at 0° for 15 min and a solution of 11.9 g (60.0 mmol) of Cu_2Cl_2 in 100 ml of 6 *N* HCl was added dropwise. After the evolution of N_2 gas had ceased, the reaction mixture was heated to boiling for 30 min. Cooling gave a solid which was collected by filtration to give 16.7 g (84.8%), mp 95–110°, of crude **36**. The crude **36** was sublimed at 120° (2 mm) and 7.40 g (37.6%), mp 128–133°, of the purified product was collected. The analytical sample was obtained by recrystallization from hexane, mp 133–135°.

6-Allyl-3,4-dihydro-5-hydroxy-1(2*H*)-naphthalenone (21). Allyl bromide (17.0 g, 140 mmol), anhydrous K_2CO_3 (19.3 g, 140 mmol), and 5-hydroxy-1-tetralone[6] (20.0 g, 124 mmol) were refluxed for 21 hr in dry Me_2CO. The reaction mixture was evaporated *in vacuo* and gave a residue which was dissolved in EtOAc (400 ml). After washing the EtOAc solution with 5% NaOH (2 × 400 ml) and H_2O (1 × 400 ml), the EtOAc was dried with $MgSO_4$ before being evaporated to give 24.6 g (97.5%) of the crude 5-allyloxytetralone.

The crude 5-allyloxytetralone (12.6 g, 61.8 mmol) was heated at reflux in diethylaniline (50 ml) for 28 hr. The reaction mixture was poured into 20% NaOH (1 l.) and extracted with Et_2O (3 × 1 l.). The alkaline phase was acidified with HCl and extracted with $CHCl_3$ (3 × 1.5 l.). The $CHCl_3$ extracts were combined, dried with $MgSO_4$, and evaporated to give 7.20 g (57.2%) of crude **21** which crystallized upon standing. The analytical sample was obtained by recrystallization from $PhCH_3$–hexane, mp 80–83°.

1,5-Dihydroxy-6-nitro-1,2,3,4-tetrahydronaphthalene (25) and 1,5-Dihydroxy-8-nitro-1,2,3,4-tetrahydronaphthalene (26). To a solution of 76.5 g (467 mmol) of 1,5-dihydroxy-1,2,3,4-tetrahydronaphthalene in 750 ml of AcOH, H_2O (150 ml) was added and the solution was cooled to 0° before 70% HNO_3 (59.0 ml) was added. The HNO_3 was added slowly maintaining a reaction temperature below 15°. After 30 min, the reaction mixture was poured onto 5 l. of ice–H_2O and extracted with $CHCl_3$ (2 × 1 l.) and CH_2Cl_2 (1 × 1 l.). An insoluble solid was collected by filtration and 10.4 g (10.7%), mp 179–184°, of homogeneous **26** was obtained. The combined organic extracts were dried with $MgSO_4$ and evaporated *in vacuo* and gave 74.2 g (76.1%) of a brown, oily residue. This crude mixture of products was placed on an acid-washed Al_2O_3 column (1 kg) and eluted with $CHCl_3$–MeOH fractions of 500 ml. Homogeneous **25** was obtained from 1% MeOH–$CHCl_3$ eluate, yield 26.4 g (27.0%). Analytically pure **25** was obtained by recrystallization from EtOAc–hexane: mp 98–100°; λ max, mμ ($\epsilon \times 10^{-3}$) 25% EtOH–0.1 *N* NaOH 237 (13.5), 298 (5.25), 435 (6.25); 25% EtOH–H_2O 215 (13.6), 295 (8.41), 358 (3.56); nmr (DMSO-d_6), aromatic region, δ 7.92 (1 H, d, J = 9.0 cps, C_7H) and 7.20 (1 H, d, J = 9.0 cps, C_8H).

The 8-isomeric product **26** was obtained from the MeOH eluate and gave a total yield of 12.3 g (13.0%), mp 179–184°, when combined with solid recovered from the extraction. The analytical sample btained by recrystallization from MeOH–H_2O: mp 183–185°; ς, mμ ($\epsilon \times 10^{-3}$) 25% EtOH–0.1 *N* NaOH 233 (6.07), 268 (4.77), 19.2); 25% EtOH–H_2O 240 (5.61), 315 (4.87); nmr (DMSO-d_6), aromatic region, δ 7.67 (1 H, d, J = 9.0 cps, C_7H) and 6.87 (1 H, d, J = 9.0 cps, C_6H).

3,4-Dihydro-5-hydroxy-6-nitro-1(2*H*)-naphthalenone (27). To an acetone solution (250 ml) containing 28.5 g (136 mmol) of **25**, a mixture of 15.0 g (150 mmol) of CrO_3 in H_2O (50 ml) and H_2SO_4 (16.5 ml) was added in a dropwise manner below 10°. The reaction was allowed to stir at 0° for 30 min and poured onto ice–H_2O (2 l.). The solid, **27**, which formed was collected by filtration: yield 26.3 g (93.6%); mp 127–128°. The analytical sample was obtained by recrystallization from EtOAc–hexane, mp 130–131°.

3,4-Dihydro-5-hydroxy-8-nitro-1(2*H*)-naphthalenone (28). Using **26** and the procedure outlined above for the preparation of **27**, a crude yield of the 8-nitro analog **28** was obtained in 77.4% yield, mp 250–251° dec. The sample of analytical purity was obtained by recrystallization from EtOAc–hexane, mp 254° dec.

6-Amino-3,4-dihydro-5-hydroxy-1(2*H*)-naphthalenone Hydrochloride (29). A suspension of 2.07 g (10.0 mmol) of **27** in MeOH (150 ml) was hydrogenated over 100 mg of PtO_2 until the theoretical uptake of hydrogen was observed. The catalyst was removed by filtration after 10 ml of 6 *N* HCl had been added to the reaction mixture The MeOH filtrate was evaporated and a solid product was obtained. The solid residue was recrystallized from 2-PrOH–MeOH and gave the analytically pure **29**: yield 1.15 g (54.0%); mp 226–230° dec.

8-Amino-3,4-dihydro-1(2*H*)-naphthalenone Hydrochloride (30). Compound **28** was catalytically reduced to **30** using the procedure outlined above for the preparation of **29**. An 88% yield of crude solid **30** was obtained, mp 204–212° dec. Recrystallization of the material from 2-PrOH gave the analytical yellow HCl salt, mp 211–213° dec.

8-Benzamido-3,4-dihydro-5-hydroxy-1(2*H*)-naphthalenone (33). To a cold suspension of 4.27 g (19.9 mmol) of **30** in 100 ml of CH_2Cl_2 and Et_3N (20 ml) was added 11.6 ml (100 mmol) of PhCOCl slowly over a period of 30 min. The mixture was refluxed for 2 hr and extracted with 6 *N* HCl (1 × 100 ml), 10% NaOH (1 × 100 ml), and H_2O (2 × 100 ml). The CH_2Cl_2 solution was dried with $MgSO_4$ and evaporated *in vacuo* to give the crude dibenzoyl intermediate.

The benzoyl dreivative was refluxed for 1 hr in a mixture of MeOH–20% NaOH (200 ml, 1:1). The reaction mixture was added to H_2O (250 ml), acidified, and extracted with CH_2Cl_2 (3 × 500 ml). The CH_2Cl_2 extracts were combined, washed with 10% Na_2CO_3 (200 ml) and H_2O (200 ml), and dried with $MgSO_4$. Evaporation of the solvent gave the crude **33**: yield 4.10 g (72.9%); mp 220–222°. One recrystallization from $PhCH_3$ gave the analytical sample: yield 2.65 g (47.2%); mp 223–225°.

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Preparation of Some 7-Oxaandrostane Derivatives

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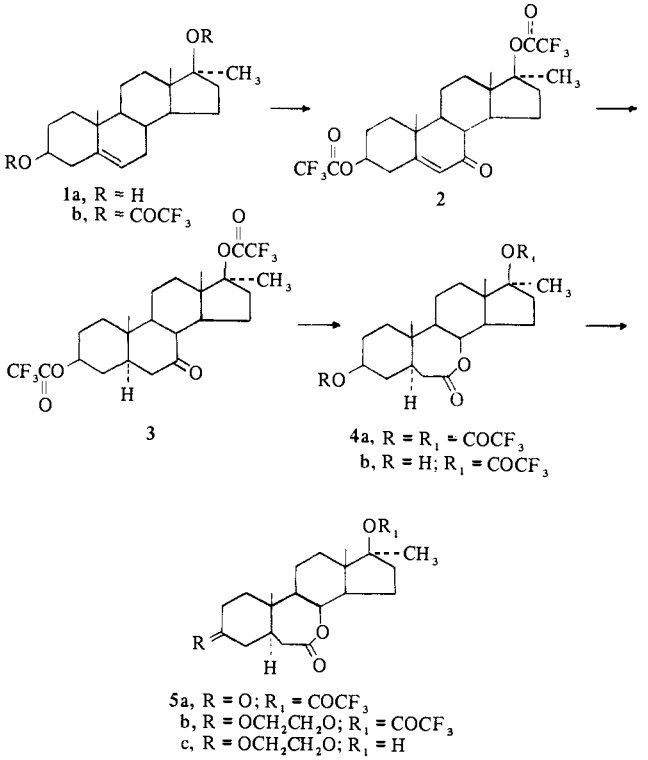
The conversion of a 5α-7-keto steroid, *via* a *B*-homo lactone, into its 7-oxa analog is outlined. The preparation and endocrinological properties of several 7-oxa derivatives are described.

In recent years, as a result of the investigations on structural modifications of naturally occurring hormones, numerous publications (for leading references, see ref 1) have described the synthesis of novel nucleo-hetero steroids, some of which have exhibited interesting biological activities.[2-4] In spite of the abundance of the various oxa and aza steroids

that have been described, those that incorporate the interesting and potentially active 5α-7-oxa grouping were hitherto unknown.† Thus, it seemed prudent to investigate this novel series of compounds.

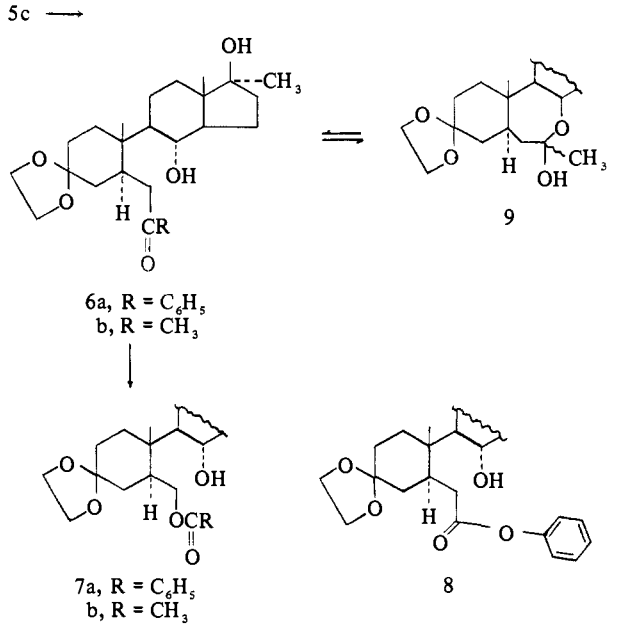
Accordingly, methylandrostenediol 1a was converted to the bisester **1b** when treated with a slight excess of trifluoroacetic anhydride in pyridine. Allylic oxidation[6] of **1b** using *tert*-butyl chromate in CCl_4 furnished the corresponding Δ^5-7-ketone **2** in just over 50% yield. Hydrogenation of **2** over 10% Pd/C gave the expected[7] 5α-dihydro derivative **3**. Baeyer-Villager oxidation of **3** using *m*-chloroperbenzoic acid in $CHCl_3$ afforded the *B*-homo lactone **4a**[8] that was readily identified by the C_8 proton at 4.23 ppm in its nmr spectrum. Selective hydrolysis of the 3β-trifluoroacetate group was accomplished using $KHCO_3$ in aqueous MeOH to give the monoalcohol **4b**. In practice it was found desirable to effect the transformation 2 → 4b without isolation of intermediates. This could be carried out in yields exceeding 80%. Reaction of **4b** with Jones reagent[9] yielded the 3-ketone **5a** which, when ketalized under standard conditions,[10] afforded the dioxolane **5b**. Saponification of **5b** using 1 equiv of KOH in aqueous MeOH–THF at 0° gave the 17β-alcohol **5c**.

Treatment of **5c** with excess PhLi[11] in THF gave a good yield of the phenyl ketone **6a** as an oil which exhibited the typical uv maximum at 243 nm (ε 9300). Prolonged reaction times or the presence of an excess of PhLi did not appear to cause the formation of the corresponding diphenylcarbinol. Oxidation of **6a** using monoperphthalic acid at room temperature affected a smooth transformation of **6a** into the benzoate **7a**. Ir and nmr analysis of the crude

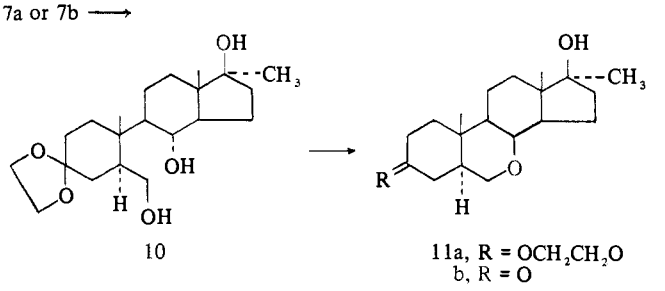


material did not show any detectable amount of the other possible oxidation product, the phenyl ester **8**. This preferential migration of a primary alkyl residue *vis à vis* an aryl group in a Baeyer-Villiger reaction is unusual but not unprecedented.[11,‡]

In a parallel sequence, the lactone **5c** was allowed to react with MeLi in THF. As before, only 1 mol of alkyllithium added to the carbonyl function. However, the product was not the expected methyl ketone **6b** but rather the hemiketal **9**. Nevertheless, this "masked" ketone readily underwent a normal Baeyer-Villiger oxidation to yield the acetate **7b**.



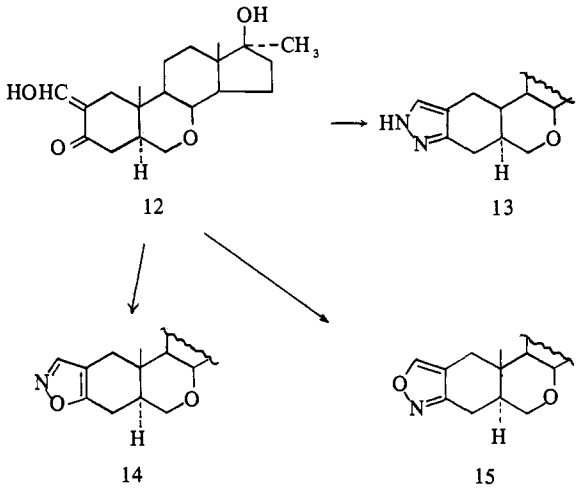
Hydrolysis of both the benzoate 7a and the acetate **7b** required unexpectedly vigorous conditions involving a large excess of base and extended reaction times. The resultant triol **10** cyclized smoothly to the 7-oxa steroid **11a** when treated with *p*-toluenesulfonyl chloride in pyridine at room temperature.[12] Thus what was, in effect, the decarbonylation of **5c** could be achieved in both series in about 50% yield. Hydrolysis of the ketal function of **11a** afforded the corresponding ketone **11b**.



Since heterocyclic fused 5α-androstane derivatives, for example, [3,2-*c*]pyrazoles,[13] and [3,2-*c*]- and [2,3-*d*]-isoxazoles,[14-16] have been shown to have superior anabolic-androgenic ratios (for leading references, see ref 17), the corresponding derivatives were made in the 7-oxa series. Treatment of **11b** with ethyl formate in pyridine in the presence of NaOMe furnished the hydroxymethylene derivative **12** which was readily converted to the [3,2-*c*]pyrazole **13** on treatment with hydrazine hydrate in PhH.[13] Reaction of **12** with $NH_2OH \cdot HCl$ in HOAc containing NaOAc[14] afforded the [2,3-*d*]isoxazole **14** while NH_2OH

†The synthesis of several 5β-7-aza- and 5β- and 5α-6-oxo-7-oxaandrostanes and -cholestanes has been reported; see ref 5.

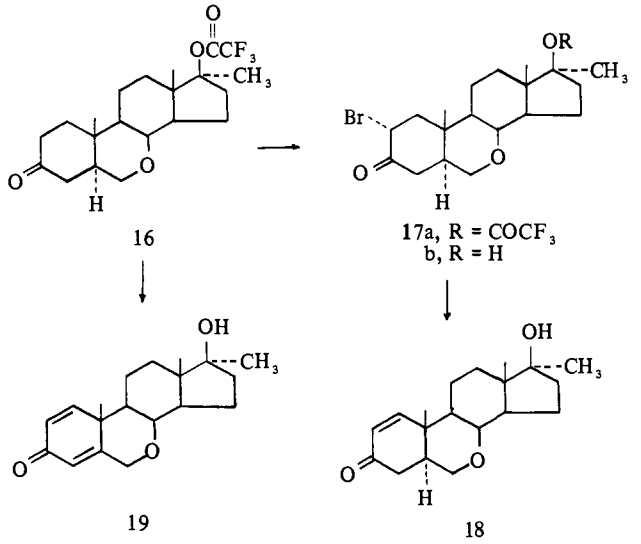
‡In a preliminary experiment using *m*-chloroperbenzoic acid as the oxidant, ir analysis suggested that the product was a mixture of 7a and 8 in the ratio of 1 : 2.



12 13 14 15

in pyridine yielded the characteristic mixture of the isomeric isoxazoles from which the [3,2-*c*]isoxazole **15** could be isolated by the prescribed method.[14-16]

The Δ^1 and $\Delta^{1,4}$ analogs of **11b** were prepared by conventional methods. Thus, the trifluoroacetyl ester **16**, derived from **11b** in the usual way, was allowed to react with pyridinium bromide perbromide[18] to give the 2α-bromo derivative **17a**. Mild saponification of the ester function followed by treatment with LiCl and Li_2CO_3 in refluxing DMF[19,20,§] furnished the Δ^1-3-ketone **18**. Dehydrogenation of **16** with DDQ in dioxane[21] and subsequent hydrolysis of the ester group gave the $\Delta^{1,4}$-3-ketone **19**. An attempted selective hydrogenation of **19** using $[(Ph)_3P]_3RhCl$[22]

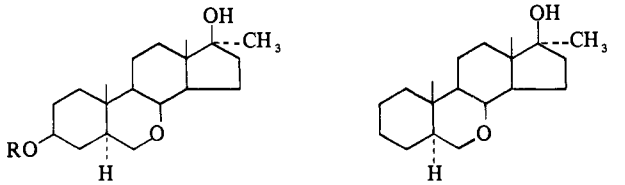


16 17a, R = $COCF_3$ b, R = H 19 18

as catalyst resulted in a mixture of the desired Δ^4-3-ketone (70%) and the product of overreduction, the saturated ketone **11b** (30%).

Reduction of **11b** using $LiAl(tert\text{-}BuO)_3H$ in THF[23] led to the corresponding 3β-hydroxy compound **20a** which was then acetylated selectively to give the monoacetate **20b**. The 3-desoxy derivative **21** was obtained by treatment of the tosylhydrazone of **11b** with $NaBH_4$ in methanol.[24]

Biological Results. The compounds were screened for various endocrinological properties. Testing for antiuterotropic activity was performed in 21-day-old rats using a procedure outlined by Boris.[25] The dosage level used was 0.5 mg/day of the test compound po and estradiol benzoate (0.1 μg/day sc) was used as standard uterine growth stimulator. Antigonadotropic activity was determined in 21-day-old rats at a level of 1 mg/day/rat po of test substance using the protocols outlined by Boris.[25] Androgenic and anabolic activities were measured according to the method of Hershberger, *et al.*,[26] at a dosage level of 1 mg/day/rat po. The only modification was that treatment began 7 days after castration of the 21-day-old rats used in the test. Compounds **4b**, **5a-c**, **11a,b**, **12**, and **13** were tested as antiestrogens and were found to be inactive. Compounds **4b**, **5a-c**, **11a,b**, **12-15**, **18**, **19**, **20a,b**, and **21** were screened for antigonadotropic, androgenic, and anabolic activities. Compounds **13** had no anabolic nor androgenic effect but it did exhibit a significant antigonadotropic effect. It caused a 16% reduction in testes weight at 1 mg/rat/day and a 51% decrease in testes weight at 4 mg/rat/day po. The remaining compounds were inactive in all three screens.



20a, R = H b, R = Ac 21

These results indicate that the incorporation of a 7-oxa function into 17β-hydroxy-17α-methyl-5α-androstane derivatives greatly diminishes their primary endocrinological properties. Nevertheless, an interesting separation of activities was noted in compound **13**. At higher dosage levels **13** retains the significant antigonadotropic properties of its "normal" steroid analog Winstrol while lacking the latter compound's anabolic and androgenic effects.

Experimental Section

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are corrected. Where elemental analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of theoretical values. The nmr spectra were determined using a Varian A-60 or HA-100 spectrometer and the chemical shifts (δ) are given in parts per million downfield from TMS. Only those resonance signals necessary for differentiating the various compounds are given. Unless indicated otherwise uv spectra were determined in EtOH solution, ir spectra in $CHCl_3$ solution, and nmr spectra in $CDCl_3$ solution.

17α-Methylandrost-5-ene-3β,17β-diol Ditrifluoroacetate (1b). Trifluoroacetic anhydride (100 ml) was added dropwise to a stirred solution of methylandrostenediol **1a** (80 g) in dry pyridine (750 ml) previously cooled to 0° at such a rate that the reaction temperature did not exceed 0-5°. After the addition was complete, the mixture was maintained at 0-5° for 1 hr; then it was poured slowly into an ice-H_2O mixture (3 l.) containing concentrated HCl (750 ml). The off-white precipitate was recovered by filtration, washed (H_2O), and air-dried to give 136 g of **1b**, mp 136-138°. Crystallization from CH_2Cl_2-MeOH gave the analytical sample: mp 140.5-141°; $[\alpha]^{25}D$ -60.7°; ir 1775 cm^{-1}. *Anal.* ($C_{24}H_{30}F_6O_4$) C, H, F.

3β,17β-Dihydroxy-17α-methylandrost-5-en-7-one Ditrifluoroacetate (2). To a stirred solution of **1b** (39.5 g) in CCl_4 (400 ml) containing HOAc (75 ml) and Ac_2O (20 ml) at 60° was added a mixture of *tert*-butyl chromate in CCl_4 (250 ml, equivalent to 46 g of CrO_3), HOAc (75 ml), and Ac_2O (20 ml). The mixture was stirred at 60-70° for 18 hr, then was cooled to room temperature and filtered, and the filtrate was added to a stirred solution of oxalic acid (100 g) in H_2O (1 l.). After 30 min the layers were separated and the organic layer was washed with H_2O (twice) and with brine (twice). The dried (Na_2SO_4) organic extract was percolated through a short column of silica gel and then was evaporated to give 29.5 g of crude product. Crystallization from Et_2O-hexane gave 19.7 g of **2**, mp 145-148°. Concentration of the mother liquors gave an additional 3.1 g, mp 139-144°. The analytical sample was obtained

§ An attempt to dehydrohalogenate **17a** before hydrolysis of the ester function resulted in the facile pyrolytic elimination of the trifluoroacetate group.

from the same solvent system: mp 147–148°; $[\alpha]^{25}D$ −105.7°; uv 233 nm (ε 13,000); ir 1777 and 1672 cm^{-1}; nmr δ 5.78 (s, 1 H, C_6H). *Anal.* ($C_{24}H_{28}F_6O_6$) C, H, F.

3β,17β-Dihydroxy-17α-methyl-5α-androstan-7-one Ditrifluoroacetate (3). A solution of 2 (61 g) in EtOAc (500 ml) containing pyridine (1 ml) was hydrogenated (23°, 760 mm) over 10% Pd/C (4.5 g). After absorption of 1 molar equiv of H_2 (1 hr) the rate of hydrogenation diminished sharply and the reaction was stopped. The catalyst was removed by filtration through Celite and the filtrate was evaporated to dryness to give 62 g of 3 as a colorless oil that was homogenous by tlc. The crystalline material, mp 118–119°, was obtained from Et_2O–hexane: $[\alpha]^{25}D$ −44.9°; ir 1780 and 1710 cm^{-1}. *Anal.* ($C_{24}H_{30}F_6O_6$) C, H, F.

3β,17β-Dihydroxy-17α-methyl-7a-oxa-5α-*B*-homoandrostan-7-one Ditrifluoroacetate (4a). A solution of 3 (33 g) and *m*-chloroperbenzoic acid (20 g) in $CHCl_3$ (300 ml) was refluxed for 4 hr. The reaction mixture was cooled and was washed in turn with $NaHSO_3$ solution (three times), cold 1 *N* NaOH solution (once), and H_2O (twice). The organic extracts were dried (Na_2SO_4) and evaporated to give 33.6 g of essentially pure **4a** as a white solid. Crystallization of a small sample from Et_2O–hexane gave the analytical specimen: mp 166–167°; $[\alpha]^{25}D$ − 38.5°; ir 1780 and 1635 cm^{-1}; nmr δ 4.26 (m, 1 H, C_6H). *Anal.* ($C_{24}H_{30}F_6O_6$), C, H, F.

3β,17β-Dihydroxy-17α-methyl-7a-oxa-5α-*B*-homoandrostan-7-one 17-Trifluoracetate (4b). A solution of $KHCO_3$ (60 g) in H_2O (200 ml) was added to a stirred solution of **4a** (33 g) in MeOH (500 ml). After 30 min at room temperature, the reaction mixture was diluted with H_2O (2 l.). The resulting percipitate was collected by filtration, washed with water, and dried *in vacuo* to give 27.1 g of 4b, mp 185–187°. Crystallization from CH_2Cl_2–Et_2O afforded the analytical material: mp 188–188.5°; $[\alpha]^{25}D$ −33.4°; ir 3610, 1775, and 1730 cm^{-1}; nmr δ 2.88 (m, 2 H, C_6H_2) and 4.23 (m, 1 H, C_8H). *Anal.* ($C_{22}H_{31}F_3O_5$) C, H, F.

17β-Hydroxy-17α-methyl-7a-oxa-5α-*B*-homoandrostane-3,7-dione Trifluoroacetate (5a). A solution of **4b** (39 g) in Me_2CO (600 ml) was cooled to 0°. To the stirred solution Jones reagent (40 ml) was added and the reaction mixture was stirred at 0–5° for 5 min. *i*-PrOH (50 ml) was added and the mixture was stirred for an additional 5 min; then it was diluted with water (4 l.). The resulting precipitate was collected by filtration and then was dissolved in CH_2Cl_2. The dried (Na_2SO_4) solution was evaporated under reduced pressure to give 36.5 g of the ketone 5a. Crystallization of a small portion from Et_2O–hexane furnished the analytical sample: mp 172–173°; $[\alpha]^{25}D$ −38.9°; ir 1778 and 1730 cm^{-1}. *Anal.* ($C_{22}H_{29}F_3O_5$) C, H, F.

17β-Hydroxy-17α-methyl-7a-oxa-5α-*B*-homoandrostane-3,7-dione Trifluoroacetate 3-Ethylene Ketal (5b). A solution of 5a (36 g) and *p*-TsOH (0.5 g) in PhH (300 ml) containing ethylene glycol (36 ml) was heated under reflux for 3 hr. The H_2O was removed as it was formed by means of a Dean-Stark trap. The mixture was cooled and poured into an ice-cold $NaHCO_3$ solution (300 ml). The layers were separated and the organic layer was washed with brine, then dried (Na_2SO_4), and concentrated to dryness. The resulting residue was crystallized from MeOH to give 32.4 g of 5b, mp 185–187°. Crystallization from CH_2Cl_2–MeOH furnished the analytical material: mp 187.5–188°; $[\alpha]^{25}D$ −29.9°; ir 1775 and 1725 cm^{-1}; nmr δ 3.95 (s, 4 H, $-OCH_2CH_2O-$). *Anal.* ($C_{24}H_{33}F_3O_6$) C, H, F.

17β-Hydroxy-17α-methyl-7a-oxa-5α-*B*-homoandrostane-3,7-dione 3-Ethylene Ketal (5c). To a stirred solution of 5b (27 g) in THF (500 ml) previously cooled to 0° was added 57 ml of 1 *N* NaOH solution. A sufficient amount of MeOH was added to make the reaction homogeneous and after 5 min 10 ml of EtOAc was added. The reaction mixture was concentrated *in vacuo* to ~100 ml and then was diluted with H_2O. The resulting precipitate was recovered by filtration, then washed with H_2O, and air-dried. Crystallization from Et_2O–hexane afforded 15.1 g of 5c, mp 211–212°. An additional 3.4 g of material, mp 211–212°, was obtained from the mother liquor. The same solvent system gave the analytical material: mp 212.5–213°; $[\alpha]^{25}D$ −42.5°; ir 3600 and 1722 cm^{-1}. *Anal.* ($C_{22}H_{34}O_5$) C, H.

7-Oxa-17β-hydroxy-17α-methyl-5α-androstan-3-one 3-Ethylene Ketal (11a). Method A. *Via* **the Phenyl Ketone 6a.** A solution of PhLi in 3:1 PhH–Et_2O (2 *N*, 70 ml) was added to a stirred solution of 5c (21.2 g) in dry THF (500 ml) that had been cooled to 0°. After the addition was complete, the mixture was stirred at room temperature for 1 hr and then it was poured into a mixture of ice and saturated Rochelle salt solution. The layers were separated and the aqueous portion was extracted with EtOAc (three times). The organic extracts were washed in turn with brine and then were combined, dried (Na_2SO_4), and evaporated. The resulting oil was dissolved in MeOH (150 ml) and diluted with hexane (150 ml) and H_2O (15 ml). After the mixture was shaken, the layers were separated and the aqueous MeOH layer was washed with hexane (3 × 50 ml). The hexane layers which primarily contained biphenyl were discarded. The aqueous methanol layer was concentrated *in vacuo* and the resulting residue was dissolved in $CHCl_3$. The dried (Na_2SO_4) solution was evaporated to give 25.1 g of crude phenyl ketone 6a as an oil, homogeneous by tlc: uv 243 nm (ε 9800); ir 3620 and 1690 cm^{-1}.

The phenyl ketone **6a** (25.1 g) was dissolved in $CHCl_3$ (300 ml) and was treated with a solution of monoperphthalic acid in Et_2O (0.6 *M*, 125 ml). The solution was left at room temperature for 3 hr and then it was washed in turn with 1 *N* NaOH solution and brine. Evaporation of the dried (Na_2SO_4) organic layer gave the benzoate ester 7a (23.9 g) as an oil: uv 228 nm (ε 12,500), 266 (730), 273 (830), and 279 (640); ir 3620, 1710, and 1280 cm^{-1}.

A mixture of 7a (23.9 g) in EtOH (300 ml) and 10 *N* NaOH solution (20 ml) was heated under reflux. The progress of the reaction was monitored by tlc and after 36 hr the starting material had been consumed. The reaction mixture was concentrated *in vacuo* and then was diluted with CH_2Cl_2. The organic solution was washed with brine (twice), dried (Na_2SO_4), and evaporated to give 15.3 g of the triol 10.

p-TsCl (10 g) was added to a cooled (~10°) solution of the triol (15.1 g) in pyridine (100 ml). After the solution was stirred at room temperature a small amount of ice (4 g) was added. The stirring was continued for an additional 10 min. The solution was poured into an ice–water mixture (1.2 l.) containing concentrated HCl (100 ml). The reaction mixture was quickly extracted with CH_2Cl_2 (three times) and the organic extracts were washed in turn with brine (once), 1 *N* NaOH solution (once), and with brine (once). Evaporation of the dried (Na_2SO_4) CH_2Cl_2 layers furnished 12.8 g of an off-white solid. Trituration of the crude material with hexane gave 10.2 g of the oxa steroid **11a**, mp 193–197°. Crystallization from MeOH gave the analytically pure specimen: mp 199–200°; $[\alpha]^{25}D$ −3.96°; ir 3610 cm^{-1}; nmr δ 3.61 (m, 3 H, C_6H_2 and C_8H) and 3.93 (s, 4 H, $-OCH_2CH_2O-$); molecular ion *m/e* 350. *Anal.* ($C_{21}H_{34}O_4$) C, H.

Method B. *Via* **the Hemiketal (9).** To a stirred solution of 5c (16.0 g) in dry THF (400 ml) previously cooled to 0° was added a solution of MeLi in Et_2O (1.9 *M*, 110 ml). The cooling bath was removed and the mixture was stirred at room temperature for 15 min and then it was poured over ice and extracted with EtOAc. The organic extracts were washed with H_2O, dried (Na_2SO_4), and evaporated to give a white solid 9 (15.7 g): molecular ion *m/e* 394; nmr δ 1.38 (s, 3 H, $CH_3C(OH)O-$).

An ethereal solution of monoperphthalic acid (0.65 *M*, 400 ml) was added to a solution of the hemiketal 9 (15.7 g) in $CHCl_3$ (400 ml). The mixture was left at room temperature for 3 hr; then it was washed with 1 *N* NaOH solution (once) and brine (once). The dried (Na_2SO_4) layer was evaporated *in vacuo* to give 12.3 g of 7b as a colorless oil.

A solution of 7b (12.3 g) in EtOH (500 ml) and 10 *N* NaOH (20 ml) was refluxed for 16 hr. The reaction mixture was concentrated *in vacuo* and then was diluted with H_2O and extracted with CH_2Cl_2 (three times). The combined organic extracts were washed with H_2O, dried (Na_2SO_4), and taken to dryness under reduced pressure to give the triol 10 (10.5 g). This material was transformed into 11a by the method described above.

7-Oxa-17β-hydroxy-17α-methyl-5α-androstan-3-one (11b). A solution of 11a (12.2 g) in THF (150 ml) was treated with 1 *N* HCl solution (20 ml) and was allowed to stand at room temperature for 24 hr. Most of the THF was removed *in vacuo* and the concentrate was diluted with H_2O (150 ml) and extracted with CH_2Cl_2 (twice). The organic layers were washed with water (twice), dried (Na_2SO_4), and concentrated under reduced pressure to give 10.5 g of the crude ketone. Crystallization from CH_2Cl_2–hexane gave 8.2 g of **11b**, mp 184–187°. Recrystallization from CH_2Cl_2–Et_2O furnished the analytical sample: mp 187–188.5°; $[\alpha]^{25}D$ +25.5°; ir 3620 and 1710 cm^{-1}; ORD in dioxane (*c* 0.254) $[\phi]_{700}$ +63°, $[\phi]_{589}$ +80°, $[\phi]_{317}$ +3120° (peak), $[\phi]_{309}$ +2736° (shoulder), $[\phi]_{298}$ 0°, $[\phi]_{266}$ −3802° (trough), $[\phi]_{232}$ −2836° (peak), $[\phi]_{208}$ −3621°. *Anal.* ($C_{19}H_{30}O_3$) C, H.

7-Oxa-2-hydroxymethylene-17β-hydroxy-17α-methyl-5α-androstan-3-one (12). A solution of 11b (4.1 g) and NaOMe (1.38 g) in dry pyridine (90 ml) containing ethyl formate (7.5 ml) was stirred at room temperature for 16 hr and then was poured into an ice–H_2O mixture containing HOAc (75 ml). The resulting mixture was extracted with CH_2Cl_2 (3 × 100 ml). The combined organic layers were washed with H_2O (once) and then with 2% KOH solution (6 × 100 ml). The combined basic extracts were washed with Et_2O (once)

and then acidified with HOAc (20 ml). The resulting precipitate was washed with H_2O and dried to give 3.2 g of the hydroxymethylene derivative, mp 229–236°. Crystallization from PhH–hexane afforded the analytical sample: mp 235–238°; $[\alpha]^{25}D$ +51.8°; uv 281 nm (ϵ 8850); ir 3600, 1640, and 1585 cm^{-1}; nmr δ 8.56 (s, 1 H, C_2H). *Anal.* ($C_{20}H_{30}O_4$) C, H.

7-Oxa-17β-hydroxy-17α-methyl-5α-androstano[3,2-*c*]pyrazole (13). A solution of **12** (2.2 g) in absolute EtOH (75 ml) containing (0.52 ml) 85% hydrazine hydrate was refluxed for 1 hr and then evaporated to dryness. The residual solid was crystallized from MeOH to give 1.76 g of the pyrazole **13**: mp 268–269°; $[\alpha]^{25}D$ +42.6°; uv 222 nm (ϵ 4710); ir 3600 and 3465 cm^{-1}; nmr (DMSO) δ 7.17 (2, 1 H, C_3H). *Anal.* ($C_{20}H_{30}N_2O_2$) C, H, N.

7-Oxa-17β-hydroxy-17α-methyl-5α-androstano[2,3-*d*]isoxazole (14). A solution of $HONH_2 \cdot HCl$ (0.565 g) and NaOAc (0.66 g) in H_2O (2 ml) was added to a solution of **12** (2.7 g) in AcOH (25 ml) and heated at 100° for 30 min. The reaction mixture was cooled and the crystalline precipitate that had formed was collected by filtration and washed with 80% HOAc and with H_2O. The dried solids (1.8 g) triturated in refluxing CH_2Cl_2 (100 ml) to give 1.3 g of pure [2,3-*d*]isoxazole, mp 301–302°. Crystallization from THF–MeOH gave the analytical sample: mp 301–301.5°; uv (MeOH) 228 nm (ϵ 5030); ir (KBr) 3400 and 1640 cm^{-1}; nmr (DMSO) δ 8.35 (s, 1 H, C_2H). *Anal.* ($C_{20}H_{29}NO_3$) C, H, N.

7-Oxa-17β-hydroxy-17α-methyl-5α-androstano[3,2-*c*]isoxazole (15). A solution of $HONH_2 \cdot HCl$ (1.13 g) in H_2O (2 ml) was added to a solution of **12** (2.7 g) in warm pyridine (10 ml) and the mixture was refluxed for 3 hr. The solvent was removed *in vacuo* and the residue was partitioned between EtOAc (500 ml) and 1 *N* HCl solution. The organic layer was washed with 1 *N* HCl solution (twice) and brine (once) and then was dried (Na_2SO_4) and evaporated. The residue (2.4 g) was dissolved in dry THF (250 ml) containing NaOMe (1 g) and the solution was stirred at room temperature for 1 hr. The reaction mixture was washed in turn with 2% NaOH in brine and with brine; then the organic solution was dried ($MgSO_4$) and concentrated under reduced pressure. Crystallization of the resulting solid from Me_2CO afforded 1.7 g of the isoxazole: mp 290–292°; $[\alpha]^{25}D$ +34.2°; uv (MeOH) 225 nm (ϵ 3900); ir 3620 and 1595 cm^{-1}; nmr (DMSO) δ 8.55 (s, 1 H, C_2H). *Anal.* ($C_{20}H_{29}NO_3$) C, H, N.

7-Oxa-17β-hydroxy-17α-methyl-5α-androst-3-one Trifluoroacetate (16). To a solution of **11b** (1.0 g) in dry pyridine (7 ml) previously cooled to 0° was added trifluoroacetic anhydride (1.0 ml). The reaction mixture was stirred at 0–5° for 30 min, then was poured into an ice–H_2O mixture, and acidified with 3 *N* HCl solution. The precipitate that formed was collected by filtratrion, washed with H_2O, and dissolved in CH_2Cl_2. The dried solution (Na_2SO_4) was evaporated *in vacuo* to give 1.1 g of **16**, ir 1775 and 1710 cm^{-1}.

7-Oxa-17β-hydroxy-17α-methyl-5α-androst-1-en-3-one (18). A mixture of **16** (3.0 g) and pyridinium bromide perbromide (2.64 g) in AcOH (45 ml) was stirred at room temperature for 10 min and then was poured into an ice–H_2O mixture. The resulting precipitate was recovered by filtration, washed well with H_2O, and then dissolved in a mixture of MeOH and THF (1:1, 150 ml). The solution was cooled to 0° and 6 ml of 1 *N* NaOH was added. After 15 min at 0–5°, most of the solvent was removed *in vacuo* and the concentrate was diluted with H_2O and extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated *in vacuo* to give 2.6 g of the crude bromo derivative 17b as a foam.

To a solution of crude **17b** (2.6 g) in DMF (30 ml) was added LiCl (0.65 g) and Li_2CO_3 (0.73 g). The stirred reaction mixture was refluxed for 2 hr under N_2; then it was cooled and diluted with H_2O. Extraction with CH_2Cl_2 furnished 2 g of crude material which when crystallized from EtOAc afforded 1.2 g of enone **18**, mp 192–196°. Recrystallization from EtOAc gave the analytical sample: mp 197–198°; $[\alpha]^{25}D$ +17.1°; uv 225 nm (ϵ 10,750); ir (KBr) 3520 and 1660 cm^{-1}; nmr δ 7.11 (d, 1 H, C_1H) and 5.90 (d, 1 H, C_2H). *Anal.* ($C_{19}H_{28}O_3$) C, H.

7-Oxa-17β-hydroxy-17α-methylandrosta-1,4-dien-3-one (19). A solution of 16 (3.0 g), *p*-TsOH (10 mg), and DDQ (3.6 g) in dioxane (65 ml) was heated under reflux for 5 hr. The cooled reaction mixture was diluted with CH_2Cl_2 (200 ml) and the precipitated hydroquinone was removed by filtration. The filtrate was washed with 1 *N* NaOH solution (three times) and with H_2O. The dried (Na_2SO_4) organic extract was evaporated. The residual oil (2.2 g) was dissolved in MeOH (50 ml) and treated with 10 ml of 1 *N* NaOH solution. After 60 min at room temperature, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2 (twice). The organic layers were washed with H_2O, dried (Na_2SO_4), and concentrated *in vacuo* to give 1.6 g of a pale yellow foam. Crystallization of the residue from MeOH furnished 1.2 g of the dienone **19**, mp 183–185°. The analytical sample was obtained from the same solvent: mp 185–186°; $[\alpha]^{25}D$ +17.4°; uv 240 nm (ϵ 14,600); ir 3610, 1660, 1622, and 1600 cm^{-1}; nmr δ 7.07 (d, 1 H, C_1H), 6.28 (m, 1 H, C_2H), and 6.16 (d, 1 H, C_4H). *Anal.* ($C_{19}H_{26}O_3$) C, H.

7-Oxa-17α-methyl-5α-androstane-3β,17β-diol (20a). A solution of **11b** (1.5 g) in dry THF (25 ml) was added to a mixture of LiAl(*tert*-BuO)$_3$H (3.0 g) in dry THF (30 ml) and the reaction mixture was stirred at 0° for 30 min. After the excess hydride was destroyed by the careful addition of H_2O, most of the solvent was removed under reduced pressure. The residue was partitioned between EtOAc and H_2O and the organic layer was dried (Na_2SO_4) and evaporated to give 1.5 g of product. Crystallization from Et_2O gave 1.4 g of diol, mp 173–175°. The analytical specimen was crystallized from the same solvent: mp 174–175°; $[\alpha]^{25}D$ −12.0°; ir 3610 and 3450 cm^{-1}. *Anal.* ($C_{19}H_{32}O_3$) C, H.

7-Oxa-17α-methyl-5α-androstane-3β,17β-diol 3-Acetate (20b). A solution of **20a** (1.2 g) in pyridine (2 ml) and Ac_2O (2 ml) was left at room temperature for 16 hr and then was poured into an ice–H_2O mixture. The resulting precipitate was collected by filtration and washed with H_2O. The dried solid was crystallized from Et_2O–hexane to give 1.1 g of the monoacetate **20b**, mp 148–149°. Recrystallization from the same solvent mixture afforded the analytical sample: mp 149–150°; $[\alpha]^{25}D$ −16.9°; ir 3610 and 1730 cm^{-1}; nmr δ 4.7 (m, 1 H, C_3H) and 2.0 (s, 3 H, CH_3CO). *Anal.* ($C_{21}H_{34}O_4$) C, H.

7-Oxa-17α-methyl-5α-androstan-17-ol (21). A solution of **11b** (3.4 g) and *p*-$TsNHNH_2$ (3.4 g) in MeOH (150 ml) was heated under reflux for 2 hr. The solution was cooled and $NaBH_4$ (2 g) was added; then the mixture was refluxed for 5 min. After the addition of H_2O (10 ml), the cooled solution was concentrated under reduced pressure until solids formed. The concentrate was diluted with CH_2Cl_2 and was washed in turn with 1 *N* HCl solution (once), 1 *N* NaOH solution (once), and brine. Evaporation of the dried (Na_2SO_4) organic layers furnished 2.7 g of a solid. Crystallization from MeOH–H_2O gave 2.1 g of **21**, mp 173–175°. The analytically pure sample was obtained from the same solvent system: mp 175–176°; $[\alpha]^{25}D$ −12.5°; ir 3610 cm^{-1}. *Anal.* ($C_{19}H_{32}O_2$) C, H; C: calcd, 73.98; found, 73.45.

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Aromatic Esters Which Inhibit Plasmin or Thrombin by Formation of Relatively Stable Acyl Enzymes

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Plasmin and thrombin were inhibited by several substituted benzoic acid esters due to the formation of a moderately stable, inactive, covalent intermediate (acyl enzyme). Although both of these enzymes are serine proteinases of trypsin-like specificity, selective inhibition is shown to be possible through the use of active esters (nitrophenyl) of benzoic acid carrying positively charged substituents such as sulfonium, isothiouronium, and pyridinium. Para substitution favored the selective inhibition of plasmin which appeared to be more susceptible to this type of inactivation than thrombin by the compounds studied thus far. However, thrombin was more extensively converted to the acyl enzyme form than plasmin by certain meta-substituted derivatives.

Thrombin and plasmin are proteolytic enzymes of the serine proteinase class for which there is a common mechanism of hydrolysis involving a covalent intermediate between the enzyme and part of the substrate.[1] The carboxyl group participating in the bond of the substrate that is cleaved becomes transferred to a serine group of the active center of the enzyme. This intermediate, an acyl enzyme, is subsequently split hydrolytically to liberate the carboxylic acid and regenerate the enzyme, completing a catalytic cycle. With typical substrates, the kinetic properties of the acylation and deacylation steps are such that in steady-state function little of the enzyme is present as the acyl enzyme, judging from the well-studied example of chymotrypsin.[2] This is true even in the case of esters for which the rate of acylation is greater than that of deacylation (in contrast to amides). For example, in the case of *N*-acetyl-L-phenylalanine ethyl ester for which the kinetic constants at pH 5 are available,[2] the steady-state level of acyl enzyme can be calculated (using eq 9 of ref 3) to be 0.55% at a substrate concentration of 10^{-5} *M* or 5.1% at 10^{-4} *M*. This is the concentration range of interest in the present investigation. On the other hand, there have been found some synthetic esters for which the deacylation step is very slow. As a consequence these "poor" substrates lead to a steady state in which much or all of the enzymes is tied up as an acyl enzyme and is therefore not available for function. For example, trypsin is inactivated, even at a pH optimal for its function, by the ethyl ester[4] or *p*-nitrophenyl ester (NPGB)[5] of *p*-guanidinobenzoic acid. In view of the fact that an increasing number of serine proteases of physiological importance are being discovered whose inhibition may be of therapeutic value, it was proposed that ester substrates might be synthesized that would provide a means for their selective inactivation.[3,6]

Since NPBG was found to convert thrombin and plasmin completely to an acyl enzyme form, although with different stabilities,[3] a number of other substituted benzoates have been synthesized and examined for their ability to provide this type of inactivation. These contained positive charges since earlier work with trypsin[4,7] as well as in the action of esters of *p*-guanidinobenzoic acid with thrombin and plasmin[3] indicated that substrate behavior was encountered with aromatic esters containing an amidino or guanidino group and having an overall geometry comparable to lysine or arginine.

With respect to the alcohol portion of the esters, the present study was confined to nitrophenyl esters which provided a twofold advantage. Enzymatic cleavage could be readily followed by spectrophotometry and the extent of conversion of thrombin or plasmin to an acyl enzyme could be determined by the subsequent addition of NPGB as a titrant[8] to the test mixture during the same analytical procedure. In addition, nitrophenyl esters are activated with respect to enzyme acylation and thus kinetically favor the accumulation of acyl enzyme. However, the deacylation rate for acyl enzyme hydrolysis is generally rate limiting[2,9] and therefore the extent of inactivation by activated or nonactivated (*viz.* ethyl) esters may be the same. The nature of the alcohol group is a structural variable that may be usefully manipulated to modulate the inhibitory properties of an ester whose main characteristics are determined by the structure of the acyl component. The esters synthesized in the present work structurally complement a group available from earlier work with trypsin[10] and are now examined for their action on thrombin and plasmin.

Chemistry. The new esters described in this study were prepared from the nitrophenyl esters of *p*- and *m*-bromomethylbenzoic acid by reaction with nucleophiles which displaced the bromine without disrupting the ester bond (Table I). The remaining compounds were obtained as described earlier.[10]

Results

The nitrophenyl esters examined were derived from benzoic acid which had a meta or para substituent, either uncharged or positively charged, and were tested at pH 8.3 in the concentration range of 10^{-5}-10^{-4} *M*. As expected, esters with uncharged substituents such as *p*-hydroxy-, methyl-, amino-, chloroacetamido-, as well as nitrophenyl benzoate itself, gave evidence of being very poor substrates for thrombin and plasmin. On the other hand, positively charged substituents such as isothiouronium, sulfonium, or pyridinium