tion (MIC) at 48 hr of incubation, or an absence of organisms in a subculture after 24 hr of incubation for the minimum lethal concentration (MLC). Cultures were incubated at 35°.

In vivo activity was determined by a mouse-mortality model. Male CF-1 mice, 18–20 g, were inoculated with 2×10^6 *T. foetus* intraperitoneally. Compounds were suspended in a carboxymethylcellulose vehicle[15] and administered orally twice daily for 2 days beginning 24 hr after infection. The median survival time and number of survivors were used as criteria to assess activity. The dose of metronidazole affording 50% survival was approximately 22 mg/kg.

References and Notes

(1) The chemotherapeutic properties of five-membered heterocycles have recently been reviewed by E. Grunberg and E. H. Titsworth, *Annu. Rev. Microbiol.*, **27**, 317 (1973).
(2) (a) G. Karmas, U. S. Patents 3,156,699 (1964), 3,244,726 (1966), 3,248,624 (1966), 3,256,279 (1966), 3,256,295 (1966); (b) Rhone-Poulenc S.A., French Patents M3093 (1965), CAM90 (1965); (c) Societa Farmaceutical Italia, Belgian Patent 666,612 (1965).
(3) D. R. Hoff, *Proc. Int. Symp. Drug Res.*, 100 (1967), and references cited therein.
(4) (a) D. W. Wright, U. S. Patent 3,014,916 (1961); (b) May and Baker Ltd., British Patent 938,726 (1963).
(5) (a) P. M. G. Bavin, *J. Med. Chem.*, **9**, 788 (1966); (b) H. A. Burch, *J. Med. Chem.*, **10**, 91 (1967).
(6) G. L. Dunn, P. Actor, and V. J. DiPasquo, *J. Med. Chem.*, **9**, 751 (1966).
(7) A. S. Tomcufcik, "Medicinal Chemistry," A. Burger, Ed., Wiley-Interscience, New York, N.Y., 1970, p 2, and references cited therein.
(8) (a) L. N. Simon, D. E. O'Brien, W. B. Jolley, R. J. Bauer, T. Novinson, R. H. Springer, K. Dimmitt, and R. K. Robins, *Pharmacol. Future Man, Proc. Int. Congr. Pharmacol., 5th, 1972*, Abstract 1282 (1973); (b) R. H. Springer, R. P. Rao, T. Novinson, M. K. Dimmitt, R. J. Bauer, L. N. Simon, R. K. Robins, and D. E. O'Brien, *ibid.*, Abstract 1321 (1973); (c) T. Novinson, R. Hanson, M. K. Dimmitt, L. N. Simon, R. K. Robins, and D. E. O'Brien, *J. Med. Chem.*, **17**, 645 (1974).
(9) G. Y. Lesher, E. J. Froelich, M. D. Gruett, J. H. Bailey, and R. P. Brundage, *J. Med. Pharm. Chem.*, **5**, 1063 (1962).
(10) P. Schmidt and J. Druey, *Helv. Chim. Acta*, **39**, 986 (1956).
(11) Y. Makisumi, *Chem. Pharm. Bull.*, **10**, 620 (1962).
(12) H. Dorn and H. Dilcher, *Justus Liebigs Ann. Chem.*, **707**, 141 (1967).
(13) Y. H. Tsai and K. E. Price, *Chemotherapy*, **18**, 348 (1973).
(14) L. S. Diamond, *J. Parasitol.*, **43**, 488 (1957).
(15) H. R. Wilson, G. R. Revankar, and R. L. Tolman, *J. Med. Chem.*, **17**, 760 (1974).

3-Substituted 2′,3′-Dihydroestra-1,3,5(10)-trieno[16α,17α-*b*]furan-17β-ols as Potential Estrogens

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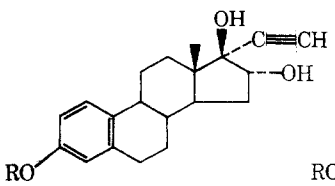
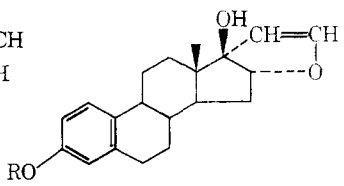
The preparation, characterization, and estrogenic activity of the two new steroids 3-(cyclopentyloxy)-2′,3′-dihydroestra-1,3,5(10)-trieno[16α,17α-*b*]furan-17β-ol and 2′,3′-dihydroestra-1,3,5(10)-trieno[16α,17α-*b*]furan-3,17β-diol are described. The compounds were found to be 0.1 and 0.002, respectively, as potent as estrone in a test designed to measure the uterine weight gain of treated immature mice relative to controls.

A potent, orally active estriol-like steroid, diol **1a**, useful in the treatment of menopausal syndrome and other conditions of estrogen deficiency has been synthesized recently in our laboratories.[1,2] In the course of studying the chemical reactivity of this diol, it was noted from thin-layer chromatographic observations that the reaction of **1a** with mild base resulted in transformation to a less polar compound.

High-resolution mass spectrometry indicated that this new compound had an identical molecular formula to the starting diol, while the mass spectrum of the silylated derivative (TMSI) of the compound showed the presence of only one trimethylsilyl group. The infrared spectrum revealed that the $-C{\equiv}CH$ moiety was absent and a double resonance nmr experiment indicated the presence of two 1-proton doublets in the olefinic region. These data are consistent with alcohol **2a**, 3-(cyclopentyloxy)-2′,3′-dihydroestra-1,3,5(10)-trieno[16α,17α-*b*]furan-17β-ol, as the structure of the less polar transformation product from **1a**.



1a, R = cyclopentyl
b, R = H



2a, R = cyclopentyl
b, R = H

An alternative structure containing a 16α,17α-methyleneoxetane moiety is mechanistically possible;[3-5] however, it does not fit the analytical data. The ir spectrum of the product assigned structure **2a** lacks the strong absorption indicative of a methylene moiety exocyclic to a cyclobutane or oxetane ring[6] and, furthermore, the chemical shifts (H_2 = 6.2, H_3 = 4.8) and J value (J = 2.6) of the olefinic protons in the nmr spectrum of the model compound 2,3-dihydrofuran[7] are in excellent agreement with the observed data.

Although there are many examples of γ-lactones fused onto the D ring of a steroid nucleus[8-10] and there are a few sapogenin derivatives that contain a furan or reduced furan ring in a similar position,[11,12] compound **2a** is the first example of a steroid containing a [16α,17α-*b*]furan moiety with an alcohol function at C_{17}.

The relative stereochemistry of **2a** was ascertained by noting the pyridine-induced solvent shift of the C_{18}-methyl group in its nmr spectrum. The observed shift (δ $CDCl_3$-C_5D_5N) indicates the C_{18} methyl moiety is syn to the C_{17} OH group.[13] Diol **2b** was made from **1b** in a manner similar to the preparation of **2a** from **1a**. The spectroscopic and analytical data for **2b** are consistent with the proposed structure.

The estrogenic activity of **2a**,**b** was measured by treating groups of ten immature female mice each, subcutaneously, with the compound under test for 3 days at different dose levels (see Figure 1). A control group is maintained which receives only the injection vehicle. The average increase in the uterine weights of the treated mice as compared to those of the untreated mice determines estrogenic activity.[14] Using groups of ten mice each yields statistically significant results. Relative to estrone, the potency of **2a** was found to be 0.1 while that of **2b** was approximately 0.002. This demonstrated biological activity of the two new compounds is not surprising. Diverse studies continue to reveal that the structural criteria for estrogenic activity are much

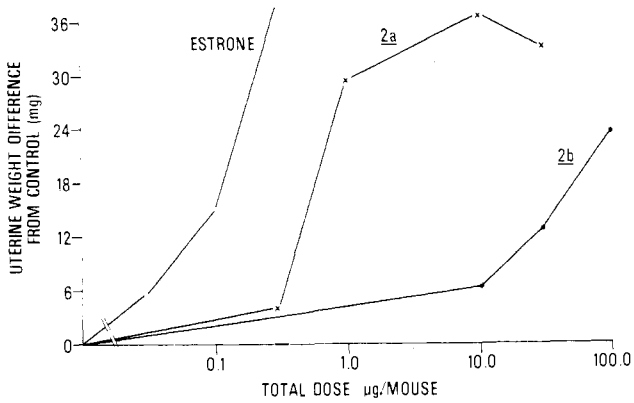


Figure 1. Uterine weight gain compared to controls of immature mice after sc injections of estrone, **2a**, and **2b**.

less exacting than for other classical hormonal activities.[15,16]

Experimental Section

Melting points were determined on a Mel-Temp apparatus. Infrared spectra, reported in reciprocal centimeters, were taken on either a Perkin-Elmer 221 or Beckman IR 12 spectrophotometer. The nmr spectra were determined on a T-60A spectrometer; chemical shifts are reported in δ from $(CH_3)_4Si$ and J values are given in Hz. Mass spectra were determined on a Hitachi RMU-6D spectrometer at 70 eV. Elemental analyses obtained were within +0.3% of the theoretical values.

3-Cyclopentyloxy-2′,3′-dihydroestra-1,3,5(10)-trieno[16α,-17α-*b*]furan-17β-ol (2a). A solution of 1.0 g (2.64 mmol) of 3-cyclopentyloxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yne-16α,17β-diol (**1a**) in 250 ml of 0.05 *M* methanolic KOH was allowed to reflux for 24 hr. The methanol was then evaporated on a rotary evaporator and the resulting yellow solid was immediately chromatographed over a column of 50 g of Woelm silica gel, activity 1, using 5% EtOAc in benzene as the eluent. Alcohol **2a** was eluted as the first band, 850 mg (85%), mp 130–131°. Further elution afforded a small amount of starting diol **1a**. Both thin-layer chromatography (Brinkmann silica F_{254}, eluent 1:1 EtOAc–benzene, R_f 0.43) and glc[17] revealed **2a** to be homogeneous: mass spectrum exact mass at *m/e* 380.2358, calcd for $C_{25}H_{32}O_3$ 380.2351; ir (KBr) 3490 (OH), 2930 and 2860 (CH), no peaks from 2800–1620; nmr (pyridine-d_5) 7.4–6.8 (3 H, m, aromatics), 6.7 (1 H, d, J = 2.5 Hz, olefinic), 5.2 (1 H, d, J = 2.5 Hz, olefinic), 5.0–4.6 (2 H, m, CH adjacent to O), 3.0–1.3 (*ca.* 22 H, m), 1.2 (3 H, s, CH_3). A double resonance experiment revealed that the doublets at 6.7 and 5.2 were coupled to one another—irradiation of either doublet caused collapse of the other to a singlet. From nmr measurements in $CDCl_3$, a (δ $CDCl_3$–pyridine-d_5) value of −0.22 for the angular C_{18} methyl moiety was obtained. This is consistent[13] with a 1,3-diaxial relationship between the C_{18} methyl function and the C_{17} OH group. *Anal.* ($C_{25}H_{32}O_3$) C, H, O.

2′,3′-Dihydroestra-1,3,5(10)-trieno[16α,17α-*b*]furan-3,17β-diol (2b). This diol was generated from **1b** in 70% yield in a manner similar to the preparation of **2a** from **1a**. Purification *via* column chromatography (eluent 7% EtOAc in benzene) afforded the diol as a pale yellow solid: mp 210–212°; ir (KBr) 3400 (OH), 2950 and 2850 (CH), no peaks 2800–1670; mass spectrum exact mass at *m/e* 312.1721, calcd for $C_{20}H_{24}O_3$ 312.1725. *Anal.* ($C_{20}H_{24}O_3$) C, H.

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References and Notes

(1) N. J. Bach and E. Farkas, U. S. Patent 3,697,558 (Oct 10, 1972).
(2) R. J. Kraay and E. Farkas, U. S. Patent 3,790,605 (Feb 5, 1974).
(3) R. D. Dillard and N. R. Easton, *J. Org. Chem.*, 31, 122 (1966).
(4) A. T. Bottini, F. P. Corson, and E. F. Bottner, *J. Org. Chem.*, **30**, 2988 (1965).
(5) A. T. Bottini, J. A. Mullikin, and C. J. Morris, *J. Org. Chem.*, **29**, 373 (1964).
(6) D. E. Applequist and J. D. Roberts, *J. Amer. Chem. Soc.*, **78**, 4012 (1956).
(7) P. K. Korver, *et al.*, *Recl. Trav. Chim. Pays-Bas*, **84**, 129 (1965).
(8) P. Kurath and W. Cole, *J. Org. Chem.*, **26**, 4592 (1961).
(9) K. Heusler, *et al.*, *Helv. Chim. Acta*, **44**, 502 (1961).
(10) L. Labler and F. Sorm, *Collect. Czech. Chem. Commun.*, **25**, 2855 (1960).
(11) C. E. Shoppee, "Chemistry of the Steroids," Butterworths, London, 1964, Chapter VI.
(12) L. Fieser and M. Fieser, "Steroids," Rheinhold, New York, N.Y., 1959, Chapter 21.
(13) P. V. Demarco, *et al.*, *J. Amer. Chem. Soc.*, **90**, 5480 (1968).
(14) B. L. Rubin, *et al.*, *Endocrinology*, **49**, 429 (1951).
(15) E. B. Astwood in "The Pharmacological Basis of Therapeutics," L. S. Goodman and A. Gilman, Ed., Macmillan, New York, N.Y., 1970, p 1538.
(16) C. G. Pitt and R. W. Handy, *Tetrahedron*, **27**, 527 (1971).
(17) R. H. Bishara, B. S. Rutherford, and A. Dinner, unpublished results.

Hypotensive Activity of 3-Alkyl-2-iminobenzothiazolines

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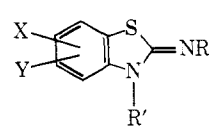
The hypotensive effects of a variety of guanidine derivatives are well documented.[1] The title compounds were investigated since they may be considered to be isosteric analogs of guanidine derivatives fused to an aromatic ring. The series of compounds investigated may be represented by formula I where R = H, aryl, aralkyl, cycloalkyl, acyl, or aroyl; R′ = alkyl; X and Y = H, alkyl, halo, or alkoxy.

The 3-alkyl-2-iminobenzothiazolines bearing an unsubstituted 2-imino moiety were synthesized by treating the appropriate phenylthiourea with bromine in chloroform solvent[2] or by alkylating the corresponding 2-aminobenzothiazole with either alkyl iodides in refluxing ethanol[3,4] or methyl fluorosulfonate in refluxing chloroform. Commerically unavailable 2-aminobenzothiazoles were synthesized by following known procedures.[5,6]



I

The displacement of the imino group of 2-imino-3-methylbenzothiazoline (1) was achieved by treating it with the appropriate primary amine at 220° under a nitrogen atmosphere.

The acylation of 1 was conveniently effected by treating it with an acylating agent in pyridine.

Unexpectedly, compound **43** was isolated during the