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.

(11) T. Urbanski and J. Falécki, *Roczniki Chem.*, **34**, 1283 (1960).

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.

(2) L. G. Hershberger, E. G. Shipley, and R. K. Meyer, *Proc. Soc. Exptl. Biol. Med.*, **83**, 175 (1953).

(3) I. Shemano, G. S. Gordan, and E. Eisenberg, *ibid.*, **78**, 612 (1951).

(4) A. Segaloff, J. B. Weeth, K. K. Meyer, E. L. Rongone, and M. E. C. Cuningham, *Cancer*, **15**, 633 (1962).

(5) D. R. Brannon, J. Martin, A. C. Oehlschlager, N. N. Durham, and L. H. Zalkow, *J. Org. Chem.*, **30**, 760 (1935).

(6) D. R. Brannon, F. W. Parrish, B. J. Wiley, and L. Long, Jr., *ibid.*, **32**, 1521 (1967).

(7) R. D. Garrett and J. T. McCurdy, unpublished data.

Experimental Section[8]

Transformation of 2α-Methyl-19-nortestosterone by *A. tamarii*.—To each of 14 erlenmeyer flasks, each of which contained 100 ml of a 3% Difco malt extract solution and a 48-hr growth of *A. tamarii*, was added 75.0 mg of 2α-methyl-19-nortestosterone[9] in 0.4 ml of DMF. After an additional 72 hr of incubation on a rotary shaker at 28°, each reaction mixture was extracted with CH_2Cl_2. The organic layers were combined, dried ($MgSO_4$), and evaporated to a dry residue (1034 mg). A portion of the latter (1005 mg) was chromatographed on a 90-g column of silica gel H with EtOAc as the eluent. Tlc of the eluent fractions indicated that three major fractions were obtained. Fraction 1 (150 mg) was starting material, 2α-methyl-19-nortestosterone. Fraction 2, after recrystallization from Me_2CO–hexane, produced 675 mg (68% yield) of 2α-methyl-19-nortestololactone: mp 191–192.5°; ν_{max}^{KBr} 1725, 1670, and 1620 cm^{-1}; nmr ($CDCl_3$), δ 1.10 (3 H), 1.38 (3 H), and 5.83 (1 H); $[\alpha]^{25}D$ +15° in $CHCl_3$. *Anal.* Calcd for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.45; H, 8.64.

Fraction 3 (180 mg) consisted of mixtures of the above steroids and trace amounts of other compounds which are probably additional oxidative metabolites of 2α-methyl-19-nortestosterone, contaminated with CH_2Cl_2-soluble cellular material.

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(8) All melting points were determined by a Kofler apparatus and are corrected.

(9) Generously supplied by Dr. Paul W. O'Connell, The Upjohn Co., Kalamazoo, Mich.

Application of 1,3-Di(4-piperidyl)propane in the Mannich Reaction. Synthesis of β-Amino Ketones

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β-Amino ketones (Mannich bases) have been reported to possess antispasmodic,[1] analgetic,[2] local anesthetic,[3-6] and antibacterial[7-10] activity. In a recent communication[11] from this laboratory we described the synthesis of a series of β-amino ketones derived from 1-(N-β-hydroxyethyl-4-piperidyl)-3-(4-piperidyl)propane. Several of these compounds have exhibited antibacterial and antiviral activity.[12] Ready availability of 1,3-di(4-piperidyl)propane (4-DI-PIP) prompted us to prepare β-amino ketones of this novel secondary amine for biological screening.

Screening Results.—The compounds of Table I were screened *in vitro* against four organisms: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Mycobacterium smegmatis*, and *Klebsiella pneumoniae*. Filter paper disks (6.35-mm diameter) saturated with the solution (20 mg/ml) of the test compound were placed on the agar. After 72 hr of incubation the zones of inhibition around the disks were measured. The results are reported in Table II.

TABLE I

No.	R	Mp, °C[a]	Yield, %[b]	Formula[g]
1	2-Thenyl	212–215	55	$C_{27}H_{40}Cl_2N_2O_2S_2$[c]
2	4-Ethoxyphenyl	184–185	44	$C_{35}H_{52}Cl_2N_2O_4$[c,f]
3	4-Hydroxyphenyl	245	43	$C_{31}H_{44}Cl_2N_2O_4$[d]
4	4-Nitrophenyl	200–203	35	$C_{31}H_{42}Cl_2N_4O_6$[e]
5	4-Chlorophenyl	209–212	81	$C_{31}H_{42}Cl_4N_2O_2$[c]
6	4-Bromophenyl	212–215	69	$C_{31}H_{42}Br_2Cl_2N_2O_2$[c]
7	4-Fluorophenyl	190–194	42	$C_{31}H_{42}Cl_2F_2N_2O_2 \cdot 1.5H_2O$[c]
8	4-Methylphenyl	195–197	65	$C_{33}H_{48}Cl_2N_2O_2 \cdot H_2O$[c]
9	3-Nitrophenyl	180–183	49	$C_{31}H_{42}Cl_2N_4O_6 \cdot H_2O$[c]
10	2-Hydroxyphenyl	211–212	28	$C_{31}H_{44}Cl_2N_2O_4 \cdot H_2O$[c]
11	Phenyl	210–212	48	$C_{31}H_{44}Cl_2N_2O_2 \cdot 0.5H_2O$[c]

[a] All compounds melt with decomposition. [b] Yields are of the product obtained after the first crystallization. [c] Prepared by method C. [d] Prepared by method A. [e] Prepared by method B. [f] Recrystallized from EtOH; the other compounds were recrystallized from EtOH–Me_2CO–H_2O. [g] All compounds were analyzed for C, H, N. Infrared absorption bands for NH^+ and C=O were as expected.

TABLE II

In Vitro ANTIBACTERIAL ACTIVITY OF β-AMINO KETONES

	Microbial spectrum[a]			
No.	*S. aureus* K257	*P. aeruginosa*	*K. pneumoniae* ATCC 8052	*M. smegmatis*
1	+	+	−	+
2	+	+	−	+
3	−	−	−	−
4	+	+	+	−
5	−	+	+	+
6	+	−	+	+
7	−	−	−	+
8	+	+	+	+
9	−	+	+	−
10	+	+	+	+
11	−	−	−	−

[a] A negative sign indicates no observable activity.

Experimental Section[13]

1,3-Di(4-piperidyl)propane Dihydrochloride.—4-DI-PIP (42 g) was suspended in 100 ml of EtOH. Concentrated HCl (40 ml) was added dropwise with cooling and stirring. After the additions were completed, Me_2CO (200 ml) was introduced into the reaction vessel. The reaction mixture on refrigeration overnight furnished the desired salt in nearly quantitative yield. The salt was recrystallized (EtOH–Me_2CO); mp 262–264° dec. *Anal.* ($C_{13}H_{28}Cl_2N_2$) C, H, N.

β-Amino Ketone Dihydrochlorides. Method A.—A mixture of 0.04 mole of the appropriate ketone, 0.02 mole of 4-DI-PIP dihydrochloride, 1.8 g of paraformaldehyde, and 50 ml of EtOH containing 2 drops of concentrated HCl was refluxed for 5 hr. The warm solution was poured into Me_2CO (100 ml). Overnight refrigeration of the contents yielded the desired product.

Method B.—Concentrated HCl (4 ml) was added dropwise to a cooled suspension of 4-DI-PIP (4.2 g, 0.02 mole) in 10 ml of EtOH with shaking. Aqueous formaldehyde (37% 6 ml) was then introduced into the reaction vessel followed by the appropriate ketone (0.04 mole). The resulting reaction mixture was heated at 90–100° for 6 hr. During this time the entire mixture went into the solution. In a few cases an amorphous solid product separated at the end of this period. The contents were then diluted with Me_2CO (100 ml) and refrigerated overnight or until a solid product separated.

Method C was similar to that of B except that paraformaldehyde was used in place of formalin.

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(13) All melting points were taken on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Infrared spectra were recorded in Nujol mull on a Perkin-Elmer Model 137 Infracord spectrophotometer and were as expected. Where analyses are indicated only by symbols of the elements analytical results obtained for these elements were within ±0.4% of the theoretical values.

(1) J. J. Denton, R. J. Turner, W. B. Neier, V. A. Lawson, and H. P. Schedl, *J. Am. Chem. Soc.*, **71**, 2048 (1949).

(2) U. S. Patent 3,030,372 (April 17, 1962); *Chem. Abstr.*, **59**, 2780 (1963).

(3) C. Mannich and D. Lammering, *Ber.*, **55**, 3510 (1922).

(4) F. F. Blicke and E. S. Blake, *J. Am. Chem. Soc.*, **52**, 235 (1930).

(5) W. Wilson and Z. Y. Kyi, *J. Chem. Soc.*, 1321 (1952).

(6) T. Teshigahara, *Nippon Yakurigaku Zasshi*, **58**, 67 (1962); *Chem. Abstr.*, **59**, 3239 (1963).

(7) H. J. Florestano and M. E. Bahler, *J. Am. Pharm. Assoc.*, **45**, 320 (1956).

(8) W. L. Nobles and B. B. Thompson, *J. Pharm. Sci.*, **53**, 1554 (1964).

(9) W. L. Nobles and C. D. Blanton, *ibid.*, **51**, 878 (1962).

(10) J. Sam and A. C. Thompson, *ibid.*, **53**, 535 (1964).

(11) R. S. Varma and W. L. Nobles, *ibid.*, **65**, 455 (1967).

(12) R. S. Varma and W. L. Nobles, unpublished work.