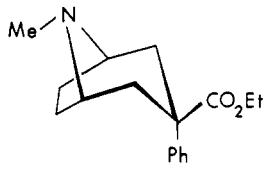
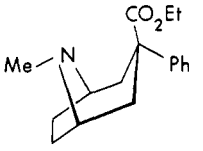
(nonbonded interactions of e-Me groups in 8-α and -γ are raised in boat forms, and models show that the *cis*-1,3-diaxial Me–Me interaction of 8-β is not relieved in the corresponding boat), the sole active member (γ) being about as active as the γ-1,2,5-trimethyl isomer.[15] Ethyl 3-α-phenyltropane-3-β-carboxylate, which is somewhat more potent than meperdine,[16] would be expected to have a significantly large skew-boat **10** population because the chair conformer **9** is destabilized



9



10

by a-Ph–bimethylene bridge interactions. Spectroscopic evidence supports this contention for related β-ethyl and phenyl ketones.[17]

In a recent analysis of stereochemical factors in narcotic analgetics, Portoghese[18] stated that the conformational requirements for most of the 4-phenylpiperidine analgetics appear to be minimal. From the present evidence, however, it is probably more accurate to state that although a fairly wide range of 4-phenylpiperidine conformations are compatible with activity, those in which the aromatic and piperidine rings approach coplanarity (as in the skew-boat with phenyl in the bow-sprit position) may be most effective in evoking a response.

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within $\pm 0.4\%$ of the theoretical values.

The pmr spectra were recorded on a Varian A-60 spectrometer operating at the normal running temperature with TMS as standard (internal with $CDCl_3$ and external with D_2O as solvent). α-1,3-Dimethyl-4-phenyl-4-propionoxypiperidine hydrochloride (α-prodine), mp 222° (lit.[19] 220–221°), and the corresponding β isomer (β-prodine), mp 199–200° (lit.[19] 195–196°), were obtained by heating the α- and β-piperidinols **1a** with propionic anhydride and pyridine.[19] New esters prepared in this way were as follows (uncorrected melting points determined with a Buchi-Tottoli apparatus in capillary tubes).

α-4-Acetoxypiperidine (**1b**) hydrochloride, mp 216–218°, from *i*-PrOH–Et_2O. *Anal.* ($C_{15}H_{22}ClNO_2$) C, H, N.

β-4-Acetoxypiperidine (**1b**) hydrochloride, mp 211–213°, from EtOH–Me_2CO. *Anal.* C, H, N.

α-4-Butyoxypiperidine (**1d**) hydrochloride, mp 196–197°, from EtOH–MeCOEt. *Anal.* ($C_{17}H_{26}ClNO_2$) C, H, N.

β-4-Butyroxypiperidine (**1d**) hydrochloride, mp 202°, from EtOH–MeCOEt; ν_{max} 3400 cm^{-1}. *Anal.* C, H, N (low C value due to water of crystallization).

All of the esters had ν_{max}^{Nujol} near 1720 cm^{-1} (ester C=O). The pK_a' values of the prodine isomers in 50% EtOH–H_2O, determined by Albert's and Sergeant's method,[20] were α, 7.68 ± 0.06, and β, 7.75 ± 0.06.

Acknowledgment.—The author acknowledges Drs. M. A. Iorio and P. Pocha for assistance in this work.

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Effects of Certain Arylhydroxamic Acids on Deoxyribonucleic Acid Synthesis by Ehrlich Ascites Tumor Cells *in Vitro*[1]

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A recent report[2] described a selective inhibition by salicylhydroxamic acid (I) of deoxyribonucleic acid (DNA) synthesis in Ehrlich ascites tumor cells. Characteristics of the inhibition were similar in some respects to the actions of hydroxyurea[3] and of oxamylhydroxamic acid,[4,5] the hydroxamic acid analogs of carbamic acid and oxamic acid, respectively. Effects on the synthesis of ribonucleic acid (RNA) and of protein were nominal and were considered to be of a secondary nature as a consequence of the lowered rate of DNA formation. The inhibition by I was further evident immediately upon adding the compound to the cells; that is, no preincubation was necessary to evoke the effect. The rate of DNA synthesis resumed the control rate upon removal of the inhibitor by washing the cells, indicating no firm binding to the cells and no irreversible alteration of the cells by the compound.

The work herein described was initiated to determine the ways in which structural features of compounds related to I may influence the course of nucleic acid synthesis in a tumor-cell test system.

Biological Data.—Table I shows the 50 and 90% inhibitory concentrations of each compound on DNA synthesis in Ehrlich ascites tumor cells. With one exception, data are presented as obtained with no preincubation (*i.e.*, inhibitor and isotopic precursor were added to the cell suspension simultaneously) and, also, following 1-hr preincubation of the cells with each compound prior to addition of the isotopic precursor. The relative potency of 9 of the 12 compounds was increased by the 1-hr preincubation period, the most striking example being that of XII. Slopes of the regression lines were fairly closely grouped when inhibitor and precursor were added simultaneously but varied erratically following the 1-hr preincubation period.

Figure 1 shows the effects of each of the 12 compounds on DNA, RNA, and protein synthesis in Ehrlich ascites tumor cells. The selectivity of action of I on DNA synthesis was confirmed; that is, the rate of DNA synthesis was depressed almost 80% after 1-hr exposure of the cells to the compound with no appreciable diminution in the rate of RNA or protein synthesis. Com-

(1) This work was aided by Grant GM-13958 from the National Institutes of Health, U. S. Public Health Service.
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TABLE I

EFFECTS OF CERTAIN ARYLHYDROXAMIC ACIDS ON DNA SYNTHESIS BY EHRLICH ASCITES TUMOR CELLS *in Vitro*

Compd	No preincubation[a]			1-Hr preincubation[a]		
	IC_{50}, *M*	IC_{90}, *M*	Slope	IC_{50}, *M*	IC_{90}, *M*	Slope
I	1.3×10^{-3}	5.0×10^{-3}	−2.13	5.9×10^{-4}	9.0×10^{-4}	−7.04
II	1.0×10^{-3}	5.7×10^{-3}	−1.91	2.0×10^{-3}	3.5×10^{-3}	−5.31
III	2.2×10^{-3}	6.3×10^{-3}	−2.79	2.8×10^{-3}	8.5×10^{-3}	−2.63
IV	1.0×10^{-3}	4.1×10^{-3}	−2.24	1.5×10^{-3}	2.6×10^{-3}	−5.69
V	1.2×10^{-3}	4.5×10^{-3}	−3.08	3.8×10^{-4}	8.2×10^{-4}	−3.88
VI	9.3×10^{-3}	9.1×10^{-2}	−1.29	4.8×10^{-3}	7.6×10^{-3}	−6.45
VII	1.1×10^{-3}	8.0×10^{-3}	−1.48	4.7×10^{-4}	9.2×10^{-3}	−0.99
VIII	9.4×10^{-4}	2.0×10^{-3}	−4.01	6.7×10^{-4}	1.5×10^{-3}	−3.64
IX	2.4×10^{-3}	7.0×10^{-3}	−2.76	1.8×10^{-3}	3.9×10^{-3}	−3.80
X	[b]			6.1×10^{-4}	9.6×10^{-4}	−6.44
XI	3.7×10^{-3}	1.0×10^{-2}	−2.94	1.7×10^{-3}	4.0×10^{-3}	−3.64
XII	1.6×10^{-3}	4.9×10^{-3}	−2.73	5.3×10^{-3}	2.7×10^{-4}	−1.82

[a] IC_{50} and IC_{90} represent 50 and 90% inhibitory concentrations, respectively. The slope is the decrease in probit units for each tenfold increase in concentration. [b] Compound X was virtually inactive with no preincubation; accurate data could not be obtained due to the insolubility of the compound at high concentrations.

pounds II, VIII, and XI were initially inhibitory to DNA synthesis, but activity was reduced upon preincubation, indicating a possible conversion of an active agent to an inactive one. The onset of depression of RNA synthesis by II which was coupled with loss of DNA-inhibiting capacity was reproducible (three experiments), suggesting conversion of II to an inhibitor of RNA synthesis. Considering the possibility that II may be reduced to the amide, benzamide was tested for its inhibitory action on RNA synthesis and was found to be totally inactive in this respect. Compounds VI and VII displayed relative selectivity against DNA synthesis but only after exposure of the cells for 1–2 hr. Compound XII showed a marked selectivity against DNA synthesis, and the extent of inhibition was greater than that obtained with I at the same concentration. Compound IX virtually completely inhibited DNA synthesis, but this was accompanied by almost 50% depression of the rate of RNA and protein synthesis. The most active and selective compounds were, thus, those with hydroxyl substituents in the 2 and 2,3 positions on the aryl ring, while addition of the same group to the 4 position yielded a virtually inactive compound. Relatively selective but less active compounds were those with amino groups in the 4 and 3,5 positions.

Reversibility of the action of each compound on DNA synthesis is shown in Figure 2. With the exception of XII when used at the higher concentration, the inhibitory action of each was readily abolished upon removal of the inhibitor by washing the cells with fresh medium, indicating no firm binding to, or irreversible change in, the cells.

Attempts to demonstrate an action of each compound on preformed DNA were done by labeling the DNA of intact cells with thymidine-^{3}H followed by exposing

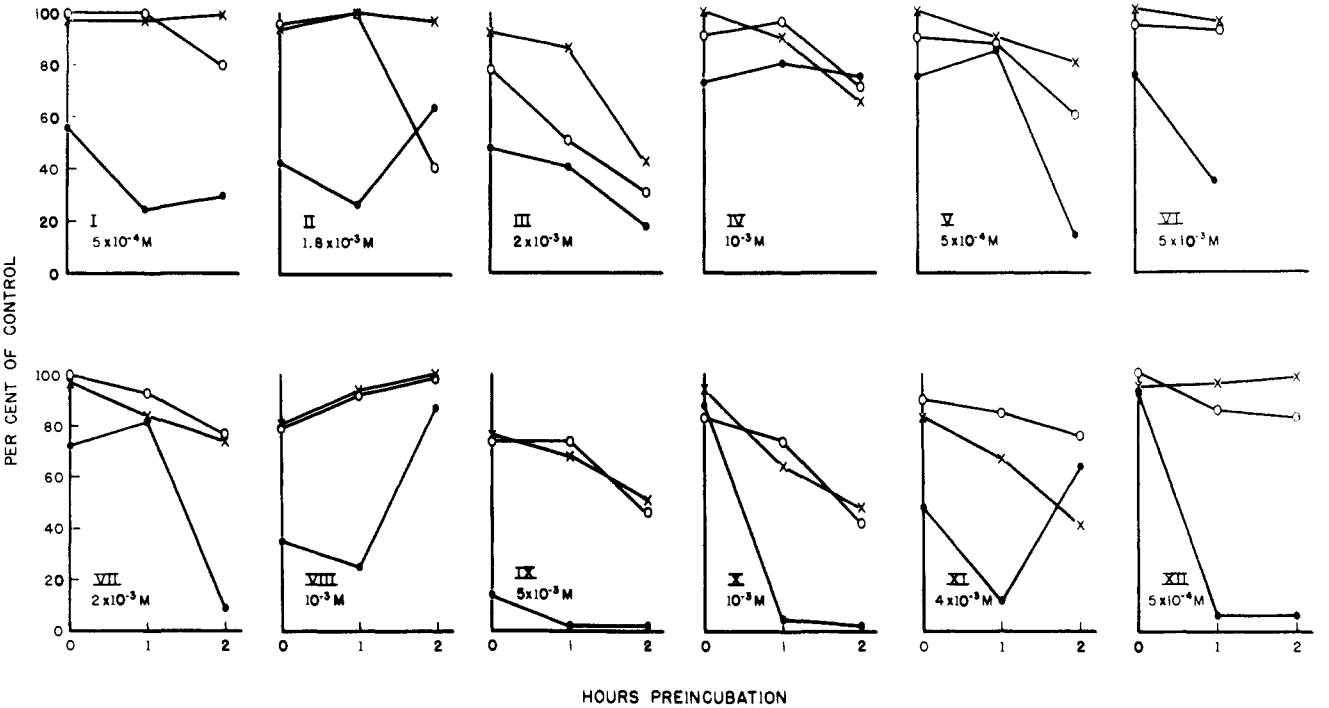

Figure 1.—Effects of certain arylhydroxamic acids on DNA, RNA, and protein synthesis by Ehrlich ascites tumor cells: ●, DNA; ○, RNA; ×, protein. Cells were incubated with each compound for the interval designated on the abscissa. Isotopic precursors were then added and samples were removed after 20 min for analysis. Other experimental details are in ref 6.

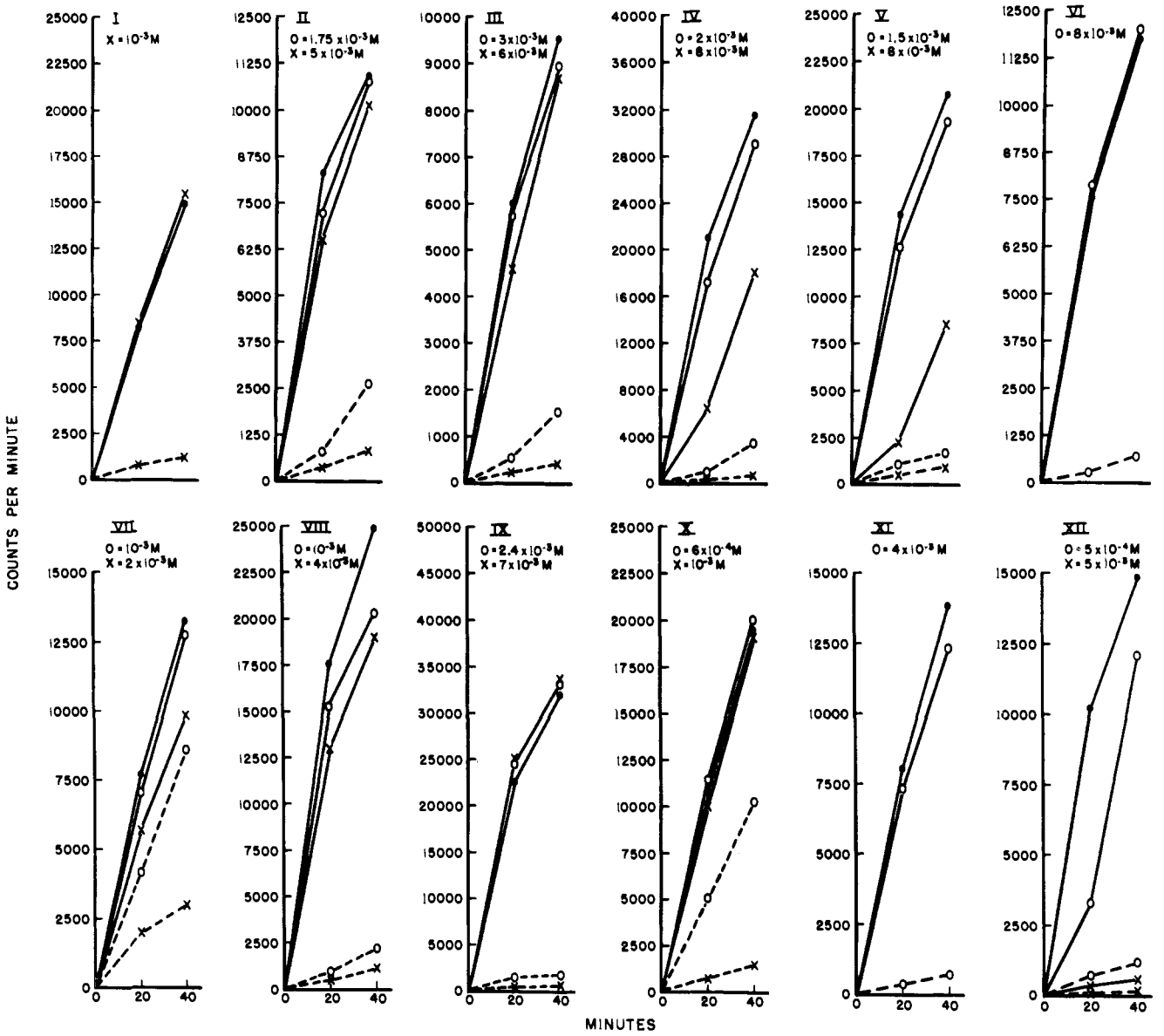

Figure 2.—Reversibility of the inhibitory actions of arylhydroxamic acids on DNA synthesis in ascites tumor cells: ●, no inhibitor ○ and ×, cells exposed to inhibitor for 30 min at indicated concentrations; solid lines, cells then washed with fresh MEM; dotted lines, cells then washed with MEM containing the inhibitor at the same concentration as during the initial 30-min exposure. Following the washings, isotopic thymidine was added, and its incorporation into DNA was assessed at 20 and 40 min. Other experimental details are in ref 6.

the cells to each compound for 3 hr at the 90% inhibitory concentration.[6] No depolymerization whatsoever of preexisting DNA could be demonstrated.

Experimental Section

Chemistry.—The previously unreported hydroxamic acids were prepared by the method of Scott and Wood[7] modified according to the solubilities of the esters and of the resulting products. The methyl or ethyl esters used were obtained commercially with the exceptions of methyl 2,6-dihydroxybenzoate, which was prepared by the method of Tomino,[8] methyl 3-amino-2-pyrazinoate, prepared according to Ellingson, *et al.*,[9] and methyl 2,3-dihydroxybenzoate, synthesized according to King, *et al.*[10] The melting points (Fisher-Johns apparatus, uncorrected) and analytical data (Galbraith Laboratories, Inc., Knoxville, Tenn.) are presented in Table II along with the literature values for the known compounds. With the exception of VII, each of the hydroxamic acids was recrystallized from H_2O using activated carbon. Compound VII was recrystallized from absolute MeOH by the addition of petroleum ether (bp 30–60°). As indicated in Table II, it was often necessary to recrystallize twice in order to obtain analytical purity.

A number of unsuccessful attempts were made to prepare pure *m*-hydroxybenzoylhydroxamic acid. Because of its tendency to form intractable oils, the reaction was performed in the following manner which yielded the product actually tested. A 16-g sample (0.4 mole) of NaOH was dissolved in 100 ml of H_2O and 16.4 g (0.2 mole) of $NH_2OH \cdot 0.5H_2SO_4$ was added with cooling and stirring in a N_2 atmosphere. Methyl *m*-hydroxybenzoate (15.2 g, 0.1 mole) was added, and the resulting mixture was left for 2 days with a slow N_2 purge. After neutralization with 5 *N* H_2SO_4, the solution was evaporated to dryness under vacuum. The solid was extracted with MeOH and dried twice over $MgSO_4$.

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TABLE II

Hydroxamic acid	No.	Mp, °C	Formula	Analyses	Yield, % (no. of recrystns)
Salicyl-	I	177–179[a]			
Benzo-	II	131–133[b]			
m-Hydroxybenzo-	III	43–45		*Cf.* Experimental Section	
p-Hydroxybenzo-	IV	185–186	$C_7H_7NO_3$	C, H, N	46 (1)
2,6-Dihydroxybenzo-	V	221–223	$C_7H_7NO_4$	C, H, N	32 (2)
3,5-Diaminobenzo-	VI	201–202 dec	$C_7H_9N_3O_2$	C, H, N	36 (2)
p-Aminobenzo-	VII	197–200[c]	$C_7H_8N_2O_2$	C, H, N	
3-Amino-2-pyrazino-	VIII	185–189[d]	$C_5H_6N_4O_2$	C, H, N	
o-Fluorobenzo-	IX	140–142	$C_7H_6FNO_2$	H, F; C,[e]N[e]	47 (1)
Acetylsalicyl-	X	136–140[f]	$C_9H_9NO_4$	C, H, N	
m-Aminobenzo-	XI	153–155	$C_7H_8N_2O_2$	H, N; C[g]	55 (1)
2,3-Dihydroxybenzo-	XII	220–223 dec	$C_7H_7NO_4$	C, H, N	39 (2)

[a] A. Jeanrenaud [*Ber.*, **22**, 1270 (1889)] reported mp 176–178°. [b] C. R. Hauser and W. B. Renfrow, Jr. ("Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p 67) reported mp 125–128°. [c] B. E. Hackley, Jr., R. Plapinger, M. Stolberg, and T. Wagner-Jauregg [*J. Am. Chem. Soc.*, **77**, 3651 (1955)] reported mp 185° dec. [d] W. B. Wright, Jr., and J. M. Smith, Jr. [*ibid.*, **77**, 3927 (1955)], reported mp 196° dec. [e] C: calcd, 54.22; found, 53.71. N: calcd, 9.03; found, 8.51. [f] Reference 11. [g] C: calcd, 55.26; found, 54.79.

The resulting oil was repeatedly dried under vacuum over fresh P_2O_5 until solidification occurred. This material was dissolved in EtOH. The solution was filtered and redried to yield a tenaciously hygroscopic solid which had to be manipulated in a dry box. The compound melted at 43–45° and gave the following analytical results, indicative of solvation. *Anal.* Calcd for $C_7H_7NO_3$: C, 54.9; H, 4.61; N, 9.15. Found: C, 52.2; H, 5.28; N, 8.20.

The acetylation of salicylhydroxamic acid was conducted as described by Urbanski and Falécki.[11] The fact that the product had a somewhat different melting point but gave an acceptable analysis suggests that this reaction produces an isomeric mixture rather than a single compound.

Biological Methods.—Methods of measuring the rates of DNA, RNA, and protein synthesis were similar to those described previously.[4-6] In general, these consisted of determining the extent of incorporation of thymidine-^{3}H, uridine-^{3}H, and L-leucine-^{14}C, respectively, into the acid-insoluble fraction of Ehrlich ascites tumor cells when incubated at 37° in Eagle's minimum essential medium (MEM) with Hank's balanced salt solution (Microbiological Associates). Compounds to be tested for inhibitory action were dissolved in DMSO which was at a final concentration of 1% in the reaction vessels, a concentration innocuous to the cells. The washed ascites cells were at a final suspension of 1% (v/v). The acid-insoluble material was solubilized in hydroxide of Hyamine (Packard Instrument Co.) and added to a toluene solution of PPO–POPOP phosphor (Packard). Radioactivity was measured with a Mark I liquid scintillation spectrometer (Nuclear Chicago Corp.). Data in Table I are averages of at least two experiments in which the rate of DNA synthesis was assessed over a range of concentrations of each compound. Least-squares analysis of probits against the log of the concentration yielded linear relationships which permitted calculations of concentrations inducing 50 and 90% inhibition. Data in Figure 1 are averages of three experiments. The concentration chosen for each experiment was approximately that shown to yield 50% inhibition as estimated from Table I. However, day to day variations in cell suspensions invariably occur, and occasional discrepancies are noted. Specifically, the pharmacological aspects of each compound which were investigated were (a) relative potency against DNA synthesis as assessed by least-squares analysis of dose–response data;[4,5] (b) slopes of the regression lines;[4,5] (c) relative selectivity for DNA synthesis;[4,5] (d) reversibility of the DNA inhibitory action upon removal of the inhibitor;[6] and (e) effect upon preformed DNA,[6] *i.e.*, depolymerization, to an acid-soluble form, of thymidine-^{3}H previously incorporated into the DNA of the cells.

Acknowledgment.—The technical skills of Alayne B. Smith and Gale B. Schmidt facilitated the present work.

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New Compounds

Microbiological Transformation of Steroids. II. The Synthesis of 2α-Methyl-19-nortestololactone[1]

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Replacement of the C_{10} angular methyl group on the nucleus of androstene-type steroids is known[2] to produce compounds which manifest a desirable enhancement of the anabolic–androgenic activity ratio, while the myotropic activities remain approximately unchanged. Since testololactone, the D-ring δ-lactone analog of testosterone, possesses good protein-anabolic activity[3] and lacks androgenicity, and Δ^1-testololactone is of chemotherapeutic efficacy[4] for the treatment of advanced human breast cancer, it was therefore felt that the lacto steroid derivative of a 19-norandrostene compound may be a tumor-regression agent. This paper describes the preparation and characterization of 2α-methyl-19-nortestololactone by fermentation with the fungus, *Aspergillus tamarii*.[5-7]

(1) Taken in part from a thesis presented by J. T. McCurdy to the University of Tennessee Graduate School for the degree of Master of Science.

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