[CONTRIBUTION FROM THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

Bacitracin A. The Nature of the Linkages Surrounding the Sulfur

BY J. R. WEISIGER, W. HAUSMANN AND L. C. CRAIG

RECEIVED DECEMBER 13, 1954

Studies with tri DNP-bacitracin A, bacitracin F, the oxidation product obtained with performic acid, and the de-sulfurized product obtained by the action of Raney nickel have been made. These studies support the theory that bacitracin A contains a thiazoline ring formed by condensation of a cysteine and an isoleucine residue. A crystalline fragment $C_9H_{11}O_3NS$ has been isolated from the hydrolysate of bacitracin F. It is thought to be 2-isovalerylthiazole-4-carboxylic acid.

Introduction

On complete hydrolysis the naturally occurring antibiotic polypeptide, bacitracin A, gives a good yield of L-cysteine.[1] Only one mole of cysteine emerges per mole of bacitracin A and this accounts for all the sulfur shown by analysis to be present. Yet the intact polypeptide does not give a nitroprusside test for SH.[1,2] In our experience all other tests for the SH group were negative and completely substituted DNP-bacitracin A did not give an S-substituted DNP derivative of cysteine following either partial or complete hydrolysis. However, after mild hydrolysis the SH can be detected readily[2] by the nitroprusside test. These data alone suggest that the SH group is covered in some way, possibly by being (1) in a thiazoline ring, a possibility suggested by Newton and Abraham[2] and Craig, Hausmann and Weisiger,[3] (2) in a thioester, or (3) tied in to the peptide chain at some point to form a ring larger than a thiazoline ring.

In this Laboratory[4] a number of peptides containing the sequence Ileu·Cys·Leu have been isolated in a state of analytical purity from partial hydrolysates. This sequence had also been suggested by the partial hydrolysis and paper chromatography studies of Lockhart, Newton and Abraham.[5] With this information at hand it should be possible to bring the question of the sulfur linkage under more careful study. Even so it has not proven easy to devise completely unambiguous experiments because no satisfactory models appear to be at hand for comparison of the stabilities and reaction behavior of such postulated linkages.

Information bearing on the problem has been obtained from a study of the transformation of bacitracin A to F. In another approach a preliminary study of the desulfurization of bacitracin A with Raney nickel has been made. Newton and Abraham have already reported on studies along this line.[2]

An unanswered question regarding the structure of bacitracin A is the nature of the second free amino group. One of the two present in the peptide is accounted for by the free δ-amino group of the ornithine residue. Although evidence was presented[3] that the second one could be covered by a DNP group it was largely split off from the residue or lost in some other way during hydrolysis. This prevented certain identification of the residue involved. Newton and Abraham[2] obtained a spot by paper chromatography following hydrolysis which coincided with the position of DNP-leucine or DNP-isoleucine. On the other hand Griffith[6] could obtain no evidence for an N-terminal leucine or isoleucine by the thiohydantoin method of Edman. Nor was Ingram able to get evidence for this group by his reductive methylation method.[7]

It was considered possible that the instability of the DNP linkage might be tied up in some way with the linkages surrounding the sulfur since DNP-cysteine is known to be largely destroyed during hydrolysis.[8] The only other DNP-amino acid present in bacitracin A which gives a DNP derivative markedly unstable to the hydrolysis conditions is histidine. However, it has been shown that the imidazole group of the histidine is free[3] since tri-DNP bactitracin A gives im-DNP histidine on hydrolysis. It has also been shown that the sequence Phe·His·Asp is present[4] and this accounts for all the functional groups of the histidine. It would therefore appear that a study of the position of the third DNP group in tri-DNP bacitracin A is properly a part of the study of the unique linkages surrounding the sulfur atom.

Still another approach to the problem can be made through a study of bacitracin A after it has been oxidized with performic acid. The present study has included preliminary work along this line.

Experimental

Preparation of Tri-DNP Bacitracin A.—One hundred mg. of bacitracin A was dissolved in 1.2 ml. of a solution made from 60 ml. of ethanol, 30 ml. of water and 10 ml. of triethylamine. One hundred mg. of fluorodinitrobenzene was immediately added. The solution was maintained at 40° for 5 minutes with shaking from time to time and then quickly evaporated under reduced pressure at a temperature not higher than 25°. The oily residue was placed immediately in the acid system used for the distribution.

The system was made from benzene, chloroform, glacial acetic acid and 0.1 *N* HCl in the volume proportions of 1, 1, 2, 1. Equal volumes of the upper and lower phases were used. After 203 transfers, Fig. 1, the main yellow band, tubes 85–110, was in a position which indicated it to travel with a K of about 2.7. The unreacted FDNB and dinitrophenol were thrown off in tubes 0–50. The weight extinction ratio at 350 mμ indicated the molecular weight to be approximately 2000 assuming 2 DNP groups attached to NH_2 groups and 1 to the imidazole of the histidine.

(1) L. C. Craig, W. Hausmann and J. R. Weisiger, *J. Biol. Chem.*, **199**, 865 (1952).

(2) G. G. F. Newton and E. P. Abraham, *Biochem. J.*, **53**, 604 (1953).

(3) L. C. Craig, W. Hausmann and J. R. Weisiger, *J. Biol. Chem.*, **200**, 765 (1953).

(4) W. Hausmann, J. R. Weisiger and L. C. Craig, THIS JOURNAL, **77**, 723 (1955).

(5) I. M. Lockhart, G. G. F. Newton and E. P. Abraham, *Nature*, **173**, 536 (1954).

(6) J. H. Griffith, Bacitracin, Ph.D. Dissertation, Iowa State College, Ames, Iowa, 1954.

(7) V. M. Ingram, *J. Biol. Chem.*, **202**, 193 (1953).

(8) R. R. Porter, "Methods in Medical Research," Vol. 3, Year Book Publishing Co., Chicago, 1950, p. 256.

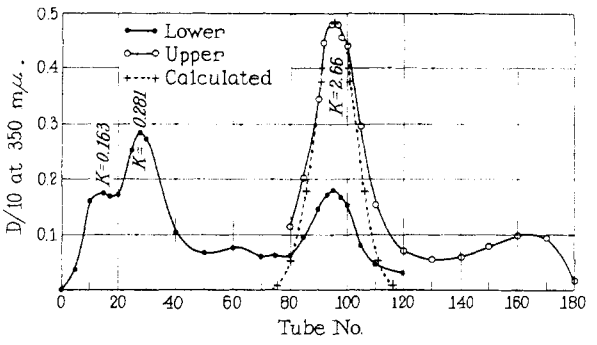


Fig. 1.—C.C.D. pattern of reaction products of bacitracin A and FDNB.

Complete Hydrolysis of Tri-DNP Bacitracin A.—Fifty mg. of tri-DNP bacitracin A was hydrolyzed in a sealed evacuated tube for 24 hr. at a temperature of 108° in 1:1 glacial acetic acid, concentrated HCl. The acid solution was evaporated to dryness and taken up in two ml. of water. The aqueous solution was extracted three times with ethyl ether. The DNP derivatives of ornithine and histidine remained in the aqueous solution and were not studied further. The ether extract was evaporated to dryness. The residue was distributed to 50 transfers in a C.C.D. apparatus in a system made from benzene, glacial acetic acid and 0.1 *N* HCl in the volume proportions 2,2,1, respectively. Phase volumes of 5 ml. upper and 10 ml. lower were used. Analysis by absorption at 350 mμ gave a major band which was in close agreement with a calculated curve for a partition ratio of 2.5. This *K* value is in good agreement with the *K* of isoleucine or leucine in the system used. A minor band occurred in the region of tube 0 to 10. No other band was found. Material isolated from the peak tubes of the major band gave partition ratios in the other systems[4] suggested for identification of DNP-amino acids also in agreement with the values for DNP-isoleucine or leucine. The decision that the derivative is from an isoleucine residue depends upon the data given further on. Summation of the total absorption under the main band indicated recovery of the DNP amino acid in an amount corresponding to 23% of the theory for one residue.

A sample of the tri-DNP derivative of bacitracin A from tubes in the peak of Fig. 1 was hydrolyzed and a quantitative amino acid analysis made by ion exchange chromatography.[9] The results expressed as molar ratios are given in Table I.

TABLE I

AMINO ACID ANALYSES OF BACITRACIN A AND DERIVATIVES

	Molar ratios found			
Amino acid	Bacitracin A[a]	Bacitracin F	Tri-DNP-bacitracin A	De-sulfurized bacitracin A
Leucine	1.02	1.00	1.00	1.00
Isoleucine	1.83	1.84	1.68	1.81
Alloisoleucine	0.5[a]	0	0	Trace ?
Phenylalanine	1.03	0.88	0.98	1.23
Cysteine (cystine × 2)	1.0	0	0.82	0
Ornithine	0.80	0.75	Trace	..
Lysine	0.89	0.75	0.98	..
Histidine	0.80	0.92	Trace	1.01
Aspartic acid	1.77	1.87	1.79	1.74
Glutamic acid	0.89	0.92	0.88	1.15
Alanine	0	0	0	0.17

[a] See ref. 1.

Desulfurization of Bacitracin A with Raney Nickel.—A suspension of Raney nickel in ethanol was made according to the directions of Covert and Adkins[10]: 230 mg. of bacitracin A in 50 ml. of absolute ethanol was treated with about 6 g. of the Raney nickel catalyst. The suspension was refluxed with stirring for 5 hr. The catalyst was filtered off but on evaporation of the filtrate practically no residue was left. The peptide which was bound to the Raney nickel was removed by successive hot extraction according to the method used by Turner, Pierce and du Vigneaud[11] with oxytocin. Three hot extractions were made with a solution containing 50 ml. of ethanol and 55 ml. of concentrated ammonia. After evaporation of each extract the weights 152, 54 and 12 mg. were obtained. The three residues were combined. A sulfur analysis made on this material was negative. It contained considerable inorganic material, presumably nickel.

The residue was fractionated by C.C.D. in the system used for purifying bacitracin A, 2-butanol–3% acetic acid. The partition ratio in this system was lower than that of bacitracin A. With phase volumes of 12/10 the weight pattern shown in Fig. 2 was obtained at 669 transfers. The curves indicated that the solutes were not behaving ideally. The right-hand band was too narrow for some unknown reason.

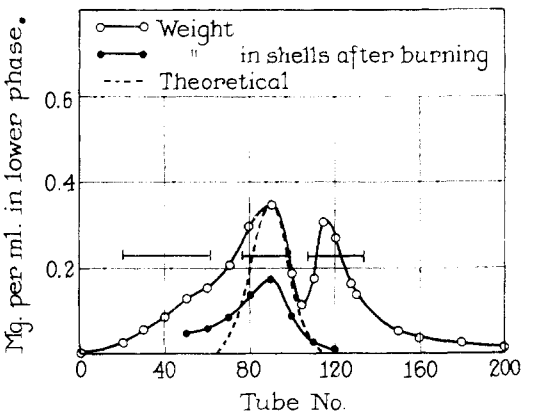


Fig. 2.—C.C.D. pattern of Raney nickel reaction products of bacitracin A.

For weight analysis platinum shells were used which are routinely cleaned by holding them in the flame of a bunsen burner. Since many of them contained inorganic residue following such treatment the residue was weighed and plotted in Fig. 2. Three cuts were taken as shown. The right-hand cut was practically free of inorganic material.

Portions of the solute from each of the cuts were completely hydrolyzed and studied by two-dimensional paper chromatography in the systems used for amino acids: 2-butanol, ammonia and 2-butanol, formic acid.[4] A sample from the right-hand cut of Fig. 2 gave strong spots for all the amino acids of bacitracin A except cysteine. A rather weak spot in the position of alanine could be seen. This hydrolysate was studied for its quantitative amino acid content by ion-exchange chromatography.[9] The result expressed as molar ratios is given in Table I. Because of a mishap during the run the values for lysine and ornithine were lost.

Conversion of Desulfurized Bacitracin A to the DNP Derivative.—Twenty-three mg. of the desulfurized resin from the right-hand cut of Fig. 2 was converted to the DNP derivative in the same way as was bacitracin A above except for the reaction time which was 10 min. The recovered residue was distributed to 49 transfers in a system made from chloroform, glacial acetic acid and 0.1 *N* HCl in the volume proportions of 2, 2, 1. The tube volumes were 10 ml. upper and 10 lower. At 49 transfers analysis was made by absorption at 350 mμ. Two well separated bands were found, one of which contained triethylamine hydrochloride in addition to the DNP derivative.

Central cuts of each of these bands were separately hydrolyzed and studied by two-dimensional paper chromatography for the amino acid content. Both gave clear spots in the region of lysine, aspartic acid, glutamic acid, phenylalanine, leucine, isoleucine, and a spot in the position of a peculiar peptide which has often been found when DNP

(9) S. Moore and W. H. Stein, *J. Biol. Chem.*, **211**, 893 (1954); **211**, 907 (1954).

(10) L. Covert and H. Adkins, THIS JOURNAL, **54**, 4116 (1932).

(11) R. A. Turner, J. G. Pierce and V. du Vigneaud, *J. Biol. Chem.*, **193**, 359 (1951).

derivatives[4] of the peptides from bacitracin containing the lysine and aspartic acid have been hydrolyzed. It was also identified by paper electrophoresis. The left-hand cut showed a very weak histidine spot but a spot in the position of im-DNP histidine. It showed a spot in the δ-DNP-ornithine position and a fast moving yellow spot which did not turn blue with ninhydrin.

The right-hand cut showed a stronger histidine spot but no im-DNP-histidine spot. It also showed no δ-DNP-ornithine spot. Under the conditions used here for paper chromatography ornithine does not separate well from lysine. Apparently the right-hand cut differs in part from the left by not having its histidine imidazole group substituted, and perhaps its ornithine may also be free. No spot corresponding to alanine was found in either fraction.

Conversion of Bacitracin A to F.—Five hundred mg. of bacitracin A was dissolved in 20 ml. of a phosphate buffer at a *p*H of 7 and 1 ml. of *N* NaOH. The latter was sufficient to neutralize the acetic acid held by the bacitracin A.[12] The solution was allowed to stand for four days at 37°. At the end of this time the *p*H had changed little and was found to be 7.05.

The *p*H of the solution was reduced by addition of 2 ml. of *N* HCl and then sufficient acetic acid to reach a *p*H approximating 4. This solution was used as the lower phases in the first three tubes of a 220 tube C.C.D. machine. The system chosen for the distribution was the 2-butanol–3% aqueous acetic acid system used for purifying bacitracin A originally.[13] After 291 transfers the pattern shown in Fig. 3 was obtained.

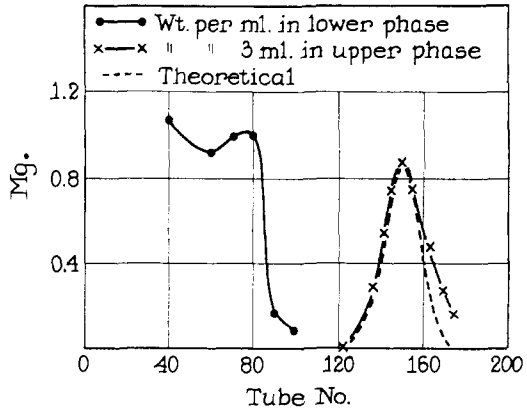


Fig. 3.—C.C.D. pattern of bacitracin F and A.

The solute in tubes 0 to 60 appeared to be inorganic but that in tubes 60 to 90 probably was unchanged bacitracin A since it moved with the proper partition ratio. This material approximated 250 mg. of peptide.

The well separated peak to the right containing the F modification had migrated as if its partition ratio were 1. The total solute in this band weighed 128 mg. A cut of tubes 138 to 160 was recovered by evaporation of the 2-butanol and lyophilization of the aqueous acetic acid solution.

Anal. Calcd. for $C_{66}H_{97}O_{17}N_{15}S$: C, 56.4; H, 6.96; N, 14.9; S, 2.26; NH_2, 1.14. Found: C, 56.07; H, 7.22; N, 14.8; S, 2.04; NH_2, 1.14.

A sample of this material was hydrolyzed completely and the quantitative amino acid content in part of the hydrolysate studied by ion-exchange chromatography,[9] Table I.

A sample of bacitracin F prepared from A as above gave an ultraviolet absorption spectrum curve which was identical qualitatively and quantitatively with that of F isolated from commercial bacitracin.

Isolation of the Chromophoric Moiety from Bacitracin F.—It was noted that the solution resulting from total hydrolysis of F showed an ultraviolet absorption spectrum not greatly different from the intact peptide. Therefore, a larger sample (600 mg. separated from commercial bacitracin) was hydrolyzed at 108° with 62 ml. of 6 *N* H_2SO_4 in a sealed tube. The hydrolysate was extracted three times with ethyl ether. The ether extract contained the fragment responsible for the absorption.

The ether extract was evaporated and the residue purified by C.C.D. in the system ethyl ether–0.05 *M* phosphate buffer at *p*H 5.6. A major band exactly coinciding with a calculated curve was found. Two small bands well separated to the left also were found.

The solute in the main band was an acid which could be moved to the ether phase by acidification of the buffer. It was recovered from the ether by evaporation. The residue crystallized readily from pentane and melted at 93 to 95°. It was purified for analysis by sublimation at 0.1 mm. pressure. The temperature of the heating bath approximated 80°. After sublimation the crystals melted at 94 to 95°.

Anal. Calcd. for $C_9H_{11}O_3NS$: C, 50.7; H, 5.22; N, 6.57; S, 15.0. Found: C, 50.68; H, 5.19; N, 6.79; S, 14.78.

A 2,4-dinitrophenylhydrazone was prepared by warming a glacial acetic acid solution of 5 mg. of the crystalline acid with 4.6 mg. of 2,4-dinitrophenylhydrazine for a short time. The solution was concentrated to about 0.1 ml. When left for several days at room temperature well-formed crystalline needles separated. After collection these weighed 3.2 mg. and melted at 230–235° with decomposition.

Anal. Calcd. for $C_{15}H_{15}O_6N_5S$: N, 17.8. Found: N, 17.8.

Oxidation of Bacitracin A with Performic Acid.—A solution of 500 mg. of bacitracin A in 20 ml. of 98% formic acid was treated with 2 ml. of 30% hydrogen peroxide. After standing at room temperature for 15 minutes 20 ml. of water was added and the solution was evaporated to dryness in the rotary evaporator at 25° *in vacuo*. The residue was taken up in the system used for purification of bacitracin A (2-butanol–3% aqueous acetic acid) and distributed to 330 transfers. The phases of the system were of equal volume. Weight analysis gave the pattern shown in Fig. 4.

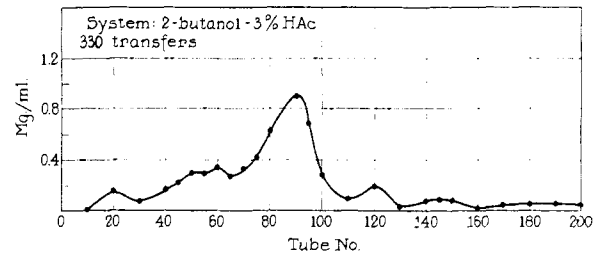


Fig. 4.—C.C.D. pattern of oxidized bacitracin A.

A cut of material which included tubes 75–105 was recovered by concentrating the solutions in the rotary evaporator at room temperature *in vacuo* and freeze drying the aqueous solutions. This was considered the oxidized derivative since on complete hydrolysis in 6 *N* HCl, paper chromatography and paper electrophoresis both indicated a spot corresponding to cysteic acid. No spot corresponding to cysteine was found. Spots corresponding to all the other amino acids in bacitracin were found.

Paper electrophoresis of the intact oxy-peptide in pyridine acetate buffer at *p*H 5.6 gave only a single spot after spraying with ninhydrin. The position of the spot indicated slightly basic properties and corresponded with that of bacitracin A.

Fifteen mg. of the oxidized peptide was converted to the DNP derivative as given above for bacitracin A. After evaporation the residue was hydrolyzed for 24 hours in 6 *N* HCl at 108° in a sealed tube. The hydrolysate was recovered and extracted with ether. The water-soluble portion was studied by two-dimensional paper chromatography. It showed all the spots shown by a hydrolysate of bacitracin A except for cysteine and ornithine. Spots corresponding to cysteic acid and δ-DNP-ornithine also were found.

The ether extract containing the yellow DNP derivative was evaporated to dryness and distributed to 30 transfers in a system containing chloroform, glacial acetic acid and 0.1 *N* HCl in 2, 2, 1 volume proportions. Only a single homogeneous yellow band of *K* 0.187 corresponding to DNP-isoleucine was found.

(12) W. Hausmann, J. R. Weisiger and L. C. Craig, THIS JOURNAL, **77**, 721 (1955).

(13) L. C. Craig, J. R. Weisiger, W. Hausmann and E. J. Harfenist, *J. Biol. Chem.*, **199**, 259 (1952).

Discussion

Rather early in the chemical investigation of bacitracin[14] it was shown that the polypeptide gives cysteine on hydrolysis. It did not, however, give a test for a thiol group until subjected to hydrolysis or reduction with stannous chloride. A sulfur analysis of 2.2%[13] and a molecular weight determination approximating 1470 by the method of partial substitution[3] showed conclusively that only one sulfur could be present in the molecule. A ring of some nature involving the sulfur was thus implied.

Newton and Abraham[2] had also noted that bacitracin gave a test for a free thiol group only after mild hydrolysis. The behavior was not that expected from a disulfide linkage and a heterocyclic ring, perhaps a thiazoline ring, was suggested as a tentative hypothesis. Direct proof, however, that both the sulfur and nitrogen of the cysteine were joined to the carboxyl carbon of the same amino acid was lacking.

Since the sequence isoleucyl-cysteinyl-leucine has now been shown[4,5] to be present in partial hydrolysates of bacitracin A, it is to be expected that the thiazoline, if present, would be that formed by loss of water from isoleucylcysteine. Accordingly experiments attempting to confirm this have been made.

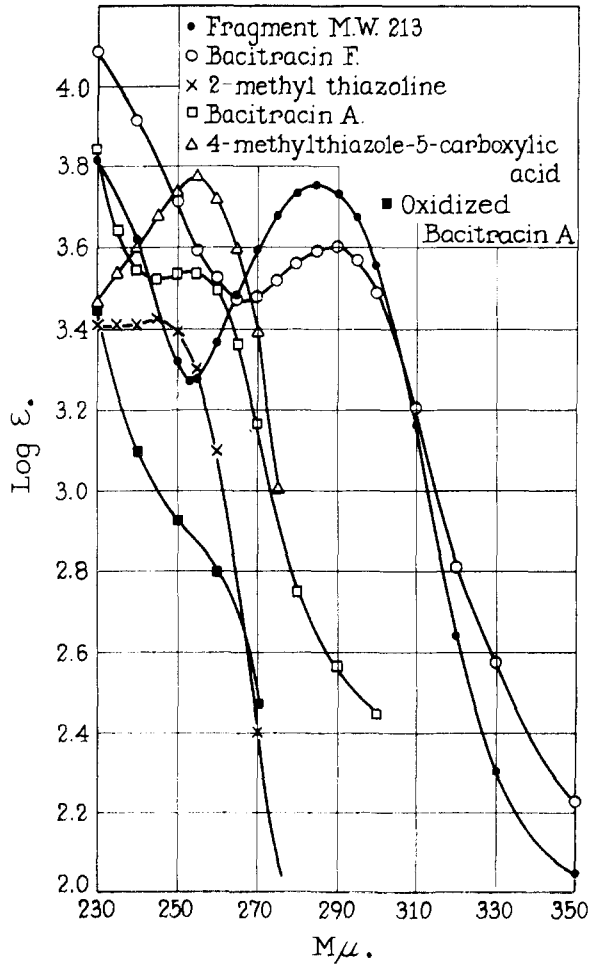
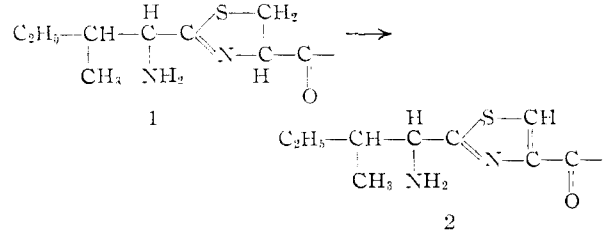


Fig. 5.—Absorption spectrum curves of substances of interest to bacitracin.

Such a ring would be expected to be unstable to hydrolysis and reduction but could be stabilized if converted to a thiazole. In fact it was considered likely that the transformation of bacitracin A to F might involve this oxidation since F did not give cysteine on total hydrolysis as is shown from the results of Table I. With this possibility in mind a careful search of the hydrolysis products of F for a chromophoric group was made. A crystalline fragment with the empirical formula $C_9H_{11}O_3NS$ was found. The substance was an acid with few or no basic properties. The ultraviolet absorption spectrum, Fig. 5, was not that of a thiazolecarboxylic acid as comparison with the spectrum of 4-methylthiazole-5-carboxylic acid[15] shows. However, a keto group or double bond conjugated with the thiazole ring could cause a shift which might explain the result of Fig. 5. Infrared studies indicated the absence of a hydroxyl group and a double bond but showed a band occurring in the ketone region. Positive evidence for the keto group was obtained by the formation of a crystalline 2,4-dinitrophenylhydrazone.

The fact that the fragment contains nine carbon atoms and must come from the cysteine residue since it contains the single sulfur atom of bacitracin A, indicates that it is derived from leucine or isoleucine and cysteine. Isoleucine is clearly shown to be the residue involved by the data of Table I. This certainly is to be expected from the Ileu·Cys sequence shown to be present in bacitracin A.

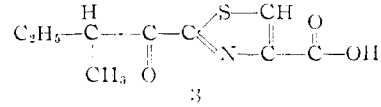
If the thiazoline ring in formula 1 should become oxidized to the thiazole, formula 2



1

2

the primary amino group would become reactive as is known to occur with aminomethylpyrrole or α-aminomethylpyridine.[16] Because of this ammonia could be lost by a further oxidation step to give the structure in formula 3 which must represent the structure of the $C_9H_{11}O_3NS$ fragment.



3

The ultraviolet absorption spectrum of this fragment is consistent with the structure proposed as can be seen from Fig. 5. A ketone group conjugated with the absorbing groups of a thiazole carboxylic acid would increase the absorption and displace it toward longer wave lengths.

The absorption spectrum of bacitracin F is sufficiently different from the fragment to indicate

(14) G. T. Barry, J. D. Gregory and L. C. Craig, *J. Biol. Chem.*, **175**, 485 (1948).

(15) A. E. Ruehle, THIS JOURNAL, **57**, 1887 (1935).

(16) L. C. Craig and R. M. Hixon, *ibid.*, **53**, 4367 (1931).

that the relationship is probably more complicated than simple incorporation of the latter into F by its carboxy group.

Attempts are being made to synthesize the compound represented by formula 3.

The ultraviolet absorption spectrum of bacitracin A[13] is not inconsistent with the presence of a thiazoline ring. In Fig. 5 is shown a comparison of the spectrum of 2-methylthiazoline[17] and bacitracin A. The rise in absorption at 240 mμ with the latter could be due to other absorbing residues which are not present in the simpler molecule. Bacitracin A and 2-methylthiazoline differ in their stabilities at various *p*H levels but this could be due to the different substituting groups present in bacitracin A. Linderstrøm-Lang and Jacobsen[18] found 2-methylthiazoline to have basic properties. However, Jacobsen[19] found carbobenzoxyaminomethylthiazoline-4-carboxylic acid to be much less basic. Therefore, the lack of evidence for such a basic group in titration[2] does not speak against a thiazoline ring in bacitracin A.

The structure shown in formula 1 would be consistent with the formation of 0.5 mole of allo-isoleucine on total hydrolysis of bacitracin A. It would explain the absence of this allo-isoleucine in the hydrolysates of tri-DNP bacitracin A and desulfurized bacitracin A, Table I. Bacitracin F gave a di-DNP derivative. Investigation of a hydrolysate of this derivative showed the δ-amino group of the ornithine and the imidazole of the histidine to have been substituted. Bacitracin A shows two NH_2 groups in the Van Slyke amino nitrogen determination but F shows only one.

It is obvious from these observations and the data of Table I that the isoleucine attached to the cysteine is the one involved in the formation of the DNP derivative. The formation of F indicates that the nitrogen of this residue has a degree of lability. This may explain the poor yield of DNP-isoleucine on hydrolysis. However, the low yield was found not to be due to the formation of dinitroaniline. A more complicated explanation must be found.

If Raney nickel should reduce the double bond in formula 1 as well as remove the sulfur, a low yield of alanine would be expected in the analysis of its hydrolysate. The result in Table I is consistent with this expectation.

It has been found by Newton and Abraham[2] that bacitracin is easily oxidized to an inactive product from which the cysteine residue is missing on hydrolysis. However, they considered the reaction to involve oxidation of the cysteine residue to cysteic acid since a spot corresponding to cysteic acid was found by paper chromatography following hydrolysis. Accordingly their oxidation must have taken a course different from the oxidation to F.

Investigations carried out in this Laboratory have shown that several different transformations can occur when bacitracin A is permitted to stand in solution at a *p*H above 7. One of these is the formation of bacitracin F which obviously involves an oxidation and loss of ammonia, as the over-all composition of bacitracin F compared to A[12] shows.

Performic acid does not accomplish a specific oxidation involving the sulfur atom, as the result in Fig. 4 shows. More extensive transformation takes place. However, the major band does give a spot by paper electrophoresis and paper chromatography after complete hydrolysis which is unmistakably that of cysteic acid. This is of interest in connection with the proposed thiazoline ring since the intact major band from Fig. 4 shows no different mobility in paper electrophoresis than does bacitracin A. It also has a similar partition ratio. These observations speak against the formation of a free sulfonic acid group by performic acid. However, it is entirely conceivable that the sulfur of the thiazoline ring is oxidized to a derivative which breaks up in part to cysteic acid on hydrolysis.

The behavior of the DNP derivative of the performic acid oxidized material is consistent with this view. On hydrolysis it gave a spot by P. C. corresponding to cysteic acid and an ether extractable DNP amino acid which behaved in C.C.D. as a single substance and showed the characteristic partition ratios of DNP-isoleucine in several different systems.[4]

Oxidized bacitracin A shows a type of absorption spectrum, Fig. 5, which would be expected on the basis of the above interpretation. The effect of the sulfur on the absorption is removed.

We are indebted to Mr. Rigakos for the microanalyses, and to Miss Elizabeth Jacobs and Miss Gerty Walker for technical assistance.

NEW YORK, N. Y.

(17) We are indebted to Prof. Linderstrøm-Lang for the absorption spectrum curve of 2-methylthiazoline.

(18) K. Linderstrøm-Lang and C. F. Jacobsen, *J. Biol. Chem.*, **137**, 443 (1941).

(19) C. F. Jacobsen, *Compt. rend. trav. Lab. Carlsberg*, **26**, 1 (1947).