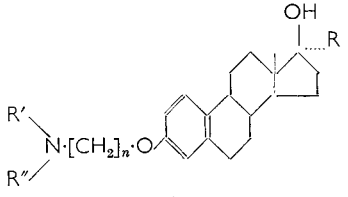
J. Pharm. Pharmacol., 1964, **16**, 717–724 Received June 1, 1964

Genotropic agents : steroid basic ethers

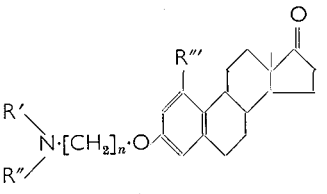
D. D. EVANS, D. E. EVANS, G. S. LEWIS, P. J. PALMER AND D. J. WEYELL

The synthesis and biological activity of a series of basic ethers of the steroid oestrogens is described. The compounds have genotropic (Black, 1961) activity coupled with low oestrogenicity.

THE problem of whether the pituitary-suppressant activity of steroidal oestrogens is due to their oestrogenicity or the result of their molecular structure has not been satisfactorily answered (Dodds, 1961). No aromatic ring-A steroid devoid of oestrogenicity has, so far, shown pituitary-suppressant activity. We have now synthesised a series of basic ethers (I, II see Tables) having low oestrogenicity coupled, in some cases, with anti-gonadotrophic activity in rats equal to that of norethisterone.



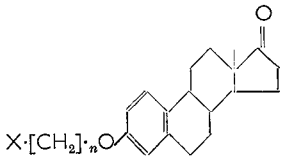
(I)



(II)

The ethers (I) (Table 1) were prepared by reaction of a suitable 17α-substituted oestradiol with the appropriate ω-dialkylaminoalkyl chloride hydrochloride in the presence of alkali, and a similar reaction using oestrone in place of the oestradiol derivatives gave compounds (II) (Table 2). An alternative method used for the synthesis of the oestrone derivatives (II) was the reaction of the appropriate amine with a ω-haloalkyl ether (III), which was obtained by reaction of oestrone with a αω-dihaloalkane. The oestradiol analogues (I; R = H) were prepared by reduction of the corresponding 17-keto-derivative with potassium borohydride in methanol.

The ethers (I, R = R″ = H; R′ = Me; $n = 2$) and (I, R = R′ = R″ = H; $n = 4$) were also prepared; the former by debenzylation of I (R = H; R′ = $C_6H_5 \cdot CH_2$; R″ = Me; $n = 2$) and the latter by reduction of 3-(ω-cyanopropoxy)oestra-1,3,5(10)-trien-17-one (IV) with lithium aluminium hydride.



III, X = I, Br or Cl
IV, X = CN; $n = 3$

From the Research Department, Parke Davis & Co., Hounslow, Middlesex.

BIOLOGICAL ACTIVITIES

The biological activities of these compounds have been assessed by our colleagues in Ann Arbor. Their findings are here summarised.

Initially the compounds described were examined for their ability to completely prevent litters being born to mice under conditions where 50–100% of the mice in a control group produced litters. Oestrogenicity was determined either by a rat vaginal cornification (Allen & Doisy, 1923) or mouse uterine weight assay (Evans, Varney & Koch, 1941). Most of the basic ethers showed an oestrogenicity equivalent to 0·05–0·5% that of stilboestrol.

The compounds most extensively examined were of type (I) and (II) in which $n = 3$, and in this series the 17-ketone (II, $R' = R'' = Me$; $R''' = H$; $n = 3$), administered orally in 0·7–1·4 mg/kg of body weight, prevented pregnancy in mice. Replacement of one *N*-methyl group by a benzyl or phenyl group produced only marginal differences in genotropic activity, whereas introduction of a methyl group in position 1 in the steroid molecule eliminated genotropic activity. Substitution at position 17 also produced some differences in degree of genotropic activity. Thus, in Table 1, the 17α-ethyl compound (4), 17α-ethynyl compound (9) and 17α-allyl compound (17) were less active than the 17α-methyl (2) and 17α-vinyl (7) compounds.

An increase in length of the alkyl chain, as in the 17-ketone (21, Table 2), produced little change in genotropic activity. Compounds of type (I) and (II) in which $n = 2$ were almost invariably less active than the corresponding compounds with $n = 3$, although the oestrogenicity of both series was similar.

Evidence suggesting an effect on the pituitary is that the compound 19 (Table 2), administered orally, had an activity greater than norethisterone in suppressing gonadotrophin-induced ovulation in rats (Callantine, Humphrey & French, 1962; Callantine, Lee, Humphrey & Windsor, 1964). This anti-gonadotrophic activity is coupled with an oestrogenicity of 0·3% that of stilboestrol. The compound 19 has no progestational or anti-oestrogenic activity.

The haloalkyl ethers (III) also showed genotropic activity coupled, however, with a relatively high oestrogenicity (1·7–7·1% that of stilboestrol).

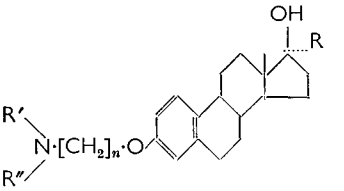
Experimental

Melting-points were determined on a Kofler block. Specific rotations are for chloroform solutions (unless indicated otherwise) at room temperature. All the compounds described exhibited infra-red spectra consistant with the assigned structures.

GENERAL METHODS FOR PREPARATION OF THE BASIC ETHERS

(*a*) *From the phenol.* A mixture of the phenolic steroid (10 mmole), ω-dialkylaminoalkyl chloride hydrochloride (11 mmole), potassium hydroxide (21–22 mmole, as 5N or 10N aqueous solution) in a convenient

TABLE 1. BASIC ETHERS OF 17α-SUBSTITUTED OESTRADIOL.



Comp. No.	R	R′	R″	n	Yield (%)	m.p.°C	Crystallising solvent	$[\alpha]_D^{\circ}$	c	Formula	Found C	Found H	Found Cl	Required C	Required H	Required Cl
1	Me	Me	Me	2	56	100–101·5	n-hexane	+42	1·12	$C_{23}H_{35}NO_2$	77·0	9·65		77·3	9·9	
2	Me	Me	Me	3	38	80–82 & 85–86	n-hexane	+44	0·92	$C_{24}H_{37}NO_2$	77·7	10·0		77·6	10·0	
3	Et	Me	Me	2	21	66–68	n-hexane	+43	0·96	$C_{24}H_{37}NO_2$	77·2	10·0		77·6	10·0	
4	Et	Me	Me	3	30	107–108	ether–n-hexane	+43	1·20	$C_{25}H_{39}NO_2$	78·2	10·2		77·9	10·2	
5	Et	Me	$C_6H_5{\cdot}CH_2$	2	54	oil	—	+28	1·06	$C_{30}H_{41}NO_2$	80·5	9·2		80·5	9·2	
6	$CH_2:CH$	Me	Me	2	41	108–110	n-hexane	+47	0·95	$C_{24}H_{35}NO_2$	77·8	9·7		78·0	9·55	
7	$CH_2:CH$	Me	Me	3	32	83·5–85·5	n-hexane	+44	1·03	$C_{25}H_{37}NO_2$	78·1	9·7		78·3	9·7	
8	CH:C	Me	Me	2	30‡	162–164·5	methanol	+10	1·09	$C_{24}H_{33}NO_2$	78·3	9·3		78·4	9·05	
9	CH:C	Me	Me	3	49‡	243–247*	ether–ethanol	−8	0·476†	$C_{25}H_{36}ClNO_2$	71·9	9·0	8·5	71·8	8·7	8·5
10	CH:C	Me	Me	3	23‡	154–156	acetone	+6	1·12	$C_{25}H_{35}NO_2$	78·8	9·2		78·7	9·25	
11	CH:C	Me	$C_6H_5{\cdot}CH_2$	2	53	217–219*	isolated direct	+3	0·915†	$C_{30}H_{38}ClNO_2{\cdot}\frac{1}{2}H_2O$	73·3	7·9	7·45	73·6	7·8	7·3
12	CH:C	Me	$C_6H_5{\cdot}CH_2$	3	75‡	89–91	acetone–n-hexane	+3·5	1·04	$C_{31}H_{39}NO_2$	81·7	8·5		81·4	8·6	
13	CH:C	Et	Et	2	70‡	120–121	acetone	+7	0·985	$C_{26}H_{37}NO_2$	78·7	9·8		78·9	9·4	
14	CH:C	Et	Et	3	53	86·5–88·5	n-hexane	−0·5	0·655	$C_{27}H_{39}NO_2$	79·35	9·5		79·2	9·6	
15	Pr^n	Me	Me	3	40	208–210*	(sublimed)	+36	1·03†	$C_{26}H_{42}ClNO_2$	71·9	9·6	8·4	71·6	9·7	8·1
16	$CH_2:CH{\cdot}CH_2$	Me	Me	2	61	216–219*	(sublimed)	+58	1·025†	$C_{25}H_{36}ClNO_2$	71·5	9·3	8·7	71·5	9·1	8·4
17	$CH_2:CH{\cdot}CH_2$	Me	Me	3	33	72–74	n-hexane	+52	0·975	$C_{26}H_{39}NO_2$	78·6	9·45		78·5	9·9	

* hydrochloride salt. † in ethanol.

volume (usually 30–100 ml) of water, ethanol or aqueous ethanol was refluxed for 30–60 min, cooled, diluted with water, and the product extracted with ether. The ethereal extract was diluted with an equal volume of benzene, and the solution washed with Claisen's alkali, washed with water until neutral, and the dialkylaminoalkyl ether extracted with aqueous citric acid solution. After washing with ether the aqueous solution was made alkaline with 2N sodium hydroxide, and the free base extracted with ether. The washed and dried ethereal extract was evaporated to dryness to yield the dialkylaminoalkyl ether. In several instances, indicated ‡ in the tables, the additional purification by extraction with citric acid was omitted.

Compounds of Table 1 and some of those in Table 2 were prepared by this method as were the following 3-ethers of 1-methyl-6-dehydro-oestrone: (*3-dimethylaminopropyl*) *ether* (14%)), double m.p. 111–112° and 123–125° (from methanol), $[\alpha]_D$–84° (*c*, 0·92). Found: C, 78·5; H, 8·8; N, 3·8. $C_{24}H_{33}NO_2$ requires C, 78·4; H, 9·05; N, 3·8%, and [*2*-(N-*benzyl*-N-*methylamino*)*ethyl*] *ether* (70%), m.p. 101–102° (from methanol), $[\alpha]_D$–74° (C, 0·975). Found: C, 81·2; H, 8·1; N, 3·4. $C_{29}H_{35}NO_2$ requires C, 81·1; H, 8·2; N, 3·3%.

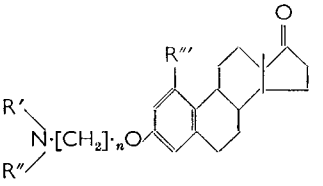
(*b*) *From the ω-haloalkyl ether.* A mixture of a 3-(ω-haloalkoxy)-oestra-1,3,5(10)-trien-17-one and a large excess of the appropriate amine was either refluxed in ethanol for 18 hr or, with the more volatile amines, heated at 50° in benzene in a sealed tube for 24–36 hr before working up as described under (*a*). In certain instances (Compounds 25; 29 and 30; Table 2) where the citrates of the product were not readily soluble in water, purification was achieved by acidifying the ethereal extract with hydrochloric acid, filtering off the hydrochloride, washing it thoroughly with ether and water, and again liberating the free base with alkali and extracting with ether.

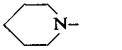
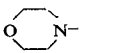
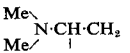
General method for 3-(*ω-haloalkoxy*)*oestra*-1,3,5(10)-*trien*-17-*one.* A solution of oestrone (50 mmole) in ethanol (150 ml) containing 5N potassium hydroxide (50 mmole) was added over 1½–2½ hr, to a refluxing solution of the αω-dihaloalkane (500 mmole) in ethanol (150 ml), and refluxing continued for a further 1–2 hr. The reaction mixture was cooled, poured into water, the product extracted with benzene, and the extract washed with water. With steam-volatile dihaloalkanes (C_3–C_6) the benzene extract was steam distilled, and the residue re-extracted with benzene. The extract was washed with Claisen's alkali, washed with water until neutral, dried, filtered and evaporated to dryness. Crystallisation of the residue from the solvents indicated (Table 3) gave the 3-(ω-haloalkoxy)oestra-1,3,5(10)-trien-17-one.

When 1,10-dibromodecane was used in the above reaction the molar excess was reduced to 225 mmole, the steam distillation omitted, and the product, obtained after washing the benzene extract with Claisen's alkali, was purified by chromatography on Woelm neutral alumina.

3-(3-*Dimethylaminopropoxy*)*oestra*-1,3,5(10)-*trien*-17β-*ol.* A solution of 3-(3-dimethylaminopropoxy)oestra-1,3,5(10)-trien-17-one(2 g) in methanol (50 ml) was stirred for 60 min with potassium borohydride (1 g), the

TABLE 2. BASIC ETHERS OF OESTRONE.



Comp. No.	R′	R″	*n*	R‴	Prep. by method	Yield (%)	m.p. °C	Crystallised from	$[\alpha]_D^{\bullet}$	*c*	Formula	Found C	Found H	Found Cl	Required C	Required H	Required Cl
18	Me	Me	2	H	(a)‡	15	113–114·5	acetone	+132	0·99	$C_{22}H_{31}NO_2$	77·45	9·3		77·4	9·15	
19	Me	Me	3	H	(a)‡	34	90–91°	n-hexane	+127	1·025	$C_{23}H_{33}NO_2$	77·9	9·5		77·7	9·4	
20	Me	Me	4	H	(b)	61	238–240*	ethanol	+119†	0·545	$C_{24}H_{36}ClNO_2$	70·8	9·1	8·6	71·0	8·9	8·7
21	Me	Me	5	H	(b)	54	214–216*	ether–ethanol	+110†	0·970	$C_{25}H_{38}ClNO_2$	71·5	9·0	8·5	71·3	9·0	8·6
22	Me	Me	6	H	(b)	50	68–69	n-hexane	+112	1·040	$C_{26}H_{39}NO_2$	78·3	9·9		78·5	9·9	
23	Me	Me	10	H	(b)	74	82–84	n-hexane	+83	0·96	$C_{30}H_{47}NO_2$	79·4	10·4		79·4	10·4	
24	Me	$C_6H_5CH_2$	2	H	(a)‡	55	86–88	n-hexane	+105	1·08	$C_{28}H_{35}NO_2$	80·2	8·4		80·5	8·5	
25	Me	$C_6H_5CH_2$	3	H	(a)	54	65–67	n-hexane	+102	1·015	$C_{29}H_{37}NO_2$	80·4	8·5		80·7	8·6	
26	Me	Ph	3	H	(b)	85	99–102	di-isopropyl ether	+104	0·98	$C_{28}H_{35}NO_2$	80·7	8·4		80·5	8·5	
27	Me	Me	3	Me	(a)	41	89–91	n-hexane	+198°	0·97	$C_{24}H_{35}NO_2$	78·0	9·5		78·0	9·55	
28	Me	$C_6H_5·CH_2$	3	Me	(a)	60	94–95·5	n-hexane	+174	0·945	$C_{29}H_{37}NO_2$	80·6	8·7		80·7	8·6	
29	$C_6H_5·CH_2$	H	3	H	(b)	20	215–222*	ethanol	+101†	0·975	$C_{28}H_{36}ClNO_2$	74·5	8·1	7·7	74·1	8·0	7·8
30	$C_6H_5·CH_2$	$C_6H_5·CH_2$	3	H	(b)	72	131–133	acetone	+87	0·935	$C_{35}H_{41}NO_2$	82·6	8·2		82·8	8·1	
31			2	H	(a)‡	58	112–115	ether	+118	0·985	$C_{25}H_{35}NO_2$	78·8	9·35		78·7	9·25	
32			2	H	(a)‡	78	107–109	ethyl acetate	+113	1·05	$C_{24}H_{33}NO_3$	75·1	9·0		75·2	8·7	
33				H	(a)‡	73	70–73	n-hexane	+125	0·97	$C_{23}H_{33}NO_2$	77·4	9·6		77·7	9·4	

* hydrochloride salt † in ethanol

reaction mixture acidified with acetic acid, then made alkaline with 2N sodium hydroxide, and extracted with ether. Evaporation of the washed and dried ethereal extract gave a white solid (2 g), which was crystallised from n-hexane to yield 3-(3-dimethylaminopropoxy)oestra-1,3,5(10)-trien-17β-ol, m.p. 91–93°, $[\alpha]_D$ + 60° (*c*, 0·9 in ethanol). Found: C, 77·4; H, 10·0, N, 3·8. $C_{23}H_{35}NO_2$ requires C, 77·3, H, 9·6, N, 3·9%.

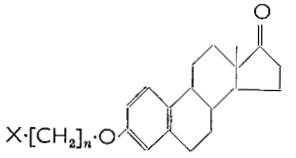
The following compounds were prepared in a similar manner: 3-[2-(N-*Benzyl*-N-*methylamino*)*ethoxy*]*oestra*-1,3,5(10)-*trien*-17β-*ol hydrochloride* (75%), m.p. 194–198° (from ether–ethanol), $[\alpha]_D$ + 50° (*c* 1·045 in ethanol). Found: C, 73·9; H, 8·5; N, 3·1. $C_{28}H_{38}ClNO_2$ requires C, 73·8; H, 8·35; N, 3·1%. 3-(2-*Diethylaminoethoxy*)*oestra*-1,3,5(10)-*trien*-17β-*ol hydrochloride* (76%), m.p. 215–220° (from ether-ethanol), $[\alpha]_D$ + 60°. Found: C, 70·9; H, 9·5; Cl, 8·6; N, 3·2. $C_{24}H_{38}ClNO$ requires C, 70·6; H, 9·4; Cl, 8·7; N, 3·4%. This compound, acetylated with acetic anhydride in pyridine, gave the corresponding 17-*acetate*, m.p. 177–181° (from ether–ethanol), $[\alpha]_D$ + 30° (*c*, 0·75 in ethanol). Found: C, 65·3; H, 9·2; Cl, 7·6. $C_{26}H_{40}ClNO_3$. $2\frac{1}{2}$ H_2O requires C, 65·1; H, 9·3; Cl, 7·4%.

3-(2-*Methylaminoethoxy*)*oestra*-1,3,5(10)-*trien*-17-*one*. 3-[2-(*N*-benzyl-*N*-methylamino)ethoxy]oestra-1,3,5(10)-trien-17-one hydrochloride (1 g) was hydrogenated in ethanol over palladised charcoal (10%, 500 mg), the catalyst filtered off, and the filtrate evaporated to dryness. The residue was made alkaline, extracted with ether, and the ethereal extract washed with water, dried, filtered and concentrated to 20 ml. Ethereal hydrogen chloride was added, and the precipitated hydrochloride filtered off. Crystallisation from ethanol gave 3-(2-methylaminoethoxy)oestra-1,3,5(10)-trien-17-one hydrochloride (90%), m.p. 258–263°. Found: C, 69·4; H, 8·2; Cl, 9·7; N, 3·8. $C_{21}H_{30}ClNO_2$ requires C, 69·3; H, 8·3; Cl, 9·7; N, 3·85%. The hydrochloride was converted to the free base, m.p. 80–82°, $[\alpha]_D$ + 134° (*c*, 0·985). Found: C, 77·3; H, 8·7. $C_{21}H_{29}NO_2$ requires C, 77·0; H, 8·9%.

3-(4-*Aminobutoxy*)*oestra*-1,3,5(10)-*trien*-17β-*ol*. A solution of 3-(3-cyanopropoxy)oestra-1,3,5(10)-trien-17-one (4 g) in ether (200 ml) and tetrahydrofuran (150 ml) was added over 60 min to a stirred solution of lithium aluminium hydride (4 g) in ether (500 ml) under nitrogen. After 60 min under reflux, the reaction mixture was cooled and treated successively with water (6 ml), 15% aqueous sodium hydroxide solution (6 ml) and water (18 ml), the precipitate filtered off, and washed with ether. The filtrate was washed with water, dried with $MgSO_4$, filtered, and evaporated to yield an oil (4 g), which on trituration with ether gave a white solid, m.p. 105–118°. The solid was converted to its hydrochloride, m.p. 210–213° (from ethanol), $[\alpha]_D$ + 61° (*c*, 0·865 in ethanol). Found: C, 69·2; H, 9·0; Cl, 9·0. $C_{22}H_{34}ClNO_2$ requires C, 69·5; H, 9·0; Cl, 9·3%.

3-(3-*Cyanopropoxy*)*oestra*-1,3,5(10)-*trien*-17-*one*. A solution of oestrone (2·7 g, 10 mmole) in dimethylformamide (100 ml) containing 5N potassium hydroxide (2 ml, 10 mmole) was heated with γ-chlorobutyronitrile (1·1 g, 11 mmole) at 100° for 1 hr and then stirred at room temperature overnight before pouring into water. The mixture was

TABLE 3. HALOALKYL ETHERS OF OESTRONE.



X	n	Yield (%)	m.p. °C	Crystallising solvent	$[\alpha]_D^{\circ}$	c	Formula	Found			Required		
								C	H	X	C	H	X
Br	3	46	99–101	methanol	+140	0·97	$C_{21}H_{27}BrO_2$	64·3	6·8	19·8	64·4	7·0	20·4
Cl	4	56	120–122	acetone	+117	1·070	$C_{22}H_{29}ClO_2$	72·6	7·9	—	73·2	8·1	—
Br	5	51	84–86	methanol–ether	+90	0·980	$C_{23}H_{31}Bro_2$	66·2	7·55	18·9	66·0	7·45	19·1
Br	6	26	74·5–76·5	n-hexane	+101	1·035	$C_{24}H_{33}BrO_2$	66·6	7·7	18·6	66·5	7·7	18·4
Br	10	41	66–68	n-hexane*	+88	1·015	$C_{28}H_{41}BrO_2$	68·9	8·7	16·3	68·7	8·4	16·5

* after chromatography.

extracted with ether, and the ethereal extract washed with Claisen's alkali, washed with water until neutral, dried, filtered, and evaporated. Crystallisation of the residue from acetone gave 3-(3-cyanopropoxy)oestra-1,3,5(10)-trien-17-one (1·2 g), m.p. 127–129°, $[\alpha]_D$ + 128° (*c*, 1·025). Found: C, 78·55; H, 8·2%. $C_{22}H_{27}NO_2$ requires C, 78·3; H, 8·1%.

17α-*Allyloestradiol.* Allyl bromide (10 ml) was added at 10° to magnesium (10 g) under anhydrous tetrahydrofuran (100 ml). Once the reaction had started, a solution of oestrone (10 g) in allyl bromide (30 ml) and anhydrous tetrahydrofuran (400 ml) was added dropwise over 2 hr and additional 1 g portions of magnesium were added at half-hourly intervals. Thereafter the temperature of the reaction mixture was allowed to rise to 20° and stirring continued for a further 2 hr. Saturated ammonium chloride solution was then added, the product isolated by extraction with ether, and crystallised from di-isopropyl ether to give 17α-allyloestradiol (10·8 g), m.p. 111–112°, $[\alpha]_D$ + 58° (*c*, 0·98). Found: C, 80·7; H, 8·9. $C_{21}H_{28}O_2$ requires C, 80·7; H, 8·9%.

17α-*Propyloestradiol.* 17α-Allyloestradiol (5 g) was hydrogenated in ethanol (60 ml) over platinum oxide (250 mg), the catalyst filtered off, and the filtrate evaporated to dryness *in vacuo*. The residue was crystallised from methanol to give 17α-propyloestradiol (3·95 g), m.p. 165–167°, $[\alpha]_D$ + 54° (*c*, 0·94). Found: C, 80·3; H, 9·5. $C_{21}H_{30}O_2$ requires C, 80·2; H, 9·6%.

Acknowledgements. The authors thank Dr R. E. Bowman for his encouragement and advice, Miss E. M. Tanner for the determination of optical rotations and spectra and Mr F. H. Oliver for the microanalyses. Thanks are also due to Doctors O. D. Bird and M. R. Callantine of Parke Davis and Company, Research Laboratories, Ann Arbor, U.S.A. for the genotropic and endocrine assays respectively.

References

Allen, E. & Doisy, E. A. (1923). *J. Amer. med. Ass.*, **81**, 819–821.
Black, M. L. (1961). *J. Pharm. Pharmacol.*, **13**, 127.
Callantine, M. R., Humphrey, R. & French, P. J. (1962). *Fed. Proc.*, **21**, 214.
Callantine, M. R., Lee, S. L., Humphrey, R. & Windsor, B. (1964). *Ibid.*, **23**, 463.
Dodds, E. C. (1961). *J. Endocrinol*, **23** (3), (i)–(xi).
Evans, J. S., Varney, R. F. & Koch, F. C. (1941). *Endocrinology*, **28**, 747–752.