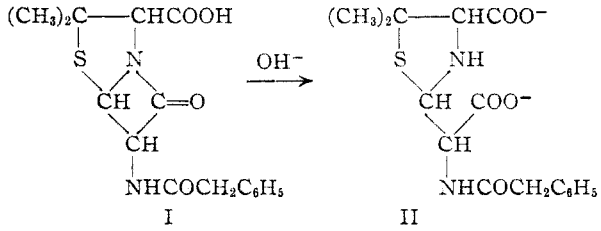
[CONTRIBUTION FROM THE NEW YORK STATE AGRICULTURAL EXPERIMENT STATION]

A β-Lactam with a Methylmercapto Substituent[1]

BY ANN D. HOLLEY AND ROBERT W. HOLLEY

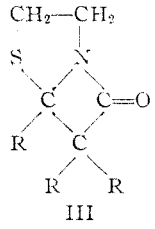
3,3-Dimethyl-1,4-diphenyl-4-methylmercapto-2-azetidinone (IV) has been synthesized. This β-lactam has a sulfur substituent in the desired position for studies relating to the reactivity of benzylpenicillin toward alkali. It has been found that in IV the sulfur substituent does not increase the reactivity of the β-lactam toward alkali.

Benzylpenicillin (I) reacts rapidly with a variety of reagents.[2] The nature of the reaction differs with the type of reagent; with alkali the β-lactam is hydrolyzed and the salt of benzylpenicilloic acid (II) is formed.[2,2a] In this reaction with alkali,



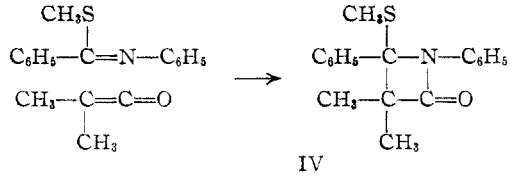
I II

benzylpenicillin is hydrolyzed much more rapidly than model β-lactams which have been studied,[2,3,4] and it is of interest to know what structural features present in benzylpenicillin make the β-lactam so reactive. Previous work[4] has indicated that the reactivity of the β-lactam in benzylpenicillin is greatly increased for the reaction with alkali by either the sulfur substituent or fusion of the β-lactam with a five-membered ring, or by both. These structural features occur together in thiazolidine-β-lactams (III),[5] and in order to estimate



III

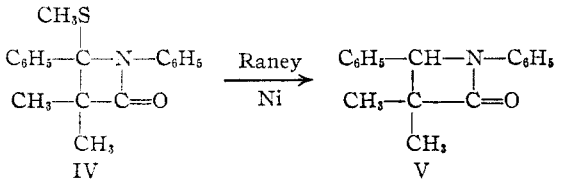
their separate effects, β-lactams having one or the other were desired for further study. Such a β-lactam, 3,3-dimethyl-1,4-diphenyl-4-methylmercapto-2-azetidinone (IV), has been synthesized from S-methylthiobenzanilide (methyl N-phenylthiobenzimidate) and dimethylketene. This preparation is analogous to the preparation of 3,3-



IV

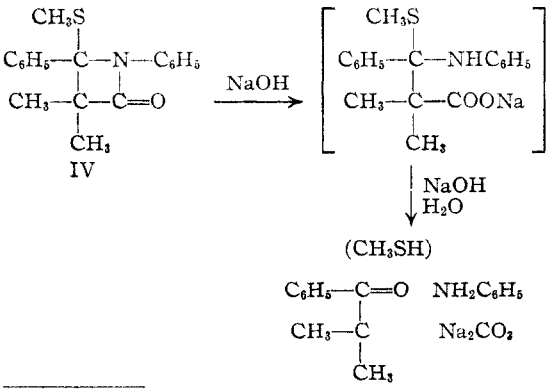
dimethyl-1,4-diphenyl-2-azetidinone (V) from benzalaniline and dimethylketene, a reaction described by Staudinger and Klever.[6] It is also similar to the preparation of substituted thiazolidine-β-lactams.[5]

Compound IV was obtained in 60% yield. It is a colorless solid which melts at 78–80°, and can be distilled under reduced pressure. Its structure was established by elementary analysis, molecular weight determination, and by desulfurization with Raney nickel to the known 3,3-dimethyl-1,4-diphenyl-2-azetidinone (V).[6] The infrared absorption spectra of IV and V show strong absorption bands at 5.73 μ.[7]



IV V

3,3-Dimethyl-1,4-diphenyl-4-methylmercapto-2-azetidinone (IV) was found to be relatively unreactive toward ethanolic hydrogen chloride and sodium hydroxide solutions. Alkaline hydrolysis



IV

(1) Presented in part at the American Chemical Society meeting, Atlantic City, N. J., 1949.

(2) H. T. Clarke, J. R. Johnson and R. Robinson, Editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949.

(2a) In the authors' view, the alkaline hydrolysis of benzylpenicillin may be explained satisfactorily by a simple mechanism involving attack of the hydroxyl ion on the amide function. The more complicated mechanism suggested by Chain for alcoholysis, involving attack on the sulfur atom, (E. Chain, F. J. Philpot and D. Callow, *Arch. Biochem.*, **18**, 171 (1948); E. Chain in H. W. Florey, *et al.*, "Antibiotics," Oxford University Press, London, 1949, p. 816) a suggestion made to explain observed metal catalysis of alcoholysis, seems unnecessary and unlikely for the reaction with alkali. Chain's mechanism involves an intermediate of the penicillenic acid type. However, it is known that methyl benzylpenicillenate, obtained from benzylpenicillin methyl ester by treatment with mercuric chloride in ether, gives, on alkaline hydrolysis, the salt of 2-benzyl-4-hydroxymethylene-5(4)-oxazolone rather than the penicilloic acid (ref. 2, p. 201). Even for alcoholysis, Chain's suggested mechanism must be accepted with caution, since alcoholysis of the sodium salt of benzylpenicillin in CH_3OD results in the incorporation of only 0.2 atom of stable deuterium per molecule (ref. 2, p. 583) rather than the one atom to be expected if an unsaturated intermediate, such as the penicillenic acid, is involved.

(3) R. W. Holley and A. D. Holley, THIS JOURNAL, **71**, 2124, 2129 (1949).

(4) A. D. Holley and R. W. Holley, *ibid.*, **72**, 2771 (1950).

(5) Ref. 2, S. A. Ballard, D. S. Melstrom and C. W. Smith, p. 996.

(6) H. Staudinger and H. W. Klever, *Ber.*, **40**, 1149 (1907).

(7) The spectra were obtained through the courtesy of Dr. Curtis W. Smith and Mr. R. R. Brattain of Shell Development Company.

of the amide link is accompanied by other reactions; isobutyrophenone, aniline and sodium carbonate were isolated, and a strong odor of methyl mercaptan was present. Presumably the amino acid is an intermediate in this reaction, as indicated in the previous equation.

The rates of reaction with alkali during hydrolysis of IV and V are given in Table I. The rate of reaction of V is greater than that of IV.

TABLE I[a]

Compound	Time, hr.	Alkali reacted, %
IV	21.0	14
	22.6	16
	48.0	38
V	4.0	13
	8.0	17.5
	10.5	22.5
	11.6	25.5
	22.6	39.5

[a] Reactions were run using 0.045 mole/l. concentrations of β-lactam and alkali in 85% ethanol at 50 ± 1°.

The reaction of V with alkali has been demonstrated to involve hydrolysis of the β-lactam to the amino acid. If, in the reaction of IV with alkali, the amino acid is the initial product, then clearly V is more reactive than IV. If, however, the reaction of IV with alkali takes place by some path other than the initial formation of the amino acid, then the rate of hydrolysis of IV to the amino acid must be even slower than that indicated by the rate of reaction with alkali. In either case it may be concluded that the β-lactam in IV is less reactive toward alkali than the β-lactam in V, and therefore the methylmercapto substituent present in IV does not facilitate the hydrolysis of the β-lactam.

Because of the differences between the structures of β-lactam IV and benzylpenicillin, conclusions about the reactivity of benzylpenicillin must be arrived at with caution. If the above results do have meaning for the study of the penicillins, they indicate that it must be the fusion of the β-lactam with a five-membered ring that greatly increases the reactivity of benzylpenicillin toward alkali. This is in agreement with the views expressed by Woodward.[8]

Experimental[9]

3,3-Dimethyl-1,4-diphenyl-4-methylmercapto-2-azetidinone (IV).—To a solution of 3.71 g. (0.016 mole) of S-methylthiobenzanilide (methyl N-phenylthiobenzimidate)[10] in 5 ml. of absolute ethyl acetate was added 11 ml. of a cold 1.5 *N* solution of dimethylketene,[5] in absolute ethyl acetate. After a few minutes the solution became quite warm. The solution was allowed to stand for two days at room temperature. The ethyl acetate solution was washed with 10 ml. of 10% sodium carbonate solution and then with water until the washes were neutral (some ether was added to break emulsions). The ethyl acetate–ether solution was dried over anhydrous sodium sulfate and the solvents were removed by distillation. The residual oil, wt. 4.29 g., did not crystallize when allowed to stand at room temperature for several days. Part of the oil was distilled, b.p. approximately 170° at 1 mm., and the following day the distillate crystallized. The remainder of the original oil (2.16 g.) was then seeded and it crystallized to a yellowish, waxy solid m.p. 72–79°. The solid was recrystallized from ligroin; yield 1.46 g., m.p. 77–80°. The yield of once recrystallized β-lactam was therefore 60%. Recrystallization from methanol gave 1.28 g., m.p. 78–80°. Further recrystallization did not change the melting point.

Anal. Calcd. for $C_{18}H_{19}NOS$: C, 72.69; H, 6.44; N, 4.71; S, 10.78; mol. wt., 297.4. Found: C, 72.36; H, 6.62; N, 5.15; S, 11.20; mol. wt. (Rast), 300.

Desulfurization of 3,3-Dimethyl-1,4-diphenyl-4-methylmercapto-2-azetidinone. Formation of 3,3-Dimethyl-1,4-diphenyl-2-azetidinone (V).—A solution of 240 mg. (0.81 millimole) of 3,3-dimethyl-1,4-diphenyl-4-methylmercapto-2-azetidinone in 25 ml. of 95% ethanol was stirred and heated under reflux for one hour with one teaspoon of Raney nickel catalyst.[11] The solution was filtered and the nickel was washed with ethanol. The solution was concentrated, at reduced pressure, to approximately 2 ml., at which point crystallization began. The mixture was cooled and the solid was collected by filtration, 62 mg. (30% yield), m.p. 140–150°. Repeated recrystallization of the product from ethanol gave 25 mg. of 3,3-dimethyl-1,4-diphenyl-2-azetidinone, m.p. 149.5–150.5°. A mixed melting point with authentic 3,3-dimethyl-1,4-diphenyl-2-azetidinone, prepared from benzalaniline and dimethylketene[6] was undepressed.

Reaction of 3,3-Dimethyl-1,4-diphenyl-4-methylmercapto-2-azetidinone with Ethanolic Hydrogen Chloride.—A solution of 254 mg. (0.85 millimole) of the β-lactam in 2 ml. of absolute ethanol was mixed with 0.43 ml. (0.86 milliequivalent) of 2.0 *N* hydrogen chloride in absolute ethanol and the solution was heated at 45° for 24 hours. The solvents were removed at reduced pressure. Absolute ether was added to the residue, and the residue crystallized, wt. 214 mg., m.p. 76–79°. A mixed melting point with starting material was undepressed.

Reaction of 3,3-Dimethyl-1,4-diphenyl-4-methylmercapto-2-azetidinone with Sodium Hydroxide in 85% Ethanol.—A solution of 948 mg. (3.19 millimoles) of the β-lactam in 10 ml. of 85% ethanol was mixed with 6.6 ml. (3.18 milliequivalents) of 0.482 *N* sodium hydroxide in 85% ethanol and the solution was kept at 30°. Aliquots were titrated at intervals with 0.44 *N* hydrochloric acid in 85% ethanol using phenolphthalein as indicator. The following amounts of sodium hydroxide had reacted: 6 days, 10%; 13 days, 16%; 35 days, 48%. The odor of methylmercaptan was noticeable from the first day. After 35 days the remainder (approximately five-sixths) was worked up. Shiny, colorless needles were present in the solution and were collected by filtration, wt. 20 mg.; these crystals were identified as sodium carbonate.

The 85% ethanol solution was evaporated to dryness at reduced pressure and a small amount of ethanol was added to the residue which crystallized. A total of 631 mg. of solid was obtained, m.p. 73–77°. After recrystallization from ethanol, 502 mg. of solid, m.p. 77.5–79.5°, was obtained. A mixed melting point with starting material was undepressed.

The ethanol mother liquors from the recovered starting material were evaporated to dryness and the residue was dissolved in ether. The ethereal solution was extracted with 1 *N* hydrochloric acid. From this acid solution was obtained 7 mg. of aniline identified as benzanilide, m.p. 162–164°, mixed melting point with authentic benzanilide undepressed. The ethereal solution was then washed with water, dried, and the ether distilled. The neutral residue, wt. 142 mg., was apparently a mixture of starting material and isobutyrophenone. From this material, 51 mg. of a 2,4-dinitrophenylhydrazone was obtained melting chiefly at 156–165° but apparently contaminated with starting material. Repeated recrystallization of the 2,4-dinitrophenylhydrazone from ethanol gave 16 mg., micro m.p. 160.5–163.5°. A mixed melting point with the 2,4-dinitrophenylhydrazone[12] of authentic isobutyrophenone was undepressed.

Alkaline Hydrolysis of 3,3-Dimethyl-1,4-diphenyl-2-azetidinone (V).—A solution of 251 mg. (1.0 millimole) of V, 8 ml. of 85% ethanol, and 2.08 ml. of 0.482 *N* sodium hydroxide (1.0 milliequivalent) was heated at reflux for 16 hours. The solution was evaporated nearly to dryness.

(8) Ref. 2, R. B. Woodward, p. 443.

(9) All melting points were determined on a micro melting point block and are corrected.

(10) P. May, *J. Chem. Soc.*, **103**, 2272 (1913).

(11) R. Mozingo, D. E. Wolf, S. A. Harris and K. Folkers, THIS JOURNAL, **65**, 1013 (1943).

(12) D. P. Evans, *J. Chem. Soc.*, 785 (1936).

The residue was taken up in water and extracted repeatedly with ether. The aqueous solution was acidified with 1.0 ml. of 0.98 *N* hydrochloric acid. The precipitated solid was extracted into ether. After drying, the ether solution was evaporated and there remained 194 mg. of crystalline solid (72% yield). This was recrystallized from 4 ml. of 50% ethanol to yield 160 mg. of β-anilino-α,α-dimethylhydrocinnamic acid, m.p. 167–169°, neutralization equivalent 265 (calcd. 269).

GENEVA, N. Y. RECEIVED DECEMBER 21, 1950

[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

A Synthesis of Crotonoside[1]

BY JOHN DAVOLL[2]

Treatment of 2,6-diamino-9-β-D-ribofuranosylpurine with nitrous acid yields 9-β-D-ribofuranosylisoguanine, identical with the natural nucleoside, crotonoside.

In 1932, Cherbuliez and Bernhard[3] isolated from croton beans (*Croton tiglium* L.) a purine derivative which they named crotonoside, and showed to yield isoguanine and D-ribose on hydrolysis with dilute mineral acid. The identification of the sugar as D-ribose was later confirmed by Spies and Drake.[4] Falconer, Gulland and Story's[5] findings that the ultraviolet absorption spectrum of crotonoside closely resembles that of 9-methylisoguanine, and that treatment of crotonoside with nitrous acid gives a ribosylxanthine with an absorption spectrum resembling that of xanthosine and 9-methylxanthine, indicate that the point of attachment of the sugar in crotonoside is position 9. However, a definite identification of the deaminated crotonoside with xanthosine was not made, and no information has so far been available on the size of the lactol ring or the configuration at the sugar-purine link in crotonoside, although the similarity of its optical rotation to that of guanosine indicated that it was probably a β-ribofuranosylpurine.

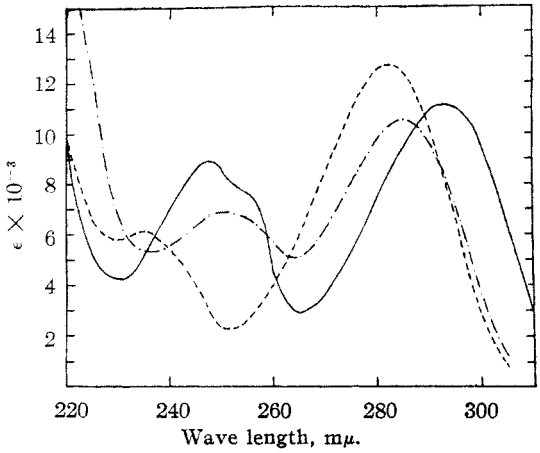


Fig. 1.—Ultraviolet absorption spectra of crotonoside, 22.9 mg. per liter: ———, water; - - - - - - -, 0.05 *N* HCl; -·-·-·-·-, 0.05 *N* NaOH.

Spies[6] has shown that isoguanine is resistant to deamination with nitrous acid, and Bendich[7] has observed that treatment of 2,6-diaminopurine with an excess of nitrous acid leads only to isoguanine, not to xanthine. This suggested that treatment of the 2,6-diamino-9-glycosylpurines reported recently[8] with nitrous acid might yield 9-glycosylisoguanines, and this proved to be the case. Treatment of 2,6-diamino-9-β-D-glucopyranosylpurine with nitrous acid for five minutes at 50° gave 9-β-D-glucopyranosylisoguanine in 55% yield. This compound has previously been prepared by treatment of 9-tetraacetyl-β-D-glucopyranosyl-2-methylsulfonyladenine with sodium hydroxide.[9] Similarly, 2,6-diamino-9-β-D-ribofuranosylpurine was converted to 9-β-D-ribofuranosylisoguanine in 57% yield.

A comparison of this compound with crotonoside was then made. The compounds were identical in melting point, optical rotation, R_f value on paper chromatograms, ultraviolet absorption spectra in water, 0.05 *N* hydrochloric acid or 0.05 *N* sodium hydroxide (Fig. 1), and in behavior toward sodium metaperiodate. Dr. Carl Clark, of the Physiology Department, Cornell University Medical College, determined the X-ray powder diffraction patterns (Table I) and infrared absorption spectra (Fig. 2) of anhydrous specimens of the natural and synthetic materials. The synthetic and natural samples show good general agreement, although the additional bands observed in the diffraction pattern and infrared absorption spectrum of the synthetic sample probably indicate the presence of a small quantity of impurity not detected by chemical methods. The decomposition points of the picrates were the same, and mixtures showed no depression. Mixtures of crotonoside and 9-β-D-ribofuranosylisoguanine showed a slight depression of melting point (one to two degrees), but since similar depressions were observed between different analytically pure preparations of the synthetic compound, this is not considered to indicate any difference in structure.

It is thus concluded that crotonoside may be fully described as 9-β-D-ribofuranosylisoguanine. Some of the properties of crotonoside, however,

(1) The author wishes to acknowledge the support of the National Cancer Institute of the United States Public Health Service and the Atomic Energy Commission, Contract AT(30-1)-910.

(2) Postdoctorate Fellow of the National Cancer Institute, United States Public Health Service.

(3) E. Cherbuliez and K. Bernhard, *Helv. Chim. Acta*, **15**, 464, 978 (1932).

(4) J. R. Spies and N. L. Drake, THIS JOURNAL, **57**, 774 (1935).

(5) R. Falconer, J. M. Gulland and L. F. Story, *J. Chem. Soc.*, 1784 (1939).

(6) J. R. Spies, THIS JOURNAL, **61**, 350 (1939).

(7) A. Bendich, unpublished.

(8) J. Davoll and B. A. Lowy, THIS JOURNAL, **73**, 1650 (1951).

(9) K. J. M. Andrews, N. Anand, A. R. Todd and A. Topham, *J. Chem. Soc.*, 2490 (1949).