(22%) of light tan needles which were recrystallized from cyclohexane and finally sublimed to give a colorless solid, m.p. 118°.

Anal. Calcd. for $C_{12}H_{19}N_5$: C, 61.77; H, 8.21; N, 30.02. Found: C, 61.48; H, 8.00; N, 30.55.

4-(3-Dimethylaminopropyl)amino-5-methylpyrrolo[2,3-d]pyrimidine (**30**). A solution of 0.1 g. of 3-(3-dimethylaminopropyl)-4(3*H*)-imino-5-methylpyrrolo[2,3-*d*]pyrimidine in 10 ml. of water was heated under reflux for 1 hr., cooled, and filtered to give 0.08 g. of product (80%) which was recrystallized from water containing a trace of ethanol to give white crystals, m.p. 170–172°.

Anal. Calcd. for $C_{12}H_{19}N_5$: C, 61.77; H, 8.21; N, 30.02. Found: C, 61.59; H, 7.80; N, 30.15.

3-Methyl-4(3H)-imino-5-phenylpyrrolo(2,3-d)pyrimidine (**28**). Treatment of 3.1 g. of 2-amino-3-cyano-4-phenylpyrrole with triethyl orthoformate followed by ethanolic methylamine, as described above for the 5-methyl analog **26**, gave 1.45 g. (38%) of crude product which was recrystallized from ethanol to give white crystals, m.p. 286.5–288°.

Anal. Calcd. for $C_{13}H_{12}N_4$: C, 69.62; H, 5.39; N, 24.99. Found: C, 69.76; H, 5.82; N, 25.13.

4-Methylamino-5-phenylpyrrolo[2,3-d]pyrimidine (**31**). A solution of 0.2 g. of 3-methyl-4(3H)-imino-5-phenylpyrrolo[2,3-*d*]pyrimidine in 20 ml. of hot ethanol was diluted with water until incipient turbidity, heated under reflux for 13 hr., and evaporated to dryness under reduced pressure. The residue was extracted with hot ethanol; the small amount of solid which separated upon cooling was discarded, and the filtrate was diluted with water. The fine white needles which separated (0.1 g., 50%) were recrystallized from aqueous ethanol to give the analytical sample, m.p. 218–219.5°.

Anal. Calcd. for $C_{13}H_{12}N_4$: C, 69.62; H, 5.39; N, 24.99. Found: C, 69.59; H, 5.40; N, 25.37.

Amide–Amide Reaction *via* Cyclols[1]

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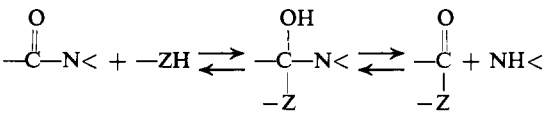
Contribution from the Department of Chemistry, University of California, Berkeley, California. *Received December 17, 1964*

Of the many recent examples of cyclols and cyclol intermediates, one type lacking was that resulting from amide–amide reaction. The most favorable geometry for finding such a reaction appeared to be present in 6,10-dioxo-1,5-diazacyclodecane and 5,10-dioxo-1,6-diazacyclodecane where the forced amide juxtaposition might lead to transannular cyclolization. To prepare these compounds, rearrangement of the dioxime ditosylate of 1,5-cyclooctanedione was investigated and found to give only the unsymmetrical product. The same compound was prepared from N-(3-aminopropyl)glutarimide via cyclolization. Similarly, the symmetrical isomer was prepared from 1-(4-aminobutyryl)-2-pyrrolidinone. Each isomer, in acid, was converted to the substituted glutarimide and pyrrolidinone, respectively. The reactions could be easily reversed at pH 8, and occurred more readily with 6,10-dioxo-1,5-diazacyclodecane (6,6-fused ring cyclol) than with 5,10-dioxo-1,6-diazacyclodecane (5,7-fused ring cyclol). Both isomers gave mass spectral fragmentation patterns best rationalized through cyclol and transannular intermediates.

Introduction

The cyclol hypothesis, in its broadest sense, postulates a reaction between an amide, or other acyl derivative, and an –OH-, –SH-, or >NH-containing group as in the general expression



Z = NH, O, or S

Attack at the carbonyl leads to the intermediate cyclol, which may then collapse to products. As originally advanced by Wrinch,[4] its purpose was to provide additional bonding and transformations in proteins.

The first clear experimental demonstration of such a reaction was furnished by the synthesis of ergotamine[5] which established the validity of a cyclol structure[6] in the peptide portion of this alkaloid. Since then, numerous examples have appeared of amide–amine, amide–alcohol, and amide–ester reactions,[7–11] including the synthesis of large-ring depsipeptides by cyclolization of β-hydroxypropionyldiketopiperazines.[12]

(1) This research was supported in part by the U. S. Army Research Office, Durham, N. C.
(2) National Institutes of Health Predoctoral Fellow.
(3) National Science Foundation Predoctoral Fellow.

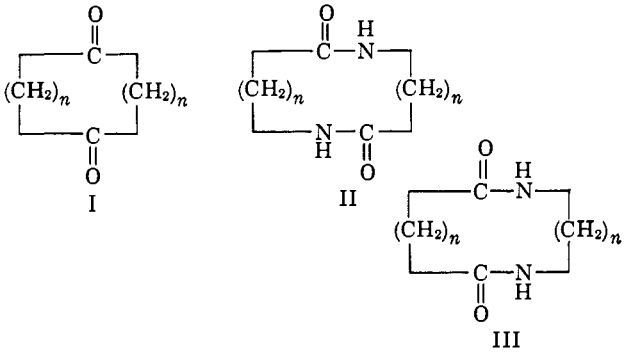
(4) D. Wrinch, *Nature*, **137**, 411 (1936); **138**, 241 (1936); recently summarized, **199**, 564 (1963).
(5) A. Hofmann, A. J. Frey, and H. Ott, *Experientia*, **17**, 206 (1961).
(6) A. Stoll, *Fortschr. Chem. Org. Naturstoffe*, 9, 114 (1952).
(7) V. K. Antonov, A. M. Shkrob, V. I. Shchelokov, and M. M. Shemyakin, *Tetrahedron Letters*, **No. 21**, 1353 (1963), and references therein to other work in this series.
(8) R. G. Griot and A. J. Frey, *Tetrahedron*, **19**, 1661 (1963).
(9) H. Ott, A. J. Frey, and A. Hofmann, *ibid.*, **19**, 1675 (1963); H. E. Zaugg and R. W. DeNet, *J. Org. Chem.*, **29**, 2769 (1964).
(10) K. Stich and H. G. Leeman, *Helv. Chim. Acta*, **46**, 1151 (1963).
(11) N. S. Vul'fson, V. I. Zaretskii, V. A. Puchkov, V. G. Zaikin, A. M. Shkrob, V. K. Antonov, and M. M. Shemyakin, *Dokl. Akad. Nauk, SSSR*, **153** (2), 336 (1963).
(12) M. M. Shemyakin, Yu. A. Ovchinnikov, V. K. Antonov, A. A. Kiryushkin, V. T. Ivanov, V. I. Shchelokov, and A. M. Shkrob, *Tetrahedron Letters*, **No. 1**, 47 (1964), and references therein.

One case of a transannular cyclolization involving amide–ester reaction has been reported.[13]

Of direct interest to peptide structure, and to the present study, are the recent cyclolizations of aminoacyl lactams and amides. N-(ε-Aminocaproyl)caprolactam gave 2 moles of caprolactam while N-glycylcaprolactam gave 5-oxo-1,2-pentamethylenimidazoline[14,15] when the protecting carbobenzyloxy group was removed. However, both N-(β-aminopropionyl)-2-pyrrolidinone and N-(β-aminopropionyl)caprolactam gave the corresponding cyclic peptides by cyclolization.[16] Also, the insertion of a new amino acid residue at a peptide bond was accomplished[16] *via* cyclolization. These reactions had to proceed through a cyclol intermediate of the general type shown where Z = NH, but in no case was the intermediate azacyclol isolated.[17]

One type of cyclolization, that involving amide–amide reaction, has not been reported.[18] Such a reaction, which might be of considerable interest in peptide chemistry, became the subject of our present investigation. The most favorable geometry for such an amide–amide reaction appeared to exist in the isomeric cyclic 10-membered diamides, II and III (n = 3), where cyclolization could proceed by a transannular reaction. Therefore, the preparation of these diamides was undertaken.

One approach to cyclic amides of the type II and III is *via* high-dilution procedures, using the dicarboxylic acid chloride and diamine for III and standard peptide syntheses for II. However, a more appealing possibility was offered by the rearrangement of cyclic diketones. The cyclic diketones I (n = 2, 4, 8, 9, and 10), when subjected to Schmidt or Beckmann rearrangements, are reported[19] in all cases to give both isomeric amides, II and III. A report[20] that rearrangement



of I (n = 4) dioxime in liquid sulfur dioxide gave only II (n = 4) was subsequently refuted.[19] The only instance where just one isomer (II, n = 2) was reported[21] was for the dioxime ditosylate of I (n = 2).

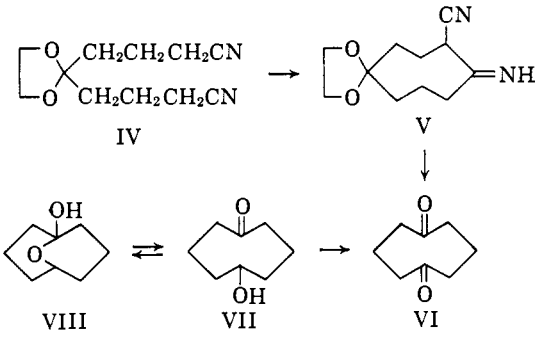
(13) R. C. Sheppard, *Experientia*, 19, 125 (1963).
(14) G. Reinisch, *Faserforsch. Textiltech.*, 13, 43 (1962).
(15) M. Rothe, *Angew. Chem.*, 74, 725 (1962).
(16) V. K. Antonov, Ts. E. Agadzhanyan, T. R. Telesnina, M. M. Shemyakin, G. G. Dvoryantseva, and Yu. N. Sheinker, *Tetrahedron Letters*, No. 13, 727 (1964).
(17) One claim has been made for the isolation and characterization of an azacyclol (D. S. Jones, G. W. Kenner, and R. C. Sheppard, *Experientia*, 19, 126 (1963)), but it has been since withdrawn (ref. 16, footnote, p. 730).
(18) Amide–amide interaction followed by elimination of water has been invoked to explain the mass spectral fragmentation pattern of two cyclic peptides.[16]
(19) M. Rothe and R. Timler, *Ber.*, 95, 783 (1962).
(20) N. Tokura, R. Tada, and K. Suzuki, *Bull. Chem. Soc. Japan*, 32, 654 (1959).
(21) H. K. Hall, Jr., *J. Am. Chem. Soc.*, 80, 6404 (1958).

In view of these considerations, we undertook the preparation of 1,5-cyclooctanedione (I, n = 3), a study of its rearrangement, and the cyclolization reactions of II and III (n = 3).[22]

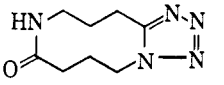
Discussion

A. Syntheses. To prepare 1,5-cyclooctanedione, the dinitrile IV was cyclized at high dilution using lithium N-ethylanilide, and the resulting cyanoimine V was hydrolyzed to the diketone VI. Cyclization proceeded well, and since the dinitrile is readily available[23] from the corresponding dichloro ketone, itself prepared from butyrolactone, this is a feasible synthesis.

However, a much more attractive possibility was presented by the availability of 5-hydroxycyclooctanone (VII).[24] This material exists almost completely in the transannular hemiketal form VIII, both as a solid and in solution. Its infrared spectrum in potassium bromide and in carbon tetrachloride shows no carbonyl absorption. Using as an extinction coefficient for the keto form VII one-half that found for 1,5-cyclooctanedione (VI), a 1 *M* aqueous solution contains about 4% hydroxyketone, and this value drops to 1% in dioxane. Oxidation to the diketone with chromic anhydride proceeded in good yield, making this a highly convenient route to 1,5-cyclooctanedione.



The first rearrangement tried was the Schmidt reaction, following exactly the conditions for homologous diketones.[19] A substance, m.p. 243–244° after crystallization from acetone, was isolated in <1% yield. It was hydrolyzed to γ-aminobutyric acid in refluxing 6 *N* alkali, and showed only end absorption in the ultraviolet. In the infrared, there were bands characteristic of *trans*-secondary amides[10] (3350, 1640, and 1550 cm.$^{-1}$), and absorption at 1095, 1050, 995, and 980 cm.$^{-1}$, in the region assigned to 1,5-dialkyltetrazoles.[25] These data and determination of the molecular formula as $C_8H_{13}N_5O$ (by C, H, N, and molecular weight analyses) led to the structural assignment IX for this material. Rearrangement *via* the



IX

Schmidt reaction was not pursued further.

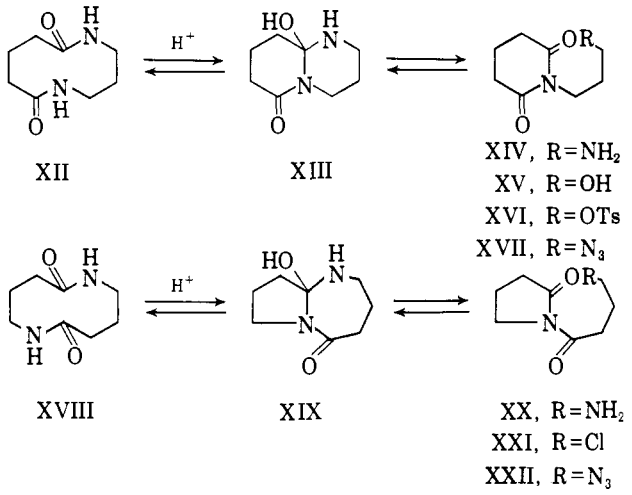
(22) A preliminary account of part of this work has appeared: G. I. Glover and H. Rapoport, *ibid.*, 86, 3397 (1964).
(23) D. Hartley, *J. Chem. Soc.*, 4722 (1962).
(24) H. Moell and O. Schlichting, German Patent 1,029,368 (May 8, 1958) assigned to Badische Anilin und Soda-Fabrik. We are indebted to BASF for a sample of this material.
(25) C. W. Roberts and M. L. Maskaleris, *J. Org. Chem.*, 24, 926 (1959). Also, we have found absorption at 1115 and 995 cm.$^{-1}$ for pentamethylenetetrazole.

The dioxime X offered a wide variety of rearrangement possibilities, and these were next examined. The dioxime itself was easily prepared from diketone and hydroxylamine hydrochloride in ether–liquid ammonia. Rearrangement in liquid sulfur dioxide containing thionyl chloride, which had been successful in some cases,[20] led to no isolable product.

Milder conditions appeared necessary, and these were available in the rearrangements of dioxime ditosylates. The dioxime ditosylate XI was obtained in excellent yield and was then subjected to a number of different rearrangement conditions[19,26] in acetic acid, acetic acid–sodium acetate, and acetic acid–acetic anhydride. In every case, complex mixtures were obtained from which no identifiable products could be isolated. Therefore, these conditions also were abandoned.

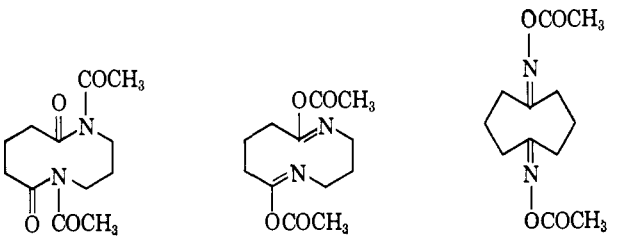
An explanation for this striking difference in behavior on rearrangement between 1,5-cyclooctanedione (VI) and all the other cyclic diketones I (n = 2, 4, 8, 9, and 10) probably resides in the marked tendency of the rearrangement products, XII and XVIII, to undergo further reactions. These would involve amide–amide reactions and result in compounds such as XIII, XIV, XIX, and XX. Since such conversions should be acid catalyzed, mild neutral rearrangement conditions were sought.

To achieve such conditions, the dioxime ditosylate XI was warmed in aqueous dioxane containing excess sodium acetate to neutralize the p-toluenesulfonic acid formed. A product was formed in nearly quantitative yield which had the molecular formula $C_{12}H_{18}N_2O_4$, contained two CCH_3 groups, and on acid hydrolysis gave 1,3-diaminopropane and glutaric acid. On the basis of this evidence, three structures (N-acetylamide XXIII, lactim acetate XXIV, and oxime acetate XXV) were considered for this product. The oxime acetate XXV was easily eliminated by preparation of an authentic sample from dioxime X. A choice between XXIII and XXIV was clear from the ultraviolet absorption [$\lambda_{max}^{H_2O}$ 221 mμ (ϵ 17,000)]. Lactim acetates have no absorption in this region, whereas values of λ_{max} 216–218 mμ (ϵ 8900–11,300) have been reported[27] for N-acetyllactams. Thus, the rearrangement product had structure XXIII.



XII XIII XIV, R=NH_2; XV, R=OH; XVI, R=OTs; XVII, R=N_3

XVIII XIX XX, R=NH_2; XXI, R=Cl; XXII, R=N_3



XXIII XXIV XXV

There are two points of interest about this reaction. The first is that rearrangement took place exclusively to give one of the two possible isomers. This was established by hydrolytic analysis and vapor phase chromatography of all the material from the rearrangement. It is in contrast with the other homologous diketones where both isomers were obtained.[19] There may be a steric preference for the corresponding configuration of the dioxime, but models fail to show any. The second point of interest is that the N-acylated amide was obtained under aqueous condition, whereas in the past[28] this has been true only when the medium was anhydrous. This may indicate a rapid intramolecular O- to N-acyl migration in the lactim acetate in preference to hydrolysis.

In order to avoid N-acetyl formation and obtain the unsubstituted diazacyclodecane XII, the rearrangement was carried out in the presence of bicarbonate as the base. Hydrolysis of the total product gave only glutaric acid and 1,3-diaminopropane, establishing that again rearrangement had proceeded in only one direction. Crystalline material, m.p. 233–234°, having the molecular formula $C_8H_{14}N_2O_2$ (elemental analyses and molecular weight), was easily isolated. This crystalline material had infrared absorption at 3400, 3050, 1650, and 1550 cm.$^{-1}$, consistent with expectations for ten-membered lactams,[10] and the 3400-, 3050-, and 1550-cm.$^{-1}$ bands were eliminated by treatment with deuterium oxide. These data allowed the assignment of structure XII to the rearrangement product.

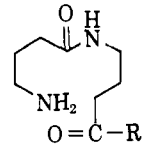
Confirmation was sought in the synthesis of XII from 1,3-diaminopropane and glutaryl chloride, using high-dilution cyclization.[29] After a number of attempts and exhaustive purification, a 1% yield was obtained of material identical with the rearrangement product.[30] The unsatisfactory nature of this reaction led us to explore other approaches to an independent synthesis of XII.

A highly satisfactory synthesis was found in the preparation of N-(3-aminopropyl)glutarimide (XIV) and its conversion to XII. The hydroxypropylglutarimide XV was first prepared from glutaric anhydride and 3-aminopropanol; this was tosylated to give XVI and then converted to the azide XVII. Catalytic hydrogenation in acid solution gave the aminopropyl glutarimide XIV as its hydrochloride, and this in bicarbonate solution was quantitatively converted to 6,10-dioxo-1,5-diazacycodecane (XII).

(26) W. Z. Heldt, *J. Am. Chem. Soc.*, **80**, 5880 (1958).
(27) C. M. Lee and W. D. Kumler; *ibid.*, **83**, 4593 (1961).
(28) L. G. Donaruma and W. Z. Heldt, *Org. Reactions*, **11**, 1(1960).
(29) H. Stetter and J. Marx, *Ann.*, **607**, 59 (1957).
(30) J. Dale and R. Coulon, *J. Chem. Soc.*, 182 (1964), reported isolation from this reaction of material in 1% yield, m.p. 268°, to which they assigned structure XII. Comparison of the infrared spectrum of this product, kindly supplied by Dr. Dale, with that of our material, m.p. 233–234°, revealed some distinct differences. Our explanation is that the 268° material may be, or may contain, a higher cyclic polymer of glutaric acid and 1,3-diaminopropane.

A synthesis of the dipeptide isomer, 5,10-dioxo-1,6-diazacyclodecane (biscyclo-γ-aminobutyryl) (XVIII), was now sought, since a comparison of the cyclolization reactions in the two series was of interest. Although no details or yield were given, the preparation of biscyclo-γ-aminobutyryl, m.p. 283°, has been reported[15] using high-dilution cyclization of an activated ester of γ-aminobutyryl-γ-aminobutyric acid at 10^{-4} *M*.

We attempted synthesis of XVIII from γ-aminobutyryl-γ-aminobutyric acid[31] (XXVI) and dicyclohexylcarbodiimide at 10^{-3} and 10^{-4} *M*, but could obtain no identifiable product. An alternative approach through S-(bis-γ-aminobutyryl)thiophenol was more promising. In this case, the dihydrobromide XXVII as a 10^{-3} *M* solution in dimethylformamide was treated with triethylamine, and a 12% yield of crude biscyclo-γ-aminobutyryl was obtained. However, since purification was very difficult and experimental manipulation quite inconvenient, this peptide approach was abandoned in favor of a cyclolization procedure, patterned on the successful preparation of XII.



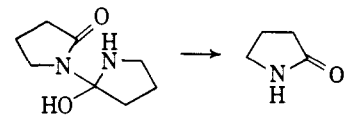
XXVI, R=OH

XXVII, R=SC_6H_5(2HBr)

In this sequence, 2-pyrrolidinone was N-acylated with 4-chlorobutyryl chloride to the 4-chlorobutyryl derivative XXI which was converted to the azide XXII by treatment with potassium iodide and sodium azide in hot dimethylformamide. Reduction of the azide in neutral aqueous solution gave some 2-pyrrolidinone and a 52% yield of pure biscyclo-γ-aminobutyryl (XVIII). Alternatively, the azide could be reduced in acid to the 4-aminobutyrylpyrrolidinone hydrochloride (XX), and this, on solution in aqueous bicarbonate, gave the cyclic dipeptide XVIII. Its structure was established by molecular formula determination, hydrolysis to γ-aminobutyric acid in acid and 2-pyrrolidinone in alkali, and consistent spectrophotometric properties.

B. Cyclolization Reactions. Both the synthesis of 6,10-dioxo-1,5-diazacyclodecane (XII) from N-(3-aminopropyl)glutarimide (XIV) and 5,10-dioxo-1,6-diazacyclodecane (XVIII) from 1-(4-aminobutyryl)-2-pyrrolidinone (XX) must proceed through cyclols XIII and XIX, respectively, since the glutarimide and acylpyrrolidinone structures are otherwise completely stable to the cyclolization conditions. These cyclols result from amide–amine reaction, which occurs very rapidly at room temperature as soon as the amine is liberated from its salt. In this regard, these reactions should be compared with the cyclolization of the hydroxypropylimide XV. This compound showed no change after several weeks under the same conditions as led to complete reaction of the corresponding amine XIV. Similar differences were found between N-(2-hydroxypropionyl)-2-pyrrolidinone[32] and N-(2-aminopropionyl)-2-pyrrolidinone.[16] These differences indicate that generalizations[8] regarding cyclolizations *via* amide–alcohol reaction do not pertain to the corresponding amide–amine case.

In the cyclolization of the 4-aminobutyrylpyrrolidinone XX, two paths are possible, *viz.*, through XIX giving biscyclo-γ-aminobutyryl (XVIII), and by addition across the butyryl carbonyl to give the cyclol XXVIII. This then collapses to 2-pyrrolidinone.



XXVIII

Products from both cyclolization pathways were found.

Of particular interest is the amide–amide reaction, previously unreported, which has been found in both the diazacyclodecanes XII and XVIII. Because of the close approach of the amide groups, invoked by the geometry of the ten-membered ring, transannular cyclol formation would be especially favorable in both compounds. It was studied using product isolation and the fact that the imide XIV and the acyllactam XX have strong absorption in the ultraviolet (208 and 217 mμ, respectively) whereas the corresponding dioxodiazacyclodecanes have none.

Interconversion *via* the cyclol could be attained from either direction. For example, in 0.1 *N* hydrochloric acid, 10 days at 20° (or 3 hr. at 90°) gave complete conversion of 6,10-dioxo-1,5-diazacyclodecane (XII) to N-(3-aminopropyl)glutarimide (XIV) hydrochloride. When the pH was adjusted to 8.75, the imide completely reverted to the cyclic diamide in 2 hr. at 20°. However, biscyclo-γ-aminobutyryl (XVIII) underwent cyclolization less readily. In 0.1 *N* acid at 20°, no change occurred. Conversion to 1-(4-aminobutyryl)-2-pyrrolidinone (XX) hydrochloride did occur at 90°, but in lower yield and accompanied by 2-pyrrolidinone. Upon adjusting the pH to 8.75, reversal to the cyclic dipeptide did occur rapidly at 20°, but again in lower yield. Warming the cyclic dipeptide XVIII in stronger alkali (pH 10.7) gave a high conversion to 2-pyrrolidinone, whereas the cyclic diamide XII was stable to these conditions.

These results clearly demonstrate amide–amide reaction and reversible cyclolization in the two systems XII ⇄ XIV and XVIII ⇄ XX. They also indicate the greater ease of formation of the 6,6-ring cyclol XIII as compared to the 5,7-ring cyclol XIX.

C. Mass Spectra. The mass spectra of XII and XVIII are in accord with previous observations reporting mass 18 peaks in the mass spectra of two cyclic dipeptides[16] and of several cyclols and cyclodepsipeptides.[11] Because of transannular reactions, the fragmentation pathways for both isomers are not analogous to those usually found in aliphatic amides[33] and cyclic dipeptides.[34]

Scheme I represents a reasonable explanation for the fragmentation pattern (Figure 1) obtained from XII. The low intensity (3%) of the parent ion indicates that dehydration of the cyclol XIII to b occurs readily.

(31) R. L. Evans and R. Irreverre, *J. Org. Chem.*, **24**, 863 (1959).

(32) M. M. Shemyakin, V. K. Antonov, A. M. Shkrob, Yu. N. Sheinker, and L. B. Senyavina, *Tetrahedron Letters*, **No. 16**, 701 (1962).

(33) H. Budzikiewicz, C. Djerassi, and D. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, Inc., San Francisco, Calif., 1964.

(34) H. J. Svec and G. A. Junk, *J. Am. Chem. Soc.*, **86**, 2278 (1964).

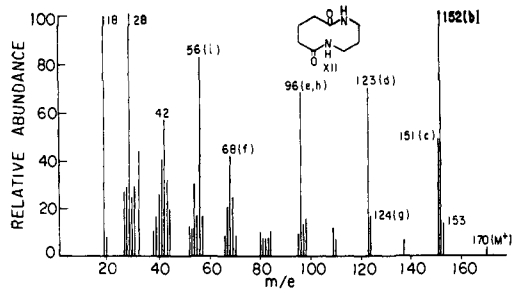


Figure 1. Mass spectrum of 6,10-dioxo-1,5-diazacyclodecane (XII) (*m/e* 18, 28 off scale).

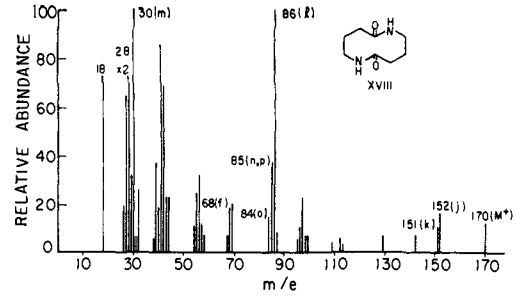
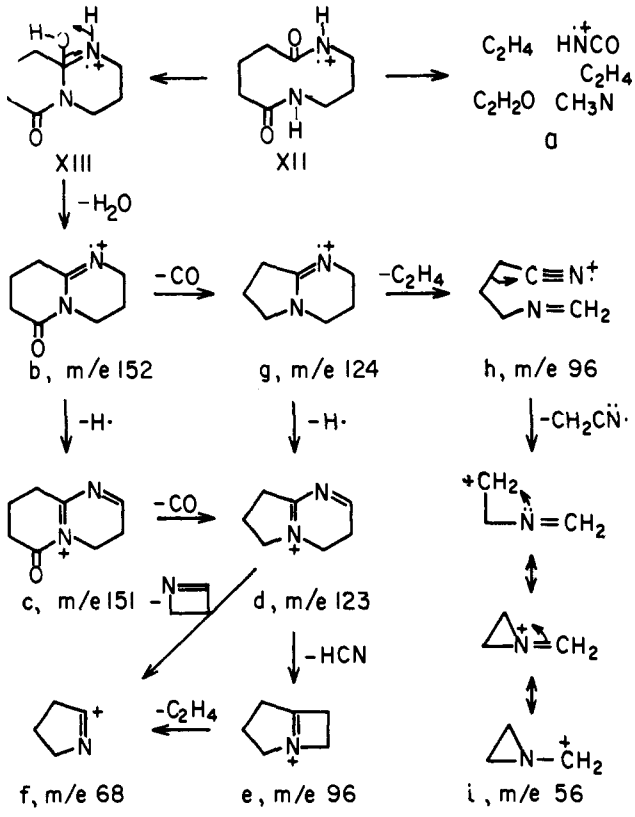


Figure 2. Mass spectrum of 5,10-dioxo-1,6-diazacyclodecane (XVIII).

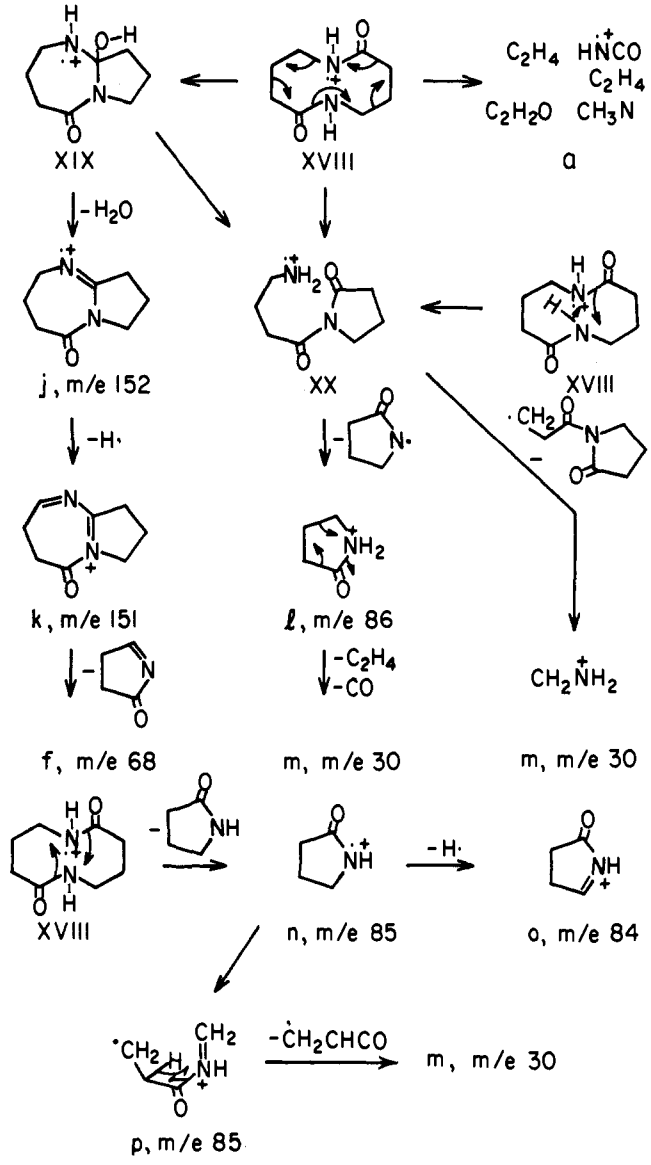
Further fragmentations, b → c → d → e → f and b g → h → i, give the other major ions in Figure 1; the large (more than twice as intense as the base peak) peaks at mass 18 and 28 substantiate the fragmentations in Scheme I, since they depend in large part on loss of H_2O (*m/e* 18) and $H_2C{=}CH_2$ and CO (*m/e* 28).

Scheme I



The spectrum of XVIII (Figure 2) differs considerably from that of XII due to the greater ease of cyclolization and dehydration of XII [compare the relative amounts of parent ion (XII, 3%; XVIII, 13%) and the amounts of dehydrated cyclol (b > j)] and to the transannular reactions available to XVIII (Scheme II) that cannot occur in XII. These factors cause cyclolization of XVIII to XIX and further reactions XIX → j → k → f to be a minor fragmentation pathway and allow the major fragmentation to proceed *via* the third form of the parent ion XX. Form XX can be formed from XVIII by transannular H and acyl shift, or from XIX. Formation of the fragment m (*m/e* 30) is commonly observed in the mass spectra of aliphatic amines and in one secondary amide[33] and the intensity of this ion (Figure 2) is equal to that of the base peak, supporting the fragmentation through XX. Another possible minor pathway is the formation of two 2-pyrrolidinone fragments by transannular cyclization of the parent ion XVIII.

Scheme II



The similarity of the patterns below *m/e* 60 suggests a parallel fragmentation of the parent ions into the five fragments, a: two C_2H_4 (*m/e* 28), $HN{=}CH_2$ (*m/e* 29), HNCO (*m/e* 43), and CH_2CO (*m/e* 42). The mass

spectrum of diketopiperazine has substantial peaks at *m/e* 43 (HNCO), *m/e* 42 (CH_2CO), and *m/e* 29 (CH_2NH), and that of alanine anhydride has minor peaks at *m/e* 43 (HNCO and CH_3CHNH) and *m/e* 56 (CH_3-$CHCO$)[34] which could also result from a similar fragmentation.

Experimental[35]

4-Cyano-5-iminocyclooctanone Ethylene Ketal (V). To freshly prepared ethereal phenyllithium (0.40 mole in 650 ml. of ether) was added 66 g. (0.54 mole) of N-ethylaniline, and the solution was boiled for 6 hr. A 200-ml. portion of ether was then added followed by addition to the refluxing and stirred solution of 9.24 g. (44 mmoles) of 1,3-dioxolane-2,2-dibutyronitrile (IV)[23] in 175 ml. of ether over a 37-hr. period, using a high-dilution adaptor.[36] Water (500 ml.) was added, the mixture was filtered, and the aqueous phase was extracted twice with ether. Evaporation of the combined ether solutions followed by distillation *in vacuo* of the N-ethylaniline (60 g. recovered) left a residue which was dissolved in methylene chloride, treated with decolorizing carbon, and crystallized from benzene after evaporating the methylene chloride, yielding 2.91 g. (14 mmoles, 32% yield), m.p. 179–181°. After sublimation at 105° (30 μ), the melting point was 181–182°.

Anal. Calcd. for $C_{11}H_{16}N_2O_2$: C, 63.4; H, 7.7; N, 13.5. Found: C, 63.1; H, 7.6; N, 13.4.

1,5-Cyclooctanedione (VI). A. By Hydrolysis of 4-Cyano-5-iminocyclooctanone Ethylene Ketal (V). A solution of 1.00 g. (4.8 mmoles) of 4-cyano-5-iminocyclooctanone ethylene ketal in 9.4 ml. of 37% sulfuric acid was boiled for 7 hr., the cooled mixture was extracted with three 25-ml. portions of methylene chloride, and the combined extracts were washed with aqueous bicarbonate solution and water and dried. Evaporation of the methylene chloride and sublimation of the residue gave 431 mg. (3.1 mmoles, 65% yield) of 1,5-cyclooctanedione, m.p. 71–72°, $\nu_{max}^{CHCl_3}$ 1698 cm.$^{-1}$, λ_{max} 287 mμ (ε 25.2).

Anal. Calcd. for $C_8H_{12}O_2$: C, 68.5; H, 8.6. Found: C, 68.5; H, 8.6.

B. By Oxidation of 5-Hydroxycyclooctanone (VII). Chromic acid oxidizing reagent[37] (5 ml.) was added dropwise during 20 min. to a rapidly stirred solution of 3 g. (21.4 mmoles) of 5-hydroxycyclooctanone (m.p. 100–102°, one peak on gas chromatography)[24] in 30 ml. of acetone (distilled from permanganate), maintained below 35°. When a persistent reddish color in the reaction mixture indicated completion of the oxidation, the supernatant solution was decanted, the precipitated salts were washed three times with acetone, and several drops of oxidant was added to ensure complete reaction. Isopropyl alcohol was added to reduce the excess oxidant, the acetone solution was neutralized with excess sodium bicarbonate (5.4 g.) and filtered, and the filtrate was concentrated to give a residue which was diluted with 20 ml. of water and extracted with four 10-ml. portions of methylene chloride. Evaporation of the washed and dried extracts and crystallization of the residue from ether–petroleum ether (b.p. 30–60°) gave 1.86 g. (62% yield) of 1,5-cyclooctanedione (VI), identical with the material prepared above. Unreacted hydroxyketone could be recovered from the mother liquors.

1,5-Cyclooctanedione Dioxime (X). Hydroxylamine hydrochloride, 7.44 g. (107 mmoles), was added to a stirred solution of 5.0 g. (36 mmoles) of 1,5-cyclooctanedione (VI) in 100 ml. of ether and 200 ml. of liquid ammonia. Stirring was continued until the ammonia had evaporated, the ether was evaporated from the steam bath, and the residue was dissolved in a minimum of hot water. Continuous extraction with ether for 12 hr. gave 4.5 g. (74% yield) of dioxime, m.p. 176–179°, $\nu_{max}^{CHCl_3}$ 3175 and 1653 cm.$^{-1}$.

Anal. Calcd. for $C_8H_{14}N_2O_2$: C, 56.4; H, 8.3; N, 16.5. Found: C, 56.1; H, 8.3; N, 16.5.

The ditosylate was prepared by the method used for the tosylation of 1,6-cyclodecanedione dioxime.[19] From 2.22 g. (13 mmoles) of 1,5-cyclooctanedione dioxime, 5.25 g. (89% yield) of *1,5-cyclooctanedione dioxime ditosylate (XI)* was obtained, m.p. 118–119°; ν_{max}^{KBr} 1625, 1585, and 1365 cm.$^{-1}$.

Anal. Calcd. for $C_{22}H_{26}N_2O_6S_2$: C, 55.3; H, 5.9; N, 5.4. Found: C, 55.5; H, 6.0; N, 5.6.

The diacetate was prepared by boiling a solution of 0.17 g. (1 mmole) of dioxime in 25 ml. of acetic anhydride for 2 hr. Evaporation of the solvent and sublimation (70° at 1 μ) of the residue gave *1,5-cyclooctanedione dioxime diacetate (XXV)*, m.p. 100°; ν_{max}^{KBr} 1760, 1630, and 1460 cm.$^{-1}$; n.m.r. absorption δ 2.5 (m, 8H) and 2.15 (m, 10 H), including the CH_3 groups as a sharp singlet at 2.15.

Anal. Calcd. for $C_{12}H_{18}N_2O_4$: C, 56.7; H, 7.1; N, 11.0. Found: C, 56.8; H, 7.0; N, 11.0.

6,10-Dioxo-1,5-diazacyclodecane (XII). A. By Beckmann Rearrangement of 1,5-Cyclooctanedione Dioxime Ditosylate (XI) in Dioxane–Water. 1. In the Presence of Sodium Acetate. Ditosylate, 479 mg. (1 mmole), was heated with 492 mg. (6 mmoles) of sodium acetate in 100 ml. of 3:1 dioxane–water for 2 hr. at 70°, with stirring. The cooled reaction mixture was evaporated to a solid residue which was digested with four 100-ml. portions of boiling ether. The combined ether solutions were concentrated to 30 ml., and petroleum ether was added to cloudiness. Cooling gave 224 mg. (88% yield) of 1,5-diacetyl-6,10-dioxo-1,5-diazacyclodecane (XXIII), m.p. 91–92° after sublimation at 50° (1 μ); λ_{max}^{KBr} 1710, 1680, and 1460 cm.$^{-1}$; λ_{max} 221 mμ (ε 17,000); n.m.r. absorption δ 3.75 (t, 4H), 2.71 (t, 4H), 2.4 (s, 6H), and 2.5-1.8 (m, 4H).

Anal. Calcd. for $C_{12}H_{18}N_2O_4$: C, 56.7; H, 7.1; N, 11.0; CCH_3, 11.8. Found: C, 56.5; H, 7.0; N, 11.0; CCH_3, 7.9.

Hydrolysis of 100 mg. of 1,5-diacetyl-6,10-dioxo-1,5-diazacyclodecane in 6 *N* hydrochloric acid at 110° in a sealed tube, evaporation of the solvent, and digestion of the residue with ether gave 41.5 mg. (theoretical yield, 52 mg.) of glutaric acid. The residue, 57.5 mg. (theoretical yield, 53.5 mg.) of 1,3-diaminopropane dihydrochloride, was crystallized from ethanol–water.

(35) All melting points are corrected, and those above 200° were taken in evacuated capillaries; microanalyses were performed by the Microchemical Laboratory, University of California, Berkeley; evaporations were made *in vacuo* using a rotary evaporator; n.m.r. spectra were taken in $CDCl_3$ with internal TMS; infrared absorptions were measured neat and ultraviolet spectra were taken in water, unless otherwise specified.

(36) N. J. Leonard and R. C. Sentz, *J. Am. Chem. Soc.*, 74, 1704 (1952).

(37) A. Bowers, T. H. Halsall, E. R. H. Jones, and A. J. Lemin, *J. Chem. Soc.*, 2548 (1953).

Both the acid and diamine were identical with authentic samples. Hydrolysis in 6 *N* hydrochloric acid of the mother liquors from the crystallization of the diacetyl compound gave only glutaric acid and 1,3-diaminopropane dihydrochloride. V.p.c. of the mother liquors (poly-*m*-phenyl ether on Fluoropak) indicated that there were only trace amounts of impurities.

2. In the Presence of Potassium Bicarbonate. Ditosylate XI, 6.9 g. (14.4 mmoles), was heated with stirring for 2 hr. at 70° in 1400 ml. of 3:1 dioxane–water containing 5.82 g. (58.1 mmoles) of potassium bicarbonate. The solvent was evaporated, the residue was dissolved in 100 ml. of water, the aqueous solution was extracted continuously with methylene chloride for 36 hr., and the methylene chloride was evaporated. Trituration of the residue with acetone left 1.14 g. (47% yield) of *6,10-dioxo-1,5-diazacyclodecane* (*XII*) which was recrystallized from ethanol to give 0.63 g. (26% yield) of material of m.p. 233–234°; ν_{max}^{KBr} 3400, 3050, 1650, 1550, and 1440 cm.$^{-1}$; the bands at 3400, 3050, and 1550 cm.$^{-1}$ were lost after treatment with D_2O; n.m.r. absorption (D_2O) broad, unresolved regions at δ 3.0–4.0 and 1.5–2.5 in the ratio of 1:2.

Anal. Calcd. for $C_8H_{14}N_2O_2$: C, 56.5; H, 8.3; N, 16.5. Found: C, 56.6; H, 8.4; N, 16.3.

Hydrolysis of a sample in 6 *N* hydrochloric acid at 110° gave only glutaric acid and 1,3-diaminopropane dihydrochloride, as did hydrolysis of the crude reaction mixture.

B. From Glutaryl Chloride and 1,3-Diaminopropane. The high-dilution cyclization technique of Stetter and Marx[29] was applied to 1,3-diaminopropane (4.45 g., 60 mmoles) and glutaryl chloride (5.07 g., 30 mmoles). The precipitate which formed in the reaction mixture was removed by filtration and sublimed at 150° (2 μ) to give a mixture of 1,3-diaminopropane dihydrochloride and the cyclized product. Resublimation at 110° (2 μ) gave a 1% yield of *6,10-dioxo-1,5-diazacyclodecane* (*XII*), identical with the material prepared above by Beckmann rearrangement.

C. From N-(3-Aminopropyl)glutarimide (*XIV*). Glutaric anhydride (114 g., 1 mole) and 3-aminopropanol (75 g., 1 mole) were heated at 150° (120 mm.) for 24 hr. so that the water formed (13.5 g., 75% of theory) slowly distilled. The residue then was distilled and *N-(3-hydroxypropyl)glutarimide* (*XV*) was collected at 122° (0.2 mm.); m.p. 59–60°; yield, 40 g., 23%; ν_{max}^{Nujol} 3400, 1725, and 1670 cm.$^{-1}$; λ_{max} 211 mμ (ε 14,600).

Anal. Calcd. for $C_8H_{13}NO_3$: C, 56.1; H, 7.7; N, 8.2. Found: C, 56.0; H, 7.7; N, 8.3.

Tosylation in the usual manner[38] followed by crystallization from methanol–water gave N-(3-tosyloxypropyl)glutarimide (XVI), m.p. 88–89°.

Anal. Calcd. for $C_{15}H_{19}NO_5S$: C, 55.4; H, 5.9; N, 4.3; S, 9.9. Found: C, 54.9; H, 5.8; N, 4.5; S, 9.9.

N-(3-Tosyloxypropyl)glutarimide (6.5 g., 20 mmoles) and sodium azide (16.3 g., 25 mmoles) in 60 ml. of acetone and 8 ml. of water were heated under reflux for 22 hr. The acetone was evaporated, 40 ml. of water was added, and the solution was extracted with six 50-ml. portions of methylene chloride. Evaporation of the combined, dried extracts gave 3.8 g. (96% yield) of N-(3-azidopropyl)glutarimide (XVII) as an oil; ν_{max} 2110, 1740, and 1680 cm.$^{-1}$; λ_{max} 208 mμ (ε 14,500).

Anal. Calcd. for $C_8H_{12}N_4O_2$: C, 49.0; H, 6.2; N, 28.6. Found: C, 49.3; H, 6.2; N, 28.8.

A solution of 982 mg. (5 mmoles) of N-(3-azidopropyl)glutarimide in 40 ml. of ethanol containing 0.5 ml. of 12 *N* hydrochloric acid was hydrogenated at 40 p.s.i., using 1.25 g. of 10% palladium-on-carbon catalyst. Hydrogen absorption ceased after 6 hr., the catalyst was removed by filtration and digested with hot aqueous ethanol, and the combined filtrate and digests were evaporated. The residue (0.85 g.) was crystallized from ethanol–ether, giving 0.61 g. (60% yield) of N-(3-aminopropyl)glutarimide hydrochloride (XIV), m.p. 194–195°; ν_{max}^{Nujol} 2025, 1720, and 1660 cm.$^{-1}$; λ_{max} 208 mμ (ε 14,400).

Anal. Calcd. for $C_8H_{14}N_2O_2 \cdot HCl$: C, 46.5; H, 7.3; N, 13.6. Found: C, 46.3; H, 7.3; N, 13.7.

N-(3-Aminopropyl)glutarimide hydrochloride (207 mg., 1 mmole) was dissolved in 50 ml. of water and 300 mg. (3 mmoles) of potassium bicarbonate was added. Continuous extraction with methylene chloride and evaporation gave a quantitative yield of *6,10-dioxo-1,5-diazacyclodecane* (*XII*), identical with material prepared above by Beckmann rearrangement.

5,10-Dioxo-1,6-diazacyclodecane (*Biscyclo-γ-aminobutyryl*) (*XVIII*). *A. From 1-(4-Aminobutyryl)-2-pyrrolidinone* (*XX*). Following the general procedure of Griot and Frey,[8] 157 g. (1 mole) of 4-chlorobutyryl chloride was added to a frozen mixture of 79 g. (1 mole) of pyridine, 126 g. of 2-pyrrolidinone, and 400 ml. of benzene. The product, *1-(4-chlorobutyryl)-2-pyrrolidinone* (*XXI*), was obtained in 98% yield (186 g.), b.p. 98° (0.1 mm.); ν_{max} 1740 and 1680 cm.$^{-1}$; $\lambda_{max}^{C_2H_5OH}$ 216 mμ (ε 12,000); n.m.r. absorption: δ 3.82 (m, 4H), 3.17 (t, 2H), and 2.86–1.8 (m, 6H).

Anal. Calcd. for $C_8H_{12}ClNO_2$: C, 50.7; H, 6.4; Cl, 18.7; N, 7.4. Found: C, 50.6; H, 6.2; Cl, 18.5; N, 7.6.

A mixture of 19 g. (0.1 mole) of 1-(4-chlorobutyryl)-2-pyrrolidinone, 13 g. (0.2 mole) of sodium azide, and 1.7 g. (0.01 mole) of potassium iodide in 100 ml. of dimethylformamide was heated at 110° for 22 hr., after which the solvent was evaporated. The residue was taken up in 200 ml. of water and extracted with methylene chloride (three 100-ml. and three 75-ml. portions), and the combined extracts were evaporated and distilled to yield 16 g. (82% yield) of *1-(4-azidobutyryl)-2-pyrrolidinone* (*XXII*), b.p. 131° (1 mm.); ν_{max} 2100, 1745, and 1685 cm.$^{-1}$; $\lambda_{max}^{C_2H_5OH}$ 215 mμ (ε 11,400).

Anal. Calcd. for $C_8H_{12}N_4O_2$: C, 49.0; H, 6.2; N, 28.6. Found: C, 49.1; H, 6.1; N, 28.6.

A mixture of 384 mg. (2 mmoles) of 1-(4-azidobutyryl)-2-pyrrolidinone, 1.7 ml. of 12 *N* hydrochloric acid, and 0.5 g. of 10% palladium on carbon in 200 ml. of methanol was hydrogenated at 40 p.s.i. for 4 hr. (hydrogen absorption ceased). Filtration, evaporation of the filtrate to 10 ml., addition of 150 ml. of tetrahydrofuran, and cooling gave 106 mg. (25% yield) of *1-(4-aminobutyryl)-2-pyrrolidinone* (*XX*) *hydrochloride*, m.p. 174–175° after sublimation at 135° (2 μ); ν_{max}^{Nujol} 3800–2150, 2060, 1730, and 1675 cm.$^{-1}$; λ_{max} 217 mμ (ε 11,800).

(38) R. S. Tipson, *J. Org. Chem.*, 9, 235 (1944).

Anal. Calcd. for $C_8H_{14}N_2O_2 \cdot HCl$: C, 46.5; H, 7.3; N, 13.6. Found: C, 46.5; H, 7.2; N, 13.5.

The hydrochloride (113 mg., 0.55 mmole) was dissolved in 50 ml. of water, 168 mg. (2 mmoles) of sodium bicarbonate was added slowly, and the solution was extracted continuously for 84 hr. with methylene chloride. Evaporation of the methylene chloride and trituration of the residue with ether left 58 mg. (62% yield) of *5,10-dioxo-1,6-diazacyclodecane (biscyclo-γ-aminobutyryl)* (*XVIII*), m.p. 287–288° (lit.[15] m.p. 283°) after sublimation at 110° (1 μ); ν_{max}^{Nujol} 3300, 3080, 1640, and 1560 cm.$^{-1}$.

Anal. Calcd. for $C_8H_{14}N_2O_2$: C, 56.5; H, 8.3; N, 16.5. Found: C, 56.5; H, 8.4; N, 16.4.

Biscyclo-γ-aminobutyryl could be obtained directly from the azide (5.11 g., 26 mmoles) by hydrogenation in water (150 ml.) with a 10% palladium-on-carbon catalyst (6 g.). Continuous extraction with methylene chloride followed by evaporating the methylene chloride and washing the residue with ether removed 0.7 g. of 2-pyrrolidinone in the ether and left 2.3 g. (52% yield) of pure dipeptide.

Heating biscyclo-γ-aminobutyryl at 110° in 6 *N* hydrochloric acid gave γ-aminobutyric acid as the only product.

B. From S-(Bis-γ-aminobutyryl)thiophenol. To a solution of 32.0 g. (0.135 mole) of carbobenzyloxy-γ-aminobutyric acid[31] and 13.7 g. (0.135 mole) of triethylamine in 300 ml. of tetrahydrofuran, cooled to −10°, was added dropwise with vigorous stirring 14.7 g. (0.135 mole) of ethyl chloroformate. Stirring at −10° was continued for 30 min., the precipitated triethylamine hydrochloride was removed by filtration, and to the filtrate at −5° was added a solution of 19.5 g. (0.14 mole) of γ-aminobutyric acid hydrochloride and 11.5 g. (0.29 mole) of sodium hydroxide in 30 ml. of water. The reaction mixture was stirred for 2 hr. at −5°, 3 hr. at 20°, and 0.5 hr. at 50°, after which the solution was evaporated, 500 ml. of water was added to the residue, and the resulting solution was added slowly to 50 ml. of 85% phosphoric acid. Crystallization of the precipitated product from ethyl acetate afforded 30.9 g. (71% yield) of *carbobenzyloxy-γ-aminobutyryl-γ-aminobutyric acid*, m.p. 111–112°.

Anal. Calcd. for $C_{16}H_{22}N_2O_5$: C, 59.6; H, 6.9; N, 8.7; equiv. wt., 322. Found: C, 59.9; H, 7.0; N, 8.5; equiv. wt., 322.

A solution of 33.3 g. (0.16 mole) of dicyclohexylcarbodiimide in 50 ml. of tetrahydrofuran was added to 47.4 g. (0.15 mole) of carbobenzyloxy-γ-aminobutyryl-γ-aminobutyric acid and 16.2 g. (0.15 mole) of thiophenol in 300 ml. of tetrahydrofuran. After 14 hr., the precipitated dicyclohexylurea (32.9 g., 98%) was removed, the filtrate was evaporated to dryness, and the residue was digested with ether and crystallized from benzene, giving 43.3 g. (71% yield) of *S-(carbobenzyloxybis-γ-aminobutyryl)thiophenol*, m.p. 71–75°. An analytical sample was prepared by crystallization from aqueous methanol and melted at 80–81°; $\nu_{max}^{CHCl_3}$ 1701, 1698, and 1658 cm.$^{-1}$.

Anal. Calcd. for $C_{22}H_{26}N_2O_4S$: C, 63.7; H, 6.3; S, 7.7. Found: C, 63.8; H, 6.1; S, 7.6.

Hydrogen bromide (1.6 g., 20 mmoles) was absorbed in a cold solution of 1.04 g. (2.5 mmoles) of S-(carbobenzyloxylbis-γ-aminobutyryl)thiophenol in 10 ml. of acetic acid. After 14 hr. at room temperature, the mixture was centrifuged, the supernatant acetic acid was removed, and the precipitate was washed with ether, giving 0.70 g. of *S-(bis-γ-aminobutyryl)thiophenol dihydrobromide* (*XXVII*), m.p. 123–128°. An additional 0.23 g. was obtained by adding ether to the original supernatant acetic acid; total yield, 0.93 g., 84%.

Anal. Calcd. for $C_{14}H_{22}Br_2N_2O_2S$: C, 38.0; H, 5.0; Br, 36.1; N, 6.3; S, 7.3. Found: C, 37.8; H, 5.0; Br, 36.2; N, 6.6; S, 7.1.

A solution of 575 mg. (1.3 mmoles) of S-(bis-γ-aminobutyryl)thiophenol dihydrobromide (XXVII) in 1300 ml. of dimethylformamide to which 263 mg. (2.6 mmoles) of triethylamine had been added, was heated under reflux for 5 hr. The solvent was evaporated, the residue was boiled with 40 ml. of water for 15 min., and the mixture was filtered. The filtrate was treated with decolorizing carbon (285 mg.), extracted continuously with ether, concentrated to 15 ml., and passed through a Dowex 50-X 1 column (4 mequiv., H^+ form) followed by a Dowex 1-X 1 column (5 mequiv., OH^- form). Evaporation of the neutral eluate and crystallization of the residue from aqueous methanol gave 28 mg. (12% yield) of *biscyclo-γ-aminobutyryl* (*XVIII*), m.p. 254–255°. This material gave the correct combustion analyses (C, H, N) and on hydrolysis with 0.1 *N* sodium hydroxide gave only γ-aminobutyric acid. However, thin layer chromatography and molecular weight determinations (osmometer) indicated contamination by larger ring polymers, which accounts for the lowered melting point (pure material above, m.p. 287–288°).

Cyclolization Experiments. A. Interconversion of 6,10-Dioxo-1,5-diazacyclodecane (XII) and N-(3-Aminopropyl)glutarimide (XIV). A solution of 85 mg. (0.5 mmole) of 6,10-dioxo-1,5-diazacyclodecane in 50 ml. of 0.1 *N* hydrochloric acid was allowed to stand at room temperature as the optical density at 208 mμ (10^{-4} *M*) increased from 0 to 0.84 during 10 days at 20° or 3 hr. at 90°, indicating complete conversion to the glutarimide. Evaporation and crystallization of the residue gave a 61% yield of N-(3-aminopropyl)glutarimide hydrochloride, identical with material prepared above. When this hydrochloride (0.005 mmole) was dissolved in 50 ml. of 0.01 *M* bicarbonate (pH 8.75), the λ_{max} at 208 mμ (ε 14,400) disappeared after 2 hr. at 20° and continuous extraction with methylene chloride gave a 90% recovery of 6,10-dioxo-1,5-diazacyclodecane.

B. Interconversion of 5,10-Dioxo-1,6-diazacyclodecane (XVIII) and 1-(4-Aminobutyryl)-2-pyrrolidinone (XX). A solution of 170 mg. (1 mmole) of 5,10-dioxo-1,6-diazacyclodecane in 100 ml. of 0.1 *N* hydrochloric acid showed no change in ultraviolet absorption (10^{-4} *M*) during a period of 10 days at 20°. Evaporation and sublimation gave only recovered cyclopeptide. An identical solution, heated at 90°, developed a λ_{max} at 217 mμ with an optical density of 0.5 (10^{-4} *M*) after 30 min. Additional heating (10 min.) caused this to decrease to 0.45; therefore, the solution was evaporated and the residue was crystallized from ethanol–ether. Sublimation of the crystals gave 10 mg. of recovered 5,10-dioxo-1,6-diazacyclodecane as sublimate and 30 mg. of 1-(4-aminobutyryl)-2-pyrrolidinone hydrochloride as residue, identical in melting point and infrared absorption with material prepared above *via* the azide.

When this hydrochloride (0.005 mmole) was dissolved in 50 ml. of 0.01 *M* bicarbonate (pH 8.75), the λ_{max} at 217 mμ (ε 12,100) disappeared after 1 hr., and continuous extraction with methylene chloride gave 5,10-dioxo-1,6-diazacyclodecane.

Treatment of 34 mg. (0.2 mmole) of the cyclodipeptide with 10 ml. of 0.1 *M* carbonate solution (pH 10.7) for 3.5 hr. on the steam bath followed by continuous extraction with methylene chloride gave 22 mg. of 2-pyrrolidinone.

Gramicidin A. V. The Structure of Valine- and Isoleucine-gramicidin A

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Contribution from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare, Bethesda, Maryland. *Received October 16, 1964*

Gramicidin A is a linear N-acylated pentadecapeptide ethanolamide. Its N-terminal L-*valine or* L-*isoleucine are blocked by a formyl group which is cleaved by mild methanolysis at room temperature and easily identified as formic acid by gas chromatography or as formaldehyde after reduction by the chromotropic acid assay. Reformylation of desformylgramicidin A, via O-formylgramicidin A, gave back gramicidin A. Desformylgramicidin A was subjected to ten successive Edman degradations under conditions modified to accommodate the insolubility of its residual peptide fragments in water. The phenylthiohydantoins, obtained by cyclization with trifluoroacetic acid, were assayed by gas chromatography up to step 10. In addition, the residual peptides up to step 10 were hydrolyzed and their amino acids determined quantitatively. Selective N-bromosuccinimide (NBS) cleavage of the bonds following the four tryptophans in gramicidin A releases aminoethanol and the NBS-oxidation product of leucyltryptophan but no dioxindolalanine spirolactone fragment expected from a Try-Try sequence. These findings combined with the older observations suggest the sequence HCO-*L-*Val*-Gly-L-*Ala*-D-*Leu*-L-*Ala*-D-*Val*-L-*Val*-D-*Val*-L-*Try*-D-*Leu*-L-*Try*-D-*Leu*-L-*Try*-D-*Leu*-L-*Try*-NHCH$_2$CH$_2$OH for valine-gramicidin A and HCO-*L-*Ileu . . . for isoleucine-gramicidin A. Thin layer chromatography, optical rotatory properties in various solvents, and molecular weight studies by ultracentrifugation and osmometry are discussed in terms of easy association of at least two molecules of gramicidin A whose unprecedented alternating sequence of (mostly hydrophobic)* L- *and* D-*amino acids permits a characteristic secondary structure dependent on the nature of the solvent.*

Gramicidin was isolated in 1941 by Hotchkiss and Dubos.[2] A cyclic structure was assumed for this peptide antibiotic because of the absence of basic and acidic functions, and because initially only neutral amino acids, but no alcohols or fatty acids were found in hydrolysates of gramicidin.[3] Later the discovery of aminoethanol[4] in gramicidin hydrolysates prompted Synge to propose an "ortho" peptide bond[5] for the aminoethanol linkage (Figure 1) in order to explain both the neutral character of gramicidin and the single hydroxyl function present in the antibiotic. However, the work of Griot and Frey[6] limits the existence of stable cyclols to systems containing a diacylimide structure. The elimination of a cyclol structure as well as results obtained from NBS oxidation of gramicidin supported a structure in which tryptophan is linked to the amino group of ethanolamine whose hydroxyl group is free. Such a linear structure can be written only if one assumes the presence of an N-terminal blocking group whose nature was of such a kind that it had escaped the attention of the previous investigators.

A. The N-Formyl Terminal Blocking Group.[7] Gramicidin A[8] represents a mixture of valine- and isoleucine-gramicidin A.[9-11] It releases a volatile acid on hydrolysis with 50% sulfuric acid. This acid was isolated by low-temperature distillation and identified as formic acid either by gas chromatography[12] or by the chromotropic acid test[13] after reduction to formaldehyde. Quantitative experiments indicated a liberation of 0.8–1 mole of formic acid per 1850 g. of gramicidin A. No other acid was found by gas chromatography except acetic acid in a sample which had been freeze dried from acetic acid and still retained 7% of this acid. However, acetic acid was no longer found after recrystallization of this sample from ethanol–water or after drying at 100° and 10^{-2} mm. Neither deformylated gramicidin A nor tryptophan liberated any trace of formic acid in control experiments. The n.m.r. spectrum[14] of gramicidin A in deuteriomethanol shows a broad peak at 8.45 p.p.m. (tetramethylsilane as internal standard), the peak area being one-twelfth to

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