means of the Wilzbach technique[6] and purified to constant specific radioactivity (21 μCi/mg). Labeled **5** used in this study showed a specific radioactivity of 4.07 μCi/mg and was chromatographically pure. Distribution of tritium was 71% in the aglycone and 29% in daunosamine residue.

Biological Activity Evaluation. The compounds were evaluated for cytotoxic, antiviral, and antitumor activity, according to methods similar to those used for daunomycin derivatives.[7-9] Drugs were dissolved in Ringer solution, except adriamycin 14-octanoate and adriamycin 14-benzoate, which were administered in aqueous solution containing 1% (v/v) of dimethyl sulfoxide. In one experiment (activity on Gross leukemia), compound **5** was dissolved in 5% aqueous ethyl alcohol.

In vitro tests were carried out on HeLa cells and on secondary mouse embryo fibroblasts (MEF), infected or not with the Murine Sarcoma virus (Moloney) (MSV-M). HeLa cells were treated for 8 hr; then the drugs were removed and replaced by normal medium; cells were counted in an haemocytometer after 48 hr. MEF were plated on 35-mm Falcon plastic dishes, infected with MSV-M and treated for 3 days with compounds under study. The number of foci was evaluated microscopically 5 days after the infection. Uninfected MEF were similarly treated; at the end of the experiment cells were counted in an haemocytometer.

The antitumor activity of derivatives under study was tested on Sarcoma 180 (solid and ascites), MSV-M induced sarcoma, intravenously transplanted Gross leukemia, and transplanted mammary carcinoma. CD 1 mice (Charles River Breeding Laboratories, Calco, Italy) were used in the test on Sarcoma 180 and MSV-M induced sarcoma; C_3H/He Dp mice were used in the experiments on the other transplanted tumors. Each experimental group consisted of at least ten mice. Compounds were administered ip or iv in a volume of 10 ml/kg of body weight, according to different schedules of treatment. Each drug was tested over a wide range of doses. For the sake of brevity, only results obtained for the optimal doses are reported (Table III).

Incubation Experiments. CD 1 male albino mice were sacrificed by decapitation under ether anaesthesia. Hearts, livers, kidneys, and spleens were excised and homogenized in 2 vol of 0.25 *M* sucrose, 10 m*M* $MgCl_2$, and 10 m*M* tris-HCl, pH 7.6, using a glass Teflon homogenizer. The suspension was centrifuged at 3000 g for 10 min and the supernatant was used for the incubation experiments. The incubation mixtures were prepared by adding 0.5 ml of an aqueous solution of labeled **5** to 3.5 ml of each supernatant. Final concentration of **5** was 85 μg/ml. Incubations were carried out at 37° in open 10-ml flasks and samples were taken at the times indicated (Table IV). Similar incubations were performed using blood serum previously diluted with 2 vol of the above-mentioned buffer solution. Aliquots of the samples were freeze-dried, extracted with a mixture of equal volumes of chloroform and methanol, and analyzed by tlc. Recovery of total radioactivity was higher than 90%. Thin-layer chromatography was carried out in silica gel plates. The following systems were used: I, chloroform-methanol-water (13:6:1 by volume); II, methylene chloride-methanol-water (100:20:2 by volume); III, chloroform-ethanol-acetic acid-water (179:4:5:12 by volume); IV, ethyl acetate-benzene-petroleum ether (bp 40-70°) (70:25:5 by volume). When systems I and II were used, plates were buffered at pH 7 with phosphate buffer. The estimation of labeled compounds involved scraping of chromatogram in sections of the same length, extracting each section with 0.5 ml of methanol in a counting vial, and adding 10 ml of the scintillation mixture to the extract. The R_f values of the compounds related to adriamycin 14-octanoate metabolism are reported in Table VI.

Table VI. Chromatographic R_f Values

Compound	Solvent system I	II	III	IV
Daunosamine	0.00	0.00		
Adriamycin	0.25	0.15		
Adriamycin octanoate	0.60	0.25		
Adriamycinone	0.90	0.70	0.35	0.25
7-Deoxyadriamycinone	0.90	0.70	0.35	0.40

Distribution Studies. CD 1 mice were injected iv with equimolar doses of tritiated adriamycin[10] (4.35 mg/kg) and of tritiated adriamycin 14-octanoate (5.31 mg/kg). Animals were killed at different times after treatment and samples of the organs were obtained and processed for liquid scintillation counting by combustion in a Packard samples oxidizer.

Measurement of Radioactivity. A Packard Tri-Carb liquid scintillation spectrometer Model 3375 and counting medium of 100 g of naphthalene, 7 g of PPO, and 0.3 g of POPOP/l. of dioxane were used. The counting efficiency of the samples was determined by the channel ratio method for quenching correction.

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(2) F. Arcamone, G. Franceschi, S. Penco, and A. Selva, *Tetrahedron Lett.*, 1107 (1969).
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(9) A. Di Marco, A. M. Casazza, T. Dasdia, G. Giuliani, L. Lenaz, A. Necco, and C. Soranzo, *Cancer Chemother, Rep.*, 57, 269 (1973).
(10) F. Arcamone, G. Cassinelli, G. Franceschi, S. Penco, C. Pol, S. Redaelli, and C. Selva, see ref 3, p 9.

Steroidal Imidazole-1-carboxylic Acid Esters

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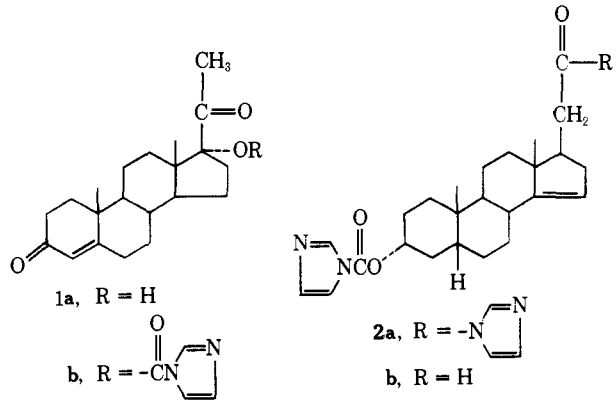
A variety of steroidal esters of imidazole 1-carboxylic acid and related acids was prepared by reaction of the steroidal alcohol with *N,N'*-carbonyldiimidazole or by displacement of phenol from steroid phenylcarbonates. One compound, **21c** (the imidazole-1-carboxylate of 19-norethisterone), was found to have an interesting separation of progestational from androgenic activity.

In connection with another project, we were interested in the preparation of the bisester of 17α-hydroxyprogesterone (**1a**) and carbonic acid. Treatment of **1a** with *N,N'*-carbonyldiimidazole (CDI) by the procedure of Staab and Mannschreck[1] gave not the desired carbonate but instead the imidazole-1-carboxylic acid ester **1b**.

Prior to our studies, no examples of characterized steroidal esters of imidazole-1-carboxylic acid had been published (although Sondheimer and coworkers had reported[2] an *in situ* preparation of **2a,b**). Since the completion of our work Kuhl and Taubert[3] have characterized two further examples of such esters.

On the other hand, nonsteroidal esters of imidazole-1-carboxylic acid and acids of related heterocyclic bases are well known; they have been prepared by a number of methods,† including the acylation of either the free

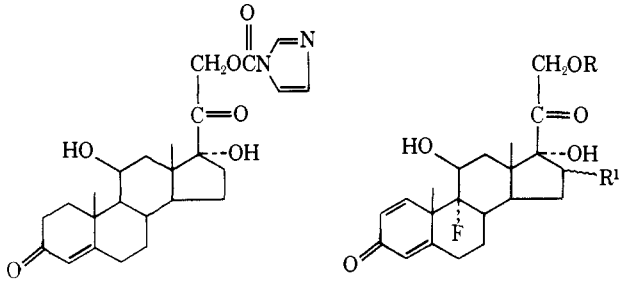
†For the excellent review on heterocyclic amides, see ref 4.



bases,[1,4-9] the corresponding trimethyl silyl derivatives,[10] or the azolide potassium salts[11] with haloformates such as carbobenzoxy chloride, ethyl chloroformate, etc. Another widely applicable synthesis of esters of imidazole-1-carboxylic acid is the reaction[1,12-14] of CDI with alcohols. By varying the reactants and ratios of starting materials in this latter reaction, either esters of imidazole-1-carboxylic acid or bisesters of carbonic acid[15] can be obtained.

Although esters of imidazole-1-carboxylic acid would be expected to be somewhat labile,[4] this substituent survived an aqueous extraction in the preparation of **1b.** This fact, in conjunction with expected differences in solubility and transport behavior relative to normal steroid esters, prompted us to prepare a variety of steroid imidazole-1-carboxylates.

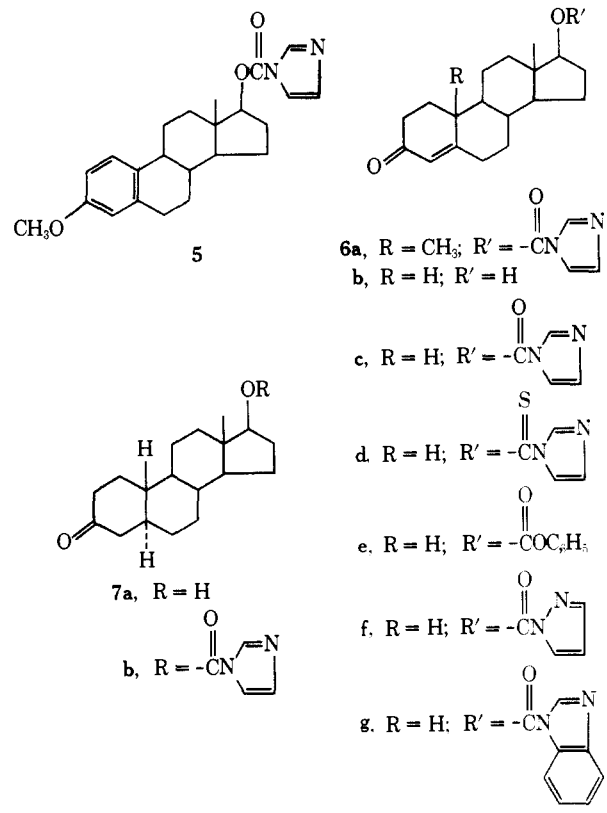
Examples of such a substituent at a primary alcohol were prepared by treating hydrocortisone, betamethasone (**4a**), and dexamethasone (**4c**) with an excess of CDI in dioxane at room temperature. Dilution of the reactions with ice water then gave the esters **3**, **4b**, and **4d**, respectively.



We also prepared a number of examples of such esters of secondary 17β-alcohols. The reaction of CDI with estradiol 3-methyl ether, testosterone, 19-nortestosterone (**6b**), and 5α-estran-17β-ol-3-one (**7a**)[16] gave **5**, **6a**, **6c**, and **7b**, respectively.

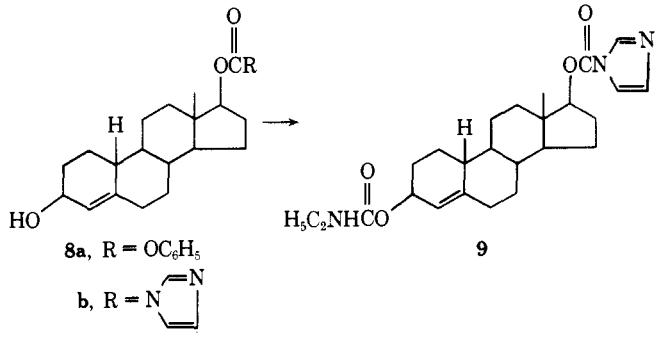
A variety of other esters related to **6c** were also synthesized. The thione analog **6d** was prepared by the analogous reaction of **6b** with *N,N'*-thiocarbonyldiimidazole.[17] Similar nonsteroidal esters of imidazole-1-thionecarboxylic acid have already been reported by the reaction[17,18] of alcohols with *N,N'*-thiocarbonyldiimidazole or the reaction of azolides with ethyl chlorothioneformate.[18]

The analogs of **6c** containing different heterocyclic bases



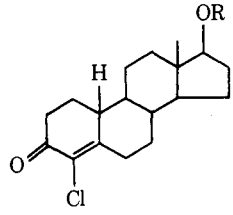
probably could be prepared by the reaction of **6b** with the requisite *N,N'*-carbonyldiazolide. However, a more convenient and versatile approach was achieved by treating **6b** with phenyl chloroformate to give **6e**, followed by treatment with the appropriate heterocyclic base. Such a preparation of azolide carboxylates by displacement of a carbonate ester does not seem to have been reported earlier, although the analogous aminolysis of phenylalkylcarbonates to form urethanes is a well-known preparative procedure.[19] Thus, treatment of **6e** with imidazole gave **6c**, identical with that prepared earlier; reaction of **6e** with pyrazole and benzimidazole then gave **6f** and **6g**, respectively.

In an attempt to prepare the 3β-hydroxy analog of **6c**, this latter compound was treated with the hindered reducing agent, lithium aluminum tri-*tert*-butoxy hydride. However, none of the desired **8b** could be isolated. Analogous reduction of the phenylcarbonate **6e** gave **8a** containing the intact phenylcarbonate group and, from this, phenol was displaced with imidazole to give **8b.** Reaction of ethyl isocyanate with **8b** then gave **9**, a molecule containing two quite different types of "carbamate" linkages.

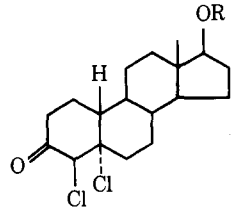


The reports that 4-chloroandrostanes[20,21] had enhanced anabolic-androgenic activity ratios prompted us to prepare **10c.** Accordingly, the reaction of 19-nortestosterone acetate with sulfuryl chloride[22] gave two products, the dichloride **11a**, which was not obtained completely pure,

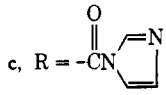
and the desired **10a**, a compound previously obtained[23] by hydrogen chloride treatment of the corresponding 4,5-epoxide. Hydrolysis of **10a** then gave **10b**. The analytical and spectral data of **10b** were in full agreement with the assigned structure, although the melting point was at variance with the literature value.[24] The reported sample of **10b** had been isolated after isocyanuric chloride treatment of **6b**, and the reported melting point is comparable to that of our **11b** prepared by hydrolysis of **11a**. Dehydrochlorination of our **11b** with triethylamine[25] gave **10b**, identical with that prepared by hydrolysis of **10a**. Reactian of **10b** with CDI then gave **10c**.



10a, R = $COCH_3$
b, R = H

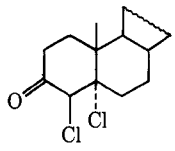


11a, R = $COCH_3$
b, R = H



c, R = -CN(=O)-imidazole

The isolation of the dichloride **11a** from the sulfuryl chloride reaction on 19-nortestosterone acetate deserves some comment. The dichloride is probably an intermediate in the reaction with sulfuryl chloride; in all the normal cases reported in the literature the combination of the 1,3-diaxial interaction between the 19-methyl and the 4β-chloro and the presence of pyridine as solvent are sufficient to cause the elimination of hydrogen chloride from **12** to give **13**. However, in **11a** the interaction with the methyl group is absent and therefore dehydrochlorination is not as favored. As noted above, it is also highly probable that the reported[23] isocyanuric chloride product **10b** is instead **11b**, as indicated by the similarity of melting points (see Experimental Section). In one case in the literature[25] the reaction of **14** with sulfuryl chloride did give a dichloride **15**. In this case, dehydrochlorination is inhibited because of the extreme peri interaction in the product **16**.



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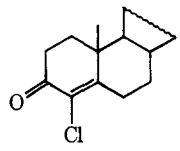
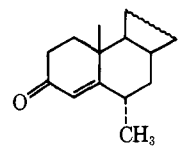
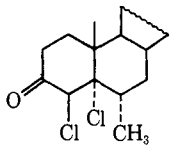


13



14



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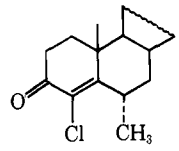
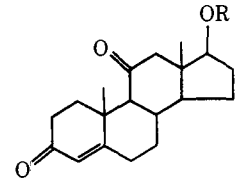


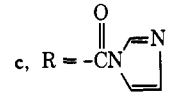
16

An analogous 4-chloro compound in the adrenosterone series was also prepared. Treatment of **17b**[26,27] with sulfuryl chloride gave **18a**, which was hydrolyzed to **18b**. The reaction of **18b** and of **17a**[28] with CDI gave **18c** and **17c**, respectively.

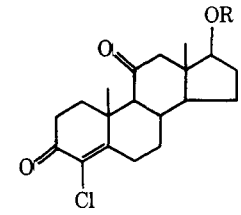
We also prepared a number of examples of imidazole-1-carboxylates of tertiary 17β-alcohols. With 17α-methyltestosterone, ethinylestradiol 3-methyl ether, ethisterone, and 19-norethisterone (**21b**), extended reaction times were needed to achieve satisfactory yields of **19**, **20**, **21a**, and **21c**, respectively.



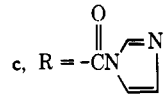
17a, R = H
b, R = $COCH_3$



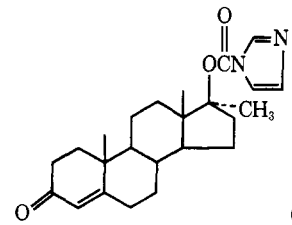
c, R = -CN(=O)-imidazole



18a, R = $COCH_3$
b, R = H



c, R = -CN(=O)-imidazole



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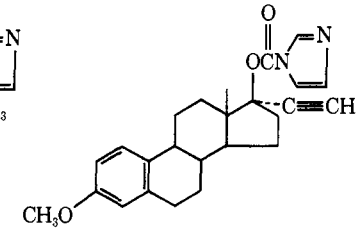

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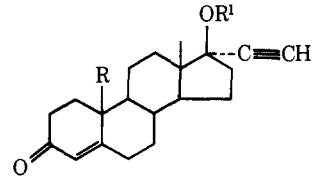
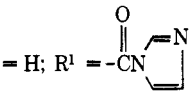
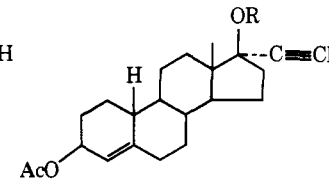
Compound **21c** was found to have an interesting separation of endocrinological effects and therefore the preparation of the corresponding 3β-acetoxy compound **22b** was achieved by the reaction of CDI with **22a**.[29]



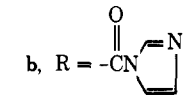
21a, R = CH_3; R^1 = -CN(=O)-imidazole
b, R = H; R^1 = H



c, R = H; R^1 = -CN(=O)-imidazole

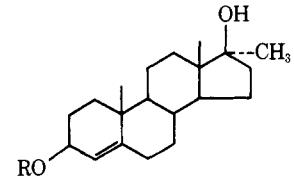


22a, R = H

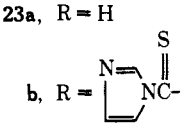


b, R = -CN(=O)-imidazole

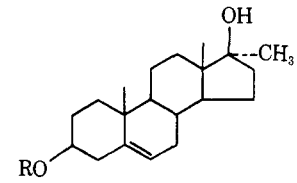
The only derivatives which we prepared at the 3 position of the steroid were the isomeric imidazole-1-thionecarboxylates **23b** and **24b** by reaction of 17α-methylandrost-4-ene-3β,17β-diol (**23a**)[30] and its Δ^5 analog **24a** with *N*,*N*′-thiocarbonyldiimidazole.



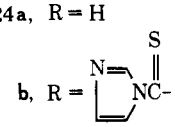
23a, R = H



b, R = -NC(=S)-imidazole



24a, R = H



b, R = -NC(=S)-imidazole

Biological Results. All of the new compounds were tested for endocrinological activity. The three corticoid imidazole-1-carboxylates **3**, **4b**, and **4d** were assayed by the thymus involution procedure and were found to have activities equal to or slightly less than the parent alcohols. This is probably due to the relatively rapid hydrolysis of these primary imidazole-1-carboxylates.

The androstanes, **6a,c-g**, **7b**, **8a,b**, **9**, **10c**, **17c**, **18a,c**,

Table I

Compd	Smallest dose, μg/day, with significant act. po	sc
17α-Acetoxyproges-terone	400	10
1b	Inactive at 400	Inactive at 400
21b, norethindrone	40	20
21c	20	10
22a	10–20	4–10
22b	40–100	10–20
Ethynodiol diacetate	100	20
Ethisterone	Inactive at 400	Inactive at 400
21a	400	400

and **19**, were tested for anabolic-androgenic activity ratios in castrated immature rats. None of these new compounds showed an interesting level of activity upon either sc or po administration. These androstanes were also tested for antigonadotropic activity in intact rats; two compounds, **6c** and **18c**, showed a significant separation of antigonadotropic from androgenic activities, although the level of activity was quite low.

The progestins were tested in a modified Clauberg-McPhail assay[31] with the results shown in Table I.

As can be seen in Table I, **1b** was devoid of activity and **22b** was less active than the parent alcohol **22a**. Although weakly active, **21a** was more active than its parent alcohol, ethisterone. However, **21c** was shown to have twice the progestational activity of **21b**. Additionally, **21c** was shown to have less androgenicity than **21b** as shown in Table II.

The estrogens **5** and **20** were found to be highly active as antigonadotropic agents as shown in Table III. Unfortunately, however, both compounds were estrogenic (Table III) at lower dosages than those required to demonstrate the antigonadotropic activity.

These results demonstrate that imidazole-1-carboxylates of secondary and tertiary steroidal alcohols have levels and ratios of activity different from the parent alcohols or simple esters, although this alteration of activity cannot yet be predicted. Particularly in the case of **21c**, a significant increase of progestational activity and a significant decrease of androgenic activity relative to **21b** were found.

Experimental Section

Melting points were determined in a Thomas-Hoover melting point apparatus and are corrected. The ir spectra were recorded on a Beckman instrument, Model IR-9, and the uv spectra were recorded on a Cary instrument Model 15. The nmr spectra were recorded with a Varian A-60 or Varian HA-100 instrument (Me_4Si). All compounds reported in this paper had ir, uv, and nmr spectra compatible with the assigned structure and only significant values are reported. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter in $CHCl_3$ at a concentration of about 1%. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements are within ±0.4% of the theoretical values.

Steroid Imidazole-1-carboxylates (Table IV). A mixture of the steroid and CDI was stirred at room temperature in an appropriate dry solvent. At the end of the reaction period, C_6H_6 reactions were washed with H_2O while dioxane reactions were drowned in 10 vol of H_2O and the resulting precipitate was collected and dissolved in CH_2Cl_2. The product solution was dried over Na_2SO_4, concentrated, and recrystallized, with the results shown in Table IV.

19-Nortestosterone Imidazole-1-carboxylate (6c). From 6e. A solution of 1.435 g (3.6 mmol) of **6e** and 0.497 g (7.3 mmol) of imidazole in 30 ml of dioxane was stirred at room temperature for 6 days. During this time, 150 mg of NaOMe was added in three portions. The reaction was poured into 400 ml of ice and H_2O, and the resulting precipitate was recrystallized from CH_2Cl_2–Et_2O to give 933 mg (70%) of **6c**, identical with the material prepared in Table IV.

Table II

Compd	Dose, mg/rat/day, and route	% difference from controls in mean organ weight: Seminal vesicles	Levator ani
21b	1.0 po	+106	+8
21c	1.0 po	+47	+8

Table III

Compd	Minimum dose, μg/day, showing significant act.: Testes reduction	Uterine enlargement
5	20	4
20	0.4	0.2

Occasionally, samples of **6c** on recrystallization from CH_2Cl_2–Et_2O gave crystals, mp 160°. Samples of each melting point could be converted to each other by judicious seeding. In addition, samples melting at 150 and 160° had identical solution ir, uv, and nmr spectra.

19-Nortestosterone Imidazole-1-thionecarboxylate (6d). To a stirring solution of 20.42 g (0.300 mol) of imidazole in 160 ml of alcohol-free $CHCl_3$ was added over 15 min a solution of 5.73 ml (8.64 g, 0.075 mol) of $CSCl_2$ in 60 ml of C_6H_6. One hour later the precipitate of imidazole hydrochloride was removed by filtration through a sintered glass funnel and washed with alcohol-free $CHCl_3$. The combined filtrates were diluted to 300 ml with alcohol-free $CHCl_3$ to give a stock solution of *N,N'*-thiocarbonyldiimidazole.

To 240 ml of the above stock solution of *N,N'*-thiocarbonyldiimidazole was added 4.00 g (0.0146 mol) of **6b** and the solution was allowed to stand at room temperature for 3 days. It was then poured into 500 ml of ice and H_2O. The aqueous layer was extracted twice with CH_2Cl_2, and the combined organic layers were washed with H_2O, dried, and concentrated to leave an amber oil which was dissolved in EtOAc. This solution was filtered through a column of 20 g of silica gel and concentrated. The residue was crystallized with charcoal from CH_2Cl_2–EtOAc to give 2.76 g (49%) of **6d** as colorless crystals, mp 142–144°. The analytical sample was obtained by recrystallization from CH_2Cl_2–Et_2O: mp 144–146°; $[\alpha]^{25}D$ +80.46°. *Anal.* ($C_{22}H_{28}N_2O_2S$) C, H, N, S.

19-Nortestosterone Phenylcarbonate (6e). To a rapidly stirred solution of 31.00 g (0.113 mol) of **6b** in 1 l. of dry pyridine was added over 3 min 63 ml (78.5 g, 0.50 mol) of phenyl chloroformate. A gummy precipitate formed immediately which soon crystallized and was broken up by the stirrer. After the reaction had stirred overnight, it was poured into 10 l. of ice and H_2O. The resulting precipitate was recrystallized from Et_2O to give 28.5 g (64%) of **6e** as colorless crystals, mp 147–149° after prior softening at 128°, suitable for use in subsequent reactions. Further recrystallization from Et_2O–pentane gave the analytical sample: mp 148–150°; $[\alpha]^{25}D$ +35.23°. *Anal.* ($C_{25}H_{30}O_4$) C, H.

19-Nortestosterone Pyrazole-1-carboxylate (6f). To a solution of 4.50 g (0.0114 mol) of **6e** and 4.68 g (0.069 mol) of pyrazole in 135 ml of THF was added 0.25 g of NaOMe. The reaction was stirred at room temperature for 1.5 hr and poured into 1.5 l. of ice and H_2O. The resulting tan precipitate was recrystallized from CH_2Cl_2–Et_2O with charcoal to give 2.81 g (67%) of **6f** as colorless crystals, mp 168–170°, with resolidification and remelting at 180–182°. Further recrystallization gave the analytical sample: mp 180.5–182° after softening at 171°; $[\alpha]^{25}D$ +99.48°. *Anal.* ($C_{22}H_{28}N_2O_3$) C, H, N.

19-Nortestosterone Benzimidazole-1-carboxylate (6g). To a solution of 1.00 g (0.00254 mol) of **6e** and 1.80 g (0.0153 mol) of benzimidazole in 30 ml of THF was added 70 mg of NaOMe. The reaction was stirred at room temperature for 3 hr and poured into 400 ml of ice and H_2O. The resulting yellow precipitate was recrystallized from CH_2Cl_2–EtOAc with charcoal to give 0.774 g (73%) of **6g** as colorless crystals, mp 226–228°. Further recrystallization gave the analytical sample: mp 226–228°; $[\alpha]^{25}D$ +93.52°. *Anal.* ($C_{26}H_{30}N_2O_3$) C, H, N.

Estr-4-ene-3β,17β-diol 17-Phenylcarbonate (8a). A solution of 8.28 g (0.021 mol) of **6e** and 11.08 g (0.044 mol) of lithium aluminum tri-*tert*-butoxy hydride in 260 ml of THF was stirred at room

Table IV. Steroid Imidazole-1-carboxylates

No.	Molar ratio of CDI to steroid	Solvent	Time	Yield, %	Mp, °C	Recrystn solvent	$[\alpha]^{25}D$, deg	Formula	Analyses
1b	2.0	C_6H_6	12 days	17[a]	203.5–205	CH_2Cl_2–Et_2O	+28.32	$C_{25}H_{32}N_2O_4$	C, H, N
3	5.6	Dioxane	1 hr	63	177 dec	CH_2Cl_2–acetone	+200.1	$C_{25}H_{32}N_2O_6$	C, H, N
4b	6.0	Dioxane	1 hr	33	190 dec	Acetone–EtOAc	+152.1[b]	$C_{26}H_{31}FN_2O_6$	C, H, F, N
4d	6.0	Dioxane	1 hr	46	197 dec	THF–C_6H_6	+123.1[b]	$C_{26}H_{31}FN_2O_6$	C, H, F, N
5	5.0	Dioxane	3 days	73	115–116	CH_2Cl_2–Et_2O	+78.77	$C_{23}H_{28}N_2O_3$	C, H, N
6a	5.0	Dioxane	18 hr	91	169–172	CH_2Cl_2–Et_2O	+146.4	$C_{23}H_{30}N_2O_3$	C, H, N
6c	5.0	Dioxane	4 hr	84	150–151.5[c]	CH_2Cl_2–Et_2O	+98.40	$C_{22}H_{28}N_2O_3$	C, H, N
7b	5.0	Dioxane	1.5 hr	49	158.5–160.5	CH_2Cl_2–Et_2O	+85.24	$C_{22}H_{30}N_2O_3$	C, H, N
10c	5.0	Dioxane	18 hr	77	197 dec	EtOAc	+112.8	$C_{22}H_{27}ClN_2O_3$	C, H, Cl, N
18c	5.0	Dioxane	5 days	47	223–224	CH_2Cl_2–EtOAc	+224.9	$C_{23}H_{27}ClN_2O_4$	C, H, Cl, N
17c	4.0	Dioxane	3 days	44	176–178	CH_2Cl_2–Et_2O	+214.5	$C_{23}H_{28}N_2O_4$	C, H, N
19	1.5	C_6H_6	13 days	42	170 dec	EtOAc	+104.4	$C_{24}H_{32}N_2O_3$	C, H, N
20	7.5	Dioxane	41 days	47	159.5–160.5	CH_2Cl_2–Et_2O	+17.28	$C_{25}H_{28}N_2O_3$	C, H, N
21a	5.0	Dioxane	55 days	23	230–231 dec	CH_2Cl_2–EtOAc	+67.16	$C_{25}H_{30}N_2O_3$	C, H, N
21c	4.4	THF	6 days	82	208 dec	CH_2Cl_2–EtOAc	+18.91	$C_{24}H_{28}N_2O_3$	C, H, N
22b	10.4	Dioxane	33 days	21	172–174	CH_2Cl_2–Et_2O	−35.92	$C_{26}H_{32}N_2O_4$	C, H, N

[a] 75% starting material recovered. [b] In dioxane. [c] Also a polymorph, mp 160°.

temperature for 2 hr. Acetone (21 ml) was added, and 10 min later the reaction was poured into 2 l. of ice and H_2O containing 10 ml of HOAc. The mixture was extracted three times with CH_2Cl_2, and the extracts were washed with 5% $NaHCO_3$ and H_2O, dried, and concentrated. The resulting yellow oil was crystallized from Et_2O to give in several crops 6.99 g (84%) of 8a as colorless crystals, mp 143–147°. Further recrystallization gave the analytical sample: mp 147–148°; $[\alpha]^{25}D$ +22.03°. *Anal.* ($C_{25}H_{32}O_4$) C, H.

Estr-4-ene-3β,17β-diol 17-(Imidazole-1-carboxylate) (8b). To a solution of 6.28 g (0.0159 mol) of 8a and 6.28 g (0.093 mol) of imidazole in 250 ml of THF was added 0.50 g of NaOMe. The reaction was stirred overnight at room temperature and then poured into 3 l. of ice and H_2O. The resulting precipitate was recrystallized from CH_2Cl_2–Et_2O to give 3.92 g (67%) of 8b as colorless crystals, mp 178–180°, with resolidification and remelting at 191–194°. Further recrystallization gave the analytical sample: mp 180–181°; $[\alpha]^{25}D$ +69.53°. *Anal.* ($C_{22}H_{30}N_2O_3$) C, H, N.

Estr-4-ene-3β,17β-diol 3-Ethylcarbamate 17-(Imidazole-1-carboxylate) (9). To a solution of 2.40 g (0.0065 mol) of 8b in 24 ml of THF was added 5.15 ml (4.60 g, 0.065 mol) of ethyl isocyanate and the reaction was stirred at room temperature for 10 days. (About 20 min after the addition of the isocyanate, a white precipitate formed which had redissolved after 6 days.) The reaction was poured into 300 ml of ice and H_2O, and the resulting precipitate was recrystallized from CH_2Cl_2–EtOAc to give 1.67 g (58%) of 9 as colorless crystals, mp 184–186°. Further recrystallization gave the analytical sample: mp 184–186°; $[\alpha]^{25}D$ +22.06°. *Anal.* ($C_{25}H_{35}N_3O_4$) C, H, N.

4-Chloro-17β-hydroxyestr-4-en-3-one Acetate (10a) and 4β,5α-Dichloro-17β-hydroxyestr-3-one Acetate (11a). A solution of 10.00 g (0.0365 mol) of 6b in 50 ml of pyridine and 50 ml of Ac_2O was allowed to stand overnight at room temperature and poured into a 2 l. of ice and H_2O. The resulting precipitate was collected by filtration and dissolved in Et_2O. The solution was dried and concentrated to yellow oily crystals of crude 19-nortestosterone acetate. To a solution of this material in 110 ml of pyridine cooled to 8° was added, over 20 min, 5.9 ml (9.8 g, 0.073 mol) of SO_2Cl_2. The temperature was maintained at 8–9° during the addition and kept at 6° for an additional 30 min. The reaction was poured into a mixture of 1.1 l. of ice and H_2O and 120 ml of concentrated HCl. The resulting precipitate was collected by filtration, washed with H_2O, and dissolved in CH_2Cl_2. The solution was washed twice with H_2O, dried, and concentrated. The residue of oily orange crystals was recrystallized from CH_2Cl_2–Et_2O to give (in two crops) 6.9 g of 10a. (The resulting mother liquor contained the crude 11a). Recrystallization of the crude 10a gave 5.80 g (45%) of 10a as colorless crystals, mp 169–173°. Further recrystallization gave mp 171–173° (reported[23] mp 168–170°).

4-Chloro-17β-hydroxyestr-4-en-3-one (10b). A. From 10a. A solution of 0.50 g (1.42 mmol) of 10a in 2 ml of CH_2Cl_2, 5 ml of THF, 20 ml of MeOH, and 5 ml of 6 *N* HCl was allowed to stir at room temperature overnight. The reaction was diluted with Et_2O and washed with H_2O, 5% $NaHCO_3$, and H_2O. The solution was dried and concentrated, and the residue was recrystallized from Et_2O–hexane to give 395 mg (90%) of 10b as colorless crystals, mp 141–144°. Further recrystallization from Et_2O gave the analytical sample: mp 144–145° (reported[24] mp 220–223°, compare the melting point of 11b, 222–224° dec); ir ($CHCl_3$) 1690 cm^{-1}; uv max (EtOH) 255 nm (ε 14,100); $[\alpha]^{25}D$ +73.92°. *Anal.* Calcd for $C_{18}H_{25}ClO_2$: C, 70.00; H, 8.16; Cl, 11.48. Found: C, 70.27; H, 7.97; Cl, 11.27.

B. From 11b. A solution of 1.00 g (0.0029 mol) of 11b in 50 ml of THF containing 4.5 ml of triethylamine was stirred at room temperature for 3 days and then heated under reflux for 4 hr. The solution was concentrated, diluted with Et_2O, washed with H_2O, 0.5 *N* HCl, and H_2O, dried, and concentrated. The residue was recrystallized from Et_2O to give 253 mg (29%) of 10b: mp 144–145°; no depression upon admixture with a sample of 10b prepared from 10a in A.

4β,5α-Dichloro-17β-hydroxyestr-3-one (11b). The crude mother liquor (from the SO_2Cl_2 reaction on 19-nortestosterone acetate) which contained 11a was mixed with 100 ml of MeOH, 10 ml of THF, and 10 ml of 6 *N* HCl and allowed to stand overnight at room temperature. The reaction was cooled to 0°; the resulting precipitate was collected by filtration, washed with 2:1 MeOH–H_2O, and dissolved in CH_2Cl_2. The solution was washed with H_2O and 5% $NaHCO_3$, dried, and concentrated to a colorless crystalline residue. Recrystallization from CH_2Cl_2–Et_2O gave 1.48 g (12% overall from 6b) of 11b, mp 218–223° dec. Further recrystallization from CH_2Cl_2–MeOH gave almost pure 11b: mp 222–224° dec; uv max (EtOH) 297 nm (ε 50); ir ($CHCl_3$) no absorption around 1690 cm^{-1} (both 10a and 10b show absorption at 1690 cm^{-1}). *Anal.* Calcd for $C_{18}H_{26}Cl_2O_2$: Cl, 20.53. Found: Cl, 21.12.

4-Chloro-17β-hydroxyandrost-4-ene-3,11-dione Acetate (18a). A solution of 3.81 g (0.011 mol) of 17β-hydroxyandrost-4-ene-3,11-dione acetate (17b) in 38 ml of pyridine was kept at 5–10° while 1.8 ml (3.0 g, 0.022 mol) of SO_2Cl_2 was added over 10 min. The reaction was stirred at 5–10° for 20 min and poured into a mixture of 400 ml of ice and H_2O and 42 ml of concentrated HCl. The resulting precipitate was collected by filtration and dissolved in CH_2Cl_2. The solution was washed with H_2O, 5% $NaHCO_3$ and H_2O, dried, and concentrated. The residue was crystallized from CH_2Cl_2–Et_2O to give 2.40 g (57%) of 18a as colorless crystals, mp 231° dec. Recrystallization from CH_2Cl_2–MeOH gave the analytical sample: mp 252–254°; $[\alpha]^{25}D$ +193.1°. *Anal.* ($C_{21}H_{27}ClO_4$) C, H, Cl.

4-Chloro-17β-hydroxyandrost-4-ene-3,11-dione (18b). To a solution of 1.50 g (0.04 mol) Of 18a in 50 ml of MeOH, 20 ml of THF, and 5 ml of CH_2Cl_2 was added 5 ml of 6 *N* HCl. After standing at room temperature overnight the solution had deposited some crystals of 18a. These were redissolved by the addition of 10 ml of CH_2Cl_2 and 10 ml of THF. Another 5 ml of 6 *N* HCl was added, and the solution was allowed to stand at room temperature for 3 days. The reaction was concentrated to 25 ml under vacuum at room temperature, diluted with H_2O, and extracted with CH_2Cl_2. The organic layer was washed with H_2O, 5% $NaHCO_3$ and H_2O, dried, and concentrated to a colorless foam. This was crystallized from Et_2O and recrystallized from CH_2Cl_2–Et_2O to give 0.64 g (48%) of 18b as colorless crystals, mp 206–208° dec. Further recrystallization gave the analytical sam-

ple: mp 209-210° dec; $[\alpha]^{25}D$ +245.9°. *Anal.* ($C_{19}H_{25}ClO_3$) C, H, Cl.

17α-Methylandrost-4-ene-3β,17β-diol 3-(Imidazole-1-thionecarboxylate) (23b). A stock solution of *N,N'*-thiocarbonyldiimidazole (100 ml) was added to a solution of 2.00 g (0.0066 mol) of 17α-methylandrost-4-ene-3β,17β-diol (23a) in 50 ml of THF, and the reaction was stirred at room temperature for 7 days. H_2O (10 ml) was added and the reaction was diluted with CH_2Cl_2, washed twice with H_2O, dried, and concentrated. The residue was adsorbed onto silica gel from 1:1 CH_2Cl_2-hexane. Elution with 2-15% EtOAc in CH_2Cl_2 gave fractions which were recrystallized from CH_2Cl_2-Et_2O-hexane to give 0.32 g (12%) of the analytical sample of 23b as colorless crystals: mp 193.5-196.5°; $[\alpha]^{25}D$ −78.64°. *Anal.* ($C_{24}H_{34}N_2O_2S$) C, H, N, S.

17α-Methylandrost-5-ene-3β,17β-diol 3-(Imidazole-1-thionecarboxylate) (24b). 17α-Methylandrost-5-ene-3β,17β-diol (24a) (2 g, 0.0066 mol) was dissolved in 50 ml of THF, 150 ml of the stock solution of *N,N'*-thiocarbonyldiimidazole was added, and the solution was stirred for 6 days. The reaction was diluted with CH_2Cl_2, washed with H_2O, dried, and concentrated. The solid residue was recrystallized from CH_2Cl_2-Et_2O to give 0.554 g (20%) of the analytical sample of 24b as colorless crystals: mp 191.5° dec; $[\alpha]^{25}D$ −70.44°. *Anal.* ($C_{24}H_{34}N_2O_2S$) C, H, N, S.

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CNDO/2 Study of the Antibacterial Activity of Penicillins and Cephalosporins

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The inhibition of bacterial growth by penicillin-like antibiotics is assumed to proceed *via* a two-step mechanism: reversible binding of drug to enzyme followed by irreversible acylation of enzyme *via* thiol attack at the β-lactam carbonyl carbon. The CNDO/2 procedure for electronic structure calculations is used to estimate relative binding strengths and reactivities for penicillins, cephalosporins, and Δ^2-cephalosporins, making simple but reasonable assumptions about the nature of these reactions. The enhanced activity of penicillins over cephalosporins is seen to be due to stronger binding. The virtual inactivity of Δ^2-cephalosporins is a result of the resistance of these molecules to thiol attack. No evidence is found for expulsion of acetate from cephalosporins during the reaction.

The modification of penicillin and cephalosporin antibiotics has been difficult because little is known about the specific enzyme process inhibited by these drugs. It is probably correct that these molecules inhibit the final step in the synthesis of the bacterial cell wall, the cross linking of the peptidoglycan strands using a pentapeptide chain. The hypothesis that the inhibition results from a structural similarity between penicillin and this pentapeptide is an attractive one.[1] However, this cross linking "transpeptidase" has not been isolated and very little is known about the active site or the reaction mechanism.

In this paper we will consider theoretically some of the experimentally unresolved questions regarding the structure-activity relationship of these antibiotics. In order to do this we will make the simplest assumptions about the nature of the reaction, those of eq 1. The first step is the reversible binding of the substrate to the enzyme with binding constant k_1'/k_1. The second step is the reaction with the enzyme to form the covalently bound enzyme-

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