attempts to subject the data to regression analysis[11] including such substituent constants as σ, π, E_s have not led to any significant correlations. However, since *p*-alkyl substituents decrease, *m*-alkyl substituents leave unchanged, and *o*-alkyl substituents increase inhibitory activity it may be that the 5-Ph group of **1** binds to a nonpolar area of restricted dimensions. The increased activity found with *o*-alkyl substituents may originate from increased binding in this area. Alternatively, binding of the azopyrimidines to a nonplanar enzyme surface may be facilitated by the *o*-alkyl substituents forcing the benzene and pyrimidine groups into a twisted configuration. The present data do not distinguish these possibilities.

The very extensive work of Baker and his colleagues[12] has already established the crucial importance to inhibitor binding of a nonpolar area on dihydrofolate reductase. The binding capacity of this area would appear to be distinctly larger than that revealed by this study; however, the well-established species differences in dihydrofolate reductase[12,13] may not make this a very definitive conclusion.

Acknowledgments.—This work was supported by grants from National Institutes of Health (CA 06645), NASA (NGR-33-015-016), and National Science Foundation (GU 1864).

(11) C. Hansch, *Accounts Chem. Res.*, **2**, 232 (1969).

(12) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," Wiley, New York, N. Y., 1967, Chapter 10; B. R. Baker, *Accounts Chem. Res.*, **2**, 129 (1969).

(13) (a) G. H. Hitchings and J. J. Burchall, *Advan. Enzymol.*, **27**, 417 (1965); (b) B. R. Baker, G. J. Lourens, R. B. Meyer, Jr., and N. M. J. Vermeulen, *J. Med. Chem.*, **12**, 67 (1969).

Medium Ring Homologs of Proline as Potential Amino Acid Antimetabolites[1]

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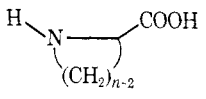
Received October 30, 1970

Hexahydro-1*H*-azepine-2-carboxylic acid (**1d**), octahydro-2-azocinecarboxylic acid (**1e**), octahydro-1*H*-azonine-2-carboxylic acid (**1f**), decahydro-2-azecinecarboxylic acid (**1g**), and azacycloundecane-2-carboxylic acid (**1h**), respectively, the 7-, 8-, 9-, 10-, and 11-membered α-imino acids that are ring homologs of the naturally occurring α-imino acid, L-proline, have been synthesized by rearrangement of α-halogenated-ω-aminolactams of ring sizes one larger than the corresponding imino acids. In addition, a number of derivatives of these imino acids which might be of use for detection of the latter in biological systems have been prepared, *viz.*, the methyl esters, phenylthiohydantoins, 1-nitroso-, 1-(2,4-dinitrophenyl)-, and 1-(6-dimethylaminonaphthalene-1-sulfonyl) derivatives. None of these α-imino acids showed growth inhibitory activities against the F-66 mouse mammary tumor in tissue culture or *Escherichia coli* in a glucose C medium, and only **1g** inhibited slightly the growth of the radicle of the mung bean, *Phaseolus aureus*. These imino acids showed no significant antitumor activity in the Cancer Chemotherapy National Service Center tumor screen, or antimalarial activity against *Plasmodium gallinaceum* or *P. berghei*.

The four-membered cyclic α-imino acid, L-azetidine-2-carboxylic acid (**1a**), the lower homolog of proline which occurs naturally in the plant kingdom as the



	n
1a	4
b	5
c	6
d	7
e	8
f	9
g	10
h	11

major nonprotein, nitrogenous component of the *Liliacae*,[2] has been shown to inhibit the growth of *Escherichia coli* in culture, as well as radicles of the mung bean, *Phaseolus aureus*.[3] This imino acid competitively substitutes for proline and is itself incorporated into the protein of *E. coli* and of *P. aureus*. The presence of **1a** also inhibits the incorporation of [^{14}C]proline into embryonic cartilage as well as the latter's conversion to [^{14}C]hydroxyproline, and results in the accumulation of abnormal protocollagen which contains incorporated **1a**.[4] When added to the culture media of *Streptomyces antibioticus* or of *S. chrysomallus*, **1a** (as well as the higher proline homolog, pipecolic acid, **1c**) is incorporated into the peptide side chain of the actinomycin molecule to produce new biosynthetic actinomycins.[5] The antimetabolic activities of proline homologs and analogs, as well as their comprehensive chemistry and biochemistry, have been the subject of

(1) This work was supported in part by Grant CA-06432 from the National Cancer Institute, United States Public Health Service.

(2) (a) L. Fowden, *Nature (London)*, **175**, 347 (1955); (b) L. Fowden, *Biochem. J.*, **64**, 323 (1955); (c) A. I. Virtanen and P. Links, *Acta Chem. Scand.*, **9**, 551 (1955); (d) A. I. Virtanen, *Nature (London)*, **176**, 984 (1955); (e) L. Fowden and M. Bryant, *Biochem. J.*, **70**, 626 (1958).

(3) (a) L. Fowden and M. Richmond, *Biochim. Biophys. Acta*, **71**, 459 (1963); (b) L. Fowden, *J. Exp. Bot.*, **14**, 381 (1963).

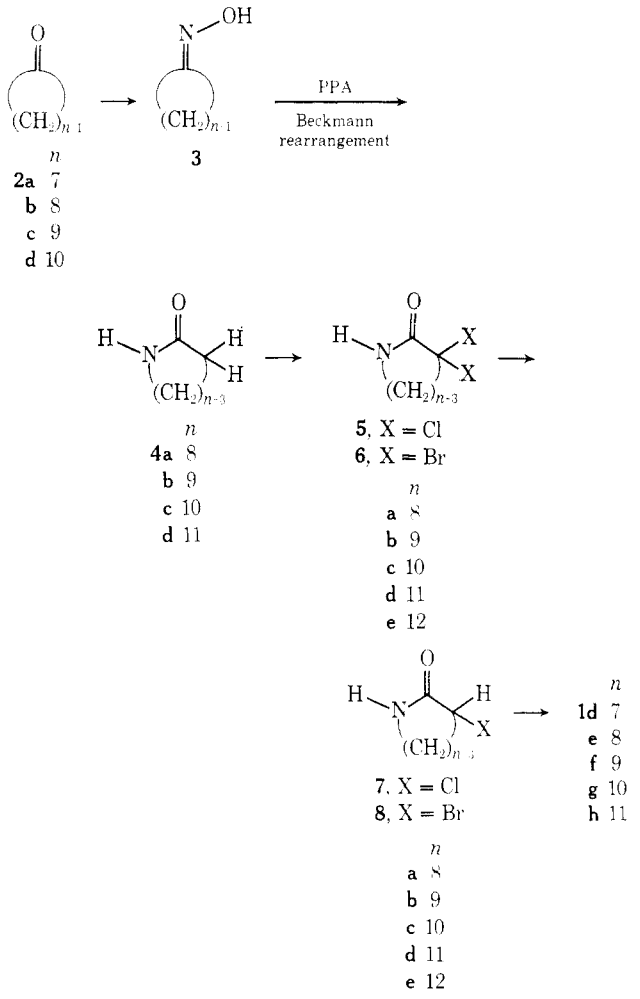
(4) T. Takeuchi and D. J. Prockop, *Biochim. Biophys. Acta*, **175**, 142 (1969).

(5) (a) E. Katz and W. A. Goss, *Biochem. J.*, **73**, 458 (1959); (b) E. Katz, *Ann. N. Y. Acad. Sci.*, **89**, 304 (1960); (c) E. Katz and H. Pugh, *Appl. Microbiol.*, **9**, 263 (1961).

recent reviews.[6] In a preliminary communication,[7] we described a novel synthesis of cyclic α-imino acids of medium ring size (**1d–1h**) representing the higher ring homologs of proline beyond pipecolic acid (**1c**). We now describe this work in detail and report some of the biological properties of these synthetic medium ring α-imino acids, as well as the physical properties of some derivatives which might be of use in biological investigations.

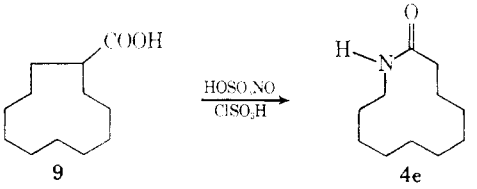
Chemistry.—The salient feature of this synthetic route to the imino acids **1d–1h** involves the base-catalyzed rearrangement of the α-halo-ω-aminolactams **7** or **8**, *viz.* **7** or **8** → **1** (Scheme I). A possible mechanism for this Favorskii-like rearrangement of α-halolactams has been presented earlier.[7] The most convenient route to the lactams **4** was *via* Beckmann rearrangement of the alicyclic ketoximes **3**; hence the cyclic ketones **2** served as the starting material in most of this work. The overall synthetic sequence involves a ring-expansion reaction with heteroatom insertion (**3** → **4**) followed later by a ring-contraction step (**7** or **8** → **1**); thus, a cyclic ketone of a given ring size is ultimately converted into an α-imino acid of the *same* ring size.

SCHEME I



Polyphosphoric acid (PPA) of 78% P_2O_5 content[8] was utilized routinely for the Beckmann rearrangement of the alicyclic ketoximes **3** to the lactams **4**, generally without resort to extensive purification of **3**. While the individual lactams have all been described in the literature,[9] use of dilute PPA for the Beckmann rearrangement of the oximes derived from the homologous series of cyclic ketones, cycloheptanone to cyclododecanone (**2a,b,c,d**) as well as cyclododecanone, cyclotetradecanone, and cyclopentadecanone (**2**, n = 12, 14, 15), is without precedent. These results are summarized in Table I. By employing continuous liquid–liquid extraction to remove the lactams from the reaction mixture after dilution with water, product isolation was facilitated. Preparative quantities of lactams were obtained routinely in good yields, and because this procedure, unlike the usual procedure for the Beckmann rearrangement does not call for neutralization of the large quantities of acid present, the greatly reduced cost adds to its attractiveness as the method of choice for large-scale preparations.

Azacyclododecan-2-one (**4e**) was prepared from cycloundecane-2-carboxylic acid (**9**) by reaction with nitrosyl sulfuric acid in chlorosulfonic acid.[10] The lactams



4 were dichlorinated to the α,α-dichlorolactams **5** with PCl_5 in a mixture of $CHCl_3$–toluene, a procedure modeled after that of Francis, *et al.*, and Wineman, *et al.*,[11] for the chlorination of caprolactam. In a few instances, restricted to the larger membered lactams (**4d**, **4e**), dichlorination became sluggish and mixtures of mono- and dichlorinated products were obtained. The dichlorolactams **5** underwent catalytic hydrogenolysis to the monochlorolactams **7** with facility, and hydrogen uptake essentially ceased at the monohalogen stage. Advantage was taken of the relative inertness of the monochlorolactams of the higher-membered series to further hydrogenolysis in separating the mixtures resulting from the halogenation of these lactams. After prior isolation of the dichlorinated products, the residues were subjected to catalytic hydrogenation until no more hydrogen was absorbed to convert any remaining dichlorolactams to the monochlorides, and the monochlorolactams were subsequently isolated in pure form.

The corresponding dibromo-(**6**) and monobromo-(**8**) lactams were prepared by bromination of the lactams in $CHCl_3$ solution with 2 or 1 molar equiv of bromine with traces of $ZnCl_2$ or I_2 as catalysts.[11b]. These halogen-

(6) (a) L. Fowden, D. Lewis and H. Tristam, *Advan. Enzymol.*, **29**, 89 (1967); (b) A. B. Mauger and B. Witkop, *Chem. Rev.*, **66**, 47 (1966); (c) L. Fowden in J. B. Pridham and T. Swain, Ed., "Biosynthetic Pathways in Higher Plants," Academic Press, New York, N. Y., 1965, pp 92–95.

(7) H. T. Nagasawa and J. A. Elberling, *Tetrahedron Lett.*, **44**, 5393 (1966).

(8) Commercially available as 105% phosphoric acid or by dilution of 115% H_3PO_4. Certain procedures described in the Experimental Section designed to moderate the vigorous exothermic reactions serve to minimize the extensive charring encountered in the usual procedure [*cf.* E. C. Horning and V. L. Stromberg, *J. Amer. Chem. Soc.*, **74**, 2680 (1952)].

(9) L. Ruzicka, M. Kobelt, O. Häfliger, and V. Prelog, *Helv. Chim. Acta*, **32**, 544 (1949).

(10) Imperial Chemical Industries, Ltd., Belgian Patent 616,544, Oct 17, 1962; *Chem. Abstr.*, **59**, 452 (1963).

(11) (a) W. C. Francis, J. R. Thornton, J. C. Werner, and T. R. Hopkins, *J. Amer. Chem. Soc.*, **80**, 6238 (1958); (b) R. J. Wineman, E.-P. J. Hsu, and C. E. Anagnostopoulos, *ibid.*, **80**, 6233 (1958).

TABLE I

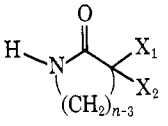
Lactam	Recrystn solvent	Mp or bp (mm), °C	Reported[a] mp or bp (mm), °C	Yield, %
4a		112–114 (0.1 mm)	162–164 (22 mm)	92
4b		127–135 (0.25 mm) [76–77]	72–73	95
4c	CH_2Cl_2–petr ether	142.5–143	138–139	80
4d	CH_2Cl_2–hexane	164–166	162	83
Azacyclotridecan-2-one	C_6H_6–petr ether	150–151	153–153.5	89
Azacyclopentadecan-2-one	CH_2Cl_2–hexane	146.5–147.5	146–147	90
Azacyclohexadecan-2-one	CH_2Cl_2–petr ether	134–135.5	133.5	92

[a] Ref 9.

ated lactams of ring sizes which range from the 8-membered hexahydro-2(1*H*)-azocinone to the 12-membered azacyclododecan-2-one are listed in Table II.

TABLE II

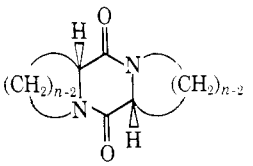
HALOGENATED LACTAMS



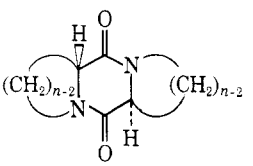
No.	n	X_1	X_2	Yield, %	Recrystn solvent	Mp, °C	Formula[a]
5a	8	Cl	Cl	61	C_6H_6–petr ether	96–97	$C_7H_{11}NOCl_2$
6a	8	Br	Br	38	CH_2Cl_2–hexane	147–148	$C_7H_{11}NOBr_2$
7a	8	H	Cl	93	Sublimed	164–165	$C_7H_{12}NOCl$
8a	8	H	Br	55	CH_2Cl_2–hexane	172–173	$C_7H_{12}NOBr$
5b	9	Cl	Cl	34	C_6H_6 and sublimed	160–162	$C_8H_{13}NOCl_2$
6b	9	Br	Br	77	CH_2Cl_2–hexane	156–157	$C_8H_{13}NOBr$
7b	9	H	Cl	94	C_6H_6–*n*-hexane	132–133	$C_8H_{14}NOCl$
8b	9	H	Br	53	$CHCl_3$–hexane	146–147	$C_8H_{14}NOBr$
5c	10	Cl	Cl	79	Acetone	172–174	$C_9H_{15}NOCl_2$
6c	10	Br	Br	74	CH_2Cl_2–hexane	168–169	$C_9H_{15}NOBr_2$
7c	10	H	Cl	74	Ethanol	159–160	$C_9H_{16}NOCl$
8c	10	H	Br	80	CH_2Cl_2–hexane	169–170	$C_9H_{16}NOBr$
5d	11	Cl	Cl	46	Ethanol–water	165–166	$C_{10}H_{17}NOCl_2$
6d	11	Br	Br	70	CH_2Cl_2	156–157	$C_{10}H_{17}NOBr_2$
7d	11	H	Cl	41	$CHCl_3$–petr ether	149–150	$C_{10}H_{18}NOCl$
8d	11	H	Br	53	CH_2Cl_2	172–173	$C_{10}H_{18}NOBr$
5e	12	Cl	Cl	51	$CHCl_3$	179	$C_{11}H_{19}NOCl_2$
6e	12	Br	Br	16	CH_2Cl_2	179–180	$C_{11}H_{19}NOBr_2$
7e	12	H	Cl	89	Hexane	142	$C_{11}H_{20}NOCl$
8e	12	H	Br	69	CH_2Cl_2–hexane	164–165	$C_{11}H_{20}NOBr$

[a] All compounds were analyzed for C, H, N.

Initial exploratory experiments indicated that a number of alkaline reagents effected the rearrangement of the α-chlorolactams **7** to the α-imino acids **1**, albeit in low yields. Thus, the reaction of 3-chlorohexahydro-2(1*H*)-azocinone (**7a**) with 3 *N* NaOH in aqueous dioxane gave rise to the 7-membered α-imino acid, hexahydro-1*H*-azepine-2-carboxylic acid (**1d**)[12] as one of the five ninhydrin chromogenic products detectable by thin-layer chromatography (tlc). The α-imino acid component was selectively isolated as the copper salt, followed by precipitation of the Cu(II) as the sulfide with H_2S, to give **1d** in 15% yield. In addition a neutral, water-insoluble product, analyzing for $C_{14}H_{22}N_2O_2$ (M^+ 250) with carbonyl absorption at 1658 cm^{-1} but no NH absorption was obtained in 6% yield. Since acid hydrolysis gave **1d**, this neutral product must be the racemic diketopiperazine **10a** or the meso isomer, **10b**.



10a, $n = 7$
11a, $n = 8$



10b, $n = 7$
11b, $n = 8$

Like treatment of 3-chlorooctahydro-2*H*-azonin-2-one (**7b**) with NaOH in aqueous dioxane gave the 8-membered octahydro-2-azocinecarboxylic acid (**1e**) in 40% yield, together with a small amount of diketopiperazine, mp 151–153° (**11a** or **11b**) which was convertible to **1e** by acid hydrolysis. Of possible mechanistic significance,[7] tlc of this crude reaction mixture revealed the presence of only two ninhydrin chromogenic components, one of which was the α-imino acid **1e**.

The smooth rearrangement of the 9-membered chlorolactam (**7b**) to the 8-membered cyclic α-imino acid (**1e**) in moderate yields under essentially hydrolytic conditions called for evaluation of other reagents that might effect the rearrangement. Potassium *tert*-butoxide in *tert*-butyl alcohol was found to be the reagent of choice. Since solvolysis of reaction intermediates can give products that are α-imino acid *tert*-butyl esters, the reaction mixtures were routinely heated in aqueous acid before isolation of the α-imino acids as their copper salts. The classical procedure for liberation of amino acids from their copper salts by precipitation of the Cu(II) with H_2S[13] was considerably simplified by utilizing 8-hydroxyquinoline as the precipitating agent. The low solubility of the copper(II) 8-hydroxyquinolinate in aqueous methanol forced the equilibrium to favor the free imino acids, and after removal of excess 8-hydroxyquinoline with ether, the α-imino acids were obtainable in an essentially pure state.

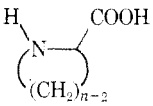
In this manner, the homologous series of α-chlorolactams (**7a**,**b**,**c**,**d**,**e**) were converted to the homologous series of α-imino acids *viz.*, hexahydro-1*H*-azepine-2-carboxylic acid (**1d**), octahydro-2-azocinecarboxylic acid (**1e**), octahydro-1*H*-azonine-2-carboxylic acid (**1f**), decahydro-2-azecinecarboxylic acid (**1g**), and azacycloundecane-2-carboxylic acid (**1h**), respectively, the 7-, 8-, 9-, 10-, and 11-membered cyclic α-imino acids homologous to proline (Table III). The yields progressively increased as the homologous series was ascended, and was maximal for the 9-membered **1f**, but the yields drastically fell with further increases in the ring size of the halogenated lactams. A limited number of the

(12) Reference to the preparation of **1d** and some of its derivatives by cyclization of α-amino-ω-haloenanthic acid derivatives has appeared as a Soviet patent by A. M. Likkosherstov and A. M. Kritsyn, USSR Patent 176,589, Nov 17, 1965; *Chem. Abstr.*, **64**, 9697*h* (1966), and subsequently in a paper by A. M. Kritsyn, A. M. Likkosherstov, and A. P. Skoldinov, *Dokl. Chem.*, **179**, 228 (1968).

(13) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 3, Wiley, New York, N. Y., 1961, p 2032.

TABLE III

PROLINE HOMOLOGS



						F-66 Mouse tumor in tissue culture[a]					*Phaseolus aureus*		
No.	*n*	Yield, %	Recrystn solvent	Mp, °C	Formula[b]	Concn, *M*	% change total cells	% change non-viable cells	pH change	Cell morphology	Growth % of control[c]	P	Concn, *M*, for 50% inhibition
1d	7	57	H_2O–acetone (or MeOH–acetone)	205–208 dec	$C_7H_{13}NO_2$	1.0×10^{-2}	+151	0	+	Normal	97.0	N.S.	
						1.0×10^{-4}	+150	0	+	Normal			
1e	8	67	H_2O–acetone (or abs EtOH–C_6H_6)	168–171 dec	$C_8H_{15}NO_2$	1.0×10^{-2}	+150	0	+	Normal	89.3	N.S.	
						1.0×10^{-4}	+150	0	+	Normal			
1f	9	80	H_2O–acetone	156–158 dec	$C_9H_{17}NO_2$						89.7	N.S.	
1g	10	37	MeOH–acetone	147–148 dec	$C_{10}H_{19}NO_2$	1.0×10^{-2}	+118	0	+	Normal	75.5	<0.01	9.2×10^{-3}
						1.0×10^{-4}	+100	0	+	Normal			
1h	11	34	Acetone	143–144 dec	$C_{11}H_{21}NO_2$	1.0×10^{-2}	+103	0	+	Normal	91.5	N.S.	
						1.0×10^{-4}	+95	0	+	Normal			
1a	4										13.2	<0.001	3.2×10^{-4}

[a] See Experimental Section for details. Under comparable conditions, the cytotoxic aminonucleoside of puromycin (1.0×10^{-4} *M*) inhibited cell growth by 47% in this system [C. S. Alexander and H. T. Nagasawa, *Biochem. Pharmacol.*, **13**, 548 (1964)]. In the CCNSC tumor screen, **1d** had a *T*/*C* of 0.59 and 0.73 against P-1798 lymphosarcoma at a dose of 100 mg/kg, but no significant activities against L-1210 lymphoid leukemia (100 mg/kg), Dunning leukemia (50 mg/kg), or KB-cells (100 μg/kg) in the dosages indicated. Against HST human sarcoma, 20 mg/kg of **1f** elicited *T*/*C* of 0.70. [b] All compounds except **1a** were analyzed for C, H, N. [c] For L-azetidine-2-carboxylic acid (**1a**) concn = 1.0×10^{-3} *M*; for all others, concn = 5.0×10^{-3} *M*.

α-bromolactams **8** were also rearranged, but the bromo compounds offered no advantage over the chlorinated analogs, and the latter were routinely used for preparative runs. Hexahydro-1*H*-azepine-2-carboxylic acid (**1d**) obtained by rearrangement of 3-chlorohexahydro-2(1*H*)-azocinone (**7a**) was identical with the α-imino acid produced by acid permanganate oxidation of 2-hydroxymethylhexahydro-2*H*-azepine.[14] Octahydro-2-azocinecarboxylic acid (**1e**) and octahydro-1*H*-azonine-2-carboxylic acid (**1f**) decarboxylated in refluxing cycloheptanone[15] to octahydroazocine and octahydroazonine, respectively, thus providing evidence for the assigned ring sizes. The latter were isolated and identified as their phenylthiuorea derivatives. Further evidence for the cyclic α-imino acid structures for **1d**–**1h** was adduced by the disappearance of the NH stretching absorptions in the ir spectra by nitrosation, dinitrophenylation, or by conversion to their phenylthiohydantoin derivatives. These as well as other derivatives of **1d**–**1h** which may be useful in the isolation and detection of these imino acids in biological systems are listed in Tables IV and V.[16]

When the chlorolactam **7a** was rearranged with potassium *tert*-butoxide in refluxing tetrahydrofuran, in addition to **1d** two isomeric diketopiperazines **10**, mp 118–119° (11%) and mp 200–201° (26%), were isolated by chromatographic work-up of the neutral fraction. The higher melting isomer was identical with the diketopiperazine **10** isolated earlier (see above). The isomeric relation of the two products with different melting points was indicated by (a) the identity of their molecular ions (M^+ 250) as well as similarity of the fragmentation patterns of their mass spectra, (b) their different retention times on glc, and (c) their infrared and nmr spectra, which were different from each other. Furthermore, acid hydrolysis of either isomer gave a single racemic α-imino acid, **1d**. The 60-MHz proton magnetic resonance spectra of the isomeric **10** in $CDCl_3$ was complex and did not permit a clearcut assignment of the structures **10a** (racemic) or **10b** (meso) to these diketopiperazines. Like treatment of chlorolactam **7b** similarly gave two isomeric diketopiperazines **11**, mp 156–158° (M^+ 278) and mp 166–168° (M^+ 278), as neutral products. Both isomers gave **1e** on acid hydrolysis.

Biological Results and Discussion.— At concentrations of 1.0×10^{-4} or 1.0×10^{-2} *M*, the homologs of proline **1d**,**e**,**g**,**h** (**1f** was not tested in this system) did not inhibit the growth of the ascites form of the F-66 mouse mammary tumor in tissue culture (Table III). Unlike L-azetidine-2-carboxylic acid (**1a**) which is inhibitory toward *E. coli* at a concentration of 1 m*M*,[2] a result which we have confirmed, the medium ring α-imino acid **1d**–**1h** did not affect the growth of *E. coli* up to concentrations of 2 m*M*, and **1d** was not inhibitory at a concentration of 5.6 m*M*. Likewise, with the single exception of **1g** which exhibited minimal activity, these ring homologs of proline did not inhibit the growth of the radicle of the mung bean, *Phaseolus aureus*, at concentrations of 5 m*M* (Table III). By comparison, **1a** when used as a standard inhibitor in our system, routinely inhibited growth of *P. aureus* by 77% at a concentration of 1 m*M*. None of these homologs of proline showed highly significant antitumor activity in the standard Cancer Chemotherapy National Service Center tumor screen (Table III), nor did they exhibit antimalarial activity against *Plasmodium gallinaceum* (mosquito test) or *P. berghei* (rodent test).

The failure of the medium ring homologs of proline described in the present communication to inhibit the growth of *E. coli* cells (as well as mung bean radicles) supports and further extends the observations of Papas and Mehler[17] regarding the rigid steric requirement for

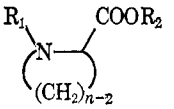
(14) F. F. Blicke, *Chem. Abstr.*, **50**, 5781 (1956).

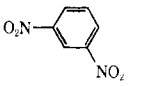
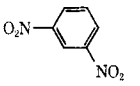
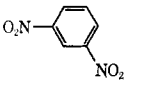
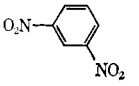
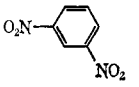
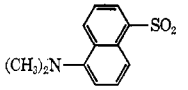
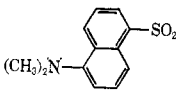
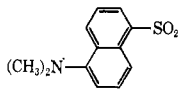
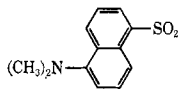
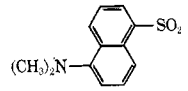
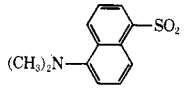
(15) G. Chatelus, *Bull. Soc. Chim. Fr.*, 2523 (1964).

(16) The chromatographic properties of **1a**–**1h** as well as their 2,4-dinitrophenyl-, 5-dimethylaminonaphthalene-1-sulfonyl-, and phenylthiohydantoin derivatives in a variety of paper and tlc systems have been reported [H. T. Nagasawa, P. S. Fraser, and J. A. Elberling, *J. Chromatogr.*, **44**, 300 (1969)]. **1a**–**1h** are eluted in order of increasing molecular weights from standard ion-exchange chromatographic columns.[7]

(17) T. S. Papas and H. H. Mehler, *J. Biol. Chem.*, **245**, 1588 (1970).

TABLE IV
α-IMINO ACID DERIVATIVES



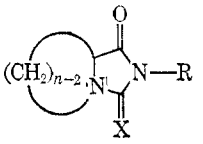
No.	n	R_1	R_2	Yield, %	Recrystn solvent	Mp, °C	Formula[g]
11a[a]	6	H·HCl	CH_3	99	MeOH–ether	213–214	$C_7H_{14}NO_2Cl$
11b[a]	7	H·HCl	CH_3	94	MeOH–ether	160.5–161.5	$C_8H_{16}NO_2Cl$
11c[a]	8	H·HCl	CH_3	97	MeOH–ether	140.5–142	$C_9H_{18}NO_2Cl$
11d[a]	9	H·HCl	CH_3	86	MeOH–ether	107–113	$C_{10}H_{20}NO_2Cl$
12a[b]	7		H	94	EtOAc–hexane	143.5–144.5	$C_{13}H_{15}N_3O_6$
12b[b]	8		H	95	EtOAc–hexane[c]	148–149	$C_{14}H_{17}N_3O_6$
12c[b]	9		H	83	$CHCl_3$–hexane	159–160	$C_{15}H_{19}N_3O_6$
12d[b]	10		H	80	CH_2Cl_2–hexane[d]	127–129.5	$C_{16}H_{21}N_3O_6$
12e[b]	11		H	84	CH_2Cl_2–hexane	136–137.5	$C_{17}H_{23}N_3O_6$
13a	6			88[e]	CH_2Cl_2–petr ether	126–136	$C_{23}H_{33}N_3O_4S$
13b	7			89	CH_2Cl_2–petr ether	119–128	$C_{24}H_{35}N_3O_4S$
13c	8			88	CH_2Cl_2–petr ether	117–122	$C_{25}H_{37}N_3O_4S$
13d	9			83	CH_2Cl_2–petr ether	125–135	$C_{26}H_{39}N_3O_4S$
13e	10			17	CH_2Cl_2–petr ether	119–124	$C_{27}H_{41}N_3O_4S$
13f	11			19	CH_2Cl_2–petr ether	120–125	$C_{28}H_{43}N_3O_4S$
14a[f]	7	NO	H		CH_2Cl_2–petr ether	106–107	$C_7H_{12}N_2O_3$
14b[f]	8	NO	H		CH_2Cl_2–petr ether	128–129.5 dec	$C_8H_{14}N_2O_3$
14c[f]	9	NO	H		CH_2Cl_2–hexane	85–86	$C_9H_{16}N_2O_3$
14d[f]	10	NO	H		CH_2Cl_2–petr ether	81–83	$C_{10}H_{18}N_2O_3$
14e[f]	11	NO	H		CH_2Cl_2–petr ether	117–118 dec	$C_{11}H_{20}N_2O_3$
15	7	$H·HOSO_2C_6H_4CH_3$-*p*	H		Abs EtOH–ether	186–188	$C_{14}H_{21}NO_5S$
16	8	$H·HOSO_2C_6H_4CH_3$-*p*	H	78	MeOH–ether	172–173.5	$C_{15}H_{23}NO_5S$
17	7	$CO_2CH_2C_6H_4NO_2$-*p*	H		C_6H_6–petr ether	138–140	$C_{15}H_{18}N_2O_6$
18	8	$SO_2C_6H_4CH_3$-*p*	H		C_6H_6	171–172	$C_{15}H_{20}NO_4S$

[a] Prepared according to the procedure of Julian R. Rachele, *J. Org. Chem.*, **28**, 2898 (1963), using 2,2-dimethoxypropane. The methyl ester hydrochlorides of the α-imino acids corresponding to n = 10 and 11 were oils which did not crystallize. [b] Procedure of K. R. Rao and H. O. Sober, *J. Amer. Chem. Soc.*, **76**, 1328 (1954). Anal. sample recrystd from CH_2Cl_2. [c] Anal. sample recrystd from C_6H_6. [d] Anal. sample recrystd from C_6H_6–hexane. [e] Coupled in H_2O–acetone. [f] By nitrosation of the imino acid with nitrous acid; *cf.* K. Heyns and W. Konigsdorf, *Z. Physiol. Chem.*, **290**, 171 (1952). [g] All compounds analyzed for C, H, N.

analog activation and incorporation into $tRNA^{Pro}$ by proline tRNA synthetase of this organism. In an extensive investigation, these authors have concluded that the naturally occurring L-proline is the best substrate for this enzyme, and the K_m values increase in the order of increasing molecular size for the following series, *viz.*, **1a** < L-thiazolidine-4-carboxylic acid < 3,4-dehydro-L-pipecolic acid ≃ L-pipecolic acid, the

TABLE V

PHENYLTHIOHYDANTOIN AND HYDANTOIN DERIVATIVES



No.	n	X	R	Recrystn solvent	Mp, °C	Formula[a]	λ_{max}^{EtOH}, mμ	Log ϵ_{max}	$\nu_{C=O}^{KBr}$, cm^{-1}	$\nu_{C=S}^{KBr}$, cm^{-1}
19a	7	S	C_6H_5	C_6H_6	165–166	$C_{14}H_{16}N_2OS$	235; 269	3.86; 4.00	1752	1505; 1495[b]
19b	8	S	C_6H_5	C_6H_6	120–121	$C_{15}H_{18}N_2OS$	236; 271	3.91; 4.05	1752	1508
19c	9	S	C_6H_5	CH_2Cl_2–petr ether	152–153	$C_{16}H_{20}N_2OS$	237; 270	4.01; 4.13	1760	1504
19d	10	S	C_6H_5	CH_2Cl_2–petr ether	126–127	$C_{17}H_{22}N_2OS$	235; 271	3.98; 4.13	1758	1501; 1483
19e	11	S	C_6H_5	CH_2Cl_2–petr ether	160–162	$C_{18}H_{24}N_2OS$	237; 270	2.98; 4.15	1755	1500; 1483
20a	7	O	H	CH_2Cl_2–petr ether	117–118	$C_8H_{12}N_2O_2$			1762; 1718	
20b	8	O	H	EtOAc–hexane	131–133	$C_9H_{14}N_2O_2$			1770; 1722; 1690 (sh)	

[a] All compounds were analyzed for C, H, N. [b] L. K. Ramachandran, A. Epp, and W. B. McConnell, *Anal. Chem.*, **27**, 1734 (1955), assign a generally weak 1425–1450 cm^{-1} band to the thiocarbonyl vibrations in the phenylthiohydantoin derivatives of α-amino acids. This assignment is in fact correct only for PTH-proline and PTH-hydroxyproline which exhibit strong bands in this region. For all other PTH-amino acids described by Ramachandran *et al.*, the C=S appear as strong bands near 1500–1550 cm^{-1}. These authors incorrectly assigned these to NH deformations, which, of course, are absent in the PTH-α-imino acids described here, permitting the unambiguous assignment of these strong 1500-cm^{-1} bands to the thiocarbonyl group.

latter two no longer being substrates for this enzyme. Although our evidence is indirect, it suggests that the proline tRNA synthetases of *Phaseolus aureus*[18] as well as of *E. coli* are highly specific and recognize the steric bulk of the alkylidene residue on the α-imino acid, since all of our proline homologs showed little or no activity in these systems. It is interesting that in the open-chain series, increasing the length of the carbon chain on the nitrogen atom from sarcosine to *N*-ethylglycine to *N*-*n*-propylglycine does not significantly affect K_m or V_{max} with the *E. coli* enzyme; while increasing the length and bulk of the alkyl residue on the α-carbon atom from *N*-methyl-L-alanine to *N*-methyl-L-valine to *N*-methyl-L-leucine and *N*-methyl-L-isoleucine abolishes binding to the enzyme. The medium ring homologs of proline may be looked upon as the cyclized form of these open-chain analogs and may therefore show little propensity for activation by proline tRNA synthetase. Additional work is obviously required to determine whether **1g** is in fact incorporated into or excluded from mung bean protein, but the evidence at hand does not favor incorporation.

The ready availability of these medium ring proline homologs makes possible their replacement for proline in polypeptide hormones and antibiotics by substituting these analogs for proline in the chemical synthetic scheme. Furthermore, since a number of α-imino acid analogs of proline inhibit actinomycin biosynthesis and are incorporated into the molecule to give new biosynthetic actinomycins,[5,19] the proline homologs described here may provide new substrates for research in this area.

Experimental Section[20]

All melting points were taken on a Fisher-Johns melting point apparatus and are corrected. Infrared spectra were taken on a Beckman IR-4 or IR-10 infrared spectrophotometer and electronic spectra on Beckman DK-2A recording spectrophotometer. Nmr spectra were run on a Varian A-60A spectrophotometer in Silanor C (Merck), and the mass spectra on a Hitachi Model RMU-6D mass spectrometer.

Tissue Culture Assay.—The cells used were F-66 mouse mammary ascites cells adapted to suspension culture. The culture medium[21] consisted of: 87 vol of Minimum Eagle's medium Spinner modified (Cultur STAT), 3 vol of 200 m*M* glutamine solution, and 10 vol of fetal bovine serum. The cells were maintained in either 50-ml tubes or 225-ml centrifuge bottles in a roller drum, 40 rpm at 37°. Weighed amounts of the proline homologs were dissolved in a given volume of culture medium, the medium was sterilized (Swinney filter), the cells were added (5×10^5 cells/ml final concn), and the cultures were incubated for 24 hr. The effect of proline homologs was noted by (a) changes in cell counts, (b) number of nonviable cells (trypan blue), (c) lactic acid production (pH change of the medium), and (d) cell morphology.

Inhibition Studies with *E. coli* and *Phaseolus aureus*.—The growth-inhibitory properties of the proline homologs were tested against *E. coli*, strain B, growing exponentially in a 0.4% glucose C medium essentially as described by Roberts, *et al.*[22] L-Azetidine-2-carboxylic acid (**1a**) served as internal standard.

Fresh seed crops of mung beans (*Phaseolus aureus*)[23] were washed once with 0.05% NaOCl soln to remove possible mold and bacterial contaminants. Failure to do this often resulted in mold and/or bacterial growth when cultured. After thorough washings with distd water, the beans were dried at room temp in a sterile environment and stored in sterile containers until used. Sample batches (25 seeds) were randomized in a manner such that the total weight of 25 seeds was uniform (±1.5%) among all test and control samples. After soaking 16–24 hr in distd water, the seeds were transferred to 150 × 75 mm crystn dishes adapted with Plexiglass covers with 13 0.125-in. holes for aeration. Distd H_2O (25 ml) was added to control samples, while soln of the α-imino acids in distd H_2O (25 ml) were added to the test samples. All tests were run in duplicate. The seeds were only partially submerged under these conditions. They were allowed to sprout in the dark at 25° for 5–6 days. The amount of H_2O lost by evapn (*ca.* 5 ml/day) was replenished daily. After the prescribed growth period, the radicles were removed by cutting at the exhausted cotyledons, blotted, and individually weighed. The occasional defective seeds that did not sprout under these conditions or those radicles that became detached from the seedlings were eliminated and the mean weights of the radicles from each growth were compared statistically using

(18) P. J. Peterson and L. Fowden, *Biochem. J.*, **97**, 112 (1965).

(19) (a) T. Yoshida, A. Mauger, B. Witkop, and E. Katz, *Biochem. Biophys. Res. Commun.*, **25**, 66 (1967); (b) J. S. Nishimura and W. F. Bowers, *ibid.*, **28**, 665 (1967).

(20) Microanalyses were by the staff of the Organic Microanalytical Laboratory, University of Minnesota, or by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

(21) Cappel Laboratories, West Chester, Pa.

(22) R. B. Roberts, P. H. Abelson, D. B. Cowie, E. T. Bolton, and R. J. Britton, "Studies of Biosynthesis in *E. Coli*," Carnegie Institution, Washington, D. C., Publication No. 607, 1957.

(23) From Polacek Mills, Enid, Okla., purchased locally from International House, Minneapolis, Minn.

the Student's t test. Differences below the 95% level of confidence were considered not significant (N.S.)[24]

Oximes (3a–3d).—The oximes were prepared from the commercially available ketones and $H_2NOH \cdot HCl$ using the pyridine method except that MeOH was used as solvent.

Lactams (4a–4d).—Commercial PPA of 82% P_2O_5 content (450 g) was dild with stirring with H_2O (53 g). (Commercially available PPA of 78% P_2O_5 can also be used in place of this H_2O dild mixt.) With the temp at 130° the oxime **3** (0.50 mole) was added at such a rate that the temp was maintained at 130–140°. The soln was kept at 130° for 1 hr and slowly cooled to 100°. The mixt was then stirred with ice and water and extd with $CHCl_3$ in a continuous liquid extractor. The $CHCl_3$ extract was decolorized with charcoal and concd *in vacuo* and the residue of lactam was crystd from $CHCl_3$-hexane. The results are summarized in Table I.

Azacyclododecan-2-one.—To a well-stirred mixt of cycloundecanecarboxylic acid[25] (0.20 mole) in $ClSO_3H$ (11.0 g) was added a soln of nitrosylsulfuric acid (0.22 mole) in $ClSO_3H$ (10.0 g) dropwise at such a rate (*ca.* 100 min) that the temp remained 70 ± 10°. The mixt was then heated at 90° for 30 min, cooled, poured onto 200 g of ice, stirred with 200 ml of $CHCl_3$ with cooling to emulsify the sticky mass, and neutralized with 40% KOH. It was then extd with $CHCl_3$ in a continuous liq extractor. The $CHCl_3$ extract was decolorized with charcoal and concd *in vacuo* and the residue was recrystd from CH_2Cl_2-petr ether to yield white needles, 24.85 g (68%), mp 156–157° (reported[9] 154–154.5°).

Dichlorolactams (5a–5e) (Table II).—The lactam **4** (0.15 mole) in 100 ml of $CHCl_3$ was slowly added to a stirred suspension of PCl_5 (62.5 g, 0.30 mole) in 250 ml of toluene. After heating under reflux for 90 min the soln was concd *in vacuo*. Ice and H_2O were then added (**caution:** a vigorous reaction sometimes occurs) followed by 200 ml of acetone and 200 ml of 10% $NaHCO_3$ soln. After stirring 18 hr, the mixture was extd with $CHCl_3$ for 6 hr in a continuous liq extractor. The $CHCl_3$ extract was decolorized with charcoal and concd *in vacuo* and the residue was crystd.

Dibromolactams (6a–6e) (Table II).—To a soln of lactam **4** (0.10 mole) in 100 ml of $CHCl_3$ was added PCl_5 (0.20 mole) in small portions while maintaining the temp at 0–5°. After addn of 0.50 g of $ZnCl_2$, Br_2 (32 g, 0.20 mole) was added dropwise with stirring. After addn was complete, the mixt was stirred at 40° for 6 hr, then concd *in vacuo*. The residue was treated with ice and H_2O (100 g) and extd twice with 100 ml of $CHCl_3$. The $CHCl_3$ extract was washed with dil $NaHSO_3$ soln to remove excess Br_2, decolorized with charcoal, dried over Na_2SO_4, and concd *in vacuo* and the residual solids were recrystd.

Monochlorolactams (7a–7e) (Table II).—To the dichlorolactam **5** (0.10 mole) dissolved in glacial AcOH (100 ml) were added 10% Pd/C (1.5 g) and NaOAc (19 g, 0.22 mole) and the mixt was hydrogenated at 3.5 kg/cm² (50 psi) starting pressure. When H_2 uptake ceased the catalyst was removed by filtration and the filtrate was evapd *in vacuo*. The residue was neutralized with 10% Na_2CO_3 soln (300 ml) and extd for 6 hr with $CHCl_3$ in a continuous liq extractor. After decolorization with charcoal, the $CHCl_3$ extract was concd *in vacuo* and the solid residue was recrystd.

Monobromolactams (8a–8b) (Table II). Method A.—A soln of lactam **4** (0.10 mole) in 50 ml of C_6H_6 was added with stirring to a mixture of Br_2 (32 g, 0.20 mole) and PBr_3 (54.1 g, 0.20 mole) at a temp of 10–15°, then diluted with 100 ml of C_6H_6 and heated at 60° for 5 hr. The layers were sepd, and the lower emulsive layer was added to ice (100 g) and then extd with $CHCl_3$. The $CHCl_3$ extract was washed with $NaHSO_3$ soln to remove excess Br_2, dried over Na_2SO_4 decolorized with charcoal, and concd *in vacuo*, and the residual solids were recrystd.

Monobromolactams (8c–8e). Method B.—The lactam **4** was reacted as in the prepn of dibromolactams above except that 0.10-mole quantities of PCl_5 and Br_2 were used for 0.10 mole of lactam, 0.2 g of I_2 replaced $ZnCl_2$, and the reaction mixt was heated at reflux for 4 hr.

Hexahydro-1*H*-azepine-2-carboxylic Acid (1d). A. By Rearrangement of 7a.—A suspension of 6.47 g (0.040 mole) of 3-chlorohexahydro-2(1*H*)-azocinone (**7a**) in 120 ml of 3 *N* NaOH and 20 ml of purified dioxane was heated under reflux for 9.5 hr. A neutralized aliquot sample, when subjected to tlc, showed the presence of 5 ninhydrin chromogenic products, one of which (yellow–violet spot) proved later to be the desired **1d**. The soln was cooled, diluted with an equal vol of water, and extd twice with 75 ml of $CHCl_3$. The combined $CHCl_3$ extract was washed and dried over Na_2SO_4, and the solvent was evapd to give 0.57 g of crude **diketopiperazine 10,** which on recrystallization from acetone afforded 0.31 g (6.2%) of **10**, mp 199–200° ($\nu_{C=O}^{KBr}$ 1655 cm^{-1}; M^+ 250).

The aq phase was acidified with concd HCl and evapd *in vacuo* to incipient dryness, and the residue was repeatedly extd with abs EtOH to remove NaCl. The EtOH extract was evapd to dryness as above and the extn with abs EtOH was repeated. The EtOH was then evapd and the residue, dissolved in 75 ml of H_2O, was warmed with 8.85 g (0.04 mole, excess) of $CuCO_3 \cdot Cu(OH)_2$ when a light blue, flocculent copper complex pptd. The solids were collected, washed thoroughly with H_2O, and repeatedly extd with hot EtOH until no more blue color was extractable. Evaporation of the EtOH extract gave 1.55 g of sky-blue copper salt of **1d**, which was suspended in 150 ml of water, and the mixt was warmed and repeatedly treated with gaseous H_2S. The ppt of CuS was removed by filtration (Celite), washed with H_2O, and the filtrate evapd *in vacuo* to give a semisolid which on trituration with hot acetone gave 0.94 g (15%) of crystalline **1d**, mp 194–198° dec (–CO_2).

The *p*-toluenesulfonic acid salt (15), mp 187–188°, and the ***N*-*p*-nitrobenzyloxycarbonyl derivative (17)** of **1d** (Table IV), mp 138–140°, were prepared by the usual methods.

B. By Oxidation of 2-Hydroxymethylhexahydro-2*H*-azepine.—To a stirred soln of 9.96 g (0.040 mole) of 2-hydroxymethylhexahydro-2*H*-azepine[14] in 40 ml of 1 *M* H_2SO_4 was added at room temp dropwise over 45 min, a soln of 8.40 g (0.53 mole) of $KMnO_4$ dissolved in 150 ml of 1 *M* H_2SO_4. After stirring for 2.5 hr, the reaction mixt was diluted with 150 ml of H_2O, and the MnO_2 was removed by filtration (Celite). The aq filtrate was made alkaline with 2 *N* KOH, extd twice with 125-ml portions of EtOAc, then neutralized to pH 7 with 1 *N* H_2SO_4, and concd to incipient dryness *in vacuo*. The residue was extd twice with 250-ml portions of abs EtOH and the EtOH extract was concd. Tlc of the residue (95% EtOH–C_6H_6–H_2O, 4:1:1; cellulose powder plates; ninhydrin spray) indicated the presence of considerable amounts of unchanged amino alcohol as well as **1d**. This material was dissolved in 25 ml of H_2O and passed through a 2.1 × 33 cm column of Amberlite IRC-50 (H^+) resin eluting with H_2O. The eluates were monitored by tlc and were found to be still contaminated with the amino alcohol. Those fractions that contained the least amount of amino alcohol together with the desired product were combined and rechromatographed on a fresh ion-exchange column, and 55 fractions of 125 drops per fraction were collected. From fractions 4–10 was obtained 2.53 g of a viscous red–yellow oil (still contaminated with amino alcohol) which was dissolved in abs EtOH and treated with *p*-toluenesulfonic acid. Dilution with ether gave after some manipulation 1.26 g of the ***p*-toluenesulfonic acid salt (15)** of **1d**, mp 186–188°[26] (recrystd abs EtOH–ether). *Anal.* ($C_{14}H_{21}NO_5S$) C, H, N.

Trituration of the residues from fractions 11–20 (1.91 g) with acetone–ether gave two crops of **1d** melting at 205–208° dec and a cruder sample, 0.78 g, mp 170–190 dec. No further attempts were made to increase the yield or to further purify the crude product. The behavior of the highest melting product on tlc was identical in R_f and ninhydrin-generated color (yellow center, violet fringe) prepared by rearrangement of the chlorolactam **7a** above.

Octahydro-2-azocinecarboxylic Acid (1e).—3-Chlorooctahydro-2*H*-azonin-2-one (**7b**) (3.51 g, 0.020 mole) when treated with NaOH in aq dioxane and subsequently processed essentially as described for **1d** above gave **octahydro-2-azocinecarboxylic acid (1e),** 1.14 g, mp 167.5–168.5° dec and 0.14 g, mp 171–173° dec, in two crops from abs EtOH–C_6H_6, total yield: 40%, as well as 187 mg (7% yield) of the diketopiperazine (**11**), mp 151–153°

(24) We describe this very useful assay in detail here since our procedure differs from that described by Fowden.[2b] The commercial method for growing these beans [H. C. Keeskow, *Mich. Agri. Exp. Sta., Spec. Bull.*, **184**, 1] as well as the procedure of Fowden allows contact of the test medium containing the inhibitors with the sprouting beans *only during the first day* of germination, whereas in the present procedure, the growing radicle is continuously exposed to the test medium for 5–6 days, thereby enhancing the sensitivity of the assay. This procedure is also more amenable to incorporation studies with radioactively labeled amino acids.

(25) W. Ziegenbein, *Chem. Ber.*, **94**, 2989 (1961).

(26) The *p*-TosOH salt of the starting amino alcohol melts at 116–117°. *Anal.* ($C_{14}H_{23}NO_2S$) C, H, N.

(recrystd hexane): $\nu_{C=O}^{KBr}$ 1650 cm^{-1}; M$^+$ 278. (Tlc of an aliquot of the initial reaction product revealed the presence of only 2 ninhydrin chromogenic products, one of which turned out to be **1e**).

The ***p*-toluenesulfonic acid salt** (**16**) and the ***N*-*p*-toluenesulfonyl derivative** (**18**) prepared by the usual methods, analyzed satisfactorily (Table IV).

Decarboxylation of 1d and 1e to Octahydroazocine and Octahydroazonine.—The imino acid (0.006 mole) was suspended in 10 g of cycloheptanone and heated at 150° for 45 min. The soln was cooled, diluted with 33 ml of 1 *N* HCl, stirred 20 min, and poured into H_2O (150 ml). After extn with Et_2O (3 × 50 ml), the aq layer was decolorized with charcoal, made basic with 6 *N* NaOH, and extd with CH_2Cl_2. The CH_2Cl_2 extract was dried over Na_2SO_4 and evapd *in vacuo*, and the residue was dissolved in hexane (10 ml) and treated with 1 g of PhNCS in 5 ml of CH_2Cl_2. The soln was evapd to yield a white ppt which was recrystd from CH_2Cl_2–hexane. The yield of 1-(*N*-phenylthiocarbamoyl)octahydroazocine from **1d** was 32%, mp 138–139°; the yield of 1-(*N*-phenylthiocarbamoyl)octahydroazonine from **1e** was 17%, mp 103–104°; both mp undepressed when admixed with authentic samples.

α-Imino Acids (1d–1h). General Procedure.—The chlorolactam **7** (0.05 mole) in 100 ml of *tert*-BuOH was heated with stirring until dissolved or finely suspended. After cooling to 50° a soln of *tert*-BuOK (11.2 g, 0.10 mole) in 60 ml of *tert*-BuOH was added and the mixt was heated under reflux overnight. The cooled mixt was acidified with 100 ml of 2 *N* HCl and boiled for 1 hr while the *tert*-BuOH distd. The residue was then neutralized to pH 8 with 6 *N* NaOH and extd with CH_2Cl_2 (4 × 50 ml). This organic phase constituted the "neutral fraction."

The aq phase was treated with $CuCO_3 \cdot Cu(OH)_2$ (4.5 g, 0.08 equiv) and the mixt was heated and agitated about 5 min. After cooling, the solids were collected and washed with 25 ml of H_2O and then leached with hot MeOH until the extract was no longer colored. If the solid still showed traces of blue complex the extn was continued with MeOH–$CHCl_3$. The aq filtrate was reheated with the unreacted $CuCO_3$ and this procedure was repeated until no more complex could be extd. The combined MeOH (or MeOH–$CHCl_3$) extract was heated to redissolve the complex and filtered through Celite to remove any excess $CuCO_3$. If the filtrate tested positive for Cl^- it was cond *in vacuo*, and the solid complex was collected and washed with H_2O, and then redissolved in MeOH (or MeOH–$CHCl_3$).

To liberate the imino acid, the dissolved Cu complex was treated with 8-hydroxyquinoline (7.26 g, 0.040 mole) and the mit was evapd *in vacuo* to yield a voluminous ppt of copper 8-hydroxyquinolinate which was removed by filtration. The filtrate was evapd to ~100 ml, diluted with 2 vol of H_2O, and again filtered. The filtrate was extd with Et_2O to remove excess 8-hydroxyquinoline, the aq phase was decolorized with charcoal and cond *in vacuo* nearly to dryness, and the residual α-imino acid was crystd (Table III).

The **phenylthiohydantoin** derivatives (Table V) were prepared by heating the solid imino acids in excess C_6H_5NCS (5 min) or with an equimolar amt of C_6H_5NCS in refluxing toluene (4 hr).

Isolation of Diasteromeric Diketopiperazines 10a, 10b and 11a, 11b.—In the reaction of **7b** with *tert*-BuOK in *tert*-butyl alcohol (see example above) neutral, $CHCl_3$-soluble by-products were formed. A 3.0-g sample of a composite mixt of "neutral fractions" from several runs was fractionated by column chromatography on alumina (Giulini, Grade II, acid washed) using the elutropic series, C_6H_6, C_6H_6–$CHCl_3$ (2:1), and $CHCl_3$. Work-up of the C_6H_6 and part of the C_6H_6–$CHCl_3$ eluate gave 202 mg of **diketopiperazine 11a** or **11b,** mp 166–168° (recrystd from C_6H_6–petr ether) and from the $CHCl_3$ eluate, 1.74 g of the **diastereomeric diketopiperazine 11b or 11a,** mp 156–158° (recrystd C_6H_6–petr ether). *Anal.* (either isomer, $C_{14}H_{22}N_2O_2$) C, H, N. The two isomers gave separate peaks with different retention times when subjected to glc (0.66% DEGS on 70–80 mesh Chromosorb W). A barely detectable trace of the 155° isomer was present in the 166° melting product, but not the reverse. Their ir spectra ($\nu_{C=O}^{KBr}$ 1650 cm^{-1}; $\nu_{C=O}^{CHCl_3}$ 1650 cm^{-1}) differed in the fingerprint region, and each isomer had a distinctly characteristic nmr spectrum. Both isomers had identical molecular ions (M$^+$ 278; 3 rings, plus 2 double bonds) and similar fragmentation patterns in the mass spectrometer. When hydrolyzed in 6 *N* HCl for 24 hr, either isomer gave rise to **1e**, as determined by identical R_f's and color reactions on tlc ($CHCl_3$–MeOH–Et_3N–H_2O; 15:6:1:1, silica gel). The higher melting isomer was identical with the diketopiperazine isolated earlier in the rearrangement of **7b** in aqueous dioxane.

Similarly, in the reaction of **7a** with *tert*-BuOK in *tert*-BuOH there was obtained from the "neutral" $CHCl_3$-soluble extract, by column chromatography, two diastereomeric diketopiperazines (**10a** or **10b**), mp 117–118° (recrystd hexane), and mp 200–201° (**10b** or **10a**) (recrystd CH_2Cl_2–hexane). *Anal.* (either isomer $C_{16}H_{26}N_2O_2$) C, H, N. When the reaction solvent was THF the lower melting (mp 118–119°) and the 200–201° melting isomers were obtained in 11 and 26% overall yields, respectively. These isomers exhibited different retention times on glc, different ir and nmr spectra, but had identical molecular ions (M$^+$ 250) and similar fragmentation patterns. The higher melting isomer was identical with the diketopiperazine isolated earlier in the rearrangement of **7a** in aqueous dioxane. When hydrolyzed as above, either isomer gave rise to **1d** as determined by tlc (95% EtOH–C_6H_5–H_2O, 4:1:1; cellulose) or by ion-exchange chromatography on the Spinco amino acid analyzer.[7]

General Procedure for the Preparation of Dansylimino Acids. 1-(5-Dimethylaminonaphthalene-1-sulfonyl)hexahydro-1*H*-azepine-2-carboxylic Acid (13b).—Methyl hexahydro-1*H*-azepine-2-carboxylate hydrochloride (**11b**) (194 mg, 1.0 mmole), dansyl chloride (270 mg, 1.0 mmole), and 1.0 g of solid $NaHCO_3$ was stirred in acetonitrile for 24 hr at room temp. The reaction mixt was diluted with H_2O until cloudy and the dansylimino acid methyl ester was extd into ether. After drying (Na_2SO_4), the solvent was evapd and the residual methyl ester was hydrolyzed by heating under reflux with 200 ml of 3 *N* HCl for 6 hr. The pH of the reaction mixt was then adjusted to 3–3.5 and the product was extd with ether using multiple extns. For further purification, it was necessary to reextract this product dissolved in ether into 5% Na_2CO_3 soln (several extns). The combined alkaline extract was coned slightly to remove dissolved ether and acidified to pH 3.5 with 6 *N* HCl and the product was reextracted into ether. After evapn of the dried ether extract, the residue was dissolved in CH_2Cl_2 excess piperidine was added, and the piperidinium salt was crystd by addition of petroleum ether.

It is necessary to use the methyl esters of the imino acids here since coupling the free imino acids with dansyl chloride in aq acetone in the presence of $NaHCO_3$[27] gave poor yields. To prepare the dansyl derivative of the 10- and 11-membered imino acids (**13e** and **13f**), reaction times of 36–48 hr were necessary, and the crude products were converted to their hydrochlorides (not the Na salts) in order to remove impurities by extn with ether.

Acknowledgments.—We are indebted to Miss I. Koehler and Mrs. O. Hamerston for the ir and electronic spectra, to Miss F. Shirota for the nmr spectra, and to Mr. A. Swanson, Mass Spectroscopy Laboratory, University of Minnesota, for the mass spectra. Dr. G. Miroff, Rockefeller University, New York, N. Y., kindly provided us with a cell culture of the P-66 mouse tumor. The Cancer Chemotherapy National Service Center and the Walter Reed Army Institute for Research furnished the results of the tumor screen and the antimalarial screen, respectively. The technical assistance of H. B. Clark and R. E. Krause is gratefully acknowledged.

(27) (a) B. S. Hartley and V. Massey, *Biochim. Biophys. Acta*, **21**, 58 (1956); (b) S. Blackburn, "Amino Acid Determination," Marcel Dekker, New York, N. Y., 1968, pp 173–174.