5.63 (2H), 2.61 (1H, s), 1.88 (6H, s), 0.86 (3H, s) δ.
MS: m/e 406(M^+), 391, 297, 283.

Anal: $C_{27}H_{34}O_3$ requires C: 79.77 H: 8.43
Found C: 79.70 H: 8.41

Norethisterone E-non-4-en-6-ynoate (V): oil, $[\alpha]_D$ -10°, c.d. (MeOH) $\Delta\varepsilon$= -1.53 (315 nm), +6.8 (240 nm).
UV: 238 nm (MeOH), ε 29600.
IR: (KBr): 3280, 1742, 1670, 1622, 1167, 965 cm^{-1}
NMR: (CCl_4): 6.06 (1H, m), 5.85 (1H, s), 5.48 (1H, d, J = 16Hz), 2.62 (1H, s), 1.15 (3H, t, J = 7Hz), 0.93 (3H, s) δ.
MS: m/e 432 (M^+).
Anal: $C_{29}H_{36}O_3$ requires C: 80.52 H: 8.59
Found C: 80.32 H: 8.38

Norethisterone E-non-2-en-4-ynoate (VI): m.p. 41-3°C, $[\alpha]_D$ + 40°.
UV: 242 nm (EtOH), ε 19300.
IR: ($CHCl_3$): 3330, 2200, 1720, 1660, 1620, 960 cm^{-1}.
NMR: ($CDCl_3$): 6.86-7.03 (1H, m), 6.10 (1H, d, J = 16Hz), 5.82 (1H, bs), 2.60 (1H, s), 0.94 (3H, t) δ.
MS: m/e 432 (M^+), 297, 283, 135.

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