[CONTRIBUTION FROM THE CHEMOTHERAPY DIVISION, STAMFORD RESEARCH LABORATORIES. AMERICAN CYANAMID COMPANY]

CRYSTALLINE PHENYLISOCYANATE DERIVATIVES FROM BENZYLPENICILLOIC ACID AND α-METHYL BENZYLPENICILLOATE

SELBY B. DAVIS, STUART D. WILLSON, AND R. BRUCE VAN ORDER[1]

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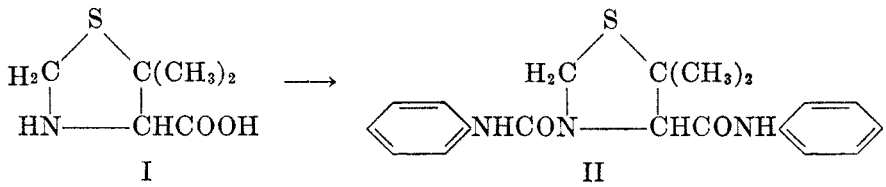
Early in the course of an investigation of the structure of penicillin[2] it was observed that treatment of the latter with cold, aqueous alkali resulted in the production of a new substance, benzylpenicilloic acid. The release of 1 mole of acid during the course of the reaction, the difference between the empirical formula of the product, $C_{16}H_{20}N_2O_5S$, and that of benzylpenicillin, $C_{16}H_{18}N_2O_4S$, and the differences between the infrared spectra of the two, indicated that the treatment with alkali had effected hydrolysis of an inner anhydride. The isolated product, however, proved refractory to crystallization, and was of indefinite melting point. Furthermore, the specific rotation of repeated preparations differed greatly, ranging from 73° to 120° (sodium bicarbonate solution).

A number of attempts were made to prepare a crystalline derivative of definite physical properties. The sodium, potassium, ammonium, and S-(*p*-bromobenzyl)thiuronium salts could not be obtained in crystalline form. Attempted acylation with ketene in acetone solution, and with benzoyl chloride in alkali or in pyridine, led to intractable oils. Crystallization of the oily picrate was not effected. The initial, partially crystalline precipitates produced by treatment with ammonium Reineckate and with ammonium rhodanilate were of inconstant composition and decomposed on attempted recrystallization.

It was then observed that treatment of the model compound, 5,5-dimethylthiazolidine-4-carboxylic acid (I), with phenylisocyanate led to a crystalline dicarbanilinodimethylthiazolidine (II), m.p. 217–217.5°, the structure of which was evident from its properties and the known reactivity of carboxyl (2) and amino groups with phenylisocyanate.



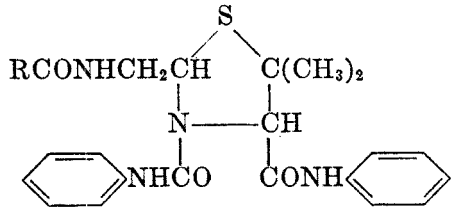
I II

When benzylpenicilloic acid was treated with phenylisocyanate a crystalline derivative was likewise obtained. The empirical formula, $C_{28}H_{30}N_4O_3S$, of the product, m.p. 221–222°, $(\alpha)_D^{22}$ −100°, indicated that two carboxyl groups had

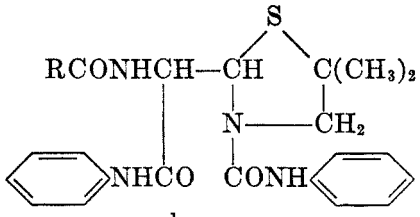
[1] Present address: Calco Chemical Division, American Cyanamid Company, 338 Wilson Avenue, Newark, New Jersey.

[2] Our crystalline isolate, upon which this work was begun (1944) has proved identical with what is now known as benzylpenicillin (1). The latter nomenclature has been; used throughout the present report.

been lost and two carbanilino groups had been introduced during the course of the reaction. The infrared spectrum (Fig. 1) resembled that of the derivative (II) of the model compound, but showed significant differences in the frequencies of several major absorption bands. Of the tentative structures (IIIa, b, and c), deducible from the above data and the previously characterized breakdown products of benzylpenicilloic acid (3, 4), structure (IIIa) appeared most probable.



a.



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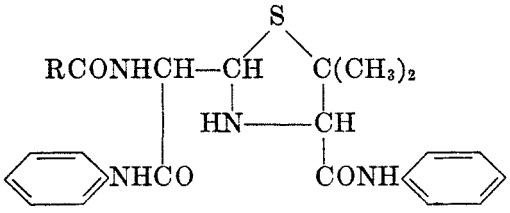
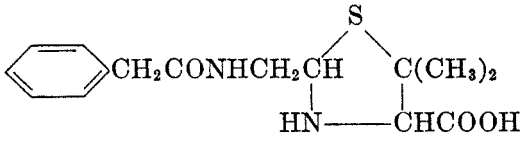


c.

III, R = benzyl

Synthesis of optically inactive 5,5-dimethyl-2-phenacetylaminomethylthiazolidine-4-carboxylic acid (IV) was



IV

accomplished by condensation of phenacetylaminoacetaldehyde with racemic β,β-dimethylcysteine. The amorphous product yielded a crystalline derivative, $C_{28}H_{30}N_4O_3S$, m.p. 207–208°, on treatment with phenylisocyanate. Comparison of the infrared spectrum of this substance with that of the phenylisocyanate derivative obtained from benzylpenicilloic acid showed the two to agree at all points (Fig. 1), establishing structure (IIIa) for the derivative from benzylpenicilloic acid.

Like the product of alkaline hydrolysis, the product of methanolysis of benzylpenicillin, α-methyl benzylpenicilloate, was ill-characterized because of its lack of crystallinity, its indefinite melting point, and the differing specific rotation of successive preparations. Application of the phenylisocyanate reaction to this substance yielded a crystalline derivative, $C_{30}H_{32}N_4O_5S$, m.p. 170–172°, $(\alpha)_D^{24}$ +46°. The properties of this substance, and the previous characterization of

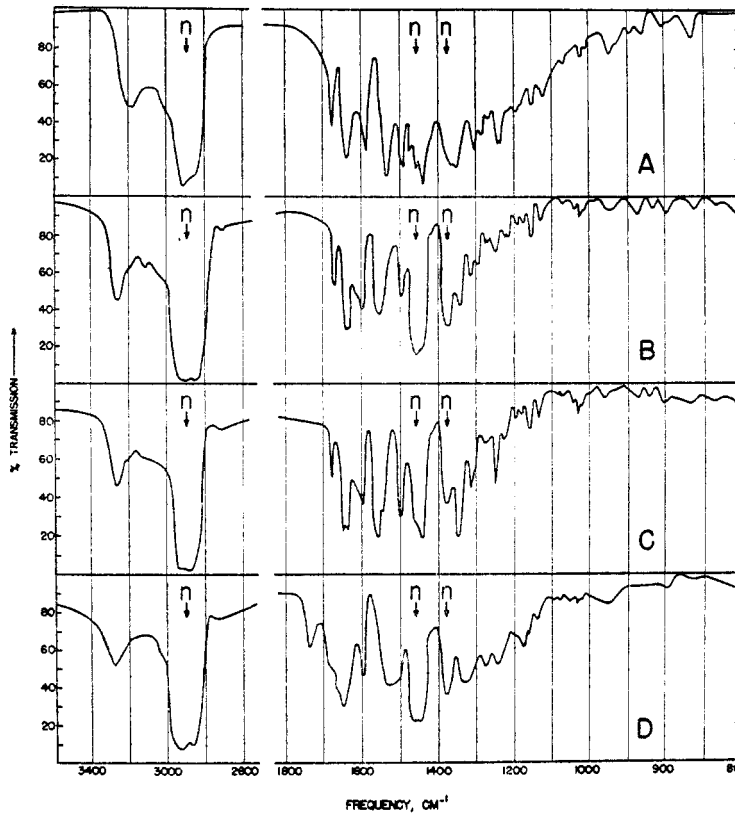
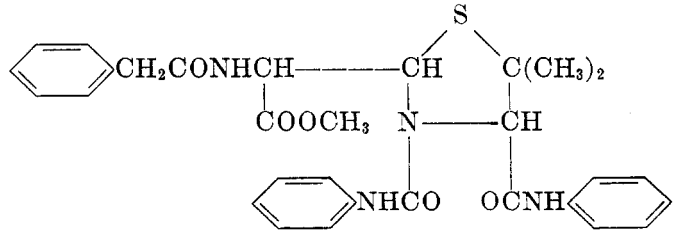


FIG. 1. INFRARED ABSORPTION SPECTRA

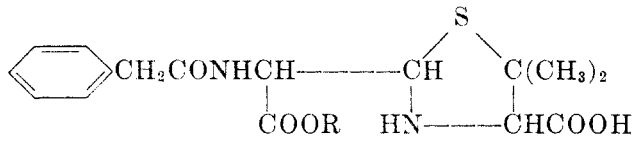
n. Absorption band due to Nujol.
A. 3,4-Dicarbanilino-5,5-dimethylthiazolidine (II).
B. Phenylisocyanate derivative from benzylpenicilloic acid (IIIa).
C. 3,4-Dicarbanilino-5,5-dimethyl-2-phenacetylaminomethylthiazolidine (IIIa).
D. Phenylisocyanate derivative from α-methyl benzylpenicilloate (V).

the breakdown products of methyl benzylpenicilloate (3, 4) served to establish its structure as (V).



V

The structures of benzylpenicilloic acid (VI) and α-methyl benzylpenicilloate (VII), deduced from the above data, agree with those published (4).



VI, R = H

VII, R = CH_3

ACKNOWLEDGMENT

We are grateful to Dr. R. O. Roblin, Jr. of these Laboratories for encouragement and suggestions, and to Misses E. P. Anderson, R. A. Rhodes, and N. E. Shakespeare for aid in the experimental work.

EXPERIMENTAL[3]

3,4-Dicarbanilino-5,5-dimethylthiazolidine (II). A mixture of 50 mg. of 5,5-dimethylthiazolidine-4-carboxylic acid,[4] with 163 mg. of phenylisocyanate was warmed gently until the evolution of gas had virtually ceased. The mixture was then heated for one and one-half hours at 100°. The cooled reaction mixture was triturated with six 0.3-cc. portions of ether, and the solid residue was dried under vacuum. Crystallization of the crude product, 93 mg., m.p. 196–199°, from a minimum quantity of absolute ethanol, yielded 44 mg. (40%) of essentially pure 3,4-dicarbanilino-5,5-dimethylthiazolidine as colorless, columnar crystals, m.p. 215–216.5°. The substance was found to be insoluble in water, 5% sodium bicarbonate, 10% sodium hydroxide, and 10% hydrochloric acid; slightly soluble in petroleum ether, benzene, carbon tetrachloride, ether, and chloroform; moderately soluble in acetone and ethanol; and readily soluble in pyridine. Recrystallization from absolute ethanol prior to analysis raised the m.p. to 217–217.5°.

[3a] Certain of the compounds described in this section have been independently prepared by various groups collaborating with the Committee on Medical Research, O.S.R.D., Washington: 5,5-dimethylthiazolidine-4-carboxylic acid, Cornell University Medical College, *D. 2*, 3, February 3, 1944; benzylpenicilloic acid, Merck & Company, *M. 33*, 2, June 30, 1944; phenacetylaminoacetal, The Upjohn Company, *U. 4*, 12, March 15, 1944; 5,5-dimethyl-2-phenacetylaminomethylthiazolidine-4-carboxylic acid, The Upjohn Company, *U. 4*, 14, March 15, 1944; α-methyl benzylpenicilloate, Abbott Laboratories, *A. 3*, 3, February 14, 1944. After completion of the present work the foregoing reports were made available to us through the courtesy of Professor Hans T. Clarke, College of Physicians and Surgeons, Columbia University.

[3b] All melting points were determined on the Fisher-Johns block.

[4] We are indebted to Dr. E. W. Cook of the Organic Research Division, these Laboratories for a sample of this substance. 5,5-Dimethylthiazolidine-4-carboxylic acid, m.p. 212–213° (dec.), was prepared from β,β-dimethylcysteine and formaldehyde following the thiazolidine-4-carboxylic acid synthesis of Ratner and Clarke (5). β,β-Dimethylcysteine, m.p. 205–206° (dec.), was synthesized by nitration of ethyl dimethylacrylate with nitric acid, addition of benzylmercaptan to the α-nitroester, reduction of the nitro group with tin and hydrochloric acid, acid hydrolysis of the ester, and removal of the benzyl group with sodium in liquid ammonia. An alternative synthesis has since been published (6).

Anal.[5] Calc'd for $C_{19}H_{21}N_3O_2S$: C, 64.20; H, 5.96; N, 11.82; S, 9.02. Mol. wt., 355.4.
Found: C, 64.62; H, 6.28; N, 11.65; S, 8.66. Mol. wt. (Rast), 356.

Benzylpenicilloic acid (VI). A solution of 199 mg. of crystalline sodium benzylpenicillin[6] in 6 cc. of distilled water at 25° was maintained at *p*H 11.4 by the intermittent addition of 0.5002 *N* sodium hydroxide for two hours. By this time the release of acid had become negligible; total alkali required at *p*H 11.4, 0.601 milliequivalents, corresponding to a release of 1.08 moles of acid per mole of penicillin. After standing for a further one-half hour at *p*H 11.4 the solution was acidified to *p*H 2 with 0.5 *N* sulfuric acid and extracted with five 2-cc. portions of butanol. The solvent was removed from the combined extract under vacuum at room temperature. The residue, a colorless glass, was taken up in 5 cc. of acetone, the solution was centrifuged to remove a small portion of insoluble material, and the supernate was evaporated in a stream of nitrogen. Trituration of the residue with three 1.5-cc. portions of ether, followed by drying at room temperature under vacuum, reduced the residue to a white powder consisting of microscopic, nonbirefringent,[7] glassy platelets of benzylpenicilloic acid. Yield, 167 mg. (85%), m.p. *ca.* 112°, $(\alpha)_D^{24}$ +120° (5% sodium bicarbonate, *c*, 0.453), $(\alpha)_D^{24}$ +83° (abs. ethanol, *c*, 0.461). Assay,[8] <0.02 units/mg. The substance was slightly soluble in petroleum ether, benzene, and carbon tetrachloride; moderately soluble in ether, chloroform, and water; readily soluble in acetone, ethanol, pyridine, 5% sodium bicarbonate, 10% sodium hydroxide, and 10% hydrochloric acid.

Anal. Calc'd for $C_{16}H_{20}N_2O_5S$: C, 54.54; H, 5.72; N, 7.95; S, 9.10. Active H (4 atoms per mole), 1.12. Equiv. wt., 176.2.
Found: C, 54.61, 54.41; H, 6.00, 5.99; N, 7.78, 7.79; S, 9.34. Active H (Zerewitinoff), 1.07, 1.09. Equiv. wt., 163, 158; pK_{a_1} = 2.4, pK_{a_2} = 5.2.

Phenylisocyanate derivative from benzylpenicilloic acid (IIIa). A mixture of 196 mg. of benzylpenicilloic acid and 640 mg. of phenylisocyanate was warmed until gas evolution had ceased and was then heated at 100° for one and one-half hours. The reaction mixture was triturated with eight 1.5-cc. portions of ether, and the residue was dried under vacuum, yielding 146 mg. of yellow, crystalline powder, m.p. 184–189° (previous softening). Continuous extraction with ether for seven hours removed most of the *sym*-diphenylurea and low-melting by-products, leaving 110 mg. of colorless crystals, m.p. 191–196°. Final purity was reached only after four crystallizations from absolute ethanol, which yielded 22 mg. (8%) of rosettes of fine, colorless needles of the phenylisocyanate derivative, m.p. 221–222°, $(\alpha)_D^{23}$ −100° (pyridine, *c*, 0.488). The solubility in various solvents was similar to that of 3,4-dicarbanilino-5,5-dimethylthiazolidine.

Anal.[9] Calc'd for $C_{28}H_{30}N_4O_3S$: C, 66.91; H, 6.02; N, 11.15; S, 6.38.
Found: C, 66.76, 66.67; H, 6.31, 6.39; N, 11.01; S, 6.24.

Phenacetylaminoacetal. Aminoacetal was prepared according to Organic Syntheses (8). A 15.5-g. portion of commercial phenacetyl chloride was added to an ice-cold solution of 13.3 g. of aminoacetal and 10.6 g. of anhydrous sodium carbonate in 100 ml. of water. After standing at room temperature for twenty-four hours with intermittent shaking, the mixture was extracted three times with 15-cc. portions of ether. The combined extract was twice

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[5] Analyses were performed by the Microanalytical Laboratory, Technical Service Division, these Laboratories, under the direction of Dr. J. A. Kuck, except as otherwise noted.

[6] These experiments were begun on a crystalline sample (1650 units/mg.) prepared by the authors (3) from crude sodium salt (150 units/mg.) furnished by the Lederle Laboratories Division, American Cyanamid Company. For later samples of crystalline sodium benzylpenicillin we are indebted to Mr. E. F. Williams of the Physics Division, these Laboratories.

[7] We are indebted to Dr. A. F. Kirkpatrick of the Physics Division, these Laboratories, for all microscopic examinations reported.

[8] We are grateful to Misses Nydia Ananenko and Marion Cook for the assays, which were performed by the paper-disc method of Vincent and Vincent (7).

[9] Huffman Microanalytical Laboratories, Denver, Colorado.

washed with 100-cc. portions of 0.05 *N* sulfuric acid, dried over sodium sulfate, and evaporated leaving 12.9 g. of orange oil. Molecular distillation of the crude product at 8 x 10^{-4} mm., bath temperature 135–140°, yielded 9.6 g. (38%) of essentially pure phenacetylaminoacetal, which crystallized on cooling to room temperature. A recrystallized sample, m.p. 35–37°, was prepared by adding several volumes of petroleum ether to a concentrated ether solution of the substance.

Anal. Calc'd for $C_{14}H_{21}NO_3$: N, 5.57. Found: N, 5.68, 5.74.

5,5-Dimethyl-2-phenacetylaminomethylthiazolidine-4-carboxylic acid (IV). A solution of 2.5 g. of phenacetylaminoacetal in 60 cc. of 95% ethanol was cooled in an ice-bath during the addition of 150 cc. of *N* sulfuric acid. The mixture was allowed to stand at room temperature for two and one-half hours and finally heated to 90° to complete hydrolysis of the acetal. After cooling to room temperature, 12.6 g. of sodium bicarbonate was added to bring the solution to neutrality and 1.5 g. of racemic β,β-dimethylcysteine,[4] was stirred in. After the addition of further sodium bicarbonate to *p*H 8 the mixture was allowed to stand at room temperature for twenty-four hours with occasional shaking. The solution was then evaporated to a volume of *ca.* 100 cc. at a maximum temperature of 50°, and extracted with three 20-cc. portions of butanol to remove unreacted aldehyde. After acidification to *p*H 2 with 6 *N* sulfuric acid, the solution was again extracted three times with 20-cc. portions of butanol. The extract was evaporated under vacuum at room temperature and the residue was taken up in a few cc. of acetone. The solution was centrifuged free of a small quantity of insoluble material and the supernate was evaporated leaving 499 mg. of a tacky, yellow-brown solid. The latter was dissolved in 10 cc. of methanol and the solution was readily decolorized with a small quantity of charcoal (Darco G-60). Evaporation of the methanol left 423 mg. of 5,5-dimethyl-2-phenacetylaminomethylthiazolidine-4-carboxylic acid as a pale yellow, glassy residue which was converted to a colorless powder by trituration with three 1-cc. portions of anhydrous ether. Yield, 335 mg. (11%), m.p. *ca.* 120°, assay <0.06 units/mg. The solubility of the substance in organic solvents was somewhat greater than that of benzylpenicilloic acid. The infrared spectrum[10] exhibited only minor differences from that of benzylpenicilloic acid.

Anal. Calc'd for $C_{15}H_{20}N_2O_3S$: C, 58.40; H, 6.54. Active H (3 atoms per mole), 0.98. Equiv. wt., 308.4.
Found: C, 57.03, 57.04; H, 6.76, 6.56. Active H (Zerewitinoff), 0.96, 1.00. Equiv. wt., 293; $pK_a = 4.6$.[11]

Further preparations, by essentially the same method, did not yield more satisfactory analyses.

3,4-Dicarbanilino-5,5-dimethyl-2-phenacetylaminomethylthiazolidine (IIIa). A mixture of 201 mg. of 5,5-dimethyl-2-phenacetylaminomethylthiazolidine-4-carboxylic acid with 618 mg. of phenylisocyanate was warmed until gas evolution had ceased and then heated for one and one-half hours at 100°. The reaction mixture was triturated with six 1.5-cc. portions of ether, yielding 166 mg. of a buff-colored, partially crystalline powder; m.p. 190–195°. The infrared spectrum of the crude product indicated the absence of an appreciable quantity of *sym*-diphenylurea. Two crystallizations from absolute ethanol yielded 80 mg. of colorless needles, m.p. 200–203° (partial dec.). Following preliminary experiments, 60 mg. of the material of m.p. 200–203° was dissolved in 30 cc. of chloroform and the solution was washed with 5 cc. of 5% sodium bicarbonate and then three times with 5-cc. portions of water. Evaporation of the chloroform, after drying over anhydrous calcium sulfate, left 55 mg. of colorless residue. Crystallization of the latter from absolute ethanol yielded 53

[10] For the infrared spectra reported we are indebted to Dr. R. C. Gore of the Physics Division, these Laboratories, under whose direction they were determined. Samples of 1 to 3 mg. were examined in Nujol mull as described in reference (9).

[11] We are grateful to Mr. J. F. Bone of the Chemotherapy Division, these Laboratories, for this titration.

mg. (22%, corrected for material removed for trial purification) of 3,4-dicarbanilino-5,5-dimethyl-2-phenacetylaminomethylthiazolidine, rosettes of colorless needles, m.p. 207–208°. The solubility behavior and infrared spectrum (Fig. 1) were identical with those of the phenylisocyanate derivative, m.p. 221–222°, from benzylpenicilloic acid. The mixed m.p. with the latter was 201–209°.

Anal. Calc'd for $C_{28}H_{30}N_4O_5S$: C, 66.91; H, 6.02; N, 11.15; S, 6.38.
Found: C, 67.18; H, 6.14; N, 11.20; S, 6.41.

α-Methyl benzylpenicilloate (VII). A solution of 202 mg. of crystalline sodium benzylpenicillin in 7.5 cc. of absolute methanol was held at reflux temperature for four hours. The solution was then evaporated in a stream of nitrogen and the residue was dried under vacuum at room temperature. A solution of the entire product in 3 cc. of water was cooled to 0° and acidified to *ca.* *p*H 2 with 1 cc. of 0.5 *N* sulfuric acid. The mixture was then extracted with one 2-cc. and four 0.5-cc. portions of butanol. Evaporation of the extract under vacuum left a glassy residue which was taken up in 2 cc. of acetone. The acetone solution was centrifuged clear and the supernate was evaporated leaving 204 mg. of a pale yellow glass. Trituration of the latter with 0.4 cc. of a 1:1 mixture of ether and petroleum ether yielded 198 mg. (95%) of α-methyl benzylpenicilloate as a colorless powder, m.p. *ca.* 75°, $(\alpha)_D^{24}$ +119° (5% sodium bicarbonate, *c*, 0.418), $(\alpha)_D^{24}$ +94° (abs. ethanol, *c*, 0.508), assay 0.27 units/mg. The substance was somewhat more soluble in organic solvents than benzyl penicilloic acid. The infrared spectra of the two were hardly distinguishable.

Anal. Calc'd for $C_{17}H_{22}N_2O_5S$: C, 55.72, H, 6.05; N, 7.65; S, 8.75. Active H (3 atoms per mole), 0.83. Equiv. wt., 366.4.
Found: C, 55.95, 56.03; H, 6.17, 5.98; N, 7.51, 7.42; S, 8.52, 8.53. Active H (Zerewitinoff), 0.84, 0.82. Equiv. wt., 365, 381; pK_a = 3.7.

Phenylisocyanate derivative from α-methyl benzylpenicilloate (V). A mixture of 161 mg. of α-methyl benzylpenicilloate and 419 mg. of phenylisocyanate was warmed until gas evolution has ceased, and was then heated at 80° for fifteen minutes. The reaction mixture was triturated with six 1-cc. portions of petroleum ether. The undissolved residue, after drying under vacuum, consisted of 272 mg. of light brown powder, m.p. 100–135° (dec.). Following preliminary experiments, a 238-mg. portion of the latter was triturated with one 0.5-cc. and two 0.25-cc. portions of methanol. The colorless, crystalline residue amounted to 46 mg., m.p. 125–155°. Retrituration of the tarry solid recovered from evaporation of the supernates, using one 0.25-cc. and two 0.1-cc. portions of methanol, yielded a further 56 mg. of similar material, m.p. 125–155°. Repetition of the recovery and trituration yielded 17 mg. more of material of similar appearance and m.p. The combined crude yield (119 mg.) was dissolved in 2 cc. of chloroform and the solution was allowed to stand at 0° for several hours, which effected the separation of a few mg. of *sym*-diphenylurea, m.p. 238–240°. More chloroform was added to the filtrate, bringing the volume to 4 cc. The solution was heated to boiling and 4 cc. of hexane was added. After standing in the ice-box for several hours the solution yielded 47 mg. of colorless, microcrystalline powder, m.p. 160–163° (previous softening). Four further crystallizations from the same solvent-pair yielded 17 mg. (8%, corrected for material removed for trial purification) of fine, colorless needles of the phenylisocyanate derivative, m.p. 170–172°, $(\alpha)_D^{27}$ +46° (abs. ethanol, *c*, 0.842). A further crystallization, from acetone-hexane, prior to analysis, removed the last trace of a highly birefringent contaminant observed in the less pure fractions, but did not raise the m.p. The substance was found to be insoluble in water, 5% sodium bicarbonate, 10% sodium hydroxide, and 10% hydrochloric acid; slightly soluble in petroleum ether; moderately soluble in benzene, carbon tetrachloride, and ether; and readily soluble in chloroform, acetone, ethanol, and pyridine. The infrared spectrum (Fig. 1) differed from that of the derivative from benzylpenicilloic acid (IIIa) in being less well resolved and in having an additional absorption band at 1736 cm^{-1} (attributed to ester carbonyl).

Anal.[9] Calc'd for $C_{30}H_{32}N_4O_5S$: C, 64.26; H, 5.75; N, 9.99; S, 5.72.
Found: C, 63.93; H, 6.24; N, 9.82, 9.92; S, 5.52.

SUMMARY

Crystalline phenylisocyanate derivatives have been prepared from 5,5-dimethylthiazolidine-4-carboxlicy acid, α-methyl benzylpenicilloate, and benzylpenicilloic acid. The derivative from the latter was shown to be an optically active form of 3,4-dicarbanilino-5,5-dimethyl-2-phenacetylamino-methylthiazolidine.

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REFERENCES

(1) DU VIGNEAUD, CARPENTER, HOLLEY, LIVERMORE, AND RACHELE, *Science*, **104**, 431 (1946).
(2) NAEGELI AND TYABJI, *Helv. Chim. Acta*, **17**, 931 (1934).
(3) Unpublished data of the authors.
(4) Committee on Medical Research, O.S.R.D., Washington, and Medical Research Council, London, *Science*, **102**, 627 (1945).
(5) RATNER AND CLARKE, *J. Am. Chem. Soc.*, **59**, 200 (1937).
(6) SAVARD, RICHARDSON, AND GRANT, *Can. J. Research*, **24B**, 28 (1946).
(7) VINCENT AND VINCENT, *Proc. Soc. Exp. Biol. and Med.*, **55**, 162 (1944).
(8) ALLEN AND CLARK, *Org. Syntheses*, **24**, 3 (1944).
(9) BARNES, GORE, WILLIAMS, LINSLEY, AND PETERSON, *Anal. Chem.*, **19**, 620 (1947).