g.) in pyridine (2 ml.) was added propionic anhydride (1 ml.), and the mixture was allowed to stand at room temperature overnight. Addition of water gave an oil which solidified on standing (ice cooling). The solid was collected by filtration and washed with water. Several crystallizations from methanol–water, followed by two crystallizations from ether–petroleum ether gave 0.05 g. of the 3-propionate IIc; m.p. 105–106.5°; ν_{max} 3509, 1745, 1727, 1667, 1205, and 1089 cm.$^{-1}$; $[\alpha]_D^{24}$ −18° (c, 1.360, chloroform).

Anal. Calcd. for $C_{23}H_{36}O_3$ (360.52): C, 76.62; H, 10.07. Found: C, 76.22, H, 10.09.

Acknowledgment. We are indebted to Messrs. Louis M. Brancone, Samuel S. Modes, John G. Heider, and Arthur A. Bodden for the microanalytical data, and to Messrs. William Fulmor and George Morton and Miss Anne Callaghan for the optical rotation data and the infrared absorption spectra.

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Esters of 17α-Ethinylandrostane-3β,17β-diol and 17α-Ethinylandrost-5-ene-3β,17β-diol

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As part of a continuing endocrinological screening program being carried out in these laboratories, an extensive investigation has been made of two series of ester derivatives based upon 17α-ethinylandrostane-3β,17β-diol and 17α-ethinylandrost-5-ene-3β,17β-diol, respectively. A number of these compounds have been shown to possess outstanding activity as pituitary inhibitors.[1]

The parent 17α-ethinyl steroid diols and their acetates were originally prepared by Ruzicka and Hoffmann;[2] the pituitary inhibiting properties of these types apparently have heretofore not been observed. In the present work it was found that while the known diols and their 3-monoacetates were active pituitary inhibitors, this activity was negated by a high degree of estrogenicity. An increase in the size of the ester group in the 3-monoesters led to a greatly increased activity ratio (defined as the ratio of pituitary inhibition to estrogenicity). The most favorable activity ratio was found with 17α-ethinylandrost-5-ene-3β,17β-diol 3-(3-cyclohexylpropionate) (Ethandrostate). The 3,17-diesters were found to be nearly inactive as pituitary inhibitors; apparently a free 17β-hydroxy is a prerequisite for activity in this series.

The new 3-monoesters were prepared by standard procedures; such minor modifications as were used were induced by the ease of diesterification with acylating agents such as the acyl halides. Frequently it was necessary to purify the esters by chromatography during the initial preparations, but it was subsequently found that experimental conditions could be worked out for each individual ester (usually an adjustment of the mole ratio of reactants and the time of reaction) which would lead to a high yield of pure product by direct recrystallization. The new esters are summarized in Tables I and II and in the Experimental Section.

EXPERIMENTAL[3]

3-Mono-esters of 17α-ethinylandrostane-3β,17β-diol and 17α-ethinylandrost-5-ene-3β,17β-diol. The 3-mono esters were prepared from the parent diols by acylation with either an acid anhydride or an acid chloride in pyridine solution, as shown in examples below.

Method A. Acid anhydrides. To a solution of 31.45 g. (0.100 mole) of 17α-ethinylandrost-5-ene-3β,17β-diol in 150 ml. of C.P. pyridine was added 44.1 g. (0.150 mole) of cyclohexylpropionic anhydride. The resulting clear solution was allowed to stand at room temperature for 60 to 70 hr., after which period it was quenched in 1500 ml. of water. After 1 hr., the mixture was extracted three times with methylene dichloride and the extracts were washed with dilute sulfuric acid and sodium bicarbonate solutions. After drying, the methylene dichloride was evaporated *in vacuo*, the residue was dissolved in 500 ml. of hot *n*-hexane, and the solution was filtered[4] and concentrated to 300 ml. for crystallization. One additional recrystallization from *n*-hexane gave 38.22 g. (86%) of the pure 3-(3-cyclohexylpropionate), crystallizing in rosettes of large, fernlike masses.

With acid anhydrides of lower molecular weight the time of reaction and the proportion of anhydride were both reduced; *e.g.*, with propionic anhydride there was used 1.3 moles of anhydride and a time of 17 hr. (83% yield).

Method B. Acid chlorides. To a cold (0°) solution of 3.16 g. (0.01 mole) of 17α-ethinylandrostane-3β,17β-diol in 20 ml. of pyridine was slowly added 1.48 g. (0.011 mole) of α-ethyl-*n*-butyryl chloride. The resulting deeply colored heterogenous mixture was held at room temperature for 5 hr. and then quenched in 500 ml. of water. After 1 hr. the resulting slurry was filtered and the semicrystalline material was washed thoroughly with water and dried at 50° *in vacuo*. The product was chromatographed on 240 g. of silica gel as usual. Elution with 5 to 10% ether—pentane mixtures gave a trace amount of the diester; further elution with a 20% ether—pentane mixture gave the 3-mono-ester. One recrystallization from methanol resulted in pure material, in about 50% over-all yield.

17α-Ethinylandrost-5-ene-3β,17β-diol 3-acid succinate. A mixture of 3.14 g. (0.01 mole) of 17α-ethinylandrost-5-ene-3β,17β-diol, 1.50 g. (0.015 mole) of succinic anhydride and 25 ml. of C.P. pyridine was refluxed for 3 hr. After quenching in 500 ml. of water, the greyish solid was filtered off, washed thoroughly with water and dried at 70°. Two recrystallizations from methanol gave 2.56 g. of material, crystallizing in clusters of flattened needles.

The *diethanolamine salt*, prepared by mixing equimolecular amounts of the components in hot acetone, formed slender,

(1) A. L. Beyler and R. O. Clinton, *Proc. Soc. Exper. Biol. Med.*, **92**, 404 (1956).

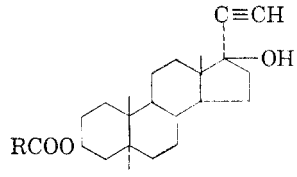
(2) L. Ruzicka and K. Hoffmann, *Helv. Chim. Acta*, **20**, 1280 (1937).

(3) All melting points are corrected; they were determined in a modified Hershberg apparatus using total immersion N.B.S.—calibrated thermometers. The analyses were done by Mr. K. D. Fleischer and staff.

(4) Insoluble material at this point is the starting diol.

TABLE I

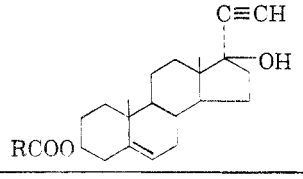
3-MONOESTERS OF 17α-ETHINYLANDROSTANE-3β,17β-DIOL



R	M.P., °C.	$[\alpha]_D^{25}$ 1% in $CHCl_3$ ± 0.4°	Formula	Analyses, % C Calcd.	C Found	H Calcd.	H Found
CH_3CH_2	174.7–177.2	−43.3	$C_{24}H_{36}O_3$	77.37	77.56	9.74	10.07
$CH_3(CH_2)_2$	149.8–154.6	−36.1	$C_{25}H_{38}O_3$	77.67	77.68	9.91	10.10
$CH_3(CH_2)_3$	164.2–166.6	−34.5	$C_{26}H_{40}O_3$	77.95	77.65	10.07	9.91
$CH_3(CH_2)_4$	118.6–121.6	−34.3	$C_{27}H_{42}O_3$	78.21	78.39	10.21	10.50
$CH_3(CH_2)_5$	89.8–91.4	−32.1	$C_{28}H_{44}O_3$	78.45	78.54	10.35	10.21
$CH_3(CH_2)_6$	67.8–70.8	−30.2	$C_{29}H_{46}O_3$	78.73	77.70	10.46	10.74
$CH_3(CH_2)_7$	91.0–92.6	−30.0	$C_{30}H_{48}O_3$	78.89	78.77	10.59	10.47
$CH_3(CH_2)_8$	72.2–74.4	−29.3	$C_{31}H_{50}O_3$	79.10	79.04	10.71	10.78
$(CH_3CH_2)_2CH$	147.6–152.0	−32.3	$C_{27}H_{42}O_3$	78.21	78.26	10.21	9.84
$(CH_3)_2CHCH_2CH_2$	142.6–144.2	−34.1	$C_{27}H_{42}O_3$	78.21	78.53	10.21	10.50
$(CH_3)_3C$	216.2–218.0	−37.9	$C_{26}H_{40}O_3$	77.95	78.09	10.07	10.19
C_6H_{11}	201.6–203.4	−30.4	$C_{28}H_{42}O_3$	78.82	78.94	9.92	10.20
$C_6H_{11}CH_2CH_2$	91.8–94.2	−30.4	$C_{30}H_{46}O_3$	79.24	79.60	10.20	10.12
$C_6H_{11}(CH_2)_5$	83.6–85.4	−28.5	$C_{33}H_{52}O_3$	79.78	79.68	10.55	10.83
$C_5H_9CH_2CH_2$	125.0–127.5	−30.0	$C_{29}H_{44}O_3$	79.04	79.31	10.07	10.14

TABLE II

3-MONO ESTERS OF 17α-ETHINYLANDROST-5-ENE-3β,17β-DIOL



R	M.P., °C.	$[\alpha]_D^{25}$ 1% in $CHCl_3$, ± 0.4°	Formula	Analyses, % C Calcd.	C Found	H Calcd.	H Found
CH_3CH_2	149.7–152.2	−108.6	$C_{24}H_{34}O_3$	77.80	78.10	9.25	9.13
$CH_3(CH_2)_2$	115.4–117.0	−102.5	$C_{25}H_{36}O_3$	78.08	78.14	9.44	9.58
$CH_3(CH_2)_3$	129.0–131.2	−98.8	$C_{26}H_{38}O_3$	78.35	78.27	9.61	9.72
$CH_3(CH_2)_4$	106.6–107.4	−94.2	$C_{27}H_{40}O_3$	78.59	78.32	9.77	9.89
$CH_3(CH_2)_6$	[a]	−93.2	$C_{28}H_{42}O_3$	78.82	78.60	9.92	9.69
$CH_3(CH_2)_6$	94.0–95.6	−87.4	$C_{29}H_{44}O_3$	79.04	79.22	10.06	10.30
$CH_3(CH_2)_7$	91.6–93.4	−84.5	$C_{30}H_{46}O_3$	79.25	79.24	10.20	10.37
$(CH_3CH_2)_2CH$	171.4–173.8	−93.5	$C_{27}H_{40}O_3$	78.59	78.58	9.77	10.07
$(CH_3)_2CH$	168.2–170.4	−102.4	$C_{25}H_{36}O_3$	78.08	78.30	9.44	9.49
$(CH_3)_2CHCH_2$	[b]	−96.9	$C_{26}H_{38}O_3$	78.35	78.52	9.61	9.84
$(CH_3)_2CHCH_2CH_2$	135.6–138.4	−92.7	$C_{27}H_{40}O_3$	78.59	78.41	9.77	9.49
$(CH_3)_3C$	200.0–202.6	−97.2	$C_{26}H_{38}O_3$	78.35	78.40	9.61	9.85
$C_5H_9CH_2CH_2$	129.7–131.2	−86.0	$C_{29}H_{42}O_3$	79.41	79.30	9.66	9.65
C_6H_{11}	175.4–178.8	−87.5	$C_{28}H_{40}O_3$	79.20	79.36	9.50	9.90
$C_6H_{11}CH_2$	191.8–194.8	−83.6	$C_{29}H_{42}O_3$	79.40	79.24	9.65	9.70
$C_6H_{11}CH_2CH_2$	115.2–116.6	−83.8	$C_{30}H_{44}O_3$	79.60	79.74	9.80	9.67
$C_6H_{11}(CH_2)_3$	108.8–110.4	−81.2	$C_{31}H_{46}O_3$	79.78	79.76	9.94	10.25
$C_6H_{11}(CH_2)_4$	98.0–101.8	−79.5	$C_{32}H_{48}O_3$	79.95	80.15	10.07	9.88
$C_6H_{11}(CH_2)_5$	106.2–107.0	−72.7	$C_{33}H_{50}O_3$	80.11	80.40	10.19	10.42
$HOOC(CH_2)_2$	203.0–204.4	−92.6	$C_{25}H_{34}O_5$	72.43	72.20	8.27	8.32

[a] Melts at 89.0–91.0°, solidifies and remelts at 97.0–97.2°. [b] Melts at 119.4–120.9°, solidifies and remelts at 126.0–127.8°.

blunt needles from absolute alcohol-ether, m.p. 154.2–157.2°; $[\alpha]_D^{25}$ −34.5° (2% in alcohol).

Anal. Calcd. for $C_{29}H_{45}NO_7$: C, 67.02; H, 8.73; O, 21.55. Found: C, 66.97; H, 8.60; O, 21.10.[5]

(5) Determined directly.

17α-Ethinylandrost-5-ene-3β,17β-diol 3,17-dipropionate. To a solution of 400 mg. of *p*-toluenesulfonic acid monohydrate in 15 ml. of redistilled propionic acid and 25 ml. of redistilled propionic anhydride was added 3.14 g. (0.01 mole) of 17α-ethinylandrost-5-ene-3β,17β-diol. The heterogeneous mixture was shaken mechanically until solution was complete

(15 min., slightly exothermic). After standing at room temperature for 18 hr. the deep lilac-colored solution was quenched in 800 ml. of water, allowed to stand for 1 hr., and filtered. The washed and dried crude product crystallized from *n*-hexane in long rods, m.p. 139.4–141.2°; $[\alpha]_D^{25}$ −99.4° (1% in $CHCl_3$). The yield was 3.18 g.

Anal. Calcd. for $C_{27}H_{38}O_4$: C, 76.02; H, 8.98. Found: C, 76.30; H, 8.75.

17α-Ethinylandrost-5-ene-3β,17β-diol 3,17-di(3-cyclohexylpropionate). A mixture of 3.14 g. (0.01 mole) of 17α-ethinylandrost-5-ene-3β,17β-diol, 8.9 g. (0.03 mole) of cyclohexylpropionic anhydride and 50 ml. of C.P. pyridine was refluxed for 18 hr. After the usual workup, the product was chromatographed on 250 g. of silica gel. The diester was eluted with 5% ether-pentane and recrystallized from alcohol, m.p. 114.0–115.6°; $[\alpha]_D^{25}$ −61.0° (1% in $CHCl_3$).

Anal. Calcd. for $C_{39}H_{58}O_4$: C, 79.27; H, 9.89. Found: C, 79.47; H, 10.06. The mixed melting point with the 3-monoester was 105–112°.

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Preparation of 2-Nitroisonicotinic Acid Hydrazide and 2-Aminoisonicotinic Acid Hydrazide

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Previous investigators[2] have shown that the introduction of a substituent in the pyridine ring of isonicotinic acid hydrazide usually causes almost complete loss of *in vitro* antituberculous activity. However, the 3-aminoisonicotinic acid hydrazide did show slight activity. For this reason, it seemed worthwhile to prepare and test the isomeric 2-amino derivative for effectiveness.

Another group, the nitro, which has not previously been tested for its effect on antituberculous activity when in the ring, was also introduced into the 2-position and tested.

EXPERIMENTAL[3]

Biological assays. The biological activity of the compounds was determined, using the biologic assay method for isonicotinic acid hydrazide.[4] The inhibitory concentration of 2-nitroisonicotinic acid hydrazide for the standard H37Rv is greater than 10 mcg./ml; that of 2-aminoisonicotinic acid hydrazide is between 2.5 and 5.0 mcg./ml. The standard test organism is inhibited by 0.03 to 0.07 mcg./ml. of isonicotinic acid hydrazide.

2-Amino-4-methylpyridine. Eastman Kodak material recrystallized from hot water was used as the starting material.

2-Nitro-4-methylpyridine. This was prepared by the method of Wiley and Hartman.[5] Oxidation of the 2-amino-4-methylpyridine with persulfuric acid gave a 52% yield of 2-nitro-4-methylpyridine, m.p. 65.5–67° (lit.[5] 61–62°).

2-Nitroisonicotinic acid. This was prepared by the permanganate oxidation of the 2-nitro-4-methylpyridine according to the procedure given by Brown.[6] The yield which was calculated after subtracting the amount of recovered starting material was 26%, m.p. 172.5–173.5° (lit.[6] 175°).

Methyl 2-nitroisonicotinate. One ml. of methanol, 0.4 g. of Victor polyphosphoric acid, and 0.168 g. (0.001 mole) of 2-nitroisonicotinic acid were mixed and heated at reflux for 6 hrs. The methanol was removed *in vacuo* and the acid was neutralized with sodium hydroxide solution. Ether was added to extract the ester. The ether was evaporated to obtain needles, 0.124 g. (68%), m.p. 80–81°. The ester was recrystallized from benzene and washed with petroleum ether before analysis.

Anal. Calcd. for $C_7H_6N_2O_4$: C, 46.16; H, 3.32. Found: C, 46.75; H, 3.27.

2-Nitroisonicotinic acid hydrazide. Methyl 2-nitroisonicotinate (0.036 g., 0.0002 mole) was refluxed with a slight excess of 85% hydrazine hydrate dissolved in 0.6 ml. of ethanol. After 10 min., crystals began to come out of the solution. Heating was continued for 1 hr. The crystals were filtered; yield 0.024 g. (67%), m.p. 181.5–183.5°.

Anal. Calcd. for $C_6H_6N_4O_3$: C, 39.56; H, 3.32. Found: C, 40.01; H, 3.41.

Methyl 2-aminoisonicotinate. The methyl 2-nitroisonicotinate (0.273 g., 0.0018 mole) was reduced by refluxing with excess iron filings in 1 ml. of a solution of 12*N* HCl in methanol (1:5). After 2 hr. the black mixture was filtered and the filtrate neutralized with methanolic sodium hydroxide solution. The solution was evaporated to dryness and extracted with ether. The ether was evaporated to obtain plates, 89 mg. (39%). After recrystallization from benzene, the crystals were pale yellow, m.p. 149.5–151°.

Anal. Calcd. for $C_7H_8N_2O_2$: C, 55.24; H, 5.30. Found: C, 55.90; H, 5.27.

2-Aminoisonicotinic acid hydrazide. Methyl 2-aminoisonicotinate (0.046, 0.0003 mole) was refluxed for 2 hr. with excess 85% hydrazine hydrate in 0.6 ml. of ethanol. On cooling, needles were obtained; yield 14 mg. (31%), m.p. 194.5–195°.

Anal. Calcd. for $C_6H_8N_4O$: C, 47.35; H, 5.30. Found: C, 47.93; H, 5.28.

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(2) J. Bernstein and coworkers, *Am. Rev. Tuberc.*, **67**, 354 (1953).

(3) Biological Assays by P. Z. Morse and B. T. Miyahara. All melting points were taken on a Fisher-Johns melting point block. Microanalyses by Galbraith Microanalytical Laboratories and Huffman Microanalytical Laboratories.

(4) Transactions of the Fifteenth Conference on the Chemotherapy of Tuberculosis, 1956, p. 581.

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(6) E. V. Brown, *J. Am. Chem. Soc.*, **76**, 3167 (1954).

Reaction of D-Glucamine with Aromatic Nitro and Halogen Compounds

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Tamm[3] has shown that *N*-glucosides of some

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