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.

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