5%, water). $K_3$ was a mixture of III and VII, whereas $K_4$ proved to be the pure hydrochloride of III.

**Preparation of Derivatives of III and VII. a. The 2,4-Dinitrophenyl Derivative of III.**—According to the method of Sanger[3] the dinitrophenyl derivative of III was prepared by treating the hydrochloride of III (239 mg.) with 2,4-dinitrofluorobenzene (0.56 ml.). After three recrystallizations from 60% methyl alcohol, the yellow, acicular crystalline derivative (223 mg.) was obtained, m.p. 148–151°.

*Anal.* Calcd. for $C_{12}H_{10}N_4O_6S$: C, 42.61; H, 2.98; N, 16.57; S, 9.46. Found: C, 42.47; H, 2.91; N, 16.49; S, 9.34.

**b. The 2,4-dinitrophenyl derivative of VII** was prepared from the hydrochloride (283 mg.) and 2,4-dinitrofluorobenzene (0.28 ml.). After three recrystallizations from 60% methanol we obtained the yellow derivative (254 mg.), m.p. 175–179°.

*Anal.* Calcd. for $C_{16}H_{15}N_3O_6$: C, 55.65; H, 4.38; N, 12.17. Found: C, 55.62; H, 4.37; N, 12.27.

**c. The Tosyl Derivative of VII.**—The hydrochloride of VII (500 mg.) was dissolved in 1 *N* sodium hydroxide (10 ml.). A solution of *p*-toluenesulfonyl chloride (1 g.) in ether (12.5 ml.) was added and the mixture was shaken mechanically for 4 hr. The ether layer was discarded and the water layer acidified with 4 *N* hydrochloric acid. A white precipitate formed, which was recrystallized several times from 60% alcohol. The acicular, white crystals (414 mg.) melted at 169–171°.

*Anal.* Calcd. for $C_{17}H_{19}NO_4S$: C, 61.25; H, 5.75; N, 4.20; S, 9.60. Found: C, 61.23; H, 5.79; N, 4.25; S, 9.73.

**d. The Acetyl Derivative of VII.**—A solution of the hydrochloride of VII (5 g.) in 1 *N* sodium hydroxide (50 ml.) was cooled in an ice-bath. Acetic anhydride (3 ml.) and 1 *N* sodium hydroxide (25 ml.) were added simultaneously and gradually under stirring over a period of 15 minutes. The mixture was stirred for an additional 30 minutes, whereupon another portion of acetic anhydride (3 ml.) and sodium hydroxide (65 ml.) were added in 13 minutes. After another 30 minutes the solution was filtered, acidified with concentrated hydrochloric acid and cooled; the precipitated solid was filtered off, washed with water and dried.

The product was recrystallized from 20% alcohol. A white, acetyl derivative (3.4 g.), m.p. 177–185°, $[\alpha]^{25}D$ +35.0° (*c* 2%, 96% ethyl alcohol), was obtained.

(3) F. Sanger, *Biochem. J.*, **39**, 507 (1945).

*Anal.* Calcd. for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.79; N, 6.33. Found: C, 64.84; H, 6.79; N, 6.42.

**Kunz-hydrolysis of VIII.**—Compound VIII (197.9 mg.) was dissolved in purified acetone (25 ml.). To this solution was added 0.1011 *N* sodium hydroxide (25 ml.). After 2.5 hours in the refrigerator, titration with 0.0906 *N* hydrochloric acid showed that 0.98 milliequivalent of alkali had been consumed per milliequivalent of VIII.

**Identification of Valine and Glycine in the Acid Hydrolyzate of Bottromycin by Means of Paper Chromatography.**—A hydrolyzate of bottromycin with concentrated hydrochloric acid (72 hr. hydrolysis) was compared with known amino acids by means of paper chromatographic methods. The one-dimensional descending method was used (Whatman no. 1 paper). The following eluent systems were used: *n*-propanol–water (70:30), *n*-propanol–water–diethylamine (85:15:3), *n*-butanol–acetic acid–water (100:12:100), isobutyl alcohol–formic acid–water (70:15:13), phenol–water–$NH_3$–NaCN, pyridine–isoamyl alcohol–water (35:35:30). In all six systems spots I and V coincided with the spots of glycine and valine, respectively. Identity was demonstrated also as follows. The hydrolyzate was evaporated to dryness under vacuum and treated with 2,4-dinitrofluorobenzene, according to the method of Levy.[4] This yielded a mixture of dinitrophenyl derivatives. By paper chromatographic technique this mixture was compared with the dinitrophenyl derivatives of glycine and valine. The following systems were used: 1, the Blackburn system[5] (phthalate buffered paper strips of *p*H 6.0 and as eluent: (a) phthalate buffer–saturated *n*-propanol–petroleum ether 3:7); (b) phthalate buffer saturated amyl alcohol; 2, the Biserte system[6] (toluene–pyridine–ethylene chlorhydrin–0.8 *N* ammonia, 5:1:3:3). In all cases the spots of dinitrophenylvaline and dinitrophenylglycine coincided with two spots of dinitrophenyl derivatives obtained with bottromycin hydrolyzate.

**Acknowledgments.**—The authors wish to thank Mr. P. J. Hubers and Mr. M. Van Leeuwen for microanalyses, Miss V. Roest and Dr. L. M. Rientsma for infrared absorption determinations and Professor W. Berends of Delft for many helpful discussions.

(4) A. L. Levy, *Nature*, **174**, 126 (1954).

(5) S. Blackburn and A. G. Lowther, *Biochem. J.*, **48**, 126 (1951).

(6) G. Biserte and R. Osteux, *Bull. soc. chim. biol.*, **33**, 50 (1951).

DELFT, HOLLAND

---

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE KON. NED. GIST-EN SPIRITUSFABRIEK]

# The Structure of the Sulfur-containing Moiety of Bottromycin

BY J. M. WAISVISZ, M. G. VAN DER HOEVEN AND B. TE NIJENHUIS

RECEIVED DECEMBER 21, 1956

The degradation of the antibiotic bottromycin by means of boiling acetic anhydride yielded two crystalline acetyl products $C_{19}H_{23}N_3O_4S$ and probably $C_{21}H_{34}N_4O_4$. The sulfur-containing moiety proved to be the methyl ester of a N-acetyl dipeptide. The two components of this peptide are two new naturally occurring amino acids, α-amino-β-phenylbutyric acid and β-(2-thiazole)-β-alanine.

Previously[1] it was reported that treatment of bottromycin with boiling acetic anhydride yields two crystalline acetyl-degradation products: $C_{19}H_{23}N_3O_4S$ (VIII) and a compound with the probable formula $C_{21}H_{34}N_4O_4$ (IX).

This paper deals with the elucidation of the structure of the sulfur-containing moiety. It has already[1] been reported that VIII contains one acetyl, one methoxyl and, in addition, one C-methyl group. Furthermore on Kunz hydrolysis one group of the molecule is split off, whereas mild acid hydrolysis yields a crystalline substance $C_{16}H_{19}N_3O_3S$ (VIIIa). Finally, vigorous hydrolysis of VIII or VIIIa yields two ninhydrin-positive compounds $C_{10}H_{13}NO_2$ (VII) and $C_6H_8N_2O_2S$ (III).

The ultraviolet spectrum of VII showed the typical absorption maxima of the phenyl nucleus (252.5–258 and 264 mμ).

By paper electrophoretic methods it could be demonstrated that VII has basic properties whereas the hydroxamic acid test[2] proved that, in addition, a carboxyl group was present in the molecule.

(1) J. M. Waisvisz, *et al.*, THIS JOURNAL, **79**, 4522 (1957).

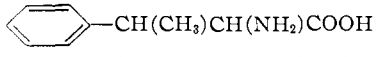
(2) F. Feigl, "Qualitative Analysis by Spot Tests," 2nd ed., Elsevier Press, London, 1939, p. 294.

The fact that crystalline acetyl, 2,4-dinitrophenyl and tosyl derivatives of VII[1] could be prepared indicated the presence of an amino grouping. Potentiometric titrations suggested the presence of an amino acid grouping.

Oxidation of VII with potassium permanganate at room temperature yields acetophenone, which was isolated and identified as its *p*-nitrophenyl- and 2,4-dinitrophenylhydrazones. This indicated a branched side chain and proved the presence of the expected phenyl nucleus. Ultraviolet spectra of VII at alkaline and acid *p*H indicated that no dissociable grouping was attached to the phenyl nucleus.

Decarboxylation and deamination of VII with ninhydrin according to the method of Van Slyke[3] yielded hydratropic aldehyde which was isolated and identified as its 2,4-dinitrophenylhydrazone.

All these data left open only this possibility



VII, α-amino-β-phenylbutyric acid

Apart from the question of the optical activity the validity of these structural deductions was confirmed by synthesis of this amino acid according to E. Fischer.[4] Fischer reported the synthesis of α-amino-γ-phenylbutyric acid, but because of the work of F. Knoop,[4] later agreed[4] that the substance was not the γ-isomer but in fact was α-amino-β-phenylbutyric acid. Although two isomers are possible, DL- and DL-allo, synthetic α-amino-β-phenylbutyric acid proved to be identical with the natural product isolated from bottromycin in respect to analyses, melting range, mixed melting range, melting and mixed melting ranges of the tosyl-, acetyl- and dinitrophenyl derivatives, $R_f$ values on paper chromatograms, ultraviolet and infrared spectra.

The other ninhydrin-positive, optically inactive hydrolytic degradation product of VIII is $C_6H_8N_2O_2S$ (III). This water-soluble compound also is unsaturated. Its ultraviolet spectrum showed maxima at 210 and 242.5 mμ. Paper electrophoretic methods demonstrated the acid properties of III. The presence of a single amino group was shown by the facts that it was possible to prepare only a mono-dinitrophenyl derivative, and that the hydrochloride salt, isolated from an acid hydrolysate, proved to be a monohydrochloride.[1]

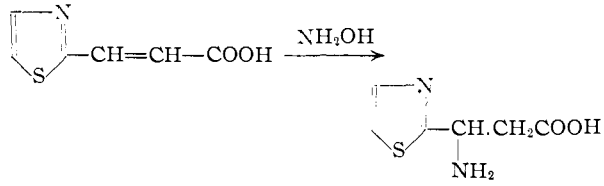
These facts suggested the presence of a heterocyclic nucleus containing sulfur and nitrogen in III. This supposition was supported by the infrared spectrum.

A literature study revealed that R. G. Jones, *et al.*,[5] synthesized a compound which has the same molecular formula and melting point as III, namely, 2-thiazolealanine. A comparative study of the two compounds revealed that they are very similar but not identical. For instance, the mixed melting point had a depression of 5°.

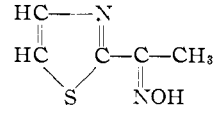
Paper chromatographic methods showed that in one of the four development solvents employed there was a slight difference in $R_f$ value and that the spot produced by compound III turned from brown to purple on exposure to daylight, whereas the spot of 2-thiazolealanine remained brown.

There were only minor differences in the ultraviolet and infrared spectra of the two compounds, the latter indicating that III possibly could have a β-amino acid structure. Paper electrophoresis, on the other hand, showed marked differences: at *p*H 8.5 compound III moved in the direction of the anode, whereas 2-thiazolealanine did not move at all. There was also a marked difference in the melting points of the dinitrophenyl derivatives, that of III melting at 148–151° dec. and that of 2-thiazolealanine at 203.5–210° dec.

Paper chromatographic studies using the method of Crumpler[6] with the aid of copper carbonate were indecisive. Desulfurization of III with Raney nickel catalyst proved unsatisfactory. Oxidation with potassium permanganate, however, yielded a crystalline compound $C_4H_4N_2OS$. This compound proved to be 2-thiazolecarbonamide.[7] This established the presence of a thiazole nucleus substituted in the 2-position. This fact and the infrared spectrum indicated III to be β-(2-thiazole)-β-alanine. We succeeded in synthesizing this compound by the following route. 2-Methylthiazole,[8] prepared from chloroacetaldehyde and thioacetamide,[9] was converted to β-2-thiazoleacrylic acid by the method of Jones, *et al.*[8,10] Condensation with hydroxylamine gave β-(2-thiazole)-β-alanine, m.p. 199–202° dec.



A by-product (m.p. 163–165.5°) was also obtained. It is thought to be methyl 2-thiazolyl ketoxime



Apart from the question of the optical activity the synthetic β-amino acid (m.p. 199–202° dec.) was identical with the natural product as is evidenced by analysis, identical melting points and mixed melting point, identical melting points and mixed melting point of the dinitrophenyl derivatives, same ultraviolet and infrared spectra, same

(3) D. D. Van Slyke, R. T. Dillon, D. A. MacFadyen and P. Hamilton, *J. Biol. Chem.*, **141**, 627 (1941); A. I. Virtanen, T. Laine and T. Toivonen, *Z. physiol. Chem.*, **266**, 198 (1940).

(4) E. Fischer and W. Schmitz, *Ber.*, **39**, 351, 2208 (1906); E. Fischer, *ibid.*, **37**, 3063 (1904); F. Knoop and H. Hoessli, *ibid.*, **39**, 1477 (1906).

(5) R. G. Jones, E. C. Kornfeld and K. C. MacLaughlin, THIS JOURNAL, **72**, 4526 (1950).

(6) H. R. Crumpler and C. E. Dent, *Nature*, **164**, 441 (1949).

(7) H. Erlenmeyer, R. Marbet and H. Schenkel, *Helv. Chim. Acta*, **28**, 924 (1945).

(8) H. Erlenmeyer, O. Weber, P. Schmidt, G. Küng, C. Zinsstag and B. Prijs, *Helv. Chim. Acta*, **31**, 1156 (1948).

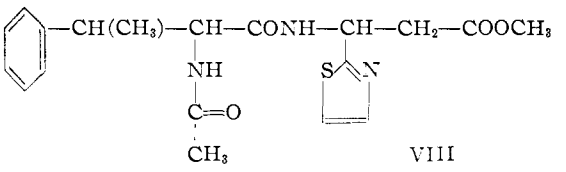
(9) A. Hantzsch, *Ann.*, **250**, 271 (1889).

(10) R. G. Jones, E. C. Kornfeld and K. C. MacLaughlin, THIS JOURNAL, **72**, 4528 (1950).

$R_f$ values on paper chromatograms, and identical behavior on paper electrophoresis.

The structure elucidation of the two amino acids III and VII being completed, there remained the question of the structure of the acetyl degradation compound of bottromycin, $C_{19}H_{23}N_3O_4S$ (VIII) from which these two amino acids were derived. Group analyses showed that VIII contained one acetyl and one methoxyl group.[1] Since neither of the two degradation products of VIII possessed a hydroxyl group, it is very likely that the acetyl group was linked to a nitrogen atom and that the methoxyl group possibly was a methyl ester grouping. The last supposition was supported by Kunz hydrolysis of VIII and by the fact that we were able to prepare a benzylamide derivative of the acetyl compound by reaction with benzylamine.

By lithium aluminum hydride reduction of VIII and acid hydrolysis of the reaction product followed by paper chromatographic study of the products formed we could establish that β-(2-thiazole)-β-alanine was the C-terminal amino acid. By synthesis of the dipeptide from the natural N-acetyl-α-amino-β-phenylbutyric acid[1] and β-(2-thiazole)-β-alanine methyl ester, by the method of Sheehan,[11] we could prove that apart from the question of the optical activity the structure of the acetyl degradation product VIII is



VIII

Analyses, ultraviolet and infrared spectra of the synthetic and natural products were identical. The mixed melting range of the two compounds was not depressed.

## Experimental

**Oxidation of VII with Potassium Permanganate. Isolation of the *p*-Nitro- and 2,4-Dinitrophenylhydrazones of Acetophenone.**—Four hundred and eighty-five mg. of the hydrochloride of VII was dissolved in 27.5 ml. of water. To this solution was added gradually with stirring and external cooling a solution of 553 mg. of potassium permanganate in 27.5 ml. of water, the addition being completed in 55 minutes. After stirring at room temperature for another 50 min. the reaction mixture was heated to a temperature of 50° and stirred for an additional 30 min. The manganese dioxide was filtered off, washed with hot water and the combined filtrates were extracted several times with ether. The combined ether layers (300 ml.) were dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residual oily product (67 mg.) was dissolved in 1 ml. of ethanol and heated on a steam-bath for five minutes with 1 ml. of a solution of *p*-nitrophenylhydrazine (1 g. of *p*-nitrophenylhydrazine dissolved in 2 ml. of 4 *N* hydrochloric acid and 10 ml. of water). After cooling, the orange-colored derivative was filtered off, washed with water and dried. Fifty-one and a half mg. of the *p*-nitrophenylhydrazone was obtained (m.p. 160–178°). After two recrystallizations from 70% ethanol the derivative melted at 177–184° (with sublimation).

The mixed melting point with synthetic material was not depressed. The ultraviolet spectra of both compounds were identical.

Repetition with 485 mg. of VII gave a similar oily product. After heating with 5 ml. of a solution of 2,4-dinitrophenylhydrazine (according to Shriner and Fuson[12]) an orange-colored 2,4-dinitrophenylhydrazone was obtained. Recrystallized twice from ethanol 56 mg. of pure product was obtained which had a m.p. of 243–246° (with sublimation), undepressed on mixture with an authentic sample. The ultraviolet spectra of both compounds were also identical.

**Decarboxylation and Deamination of VII with Ninhydrin. Isolation of Hydratropicaldehyde 2,4-Dinitrophenylhydrazone.**—To a solution of 234 mg. of the hydrochloride of VII 42 g. of sodium chloride and 28 g. of primary potassium phosphate in 280 ml. of water was added 56 ml. of a 1% ninhydrin solution in water. Over a period of 30 minutes the reaction mixture was heated to its boiling point, then steam distilled. To the distillate (80 ml.) was added 10 ml. of a 2,4-dinitrophenylhydrazine solution and the solution was heated briefly on a steam-bath. After cooling the yellow crystals were collected, washed with water and recrystallized from 4 ml. of ethanol. Recrystallized once more 25 mg. of pure 2,4-dinitrophenylhydrazone (m.p. 133–136°) was obtained, not depressed on mixture with an authentic sample. The ultraviolet spectra of both products also proved to be identical.

**The Synthesis of α-Amino-β-phenylbutyric Acid.**—This α-amino acid was prepared by the method of E. Fischer.[4] The starting product, 1-bromo-1-phenylethane, was prepared from acetophenone by hydrogenation.[13] This yielded phenylmethylcarbinol, which was brominated according to the method of J. B. Conant, *et al.*[14] The amino acid obtained was recrystallized from water as a monohydrate and melted at 162–166°. After drying *in vacuo* for four hours at 110° the product had lost its water content and melted at 234° dec.

*Anal.* Calcd. for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82. Found: C, 67.00; H, 7.24; N, 7.75.

**Preparation of Derivatives of α-Amino-β-phenylbutyric Acid. a.—The 2,4-dinitrophenyl derivative** was prepared from 239 mg. of its monohydrate and 0.28 ml. of 2,4-dinitrofluorobenzene according to the method of Sanger.[15] After two recrystallizations from 68% methanol it showed a melting range of 185–213° dec.

**b.—The tosyl derivative** of synthetic α-amino-β-phenylbutyric acid was prepared according to the method described in the previous paper.[1] The product melted at 147–152.5°.

**c.—The acetyl derivative** of the synthetic α-amino-β-phenylbutyric acid was prepared according to the method described in the previous paper.[1] The derivative melted at 185–190°.

**Oxidation of III with Potassium Permanganate. Isolation of 2-Thiazole-carbonamide.**—Four hundred and ninety-one mg. of the hydrochloride of III was dissolved in 27.5 ml. of water. The *p*H of the solution was adjusted to 8 by addition of concentrated sodium hydroxide solution. Gradually with stirring and external cooling (with running water) a solution of 1.24 g. of potassium permanganate in 40 ml. of water was added over a period of 45 min. The reaction mixture was heated to 50° and stirred for an additional 15 min. After cooling the manganese dioxide was filtered off, washed with hot water and the combined filtrates extracted continually with ether. The ether extract was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residual crystalline product (122 mg.) melting at 116–118° was recrystallized from 75 ml. of heptane; obtained, 95 mg. of the acicular crystalline product, melting at 117–118°. A molecular weight determination (Rast-camphor) gave a value of approximately 124.

*Anal.* Calcd. for $C_4H_4N_2OS$: C, 37.51; H, 3.15; N, 21.87; S, 25.00. Found: C, 37.61; H, 3.13; N, 21.66; S, 27.39.

**Synthesis of β-(2-Thiazole)-β-alanine.**—Chloroacetaldehyde was prepared by direct chlorination of paraldehyde according to the method of H. Guinot[16] (yield 46%). Thio-

(11) J. C. Sheehan and G. P. Hess, THIS JOURNAL, **77**, 1067 (1955).

(12) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," 3rd ed., John Wiley and Sons, Inc., New York, N. Y., 1948, p. 174.

(13) H. Adkins and R. Connor, THIS JOURNAL, **53**, 1093 (1931).

(14) J. B. Conant and A. H. Blatt, *ibid.*, **50**, 554 (1928).

(15) F. Sanger, *Biochem. J.*, **39**, 507 (1945).

(16) H. Guinot and J. Tabuteau, *Compt. rend.*, **231**, 234 (1950).

acetamide was obtained by treating acetamide with phosphorus pentasulfide[9] (average yield 30%). 2-Methylthiazole was prepared from above-mentioned components by the method of Erlenmeyer[8] (average yield 45%). 2-(β-Oxy-γ-trichloro)-*n*-propylthiazole and 2-thiazoleacrylic acid were prepared from 2-methylthiazole and trichloroacetaldehyde by the method of Jones.[10] Yields obtained were in agreement with those given by the authors. β-(2-Thiazole)-β-alanine was prepared as follows: 0.46 g. of sodium was dissolved in 16 ml. of absolute ethanol. To this solution was added a hot solution of 1.39 g. of hydroxylamine hydrochloride in 1 ml. of water. After cooling in an ice-bath the precipitated sodium chloride was filtered with suction and the filtercake washed with three portions of 1 ml. each of absolute ethanol. To the combined filtrates was added under shaking 1.55 g. of 2-thiazoleacrylic acid. The solution obtained was heated under reflux for 9 hours on a steam-bath, followed by an additional 14 hours at room temperature. On concentration *in vacuo* to a small volume crystallization occurred. The crystals were collected, washed with small quantities of alcohol and hexane, respectively; obtained, 600 mg. of a cream-colored product (m.p. 195–200°). After recrystallization from 280 ml. of 96% ethyl alcohol 475 mg. of the acicular crystalline compound was isolated (m.p. 199–202° dec.).

*Anal.* Calcd. for $C_6H_8N_2O_2S$: C, 41.86; H, 4.68; N, 16.28; S, 18.59. Found: C, 41.73; H, 4.68; N, 16.12; S, 18.60.

A second crop of crystals (155 mg.) was obtained from the mother liquors by concentration. The melting point of this fraction was 155–162° (under sublimation). After recrystallization from water 80 mg. of a white product was isolated melting at 163–165.5°. Supposedly this compound is methyl 2-thiazolyl ketoxime.

*Anal.* Calcd. for $C_6H_6N_2OS$: C, 42.25; H, 4.26; N, 19.71; S, 22.52. Found: C, 42.24; H, 4.30; N, 19.58; S, 22.56.

**The Dinitrophenyl Derivative of β-(2-Thiazole)-β-alanine.**—Similarly as described for this derivative of III[1] we prepared the 2,4-dinitrophenyl-β-(2-thiazole)-β-alanine from 260 mg. of synthetic β-amino acid and 0.84 mg. of 2,4-dinitrofluorobenzene. After two recrystallizations from 60% methanol 300 mg. of a yellow derivative was obtained. The melting point was 148–151°.

*Anal.* Calcd. for $C_{12}H_{10}N_4O_8S$: C, 42.61; H, 2.98; N, 16.57; S, 9.46. Found: C, 42.51; H, 3.06; N, 16.44; S, 9.52.

**Synthesis of the Benzylamide of VIII.**—A hundred and ninety-nine mg. of VIII was dissolved in 1 ml. of benzylamine on a steam-bath. After an additional 18 hr. at room temperature crystallization occurred. The reaction mixture was heated for another 2 hr. under reflux on a steam-bath and stored for an additional 24 hr. at room temperature. To the crystalline mass was added a mixture of 10 ml. of ethyl acetate and 2 ml. of alcohol, whereupon the suspension was heated to the boiling point, then filtered with suction while still hot; obtained, 117 mg. of a white solid of melting point 286° (dec. after sublimation). After recrystallization from 200 ml. of ethanol 80 mg. of the crystalline compound was isolated. The melting point was 290–291° (dec. under sublimation).

*Anal.* Calcd. for $C_{25}H_{28}N_4O_3S$: C, 64.64; H, 6.08; N, 12.06; S, 6.89. Found: C, 64.36; H, 6.13; N, 12.14; S, 7.41.

**Lithium Aluminum Hydride Reduction of VIII.**—A finely divided suspension of 97 mg. of lithium aluminum hydride in 25 ml. of dry ether was treated with 66 mg. of VIII introduced by extraction (7 hours) from an extraction thimble. After the thimble was removed the heating was continued for another 30 min. and the reaction mixture thereafter kept at room temperature for 16 hours. The excess of lithium aluminum hydride was destroyed by careful addition of 1 ml. of water. The precipitated hydroxides were filtered off and extracted several times with small portions of boiling alcohol. The combined ethereal alcoholic filtrates were evaporated to dryness and the residual oily product was hydrolyzed in a Carius tube for 72 hr. with 2 ml. of concentrated hydrochloric acid at 100–110°. Paper chromatograms run in butanol–acetic acid–water (100:12:100) showed that only the unchanged VII was present in this hydrolyzate, while the spot due to III was no longer observed.

**Methyl Ester of III.**—Two grams of the hydrochloride of III was suspended in 75 ml. of absolute methanol. The suspension was saturated with a stream of dry hydrogen chloride whereupon the compound dissolved. The reaction mixture was heated under reflux for 2.5 hours, then kept for 15 hours in the refrigerator. After evaporation to dryness the residual crystalline mass was dissolved in 10 ml. of ice-water, the solution adjusted to a *p*H of 8.5 by careful addition of a saturated solution of potassium carbonate and extracted several times with ether (180 ml.). The combined extracts were dried over anhydrous sodium sulfate, filtered, then evaporated to dryness; obtained, 1.27 g. yellow colored, oily methyl ester of III.

**Synthesis of VIII.**—One and a half g. of the methyl ester of III, 1.89 g. of the N-acetyl compound of VII[1] and 2.05 g. dicyclohexyl carbodiimide[17] were suspended in 25 ml. of purified tetrahydrofuran. The reaction mixture was shaken occasionally and kept at room temperature for 4 hr. The precipitate (1.9 g., dicyclohexyl carbamide) was removed by filtration and washed with a small quantity of tetrahydrofuran. After addition of a few drops of glacial acetic acid the filtrates were evaporated *in vacuo*, and the tarry residue dissolved in 25 ml. of boiling ethyl acetate. This solution was decolorized with active carbon. After cooling another crop (129 mg.) of dicyclohexyl carbamide was obtained. This was removed by filtration. The filtrate was extracted with three portions of 5 ml. each of 1 *N* hydrochloric acid. The *p*H of the combined extracts was adjusted to 5 by addition of a few drops of 33% potassium hydroxide solution. The resulting solution was re-extracted twice with 10-ml. portions of ethyl acetate. The extracts were dried and concentrated *in vacuo* to a small volume. After cooling crystallization took place. The product was filtered with suction and dried (96 mg.). The melting point was 195–202°. After two recrystallizations from ethyl acetate 34 mg. of synthetic VIII was obtained (m.p. 202–208.5° with sublimation).

*Anal.* Calcd. for $C_{19}H_{23}N_3O_4S$: C, 58.61; H, 5.91; N, 10.80; S, 8.23. Found: C, 58.65; H, 5.85; N, 10.63; S, 8.13.

**Acknowledgments.**—The authors wish to thank Mr. P. J. Hubers and Mr. M. Van Leeuwen for microanalyses, Miss V. Roest and Dr. L. M. Rientsma for infrared absorption determinations, and Professor W. Berends of Delft for many helpful discussions. We are also indebted to the Eli Lilly Research Labs. for a gift of 2- and 4-thiazolealanine, and to Mr. A. K. J. Mulder for the synthesis of α-amino-β-phenylbutyric acid.

DELFT, HOLLAND

(17) E. Schmidt, F. Hitzler and E. Lahde, *Ber.*, **71**, 1938 (1938).