methylacetamide became complete after addition of 0.5 g. (5 mmoles) of acetic anhydride. The mixture was allowed to stand for 3 days and was poured into 400 ml. of ether. Filtration and air drying gave 1.68 g. of yellow material which assayed 220 units/mg. A sample of vancomycin treated in the same fashion was devoid of antibiotic activity.

Reaction of Vancomycin with Diethylaminopropylene Oxide.—A solution of 1.6 g. (0.5 mmole) of vancomycin c.r. was prepared in 25 ml. of water acidified to pH 2 with 2 drops of concentrated sulfuric acid. The addition of 1.29 g. (10 mmoles) of diethylaminopropylene oxide raised the pH to about 9 but solution remained complete. After 20 hr., some barium carbonate was added; the mixture was filtered, and the filtrate was taken to dryness. The residue was triturated with acetone to help remove excess epoxide. After air drying, the product weighed 1.6 g. The antibiotic activity was almost entirely destroyed. A sample was retriturated with acetone prior to analysis.

Anal. Found: Cl, 3.15; N, 9.04.

The infrared spectrum no longer indicated the presence of any phenolic groups.

Acknowledgment.—The author is grateful to Dr. H. E. Boaz and D. O. Woolf for their invaluable assistance with the physical-chemical data, to Dr. J. C. Martin of the University of Illinois for the n.m.r. data on the vancomycin acid, to H. L. Bird, Jr., for paper chromatography, to Miss Ann Van Camp for X-ray crystallography, and to W. L. Brown, G. M. Maciak, H. L. Hunter, and R. M. Hughes for the microanalyses.

The Chemistry of Cephalosporin Antibiotics. III. Acylation of Cephalosporadesates

EARLE VAN HEYNINGEN

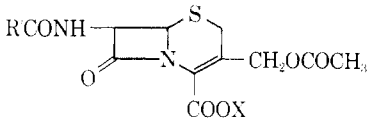
The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis 6, Indiana

Received July 23, 1964

Deacetylated cephalosporins were prepared by enzymatic cleavage of the acetoxyl group. Although many usual methods of acylation failed, aroylation of the cephalosporadesic acids was possible in basic aqueous acetone. The biological activities of several derivatives are reported.

As indicated in other reports, 7-acylamidocephalosporanic acids prepared from 7-aminocephalosporanic acid and acylating agents have shown improved antibiotic activity when compared with naturally produced cephalosporin-C (I), where the 7-acyl function is an aminoadipyl group.[1] Cephalosporins have several points where modification of substituents might produce improved biological activity: one is the acetoxyl substituting the 3-methyl group.[2] This paper reports the chemistry involved in attempted variation of the acetoxyl and biological activity of some new derivatives. Cephalosporins in which the 7-acylamido group is either the 2-thiopheneacetamido group (II)[3] or the phenylmercaptoacetamido group (III) served as starting materials for this investigation.

Several routes to new 3-acyloxymethyl derivatives were explored. Nucleophilic displacement of the acetoxyl group of cephalosporin C has been reported.[4] Attempts to replace the acetoxyl group by nucleophilic displacement with an acid salt, in this case sodium butyrate, in water solution yielded no new product other than those of hydrolysis. Bioautographs of paper chromatograms of the product showed no new biologically active materials of expected mobility. This result is not surprising since aliphatic acid salts do not differ greatly in nucleophilicity.



I, R′ = $HO_2CCH(NH_2)(CH_2)_3$; X = H
II, R′ = C_4H_3S—2—CH_2; X = Na
III, R′ = $C_6H_5SCH_2$; X = Na

Another approach to the desired products would be to remove the acetoxyl group of a cephalosporin and acylate the resultant hydroxymethyl group. Abraham[1b] has shown that desacetylcephalosporins can be prepared by acid hydrolysis, but the concomitant hydrolysis of the β-lactam ring disqualified this method as one of practical value. Jensen, *et al.*,[5] have demonstrated that enzymatic hydrolysis of an acetoxyl can be performed with the orange peel enzyme, citrus acetylesterase. Using this technique desacetylcephalosporins were prepared in good yield. The latter have been assigned the trivial name of cephalosporadesic acids for convenience.

The anticipated simple acylation of the hydroxymethyl group was not realized in practice. One barrier to acylation was the easy formation of cephalosporadesolactones. The lactone was quite readily formed in acid solution although it was possible to isolate the free acid in an analytically pure state. It was found that acylation with an acidic reagent such as acetic anhydride is also precluded by the more facile lactone formation; in the case of 7-phenylmercaptoacetamidocephalosporadesic acid, treatment of the acid with acetic anhydride is a good method for the preparation of the corresponding lactone. Ketene at room temperature, likewise, did not acylate the hydroxyl group; lactone and cephalosporadesic acid were the only materials isolated.

(1) (a) R. R. Chauvette, E. H. Flynn, B. G. Jackson, E. R. Lavagnino, R. B. Morin, R. A. Mueller, R. P. Pioch, R. W. Roeske, C. W. Ryan, J. L. Spencer, and E. Van Heyningen, *J. Am. Chem. Soc.*, **84**, 3401 (1962); (b) B. Loder, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, **79**, 408 (1961).

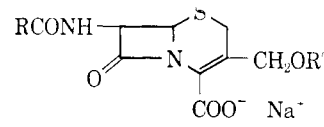
(2) For the convention adopted for naming and numbering the cephalosporin nucleus, see R. B. Morin, B. G. Jackson, E. H. Flynn, and R. W. Roeske, *J. Am. Chem. Soc.*, **84**, 3400 (1962).

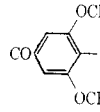
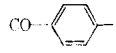
(3) Keflin® (cephalothin, Lilly).

(4) C. W. Hale, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, **79**, 403 (1961).

(5) E. F. Jensen, R. Jang, and L. R. MacDonnell, *Arch. Biochem.*, **15**, 415 (1947).

TABLE I



R	R′	Penicillin G, units/mg.	*S. aureus*,[a] γ/ml.	Penicillin-resistant[b] *S. aureus*, γ/ml.	Hemolytic streptococci[c] ED_{50}, mg./kg. × 2	Gram negative,[d] γ/ml. *Shigella* sp.	Gram negative,[d] γ/ml. *E. coli*	Gram negative,[d] γ/ml. *Klebsiella* sp.
	$COCH_3$	300	0.02	0.8–1.0	41.2	7	7	6
	COC_6H_5	470	0.025	0.1–9.0	>41.2	18	37	25
		163	0.2	6–13	24.4	53	63	49
$C_6H_5SCH_2$		266	0.1	0.3–3.0	>41.2	192	110	160
$C_6H_5SCH_2$		850–990	0.006	<0.1–>8	>41.2	49	54	20

[a] M.I.C. value obtained in a serial dilution test. [b] Gradient-plate M.I.C. value; the first value is without serum, the second, with human serum; C. W. Godzeski, G. Brier, and D. E. Pavey, *Appl. Microbiol.*, **11**, 122 (1963). [c] Drug administered orally to mice, 1 and 5 hr. post infection with 2500–6000 × LD_{50} of inoculum; W. S. Boniece, W. E. Wick, D. H. Holmes, and C. E. Redman, *J. Bacteriol.*, **84**, 1292 (1962). [d] Gradient-plate M.I.C. test; see ref. 7.

Because lactone formation is preferred to O-acylation, it was thought feasible to protect the carboxyl group as an ester and then acylate the hydroxyl and finally cleave the ester. The β-lactam ring in cephalosporins is quite stable to acid but labile in base above pH 9.5; consequently, base saponification would not be practicable. An attempt to cleave methyl 7-phenylmercaptocephalosporanate with lithium iodide in the usual fashion in collidine or pyridine gave black tars. The preparation of the acid hydrolyzable methoxymethyl ester of cephalosporadesic acid (III) was not successful, only lactone being isolated. The method employed, however, would not have produced much ester since the cephalosporanic acid, itself, gave only a trace of its methoxymethyl ester under similar conditions.

It was found possible, however, to obtain aroyl derivatives of the cephalosporadesates by a Schotten–Baumann reaction using aroyl chlorides and sodium hydroxide in aqueous acetone. Under the same conditions aliphatic acid chlorides reacted with water preferentially and no acylation took place. Even with aroyl halides, a large excess of aroyl halide was necessary to obtain even moderate yields. Some variation was made in the pH of the acylating mixture, but best yields seemed to result by maintaining the pH relatively high. Evidently, the acid halides are much more readily attacked than the β-lactam ring of the cephalosporadesates and they consume any local excess alkali before it can destroy the β-lactam ring, at least to a large degree. Much aroyl anhydride is formed in the reaction, but it is readily soluble in ethyl acetate and can be extracted from the water solution or precipitated salt of the new cephalosporin.

The acylation with aroyl halides seems to be fairly general, and two examples with two different 7-acylamidocephalosporadesic acids have been included. Analyses and titrations are of greater utility for characterization of products than melting points which were over rather long ranges and varied somewhat with the temperature at which the sample was placed on the melting point block.

The biological activities of the aroyl derivatives are listed in Table I. It is quite apparent that in this limited series there has been little or no improvement, on the average, over the activity shown by cephalothin. The most serious problem may be the strong serum-binding effect which causes "skip-zones"[6] in the gradient-plate test[7] against penicillin-resistant *Staphylococcus aureus*. The *in vivo* activity of the thenoyl derivative is against the admittedly sensitive *Streptococcus pyogenes*, but the result does question the practical significance of the serum-binding effect.

Experimental[8]

Potassium 7-Phenylmercaptoacetamidocephalosporanate (III).—A solution of 3.89 g. (0.0143 mole) of 7-aminocephalosporanic acid[2] (7-ACA) and 3.89 g. of sodium bicarbonate in 200 ml. of water and 160 ml. of acetone was cooled in an ice bath and stirred as 2.54 g. (0.0136 mole) of phenylmercaptoacetyl chloride in 25 ml. of acetone was added dropwise over 1 hr. It was then stirred an additional 1.5 hr. The acetone was removed by evaporation and the chilled water solution, layered with a small amount of ethyl acetate, was adjusted to pH 2. More ethyl acetate was used to extract the cephalosporanic acid. The acid was back-extracted into 150 ml. of water by adjusting the mixture to pH 5.75 with 1 *N* KOH solution. This extraction was repeated with two 25-ml. portions of water. The combined water layers were evaporated to dryness and the residue was dried in a vacuum desiccator. The yellow solid was recrystallized by solution in 150 ml. of hot methanol, filtration and dilution to cloudiness with 2-propanol, and refrigeration. The near white, dry product weighed 3.83 g. (0.0083 mole), 58% yield, m.p. 162–165° dec. The ultraviolet spectrum showed a maximum at 250 mμ (ε 12,100).

Anal. Calcd. for $C_{18}H_{17}KN_2O_6S_2$: C, 46.94; H, 3.72; N, 6.08. Found: C, 46.65; H, 3.70; N, 5.97.

(6) C. W. Godzeski, R. M. Hisker, and G. Brier, "Antimicrobial Agents and Chemotherapy," American Society for Microbiology, Detroit, Mich., 1963, p. 507.

(7) V. Bryson and W. Szybalski, *Science*, **116**, 45 (1952).

(8) All melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. All evaporations have been performed at temperatures only slightly above room temperature in a rotary evaporator *in vacuo*. The analyses of the salts of the acids were obtained on samples dried for 1–2 min. on a block heated to 100–130°. The paper chromatograms were performed with ethyl methyl ketone saturated with water as the eluting solvent.

Potassium 7-(2-Thiopheneacetamido)cephalosporanate (II).—In a preparation similar to that above, 7-ACA was acylated with 2-thiopheneacetyl chloride. The product melted at 140–143° dec. The ultraviolet maxima were 236 mμ (ε 12,900) and 260 mμ (ε 8030).

Anal. Calcd. for $C_{16}H_{15}KN_2O_6S_2$: C, 44.22; H, 3.47; N, 6.45. Found: C, 44.46; H, 3.86; N, 6.49.

Potassium 7-Phenylmercaptoacetamidocephalosporadesate.—Potassium 7-phenylmercaptoacetamidocephalosporanate (3.83 g., 0.0083 mole) was deacetylated by the use of orange peel enzyme according to Jensen, *et al.*[5]

The solution from the enzymolysis (1 l.) was chilled. Then, 100 g. of finely powdered NaCl was added and dissolved. The solution was layered with 500 ml. of ethyl acetate and, while being stirred, adjusted from pH 8 to 2 with HCl. A partial emulsion formed which could be broken by suction filtration through several large Büchner funnels. The protein collecting on the filter paper quickly clogged the pores of the paper so filtration through a large area of paper was necessary. The sticky material on the paper was washed with cold ethyl acetate. The ethyl acetate layer was separated, washed with a small amount of cold water, then layered over 200 ml. of water, and adjusted to pH 5.5 with 6 *N* KOH solution. The water layer was evaporated at less than 40° and the residue was finally dried over KOH pellets in a vacuum desiccator. The product was recrystallized by solution in boiling hot methanol, dilution to turbidity with 2-propanol, and chilling. The light yellow product weighed, after drying, 2.2 g. (0.00525 mole), 63.5%, m.p. 190–192° dec., violet coloration starting at 130°.

Anal. Calcd. for $C_{16}H_{15}KN_2O_5S_2$: C, 45.91; H, 3.61; N, 6.69. Found: C, 45.95; H, 3.84; N, 6.63.

A 200-mg. sample of the salt in water was converted into the acid by acidification to pH 2. The acid was extracted into ethyl acetate. Evaporation gave a yellow glass that was dissolved in ethyl acetate with mild heating. The solution was diluted with petroleum ether (b.p. 60–71°) and chilled. The flocculent yellow-white product after vacuum drying weighed 110 mg. (55%), m.p. 105° (softened at 98°).

Anal. Calcd. for $C_{16}H_{14}N_2O_5S_2$: C, 50.51; H, 4.24; N, 7.36. Found: C, 50.49; H, 4.33; N, 7.54.

Potassium 7-(2-Thiopheneacetamido)cephalosporadesate.—In a preparation similar to that above, 10 g. (0.0231 mole) of sodium 7-(2-thiopheneacetamido)cephalosporanate was converted to the cephalosporadesate in a 6.67-g. (0.017-mole, 73.5%) yield, m.p. 194–198° dec. The bioautograph of the paper chromatogram showed only one biologically active spot. The ultraviolet maxima were 235 mμ (ε 12,100) and 260 mμ (ε 7110).

Anal. Calcd. for $C_{14}H_{13}KN_2O_5S_2$: C, 42.84; H, 3.33; N, 7.14. Found: C, 42.58; H, 3.46; N, 6.82.

Methyl 7-Phenylmercaptoacetamidocephalosporadesate.—The potassium salt (400 mg., 0.000955 mole) was dissolved in water and layered with ethyl acetate, and the solution was adjusted to pH 2 with 1 *N* HCl in the cold. The ethyl acetate solution was washed with water and then chilled while an excess of diazomethane in ether was added (0.002 mole). After 15 min. the solution was evaporated to yield a white solid. A thin layer chromatogram on silica, ethyl acetate as eluent, showed two spots, one following immediately behind the other. When the chromatogram was run with lactone (see below) on the same plate, the leading spot proved to be the ester and the trailing spot the lactone. The product was recrystallized three times from ethyl acetate, m.p. 171–172° (80 mg.). Thin layer chromatography of this material showed a very faint trace of lactone.

Anal. Calcd. for $C_{17}H_{18}N_2O_5S_2$: C, 51.76; H, 4.60; N, 7.10. Found: C, 51.62; H, 4.61; N, 6.88.

The ultraviolet maximum was 250 mμ (ε 11,200); the infrared spectrum clearly showed the ester absorption band at 1730 cm.$^{-1}$.

Methyl 7-Phenylmercaptoacetamidocephalosporanate.—Potassium 7-phenylmercaptocephalosporanate (1.0 g.) was converted as above into the methyl ester in a 0.58-g. (57%) yield. Twice recrystallized from ethyl acetate, it melted at 153–154°, ultraviolet absorption at 252 mμ (ε 11,850).

Anal. Calcd. for $C_{19}H_{20}N_2O_6S_2$: C, 52.29; H, 4.62. Found: C, 52.16; H, 4.83.

Methoxymethyl 7-Phenylmercaptoacetamidocephalosporanate.—In a 50-ml. flask, a suspension of 200 mg. (0.433 mmole) of potassium 7-phenylmercaptoacetamidocephalosporanate in 20 ml. of dry tetrahydrofuran was stirred, and 42 mg. (0.52 mmole) of chloromethyl ether in 5 ml. of tetrahydrofuran was added. The flask was stoppered and the suspension was stirred for 18 hr. The reaction mixture in thin layer chromatography (silica and ethyl acetate) showed a spot with an R_f value like that of the methyl ester. The solvent was evaporated and the residue was extracted with hot ethyl acetate (10 ml.). Dilution of the extract with petroleum ether (60–71°) gave a sticky product which was removed by filtration. Further dilution of the filtrate and chilling yielded about 10 mg. of fine, feathery crystals, m.p. 99.5–101.5°.

Anal. Calcd. for $C_{20}H_{22}N_2O_7S_2$: C, 51.49; H, 4.87; N, 6.01. Found: C, 51.07; H, 5.08; N, 6.23.

7-Phenylmercaptoacetamidocephalosporanolactone. Acid Method.—A solution of 7-phenylmercaptoacetamidocephalosporadesic acid was obtained from its potassium salt (500 mg.) by acidification to pH 2 of a solution of the latter in 20 ml. of 1:1 acetone–water. After standing for 2 hr., the solution was adjusted to pH 7 with 1 *N* NaOH solution and evaporated to remove the acetone. The water was extracted with ethyl acetate and the ethyl acetate solution was dried with sodium sulfate. The residue from the evaporation of the ethyl acetate solution was recrystallized from ethyl acetate and petroleum ether to give 0.0272 g. of crude lactone. An ethyl acetate solution of the lactone was filtered through a column of Florisil and the eluates were concentrated to dryness. The lactone was recrystallized from ethyl acetate, m.p. 181.5–183.5°, ultraviolet absorption maximum at 250 mμ (ε 12,050).

Anal. Calcd. for $C_{16}H_{14}N_2O_4S_2$: C, 53.02; H, 3.89; N, 7.73. Found: C, 53.27; H, 3.89; N, 7.91.

Acetic Anhydride Method.—Potassium 7-phenylmercaptoacetamidocephalosporadesate (500 mg.) in 20 ml. of water was layered with 20 ml. of ethyl acetate and adjusted to pH 2. The ethyl acetate solution was evaporated to dryness at room temperature, and the residue was dissolved in 20 ml. of acetic anhydride and allowed to stand at room temperature overnight. The acetic anhydride was removed by evaporation, then 40 ml. each of water and ethyl acetate was added, and after being well shaken the layers were separated. The ethyl acetate layer was extracted with water to pH 6 and on evaporation gave the crude lactone. It was recrystallized by solution in ethyl acetate and addition of petroleum ether (60–71°), m.p. 182–182.5°, 0.3-g. (0.00827-mole, 69%) yield. The infrared spectra of samples from the two preparations were identical.

Sodium O-3,4,5-Trimethoxybenzoyl-7-phenylmercaptoacetamidocephalosporadesate.—A solution of 500 mg. (0.0012 mole) of potassium 7-phenylmercaptoacetamidocephalosporadesate in 15 ml. of water and 15 ml. of acetone was placed in a beaker chilled in an ice bath and stirred with a magnetic stirrer; the electrodes of a pH meter were placed in the solution so that the pH could be continuously monitored. Then 1.85 g. of 3,4,5-trimethoxybenzoyl chloride (0.008 mole) was added in several portions. After each portion was added, 1 *N* NaOH solution was added from a buret so that the solution was at approximately pH 8. Initially, the rate of addition had to be quite rapid. The next portion of acid chloride was only added after the pH seemed to be fairly constant for several minutes. At the end of the addition acetone was removed from the clear solution by evaporation. An oil separated from the water layer which was removed by extraction with ethyl acetate to give a clear, yellow solution. About 5.0 ml. of saturated NaCl solution was added, which caused an immediate turbidity and soon a solid separated on chilling. The filtered product, when dried in a vacuum desiccator, weighed 600 mg. It was recrystallized by solution in warm methanol and the addition of 2-propanol to cloudiness and chilling. The filtered, dried product weighed 250 mg. (0.00043 mole, 34.5%), m.p. 138–142° dec.

Anal. Calcd. for $C_{26}H_{25}N_2NaO_9S_2 \cdot 0.5H_2O$: C, 51.56; H, 4.33; N, 4.63. Found: C, 51.60; H, 4.59; N, 4.30.

The infrared spectrum was consistent; the ultraviolet maximum was at 262 mμ (ε 18,000). The titration gave a pK_a = 4.83 and an apparent molecular weight of 625 (calcd., 605).

Sodium O-*p*-Toluoyl-7-phenylmercaptoacetamidocephalosporadesate.—In the same manner as above, 300 mg. of the desacetyl acid salt was converted into the ester derivative with 330 mg. of *p*-toluoyl chloride. The sodium salt separated after the removal of the acetone and was triturated in hot ethyl acetate before being dried. There was ultimately obtained 200 mg. of pure product (0.000385 mole, 57%), m.p. 147–150° dec.

Anal. Calcd. for $C_{24}H_{21}N_2NaO_6S_2 \cdot 0.5H_2O$: C, 54.33; H, 4.18; N, 5.28. Found: C, 54.47; H, 4.37; N, 5.24.

The water of solvation was not removed when the sample was block-dried at 135° for several minutes. The ultraviolet maxi-

mum was at 240 mμ (ε 25,400), the infrared spectrum was consistent, and the titration gave a $pK_a = 4.75$ (apparent mol. wt., 535; calcd., 529).

Sodium O-2-Thenoyl-7-(2-thiopheneacetamido)cephalosporadesate.—As described above, 1.0 g. (0.00255 mole) of potassium 7-(2-thiopheneacetamido)cephalosporadesate was treated with 2.93 g. (0.02 mole) of 2-thenoyl chloride. After evaporation of the acetone and extraction with ethyl acetate, a yellow precipitate of the sodium salt separated from the water solution. Additional salt was obtained by the addition of saturated NaCl solution. The combined precipitates when dried weighed 800 mg. Recrystallization of this solid from methanol–2-propanol gave 400 mg. of the product (32%), m.p. 137–142° dec.

Anal. Calcd. for $C_{19}H_{15}N_2NaO_6S_3$: C, 46.90; H, 3.10; N, 5.76. Found: C, 47.11; H, 3.28; N, 5.55.

The ultraviolet maximum was at 240 mμ (ε 18,400), the infrared was consistent, and the titration indicated a $pK_a = 4.7$ (apparent mol. wt., 482; calcd., 486).

Sodium O-Benzoyl-7-(2-thiopheneacetamido)cephalosporadesate.—In the usual fashion, 3.3 g. (0.00842 mole) of potassium desacetyl acid salt was treated with 8.0 g. (0.057 mole) of benzoyl chloride and NaOH. The sodium salt was precipitated from the water solution after acetone evaporation by the addition of saturated NaCl solution. The crude, dried solid weighed 2.54 g. and an infrared spectrum indicated it was of fair quality. Purification, however, was quite difficult. The solid was dissolved by suspension in water and addition of acetone until solution was effected except for a trace which was filtered off. Some acetone was then evaporated until the product just started to precipitate. The mixture was chilled and a gelatinous precipitate separated which was filtered and dried (1 g.). It had darkened in color. It was then dissolved in hot methanol, a dark fraction was filtered off, and the solution was concentrated and then diluted with 2-propanol to cloudiness. The solution deposited a light cream solid on chilling which was centrifuged and vacuum dried, m.p. 148–150° dec., 220-mg. yield. The product, chromatographed on paper (70% 2-propanol–water), gave one spot by bioautography. The ultraviolet spectrum gave a maximum absorption at 233 mμ (ε 22,100) and the titration showed a pK_a of 4.85 (apparent mol. wt., 526; calcd., 480).

Anal. Calcd. for $C_{21}H_{17}N_2NaO_6S_2$: C, 52.49; H, 3.56; N, 5.83. Found: C, 52.36; H, 3.66; N, 5.84.

Acknowledgments.—The contributions of the following toward the experimental data are gratefully acknowledged: C. W. Godzeski and W. E. Wick for the microbiological data; H. L. Hunter, G. M. Maciak, D. Cline, and W. L. Brown for the microanalyses; L. A. Spangle, L. G. Howard, and D. O. Woolf for the titrations and spectrometry; and M. Kory, C. P. Walters, and E. Mingioli for the enzymic deacetylations.

Syntheses with 5-Nitro-2-furonitrile

W. R. SHERMAN[1] AND ANNE VON ESCH

Organic Chemistry Department, Research Division, Abbott Laboratories, North Chicago, Illinois

Received July 9, 1964

5-Nitro-2-furonitrile has been prepared by the dehydration of 5-nitro-2-furamide with phosphorus oxychloride. Reaction of the nitrile with thioacetamide in dimethylformamide saturated with HCl has provided 5-nitro-2-thiofuramide. The thiofuramide with α-bromo ketones has given 2-(5-nitro-2-furyl)-4-methyl-, -phenyl-, and -(5-nitro-2-furyl)thiazoles. Oxidation with iodine has provided 3,5-bis(5-nitro-2-furyl)-1,2,4-thiadiazole. 5-Nitro-2-furamidoxime is obtained by the addition of hydroxylamine to the nitrile. This amidoxime is O-acylated by acetyl, benzoyl, and chloroacetyl chloride as well as phosgene and ethyl chloroformate. O- as opposed to N-acylation is established by the infrared spectra of typical members of this group. Heating the acetyl, benzoyl, and chloroacetyl esters at their melting points has provided the corresponding 5-substituted 3-(5-nitro-2-furyl)-1,2,4-oxadiazoles. Reaction of the amidoxime with acetaldehyde and benzaldehyde has given the corresponding 5-substituted 3-(5-nitro-2-furyl)-Δ^2-1,2,4-oxadiazolines. 5-Nitro-2-furamidine is obtained from the nitrile *via* the ethyl imidate ester by reacting the imidate with methanolic ammonium chloride. The amidine has been condensed with the appropriate β-diketones to give 2-(5-nitro-2-furyl)-4,6-dimethyl-, -4,6-di(trifluoromethyl)-, -4-methyl-6-trifluoromethyl-, -4-trifluoromethyl-6-(2-furyl)-, and -4-trifluoromethyl-6-(2-thienyl)-pyrimidines. The antibacterial properties of the most active members of this series of compounds are presented.

As a means of extending our previous studies of 5-nitrofuran antibacterials[2] we looked on 5-nitro-2-furonitrile (I) as a potentially versatile starting material. This simple compound presented the opportunity of developing a variety of heteroaliphatic and heterocyclic systems at the 2-position of 5-nitrofuran.

At the outset of this work two methods for the preparation of nitrile I had been described: the nitration of 2-furonitrile[3] and the dehydration of 5-nitro-2-furaldoxime by means of acetic anhydride.[4] A more accessible starting material for us was methyl 5-nitro-2-furoate. This remarkably reactive ester is converted in 87% yield to 5-nitro-2-furamide by dissolving it in liquid ammonia at −33°. The amide may then be converted to the nitrile in 63% yield by the action of phosphorus oxychloride.

We have found that I undergoes many of the usual reactions of nitriles, its possibilities as a starting material being limited mainly by the base instability of the 5-nitrofuran system. The three principal intermediates (III–V) which are used in this work are prepared from I. Thus, when I is treated with ethanolic HCl in the manner of Pinner,[5] ethyl 5-nitro-2-furimidate hydrochloride (II) is formed in 92% yield. After conversion to the free base by liquid ammonia at −33°, with which it does not react further, the imido ester is heated with methanolic ammonium chloride, after Barber and Slack,[6] to form 5-nitro-2-furamidine hydrochloride (V) in 73% yield. Another useful intermediate, 5-nitro-2-thiofuramide (III), is formed in 52% yield by

(1) Department of Psychiatry, Washington University School of Medicine, St. Louis, Mo.

(2) W. R. Sherman, *J. Org. Chem.*, **26**, 88 (1961); W. R. Sherman and D. E. Dickson, *ibid.*, **27**, 1351 (1962); W. R. Sherman and A. A. Alter, *ibid.*, **27**, 2237 (1962); W. R. Sherman and A. Von Esch, *ibid.*, **27**, 3472 (1962).

(3) J. M. Straley, *Iowa State J. Sci.*, **11**, 115 (1936).

(4) C. D. Nenitzescu and C. Bucur, *Rev. chim., Acad. rep. populaire Roumaine*, **1**, 155 (1956).

(5) A. Pinner, *Ber.*, **25**, 1414 (1892).

(6) H. J. Barber and R. Slack, *J. Am. Chem. Soc.*, **66**, 1607 (1944).