STEROIDS CCLXX.[1] BIOLOGICALLY-ACTIVE LABILE ETHERS II.[2] A NEW GROUP OF POTENT ORALLY-ACTIVE ESTROGENS

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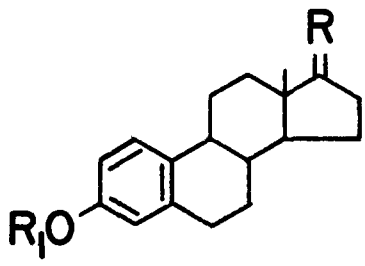
Received July 29, 1964

ABSTRACT

A range of labile ethers of estrone, 17β-estradiol, ethynylestradiol, and ethynylestradiol-3-methyl ether bearing 2'-tetrahydropyranyloxy (THP) substitutents at one or both of positions C-3 and C-17 have been prepared. The 3-THP ethers of both estrone and 17β-estradiol were considerably less estrogenic by subcutaneous injection than the corresponding free estrogens. The 17-THP ether of ethynylestradiol-3-methyl ether showed a dramatic 8-fold increase in activity by the same route. By the oral route the 3-THP and 17-THP ethers of 17β-estradiol were 12 and 15 times more estrogenic, respectively, than the corresponding free 17β-estradiol. Estrone-3-THP ether and 17β-estradiol-17-THP ether had ratios of relative potencies by the gavage to subcutaneous routes of 8.0 and 6.7, respectively, while the estrogenicity of 17β-estradiol-3-THP ether by gavage was 16.1 times estrone and only 0.04 times estrone by subcutaneous injection resulting in the remarkable gavage-subcutaneous injection ratio of 403.

Public acceptance of fertility control by means of an estrogen-progestin combination has led to a revival of interest in highly active estrogens by the oral route. Ercoli's discovery that the oral efficacy of steroids could be enhanced when administered in the form of labile ethers[3] led us to develop a new class of anabolic steroids in which

oral potentiation was conferred by an acid-labile 17β-(2'-tetrahydropyranyloxy) group[2]. An extension of this approach suggested that the oral activity of the corresponding tetrahydropyranyl ethers of estrogens might be similarly increased. We now report experimental verification of this supposition for both estrogen 3-THP and 17-THP ethers. Estrone (I, Fig. I), 17β-estradiol 3-methyl ether (II), and ethynylestradiol-3-methyl ether (III) were all readily etherified to yield ethers IV-VI, respectively, by dihydropyran in dry benzene containing a small amount of p-toluenesulfonic acid catalyst. Similarly, 17β-estradiol (VII) and ethynylestradiol (VIII) were converted to the corresponding 3,17β-bis-tetrahydropyranyl ethers (3,17β-bis THP ethers) IX and X, respectively. For the bis-ethers the possible formation of four stereoisomers during the reaction was reflected in lower amounts of the one crystalline stereoisomer isolated in each case when compared with yields in syntheses of the mono-ethers IV-VI. Both estradiol 3-THP (XI) and 17-THP (XII) mono-ethers were prepared. After treatment of 17β-estradiol (VII) with one mole of dihydropyran and acid there could be isolated 17β-estradiol 17-THP ether (XII). The isomeric ether (XI) was prepared by borohydride reduction of estrone 3-THP ether. Physical constants and analytical data for the various ethers are summarized in Table I.



I. R = O; R_1 = H	VII. R = αH,βOH; R_1 = H
II. R = αH,βOH; R_1 = Me	VIII. R = αC≡CH,βOH; R_1 = H
III. R = αC≡CH,βOH; R_1 = Me	IX. R = αH,βOTHP; R_1 = THP
IV. R = O; R_1 = THP	X. R = αC≡CH,βOTHP; R_1 = THP
V. R = αH,βOTHP; R_1 = Me	XI. R = αH,βOH; R_1 = THP
VI. R = αC≡CH,βOTHP; R_1 = Me	XII. R = αH,βOTHP; R_1 = H

Fig. 1. Structural modifications of estrone and 17β-estradiol.

Spectral analyses (UV, IR, and NMR) were all in accord with the assigned structures. Considerable discrepancies were noted however between the physical constants determined for the 3-THP ethers and those disclosed in the patent literature[4].

EXPERIMENTAL[5]

THP ethers were prepared by the general procedure described for making 17β-estradiol bis THP ether.

17β-Estradiol bis THP ether (IX)

17β-Estradiol (120 g.) in 2.1 of benzene was dried by azeotropic distillation of 100 ml. solvent. A solution of 1 g. of p-toluene-sulfonic acid in 500 ml. of benzene was similarly dried. The two solutions were mixed at 20° and

TABLE I

TETRAHYDROPHYRANYL ETHERS

THP Ether	m.p.	$(\alpha)_D$	Molecular Formula	Microanalysis Found (Calculated) C	H	O
Estrone THP ether (IV)	146-149°	+114° (C_6H_6)	$C_{23}H_{30}O_3$	78.31 (77.93	8.68 8.53)	
17β-Estradiol 3-methyl ether 17-THP ether (V)	84-85°	+ 56° ($CHCL_3$) + 63° (EtOH)	$C_{24}H_{34}O_3$	77.80 (77.84	9.25 9.28)	
Ethynylestradiol 3-methyl ether 17-THP ether (VI)	87-90°	- 47° ($CHCL_3$)	$C_{26}H_{34}O_3$	79.49 (79.15	8.83 8.69)	
17β-Estradiol 3,17-bis THP ether (IX)[a]	105-106°	+ 90° (C_6H_6) -103° (EtOH)	$C_{28}H_{40}O_4$	76.06 (76.32	9.04 9.15)	14.65 14.53
Ethynylestradiol 3,17-bis THP ether (X)[b]	160-162°	- 17° ($CHCL_3$) - 32° (C_5H_5N)	$C_{30}H_{40}O_4$	77.70 (77.55	8.78 8.68)	
17β-Estradiol 17-THP ether (XII)	212-214°	+ 1° ($CHCL_3$) + 12° (C_5H_5N)	$C_{23}H_{32}O_3$	77.61 (77.49	9.10 9.05)	
17β-Estradiol 3-THP ether (XI)[c]	148-150°	+ 21° ($CHCL_3$) + 39° (C_5H_5N)	$C_{23}H_{32}O_3$	77.07 (77.49	9.29 9.05)	

a) Reported[4] m.p. 76-81°, $(\alpha)_D$ + 21.8° (ethanol); b) Reported[4] m.p. 153-155°, $(\alpha)_D$ -24° (pyridine); c) Reported[4] m.p. 146-148°, $(\alpha)_D$ + 77° (pyridine).

150 ml. of dihydropyran was added. After 5 hr. the solution was washed with dilution sodium bicarbonate solution and with water, and the solvent removed in vacuo. Following chromatography over alumina the product was crystallised twice from hexane at 0° and once from methanol-acetone to afford 21 g. of 17β-estradiol bis THP ether, m.p. 105-106°; $(\alpha)_D$ +90° (benzene); λ max 276-278 mu.

17β-Estradiol 17-THP ether (XII)

To a dry solution of 5 g. of 17β-estradiol in 100 ml. of dry benzene was added 10 mg. of p-toluenesulfonic acid, and 1.2 g. of dihydropyran (less than 1 molar equivalent). After being kept under reflux overnight the solution was decanted from unchanged estradiol and the product chromatographed over alumina. Crystallization of the eluate from hexane-acetone gave 1.1 g. of 17β-estradiol 17-THP ether, m.p. 212-214°; $(\alpha)_D$ + 1° ($CHCL_3$); λ max 282 mu (log ε 3.32).

17β-Estradiol 3-THP ether (XI)

Estrone-3-THP ether (43.8 g.) was dissolved in a hot misture of 235 ml. of dioxan and 445 ml. of methanol, then cooled to room temperature. Sodium borohydride (9.0 g.) was added in portions and the mixture then heated under reflux for 2 hours before being precipitated into 1.5 l. of cold water. The solids were collected by filtration, dried (47 g.), and crystallized from hexane-acetone to afford

17β-estradiol-3-THP ether; m.p. 148-150°; $(\alpha)_D$ + 21° ($CHCL_3$); λ max 277 mu (log 3.19).

Biological Studies:

The bioassay procedure was that described by Rubin et al[6]. Swiss albino female mice 21 to 23 days of age were treated either by subcutaneous injection or by gavage once daily for three days with a sesame oil solution of the test material. The daily dose was contained in 0.1 ml. of oil for injection and 0.2 ml. when administered by gavage. Twenty-four hours after the last injection the mice were sacrificed and the uterine and body weights were determined. In each assay estrone standard groups were run in parallel with the unknown. Statistical calculations were performed by the method of Emmens[7].

RESULTS AND CONCLUSIONS

The relative potencies of various subcutaneously administered tetrahydropyranyl ethers and certain corresponding free steroids are presented in Table II. The formation of the 3-THP ether of estrone leads to a compound with a 75% loss of activity and similarly the 3-THP ether of 17β-estradiol was only about 5% as active as the free compound.

Formation of the 17-THP ether derivatives of the steroids lead to substances with equal or increased estrogenic

TABLE II

THE RELATIVE POTENCY OF VARIOUS SUBCUTANEOUSLY ADMINISTERED TETRAHYDROPYRANYL (THP) ETHERS AND CORRESPONDING FREE COMPOUNDS

Steroid	No. of Mice Test Compound	No. of Mice Estrone	Relative Potency Estrone = 100	95% Confidence Limits
Ethynylestradiol--17-THP 3-methyl ether	27	29	412	374 - 459
Ethynylestradiol--3-methyl ether	30	30	35	30 - 40
	60	100	22	20 - 24
	40	40	17	14 - 20
Estrone 3-THP	36	36	18	16 - 20
	80	69	31	26 - 31
17β-Estradiol 3-THP	30	40	3	2 - 4
	30	40	6	5 - 7
17β-Estradiol 17-THP	30	40	280	260 - 310
17β-Estradiol	20	20	280	240 - 330

potency. The biological activity of 17β-estradiol remained unchanged when the ether was formed (Table II). Both steroids had relative potencies of 280. A rather dramatic increase in activity was found when ethynylestradiol-3-methyl ether was converted to the 17-THP derivative. The change was from 25% of the standard estrone to 412%, or an increase of over 8-fold in activity.

The relative potencies of various tetrahydropyranyl ethers and corresponding free compounds administered by

TABLE III

THE RELATIVE POTENCY OF VARIOUS TETRAHYDROPYRANYL (THP) ETHERS AND CORRESPONDING FREE COMPOUNDS ADMINISTERED BY GAVAGE

Steroid	No. of Mice Test Compound	No. of Mice Estrone	Relative Potency Estrone = 100	95% Confidence Limits
17β-Estradiol	50	55	130*	
17β-Estradiol	20	30	1580	1160 - 2150
17-THP	24	24	2410	2240 - 2840
17β-Estradiol 3-THP	40	40	1610	1480 - 1780
Ethynylestradiol	40	40	490	450 - 550
3,17-bis THP	18	36	440	340 - 590
	20	30	400	360 - 440
Ethynylestradiol	23	32	2340	2020 - 2710
Estrone 3-THP	60	40	400*	
17β-Estradiol 3,17-	36	36	28	21 - 37
-bis THP	18	29	25	15 - 41
Ethynylestradiol	37	39	140	123 - 153
17-THP 3-methyl-	30	29	270	238 - 300
ether				
Ethynylestradiol-3-	39	40	1350	1180 - 1550
-methyl ether				
17β-Estradiol	36	27	31	28 - 34
3-methyl ether				
17-THP				

* Graphic estimate

gavage are summarized in Table III. Both the 3- and 17-THP ethers of 17β-estradiol showed remarkable increases in relative potencies compared to the activity of the free

compound. The 3-THP ether had an activity of 1610 which was an increase of more than 12-fold over that of the free compound. The relative potency of the 17-THP of 17β-estradiol was 2000, some 15 times more potent than the free compound. The 3,17-bis THP was considerably less active than the free compound with a relative potency of only 27.

The 3-THP ether of estrone was assayed by gavage and the data, assessed graphically, indicated a four-fold gain in relative potency. The 3,17-bis THP of both ethynylestradiol and the 17-THP ether of ethynylestradiol-3-methyl ether showed significant reductions in biological activity. The biological activity of ethynylestradiol was reduced from 2340 to 443 when the 3,17-bis THP ether was made. The biological activity of the 17-THP ether of ethynylestradiol-3-methyl ether was 205, whereas the original compound had an activity of 1350 (Table III).

Table IV illustrates the relatively high estrogenic potencies of THP ethers of estrone and 17β-estradiol by gavage as compared to their respective activities by the subcutaneous route. The ratio of gavage to subcutaneous injection relative potencies shows that estrone 3-THP ether had a value of 8.0 and 17β-estradiol-17-THP ether had a similar ratio of 6.7. The remarkable ratio of 403 was

TABLE IV

THE COMPARATIVE ESTROGENIC ACTIVITIES OF CERTAIN TETRAHYDROPYRANYL ETHERS WHEN ADMINISTERED BY SUBCUTANEOUS INJECTION AND BY GAVAGE

Steroid	Relative Potencies Estrone = 100 Gavage A	Subcutaneous Injection B	Ratio A/B
Estrone 3-THP ether	400	25	8.0
17β-Estradiol-17-THP ether	1880	280	6.7
17β-Estradiol-3-THP ether	1610	4	403

found for 17β-estradiol-3-THP ether and reveals a profound difference from 17β-estradiol-17-THP ether.

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