Synthesis and Biological Activity of 9α-Hydroxy-11β-Nitro-1,3,5(10)-Estratrienes; a New Class of Potent Estrogenic Steroids

In the course of our investigations on nitro steroids, we prepared a new series of estrogens with a hydroxy group in the 9α- and a nitro group in the 11β-position. We report in this communication the preparation, the structure and the biological properties of some of the compounds of this series.

The 3-acetoxy-1,3,5(10),9(11)-estratetraen-17-one (I)[1] dissolved in anhydrous ethyl ether, was treated for 30 min at 0 °C with nitric acid ($d = 1.52$); after washing the organic layer with aqueous sodium bicarbonate solution and usual work-up, 11β-nitro-1,3,5(10)-estratriene-3,9α-diol-17-one 3-acetate[2] (II; mp 235–240 °C; $[\alpha]_D$ + 36°; λ_{max} 268, 273 nm; ε = 800, 700) was obtained. Its structure follows from elemental analysis (addition of 1 molecule of HNO_3 to the starting compound), the IR-spectrum (3740 cm^{-1}, hydroxy group: 1760 cm^{-1}, 3-acetate: 1740 cm^{-1}, 17-ketone: 1550 cm^{-1}, nitro group) and the NMR-spectrum which supports the β-axial position of the nitro group at C-11 (Chart 1).

Dehydration of II with acetic anhydride and *p*-toluene-sulphonic acid in acetic acid for 3 h at room temperature gave 11β-nitro-1,3,5(10),8(9)-estratetraen-3-ol-17-one acetate[3] (III; mp 190–193 °C; λ_{max} 271 nm; ε = 12,900); its IR-spectrum (no hydroxyl bands and a band at 1550 cm^{-1} due to a nitro group in a saturated position) and NMR-spectrum (Chart 1) demonstrate that elimination of the hydroxy group introduces a double bond between carbon atoms 8 and 9.

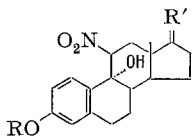
This easy dehydration points to an α-axial configuration of the 9-hydroxy group (trans-diaxial elimination with the axial 8β-hydrogen)[4].

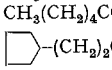
Alkaline hydrolysis of II gave the corresponding 3-hydroxy derivative IV (mp 225–228 °C; λ_{max} 279, 285 nm; ε = 1940, 1700) whereas $NaBH_4$ reduction furnished the 3,17β-diol derivative V[5], which by acetylation with acetic anhydride in pyridine furnished the diacetate VI (mp 215–218 °C; λ_{max} 268, 275 nm; ε = 1230, 1060).

Chart I

Compound	τ	H	Multiplicity	J (c/s)	Assignment
II	2.82	1	Doublet (half of AB)	6.5	CH-1 *orto* splitting to CH-2
	3.07	1	singlet (overlaps inner line of B)	–	CH-4
	3.13	1	doublet (half of AB)	6.5	CH-2 *orto* splitting to CH-1
	4.77	1	quartet (X of ABX)	8 $J_{11eq,12eq} + J_{11eq,12ax}$	CH-11eq
III	3.00	3	singlet	–	–
	3.92	1	quartet (X of ABX)	9 $J_{11eq,12eq} + J_{11eq,12ax}$	CH-11eq

Table I



Compound	R	R'
II	CH_3CO	O
IV	H	O
V	H	αH, βOH
VI	CH_3CO	αH, β$OCOCH_3$
VII	$CH_3(CH_2)_4CO$	O
VIII		O
IX	$ClCH_2CH_2$	O
X		O
XI	CH_3	O
XII	CH_3	αC≡CH, βOH
XIII	CH_3	αH, β$OCOCH_3$
XIV	CH_3	αH, β$OCO(CH_2)_2$ 

With the same reaction described above, starting from the corresponding 1,3,5(10),9(11)-estratetraenes[6], the following 9α-hydroxy-11β-nitro derivatives were prepared; 11β-nitro-1,3,5(10)-estratriene-3,9α-diol-17-one 3-hexanoate (VII; mp 151–153 °C; λ_{max} 268, 275.5 nm; ε = 870, 750; $[\alpha]_D$ + 26°); 11β-nitro-1,3,5(10)-estratriene-3,9α-diol-17-one 3-cyclopentylpropionate (VIII; mp 193 to 195 °C; λ_{max} 267, 275.5 nm; ε = 910, 750; $[\alpha]_D$ + 25°); 3-(2'-chloroethoxy)-11β-nitro-1,3,5(10)-estratrien-9α-ol-17-one (IX; mp 199–200 °C; λ_{max} 277, 285 nm; ε = 1600,

[1] K. TSUDA, E. OHKI and S. NOZOE, J. org. Chem. *28*, 786 (1963).

[2] Satisfactory analytical values have been obtained for all the compounds described; melting points were taken on a Fisher-Johns block and are uncorrected; rotations were determined at 20–22° in $CHCl_3$ at the sodium D-line; UV-spectra were run in 95% ethanol.

[3] For 3-methoxy-1,3,5(10), 8-estratetraenes, λ_{max} of 273–275 nm with ε = 15,900–18,500 are reported by L. DORFMAN, Chem. Rev. *53*, 47 (1953).

[4] K. TSUDA, S. NOZOE and Y. OKADA, Chem. pharm. Bull, Tokyo *11*, 1022 (1963), reported that addition of HBrO to 3-acetoxy-1,3,5(10),9(11)-estratetraene gives the 9α-hydroxy-11β-bromo derivative.

[5] Compound V did not crystallize but gave a single spot in thin-layer chromatography; it was directly converted to VI.

[6] All the starting compounds were prepared from 3-hydroxy-1,3,5(10),9(11)-estratetraen-17-one by employing the usual reactions; the intermediates were checked by UV- and IR-spectra and by thin-layer chromatography and used without further purification for the nitration reaction.

1340; $[\alpha]_D$ + 28°); 3-cyclopentoxy-11β-nitro-1,3,5(10)-estratrien-9α-ol-17-one (X; mp 218–223 °C; λ_{max} 277 nm; ε = 1540; $[\alpha]_D$ + 27°); 3-methoxy-11β-nitro-1,3,5(10)-estratrien-9α-ol-17-one (XI; mp 218–221 °C; λ_{max} 277.5, 285 nm; ε = 1860, 1640; $[\alpha]_D$ + 32°); 3-methoxy-17α-ethynyl-11β-nitro-1,3,5(10)-estratriene-9α,17β-diol (XII; amorphous; λ_{max} 277.5, 285 nm; ε = 2480, 2200; $[\alpha]_D$ −83°); 3-methoxy-11β-nitro-1,3,5(10)-estratriene-9α,17β-diol 17-acetate (XIII; mp 180–182 °C; λ_{max} 277, 284 nm; ε = 2030, 1770; $[\alpha]_D$ − 49°); 3-methoxy-11β-nitro-1,3,5(10)-estratriene-9α,17β-diol 17-cyclopentylpropionate (XIV; mp 145–146 °C; λ_{max} 277.5, 285 nm; ε = 1650, 1470; $[\alpha]_D$ − 28°).

Table II. Estrogenic activity of 9α-OH, 11β-NO_2-steroids

Compound	Minimal effective dose (μg)		
	s.c.	os	os/s.c.
Estrone	3	100	33
Estradiol	0.3	30	100
Ethinylestradiol	0.3	3	10
II	3	3	1
IV	3	1	0.3
V	30	30	1
VII	3	3	1
VIII	3	3	1
IX	3	3	1
X	1	1	1
XI	3	3	1
XII	1	1	1
XIII	3	1	0.3
XIV	3	3	1

The compounds listed in Table I were assayed for estrogenic activity in castrated female rats either by s.c. or by oral route. The estrogenic effect was determined by the modifications of the vaginal cytology changing from the diestrous pattern (negative smear) to the proestrus-estrus type (positive smear). The minimum effective dose (MED) was considered as the one producing a positive smear in 1 out of 3 rats or in 2 out of 5.

As shown in Table II, all the compounds of the series display a high estrogenic activity and show MED of the same order by s.c. and by oral route (os) (ratio os/s.c. $\simeq$ 1). The reference compounds are, instead, much less active by oral than by s.c. administration, thus giving higher os/s.c. ratios.

In conclusion, the 9α-OH, 11β-NO_2-steroids are potent estrogenic agents by oral route, despite the fact that they do not possess the 17α-ethynyl group which is generally introduced into a steroidal molecule in order to achieve oral activity.

The absence of the 17α-alkyl group seems to be interesting in view of a possible clinical application, since 17α-alkyl steroids are generally considered to impair the liver function.

Riassunto. Per azione dell'acido nitrico su 1,3,5(10),9(11)-estratetraeni sono stati ottenuti 9α-idrossi-11β-nitro-1,3,5(10)-estratrieni, i quali hanno mostrato una interessante attività estrogena sia per via orale che per via sottocutanea.

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Anaesthetic Properties of Some Esters of 2-Piperidylcarbinols

Anaesthetic activity was unexpectedly found among some diphenylacetyl esters of 2-piperidylcarbinols synthetized for pharmacological screening: subsequent work on these new and related compounds, reported in the Table shed some light on the structure-activity relationship and the conclusions are briefly reported here.

Chemistry. The amino alcohols employed in the synthesis of the compounds reported in the Table were obtained according to one of the following procedures: (a) Condensation between pyridine and acetone[1], cyclopentanone[2] or cyclohexanone[2] in the presence of Mg/$HgCl_2$ afforded the 2-pyridylcarbinols which were reduced (PtO_2 in EtOH) to give the corresponding 2-piperidylcarbinols. Equimolar amounts of a 2-piperidylcarbinol and an aldehyde (mostly formaldehyde) were hydrogenated at 30 atm in EtOH with Pd/C catalyst to yield the N-alkyl-2-piperidylcarbinols used in the preparation of compounds 1,4÷11. (b) The methyl ester of D or L 1-methylpipecolic acid[3], *cis* or *trans* 1,6-dimethylpipecolic acid[4] or 1-methylproline[5] was added to a large excess of an ethereal solution of CH_3MgBr. In these conditions, inversion at C_2 was minimized and in this way the aminoalcohols employed for the preparation of compounds 2, 3, 14, 15, 16 were obtained. (c) 1-methyl-2-piperidylcarbinol[6] and 1α-dimethylpiperidylcarbinol[7] (unknown isomer) used in the synthesis of compounds 13 and 12, were prepared according to the literature.

The aminoalcohols were esterified in pyridine with the appropriate acyl chloride to give the esters reported, with the exception of compound 7 which was obtained by reduction of the nitrobenzoyl ester and compound 9 which was obtained by brief hydrolysis of the diphenylchloroacetyl ester as reported by King and Holmes[8].

Pharmacology. Local anaesthetic activities of the compounds reported in the Table have been evaluated according to the Chance-Lobstein[9] test (corneal anaesthesia). Each substance was tested at varying concentrations by the administration of 0.1 ml of a 0.85% saline solution under the eyelids of guinea-pigs.

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