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Steroid Antifertility Agents. Ionic Complexes of Basic Derivatives for Prolonged Action

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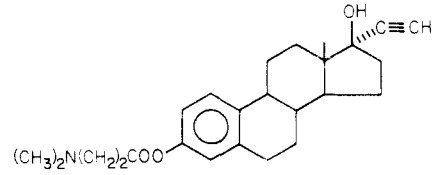
Ethynylestradiol 3-dimethylaminopropionate (1), norethindrone 3-(*O*-dimethylaminopropyl)oxime (syn and anti isomers, **2a** and **2b**), and testosterone 3-(*O*-dimethylaminopropyl)oxime (**3**) have been prepared and converted to zinc and aluminum tannate complexes as potentially long-acting prodrug forms of the parent steroids. The basic derivatives and the complexes showed the appropriate hormonal activities although they were less active in acute tests than the respective parents. The complexes of 1 showed prolonged activities and, in particular, the zinc tannate showed a prolonged duration of antifertility activity in the rat on subcutaneous administration in an aluminum monostearate gel.

In earlier reports we described the preparation of ionic complexes of narcotic antagonists with polyvalent metal ions and polybasic acids.[1-3] Several of these, notably naltrexone zinc tannate and naltrexone aluminum tannate, showed low aqueous solubility, a markedly prolonged duration of narcotic antagonist action, and a slowed rate of elimination after intramuscular injection in the form of fine suspensions in oil to mice,[1-4] rats,[4] guinea pigs,[4] and monkeys.[5] These results made it of interest, in light of the widely expressed need for long-acting antifertility agents, to investigate the feasibility of extrapolating our approach to the contraceptive steroids. Since to do this would require the use of steroids bearing basic substituents, we envisaged achieving our objective by attaching tertiary amino groups to steroids via biologically labile linkages. Conceptually then, our preparations would have a two-stage slow release process: (a) release of the base-substituted steroid from the complex; and (b) cleavage of the labile linkage and release of the pharmacologically active agent.

As a preliminary test of this strategem, we have synthesized basic derivatives of three representative steroids, specifically the estrogen ethynylestradiol, the progestin norethindrone, and the androgen testosterone. Derivatization involved attachment of a dimethylamino group via an ester or oximino function to provide ethynylestradiol 3-dimethylaminopropionate (**1**), norethindrone 3-(*O*-dimethylaminopropyl)oxime, syn and anti isomers (**2a** and **2b**), and testosterone 3-(*O*-dimethylaminopropyl)oxime (**3**). Both 1 and **2a** were found to have the appropriate hormonal activity (testing carried out by the National Institute of Child Health and Human Development) and were converted to zinc and aluminum tannate complexes.



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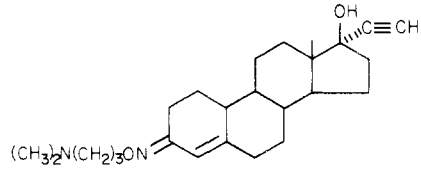
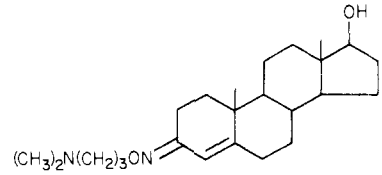


2a, syn or anti
b, anti or syn



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Prolongation of antifertility activity was demonstrated particularly with the zinc tannate complex of 1.[6]

Considerable precedent exists for the use of ester derivatives of steroids as prodrugs subject to hydrolysis by plasma esterases to release the active principle.[7-9] Oxime derivatives of steroids have also shown hormonal activity. Oximes and O-substituted oximes derived from norethindrone have shown progestational activity comparable to

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Table I. Hormonal Activities[a]

Compd	Total dose[f]	Uterotropic[b]	Clauberg[c]	Androgenic[d]
1	0.1-1.0 μg	0.23 ± 0.02		
1 Zn tannate	1.0-10 μg	0.025 ± 0.004		
1 Al tannate	1.0-10 μg	0.039 ± 0.011		
2a	6.0 mg		2.5	
2b	3.0 mg		1.7	
	6.0 mg		2.5	
2a Zn tannate	6.0 mg		Inactive	
	20.0 mg		3.4	
2a Al tannate	6.0 mg		1.0	
3	4.0 mg			Weak[e]

[a] Tests carried out by NICHD. [b] Compounds administered in sesame oil subcutaneously to rats; activity expressed on a relative scale on which the activity of ethynylestradiol = 1; range indicates 95% confidence limits. [c] Compounds administered in sesame oil subcutaneously to rabbits; activity expressed in terms of the McPhail index where 1.1 mg of norethindrone shows an index of 2.5. [d] Compounds administered subcutaneously in sesame oil to rats. [e] Androgenic activity is less than that shown by 0.6 mg of testosterone. [f] Dose of complex expressed in terms of content of steroid derivative.

Table II. Duration of Activity[a]

Compd	Total dose, mg[g]	Vehicle	Cornification, days[b] Mean incidence	Cornification, days[b] 100%	Clauberg test[d] on day 5	Clauberg test[d] on day 20	Clauberg test[d] on day 25	Antifertility act.[e] Days	Antifertility act.[e] Animals
1 Zn tannate	1.5	AMS-oil gel[c]	19.2	4				10	0/9
	1.5	AMS-oil gel[c]						30	2/10
	10	AMS-oil gel[c]						60	0/10
	20	AMS-oil gel[c]						90	0/10[f]
1 Al tannate	1.5	AMS-oil gel[c]	23.8	3				10	0/10
	1.5	AMS-oil gel[c]						30	10/10
–	0	AMS-oil gel[c]	0	0				10	10/10
–	0	AMS-oil gel[c]						30	7/8
–	0	AMS-oil gel[c]						60	9/10
–	0	AMS-oil gel[c]						90	8/8
Ethynylestradiol	32 μg	Sesame oil	0.5	0					
	1.5	AMS-oil gel[c]	9.5	7				10	7/9
	20	AMS-oil gel[c]						60	5/9
2a Zn tannate	25	Sesame oil			1.5	0.6	0.1		
Norethindrone	25	Sesame oil			3.3	0.5	1.0		

[a] Tests performed by NICHD. [b] Compounds administered in a single dose subcutaneously to rats. Data expressed as the mean incidence of cornification in days and as the number of days during which 100% of the animals remained cornified. [c] Aluminum monostearate-oil gel. See ref 2 and 3. [d] Compounds administered in a single dose subcutaneously to rabbits. Data expressed in terms of the McPhail index after the indicated number of days. [e] Compounds administered in a single dose to female rats. Data expressed as the number of animals with implantations per number of animals mated for 1 week after indicated number of days. [f] Histopathologic examination of autopsied animals revealed changes in the appearance of the uteri, suggestive of prolonged estrogenic stimulation. [g] Dose of complex expressed in terms of content of steroid derivative.

that of the parent.[10] The rates of hydrolysis in vitro have been examined of 3-oximino steroids that had been shown to be potent progestational agents.[11,12] More to the point, after intragastric administration to female rhesus monkeys, *d*-norgestrel 3-oxime 17-acetate was largely converted to *d*-norgestrel in 3–6 h and it was suggested that the oximino acetate was serving as a prodrug of *d*-norgestrel.[13]

Compounds **1**–**3** were synthesized by conventional methods as described in the Experimental Section. Use of moderate temperatures and a relatively polar hydroxylic (2-propanol) solvent helped to minimize the competing dehydrohalogenation in the reaction of dimethylamine with the β-chloropropionate ester of ethynylestradiol. The only products isolated from the alkylations of the ambident anions of the oximes of norethindrone and testosterone with dimethylaminopropyl chloride were O- rather than N-alkylated as indicated by their IR spectra and general properties. Two isomeric O-alkylated products, one (**2b**) in very small amount, were isolated from the reaction with norethindrone oxime. Their close similarity in IR spectra and other properties indicates they are the syn and anti stereoisomers but we have not determined which is which.

Conversions to the zinc and aluminum tannate complexes were carried out as previously described.[1-3] As we have noted,[1,3] these complexes do not show simple stoichiometry; nevertheless, repeat preparations afforded products of uniform composition.

Hormonal activities and durations of hormonal and antifertility activities are indicated in Tables I and II, respectively. The derivatives **1**, **2a**,**b**, and **3** all showed the appropriate hormonal action but were less active than the parent compounds (Table I). This was to be expected if the derivatives were slowly releasing the active agents in vivo, thus never providing the blood levels given by the parent compounds. The complexes were even less active which could reflect a further slowing of release as a result of their depot effect. It should also be noted that the complexes contain no more than about 30% of the active agent.

Durations of activity (Table II) were generally determined with the complexes suspended in an aluminum monostearate–oil gel because we had found this vehicle to improve the performance of our narcotic antagonist complexes,[2,3] further reducing the rate of drug release and enabling the maintenance of effective drug levels over a longer period of time. As indicated by the Clauberg test, the zinc tannate of **2a** apparently did not show an increase in duration of action in comparison with norethindrone,

both administered at the same dose level in sesame oil. However, both the zinc and aluminum tannates of **1** showed an increase in the mean incidence of cornification compared to ethynylestradiol administered at the same dose in the gel vehicle. With all preparations administered at the same dose in this vehicle, moreover, both complexes of **1** completely protected female rats mated for 1 week starting 10 days after being dosed, whereas ethynylestradiol was ineffective. Indeed, the zinc tannate complex of **1** was completely effective for up to 90 days, whereas ethynylestradiol was ineffective 60 days after administration of the same dose. Histopathologic examination of the 90-day test animals treated with the zinc tannate complex of **1** revealed changes in the appearance of the uteri, suggestive of prolonged estrogenic stimulation.

Continued testing has demonstrated the complete retention of antifertility activity 1 year after administration of a single 20 mg/kg dose of **1** zinc tannate to female rats.[15] It is recognized that further study will be required to establish whether the complex of **1** is, in fact, serving as a prodrug of ethynylestradiol or is acting in some other, unexpected fashion.

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were obtained with a Perkin-Elmer Model 137 spectrophotometer and UV spectra with a Cary Model 14 instrument, and quantitative UV determinations were carried out with a Beckman DU-2 spectrophotometer. Spectral data were consistent with assigned structures. Compounds were checked for purity by TLC on silica gel plates. Microanalyses were performed by the Micro-Tech Laboratories, Inc., Skokie, Ill., and agreed within ±0.3% of calculated values. All analyses of complexes for steroid and metal content were carried out in duplicate.

3-(Dimethylaminopropionoxy)-17α-ethynyl-1,3,5-estratrien-17β-ol (1). This synthesis was repeated several times. The following is representative. A benzene solution of 5.27 g (17.8 mmol) of ethynylestradiol and 2.55 g (20.1 mmol) of chloropropionyl chloride was heated at reflux for 5 h. The solution was concentrated to dryness and the residue was taken up in ether. The ether solution was washed with dilute alkali and water, dried, and evaporated to yield 6.2 g of a residual solid which resisted crystallization. Examination by IR and TLC indicated the material to be primarily the ester derivative.

An isopropyl alcohol solution of 5.9 g of the crude ester and anhydrous dimethylamine (3.6 g, 80 mmol) in a securely stoppered flask was magnetically stirred at room temperature for 6 days and then heated at 55 °C for 30 min. The solution was evaporated to dryness under N_2, the residue was washed with water and extracted with 2% HCl, and the acid solution was made basic and reextracted with ether. Drying and removal of the ether left a residue which was recrystallized from benzene to give 1.39 g (21% overall yield) of **1**: mp 154–155 °C; mp 158.5–160 °C after further recrystallization; TLC (silica gel) ether–petroleum ether–ammonia (9:1:0.1) R_f 0.16, chloroform–methanol–ammonia (9:1:0.2) R_f 0.63; UV max (0.1 N HCl) 278.5 nm (ε 1760). Anal. ($C_{25}H_{33}NO_3$) C, H, N.

3-(3-Dimethylaminopropoximino)-17α-ethynyl-4-estren-17β-ol (2a and 2b). The following is a representative procedure. To a stirred solution of 5.39 g (17.2 mmol) of norethrindrone oxime[6] and 1.33 g (19.0 mmol) of potassium methoxide in absolute ethanol was added, dropwise, 2.77 g (21.7 mmol) of dimethylaminopropyl chloride which had been obtained by neutralization of the hydrochloride salt. Stirring was continued and the reaction mixture was heated for 16 h at 60–70 °C. The cooled, filtered reaction mixture was diluted with ether and extracted with 2% hydrochloric acid. The acid extract was made alkaline and extracted with ether. Drying and removal of the ether and recrystallization of the residue from isopropyl alcohol afforded 1.77 g (26%) of product, mp 138–144 °C. Further recrystallization from isopropyl alcohol (charcoal) gave the predominant, low-melting oxime isomer **2a**: needles; 1.15 g; mp 142–142.5 °C; structural assignment and purity supported by IR and TLC, respectively; UV max (0.1 N HCl) 249 nm (ε 19100). Anal. ($C_{25}H_{38}N_2O_2$) C, H, N.

Concentration of the recrystallization mother liquors to a small volume and fractional crystallization of the resultant precipitate from isopropyl alcohol afforded 44 mg of the high-melting oxime isomer **2b**: mp 162.5–164.5 °C; the IR spectrum supported the structural assignment; purity was indicated by TLC. Anal. ($C_{25}H_{38}N_2O_2$) C, H, N.

TLC (silica gel), chloroform–methanol–ammonia (9:1:0.1), showed R_f of **2a** = 0.47 and of **2b** = 0.51. Both **2a** and **2b** showed IR bands at 1640 and 970 cm^{-1}.

3-(3-Dimethylaminopropoximino)-4-androsten-17β-ol (3). To an absolute ethanol solution of 595 mg (1.96 mmol) of testosterone oxime, mp 207–210 °C, and 160 mg (2.28 mmol) of potassium methoxide was added, dropwise with stirring, 305 mg (2.5 mmol) of dimethylaminopropyl chloride (obtained from the hydrochloride salt). After being allowed to stand 4 days at room temperature, the reaction mixture was filtered and concentrated, and the residue was taken up in ether. The ether solution was extracted with 2% hydrochloric acid; the acid layer was made alkaline and shaken with ether. Drying and removal of the ether left 213 mg of oil which was chromatographed on silica gel with chloroform–methanol (94:6) to yield 154 mg (20%) of **3**: mp 114–115 °C after recrystallization from isopropyl alcohol–hexane; structure and purity supported by IR and TLC. Anal. ($C_{24}H_{40}N_2O_2$) C, H, N.

Zinc Tannate Complex of 1. To a magnetically stirred solution at room temperature of 0.68 g of gallotannic acid (Mallinckrodt, AR powder, 2.0 mequiv or 0.4 mmol, assuming an approximate molecular weight of 1700 and 5 equiv per mol[1]) in 10 mL of 0.2 N sodium hydroxide was added 9.2 mL of an aqueous solution containing 2.2 mequiv of zinc sulfate followed, after 10 min, by a solution of 396 mg (1.0 mequiv) of analytically pure **1** in 11 mL of 0.1 N hydrochloric acid. Stirring was continued for 1 h after which time the reaction mixture was allowed to stand for 16 h. The precipitate was thoroughly washed with distilled water and dried to a constant weight at 60 °C in vacuo to yield 980 mg of the zinc tannate complex of **1** as a tan powder, mp 198–208 °C dec.

To analyze for **1** content, a weighed amount of complex was dissolved in 0.1 N HCl. The acid solution was treated with a pH 10.4 phosphate buffer, sodium chloride was added, and the pH was adjusted to 10 with 1 N NaOH. The aqueous layer was extracted with ether and the ether extract was shaken with 0.1 N HCl. The acid solution was diluted to standard volume, the absorbance at 278.5 nm was determined, and the amount of **1** was read off a standard curve. The complex was found to contain 31.9% **1** which represented a 79% recovery of **1** in conversion to complex.

Zinc content was determined as previously described[1] by dissolving a weighed amount of complex in 0.1 N HCl, serially diluting the solution with 0.1 N HCl, and measuring the responses of a Jarrell-Ash Model No. 82-528 atomic absorption-flame emission spectrophotometer in comparison with the dose response of standard zinc solutions. Zinc content was found to be 3.6%.

With the tannic acid content assumed by difference, the data indicated 2.1 equiv of **1** and 2.9 equiv of zinc per mole of tannic acid.

Aluminum Tannate Complex of 1. In similar fashion, a magnetically stirred solution of 510 mg (1.5 mequiv) of gallotannic acid in 7.5 mL of 0.2 N NaOH was treated with a solution of 1.5 mequiv of aluminum nitrate followed, after 10 min, by a solution of 298 mg (0.75 mmol) of **1** in 8.25 mL of 0.1 N HCl. Workup as before gave 732 mg of complex as a grey powder.

Determination of **1** content as before showed the complex to contain 33.4% **1**, indicating an 82% recovery in the conversion.

Aluminum content was determined by the eriochrome cyanine R dye method.[3,14] A weighed amount of aluminum complex was first subjected to wet oxidation with a mixture of nitric and sulfuric acids to destroy interfering organic materials (tannic acid was found to complex with aluminum more strongly than the dye). The pH of the final solution was adjusted to 6.0 with an acetate buffer, the eriochrome cyanine R dye was added, and the absorbance at 535 nm was read and compared with the absorbance of standard aluminum solutions. Aluminum content was found to be 1.4%.

The data indicated 2.2 equiv of 1 and 4.1 equiv of aluminum per mole of tannic acid.

Zinc Tannate Complex of 2a. This was prepared in the same way as the complex of 1. Determination of 2a content by measurement of absorbance of a 0.1 N HCl extract at 249 nm indicated the complex to contain 36.4% of 2a. Zinc content was found to be 2.8%. This represents 2.6 equiv of 2a and 2.4 equiv of zinc per mole of tannic acid.

Aluminum Tannate Complex of 2a. Prepared in the same way, this complex was found to contain 27.1% of 2a and 4.2% of aluminum or 1.7 equiv of 2a and 11.5 equiv of aluminum per mole of tannic acid.

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Solution Conformations of Muscarine and Some Analogues

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Proton magnetic spectra have been recorded for muscarine and two biologically active cyclopentane analogues. In order to observe homonuclear intramolecular nuclear Overhauser effects, the $-N^+(CH_3)_3$ signal was irradiated and increases in integrated intensities for other key signals in the molecule were observed. The results indicate that the quaternary side chain in these compounds is in an extended conformation in aqueous solution.

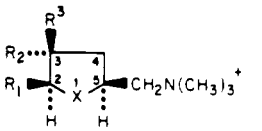
Muscarine (1) displays remarkable stereospecificity toward the cholinergic receptor system.[1] The solution conformation of muscarinic agents has long been of interest. Pullman[2] has calculated that the positive charge on the quaternary nitrogen atom is distributed over the *N*-methyl groups, and Waser[3] proposed that this cationic globe was held in position over the tetrahydrofuran ring system by electrostatic interaction with the ether oxygen. Jellinek[4] has determined the crystal structure of muscarine and Pauling and Petcher[5] that of muscarone. In both, the cationic center is directed away from the ring. Kier[6] has suggested on the basis of calculations that a similar conformation is likely in the gas phase. Furthermore, Melchiorre[7] and Givens[8] have demonstrated that the ether oxygen is not essential for high-level muscarinic activity, since cyclopentane analogues of muscarine are quite active. In cyclopentane analogues, cation–ether interaction is impossible.

The conformations of acetylcholine and some of its open-chain analogues have been previously investigated in aqueous solution by ^1H NMR[9,10] and by Raman–infrared spectroscopy.[11] Although ^{13}C NMR studies on similar systems have been published,[12] these do not afford conformational information; no previous ^1H NMR studies on muscarine analogues appear to have been carried out, although Belleau has recorded the ^1H NMR spectrum of muscarine itself.[13]

It is the purpose of this study to determine the approximate position of the cationic center in muscarine and several cyclopentane analogues in aqueous solution. This is accomplished by the homonuclear intramolecular nuclear Overhauser effect (NOE), in which the effect of irradiation of the *N*-methyl groups on the integrated intensities of other observable protons is measured.

Results and Discussion

^1H NMR signals for key protons in compounds 1–3 were



Compd	X	R_1	R_2	R_3
1	O	CH_3	OH	H
2	CH_2	CH_3	OH	H
3	CH_2	CH_3	=O	
4	CH_2	H	OH	H
5	CH_2	H	=O	

identified by comparison with ^1H NMR spectra for analogous molecules.[14] These are shown in the Experi-

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