6-Benzylmercapto-9-β-D-ribofuranosylpurine (V).—To a suspension of 500 mg. (1.74 mmoles) of 6-chloro-9-β-D-ribofuranosylpurine in 80 ml. of methanol was added 3.48 mmoles of sodium benzylmercaptide (prepared by adding benzylmercaptan to 1 *N* sodium methoxide). The mixture was heated under reflux for one hour; solution occurred after the first few minutes of heating. The reaction solution was neutralized with 1 *N* hydrochloric acid and evaporated *in vacuo*. The residue was extracted with ethyl ether or di-*n*-butyl ether; concentration of the extract gave the crystalline product. One recrystallization from di-*n*-butyl ether gave the pure material; yield 265 mg. (41.0%), m.p. 156–158°, $[\alpha]^{32}D$ −49 ± 2° (0.65% in methanol). Spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 293 (19.1); *p*H 7, 292 (20.4); *p*H 13, 292 (20.5); $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3400 (broad, OH); 1570 and 1500 (C=C and C=N); 1120, 1080 and 1050 (C–O–).

Anal. Calcd. for $C_{17}H_{18}N_4O_4S \cdot {}^1/_2H_2O$: C, 53.30; H, 5.00; N, 14.63. Found: C, 53.66; H, 4.93; N, 14.83.

6-Hydrazino-9-β-D-ribofuranosylpurine (VI).—6-Chloro-9-β-D-ribofuranosylpurine (200 mg.) was added in three portions over a period of ten minutes to 1.0 ml. of anhydrous hydrazine which was kept in a water-bath at 25°. The reaction solution was allowed to stand at room temperature for 30 minutes, then 5 ml. of isopropyl alcohol was added. The oil which separated crystallized, and the solid was collected by filtration; yield 189 mg. (95.0%), m.p. 176–181° dec. One recrystallization from absolute ethanol gave the pure compound; yield 123 mg. (61.8%), m.p. 214–216° dec. Spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 261 (15.0); *p*H 7 and *p*H 13, unstable; $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3250 (broad, OH and NH); 1635 (shoulder), 1610 and 1580 (C=C and C=N); 1095, 1075 and 1055 (C–O–).

Anal. Calcd. for $C_{10}H_{14}N_6O_4$: C, 42.54; H, 5.00; N, 29.79. Found: C, 43.04; H, 5.21; N, 29.86.

Treatment of 6-Hydrazino-9-β-D-ribofuranosylpurine (VI) with Nitrous Acid.—To a solution of 0.50 g. (1.8 mmoles) of 6-hydrazinopurine riboside (VI) in 10 ml. of 5% acetic acid, cooled in an ice-bath, was added a solution of 0.14 g. (2.0 mmoles) of sodium nitrite in 25 ml. of water. Crystals began to separate after 10 minutes; the reaction mixture was kept cold for one hour, and the solid was collected by filtration; yield 0.48 g. (92%), m.p. 218° dec. Recrystallization from water gave the pure product; yield 0.36 g. (69%), m.p. 222° dec., $[\alpha]^{32}D$ −12 ± 3° (0.51% in 0.1 *N* HCl). Spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 252 (4.78), 260 (4.82), 287 (8.29); *p*H 7, 251 (4.97), 260 (4.90), 287 (7.93); *p*H 13, unstable; $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3420–3250 (broad, OH); 1640 (unassigned); 1500 (C=C or C=N); 1105, 1080 and 1045 (C–O–).

Anal. Calcd. for $C_{10}H_{11}N_7O_4$: C, 40.96; H, 3.78; N, 33.44. Found: C, 41.05; H, 3.65; N, 33.28.

2-Azidopurine.—To a solution of 300 mg. (2.00 mmoles) of 2-hydrazinopurine[13] in 12 ml. of 10% acetic acid, which was cooled in an ice-bath, was added a cold solution of 165 mg. (2.40 mmoles) of sodium nitrite in 15 ml. of water. The reaction mixture immediately began to deposit a solid; the mixture was kept cold for one hour, and the solid was collected by filtration, washed with cold water and dried; yield 301 mg. (93.0%), m.p. 245° dec. One recrystallization from water gave 250 mg. (78.0%) of pure product, m.p. 240–250° dec. Spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 7,280 (5.06); *p*H 1 and *p*H 13, unstable; $\bar{\nu}$ in cm.$^{-1}$ (KBr): 2135 (azido group), 1610 and 1565 (C=C and C=N).

Anal. Calcd. for $C_5H_3N_7$: C, 37.27; H, 1.87; N, 60.75. Found: C, 37.40; H, 2.12; N, 60.81.

2,4-Diazidopyrimidine.—This compound was prepared by the method described in the literature.[14] From 300 mg. of 2,4-dihydrazinopyrimidine, there was obtained 64 mg. of pure product, m.p. 88–90.5°. Spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): ethanol, 239 (21.5), 284 (7.93); $\bar{\nu}$ in cm.$^{-1}$ (KBr): 2160 and 2130 (azido group), 1620 and 1530 (C=C and C=N).

BIRMINGHAM 5, ALABAMA

[COMMUNICATION NO. 1919 FROM THE KODAK RESEARCH LABORATORIES, EASTMAN KODAK CO.]

Preparation of Thiazolidines and Related Compounds: Lactams and Lactamidines

BY G. L. OLIVER, J. R. DANN AND J. W. GATES, JR.

RECEIVED AUGUST 8, 1957

The reaction of cysteine and analogous aminothiols with *o*-cyanobenzaldehyde has been shown to produce cyclic amidine or "γ-lactamidine" structures. This cyclization reaction is similar to the lactam formation encountered in the reaction of these aminothiol-type compounds with phthalaldehydic acid and levulinic acid. The structures of these lactamidine compounds have been confirmed by potentiometric titration and by infrared absorption spectra studies.

The preparation of the γ-lactam of benzylhomopenicilloic acid (I) and of many related compounds having the γ-lactam configuration has been presented in detail.[1] The γ-lactam of benzylhomopenicilloic acid (I) has been prepared by ring closure of either the ethyl ester or free acid of α-phenylacetamido - 4 - carboxy - 5,5 - dimethyl - 2 -thiazolidinepropionic acid (II). In a similar manner, α - benzamido - 4 - carboxy - 2 - thiazolidinepropionic acid (III) produced the γ-lactam IV by ring closure.

In the continuation of an investigation presented in previous publications[2,3] involving the isolation and preparation of related thiazolidine structures, examples of γ-lactams and γ-lactamidines have been prepared. In this present work, the reaction of cysteine (V) with phthalaldehydic acid (VI) and with levulinic acid (VII) produced the free acids VIII and IX which readily lost water and produced the γ-lactams X and XI by ring closure. The free acids lost water to form the lactams, even on drying the samples for analysis.

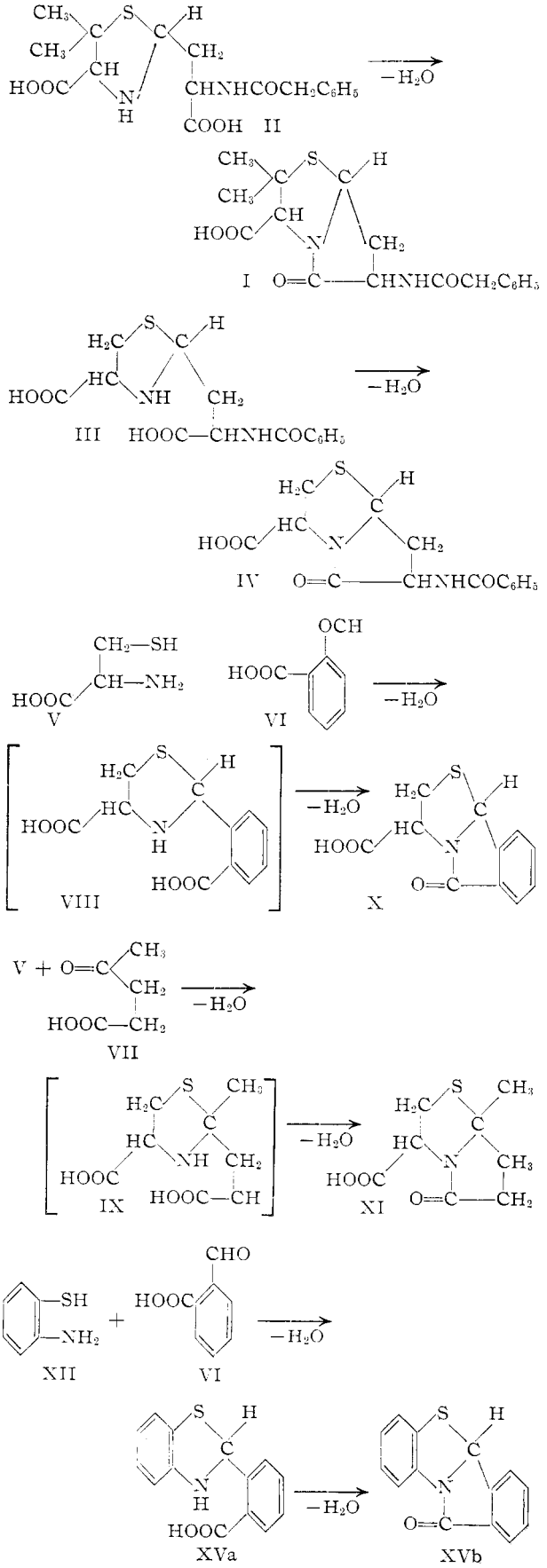
Additional examples of this type of structure were prepared by reaction of *o*-aminobenzenethiol (XII), of β-mercaptoethylamine (XIII) and of homocysteine (XIV) with these reactive carbonyl reagents, as illustrated in Chart I. The free acids were not isolated in the reactions of phthalaldehydic acid or levulinic acid with β-mercaptoethylamine or homocysteine; only the lactams were obtained. The lactam structure is confirmed by the titration curve which resembles that of the N-acetylthiazolidine-4-carboxylic acid (XXIV), rather than that of 2-methylthiazolidine-2,4-dicarboxylic acid[2] (Fig. 1).

In the course of examination of thiazolidine-4-carboxylic acid derivatives, the reaction of cysteine with *o*-cyanobenzaldehyde was carried out as indicated here.

(1) H. T. Clarke, J. R. Johnson and R. Robinson, editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 1012.

(2) J. R. Dann, G. L. Oliver and J. W. Gates, Jr., THIS JOURNAL, **79**, 1644 (1957).

(3) J. R. Dann and J. W. Gates, Jr., *ibid.*, **79**, 1650 (1957).



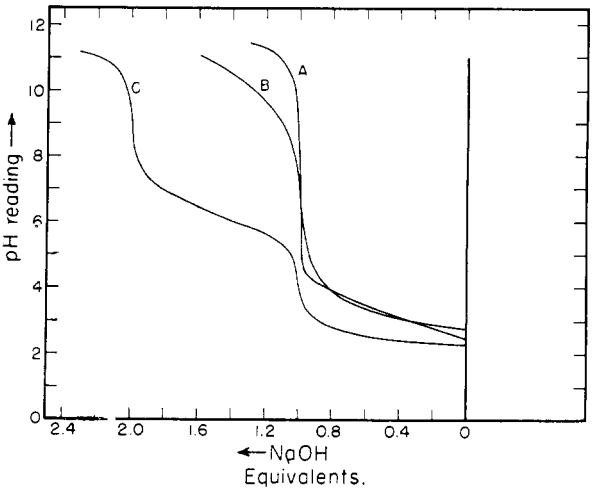
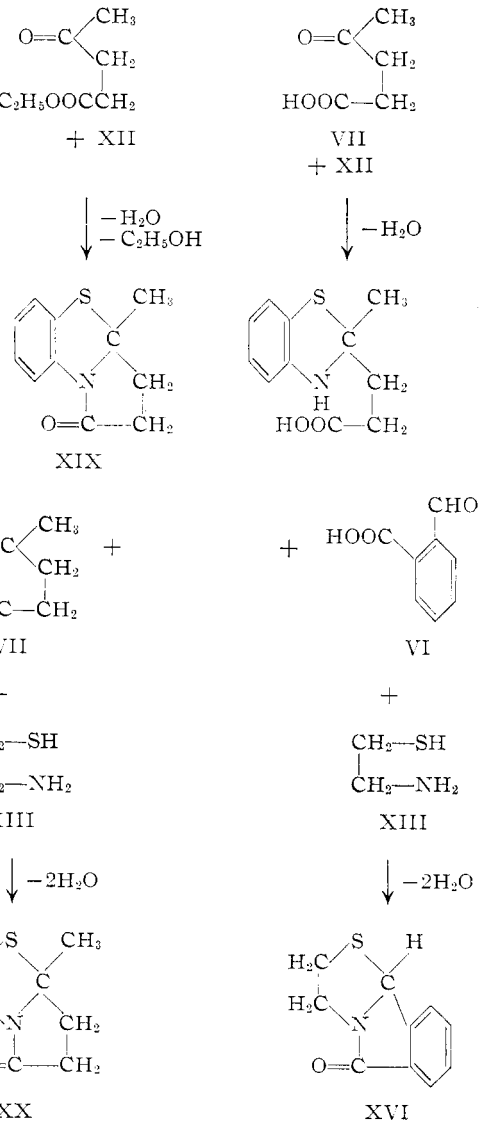
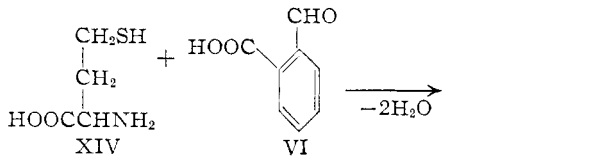
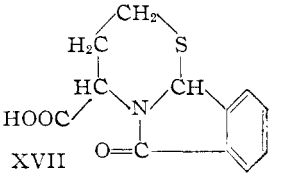


Fig. 1.—Titration curves of amides and lactams: A, lactam of 2-*o*-carboxyphenylthiazolidine-4-carboxylic acid; B, N-acetylthiazolidine-4-carboxylic acid; C, 2-methyl-2,4-thiazolidinedicarboxylic acid.

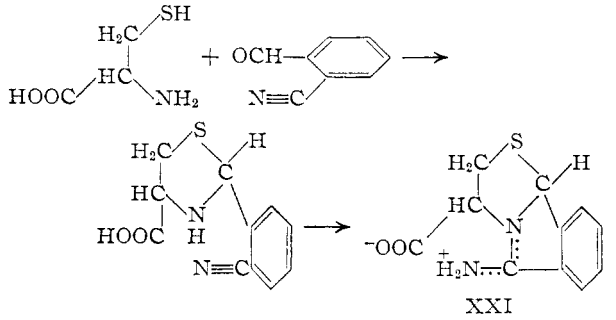




XIV VI



XVII



XXI

The titration curve (B, Fig. 2) of the crystalline derivative XXI, which was expected to be the thiazolidine derivative, indicated the presence of a

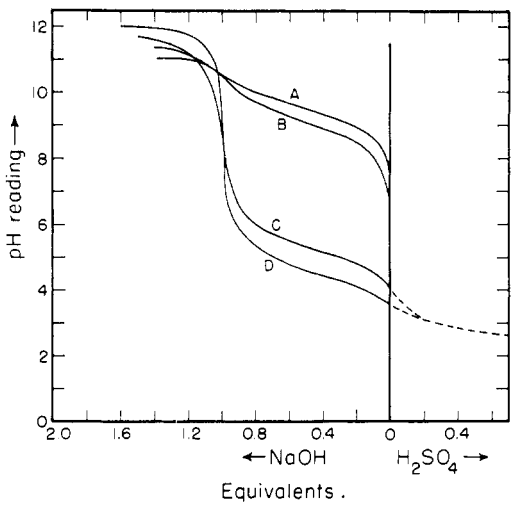
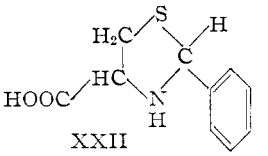


Fig. 2.—Titration curves of amino acid types: A, glycine; B, "γ-lactamidine" of 2-*o*-cyanophenylthiazolidine-4-carboxylic acid; C, 2-phenylthiazolidine-4-carboxylic acid; D, 2-*p*-cyanophenylthiazolidine-4-carboxylic acid.

basic group stronger than that of the >NH group in the thiazolidine-4-carboxylic acids and more nearly resembling that of the more strongly basic group of glycine (A, Fig. 2). The titration curves of 2-phenylthiazolidine-4-carboxylic acid (XXII) and its 2-*p*-cyanophenyl analog XXIII (C and D, Fig. 2) are characteristic of the very weakly basic 2-substituted thiazolidine-4-carboxylic acids and actually resemble in shape those of the non-basic N-acetylthiazolidine-4-carboxylic acid (XXIV)[4] and the lactam (Fig. 1, B and A) more than those with the more strongly basic groups.

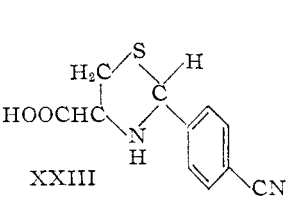
(4) S. Ratner and H. T. Clarke, THIS JOURNAL, **59**, 200 (1937).

Two factors, the much greater basicity of the 2-*o*-cyanophenylthiazolidine-4-carboxylic acid and the obvious structural similarity to the easily formed lactams derived from phthalaldehydic and levulinic acids, suggest the formation of a cyclic amidine or γ-iminolactam structure XXI, which we

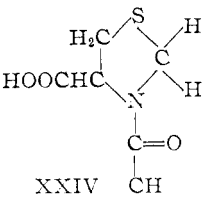


XXII

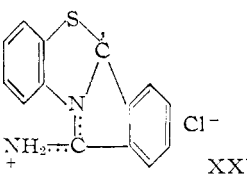
have elected to call a "γ-lactamidine." The *o*-cyanobenzaldehyde also was found to react readily with the hydrochlorides of *o*-aminobenzenethiol and β-mercaptoethylamine and with homocysteine to produce the lactamidine salts XXV, XXVI and XXVII.



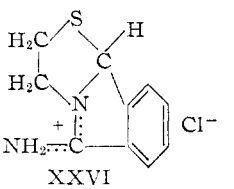
XXIII



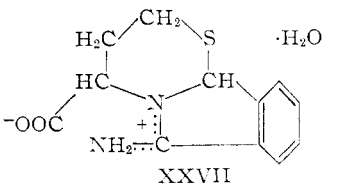
XXIV



XXV



XXVI



XXVII

The "γ-lactamidines" derived from cysteine and homocysteine undoubtedly exist as zwitterions like glycine, the cation being a resonance-stabilized

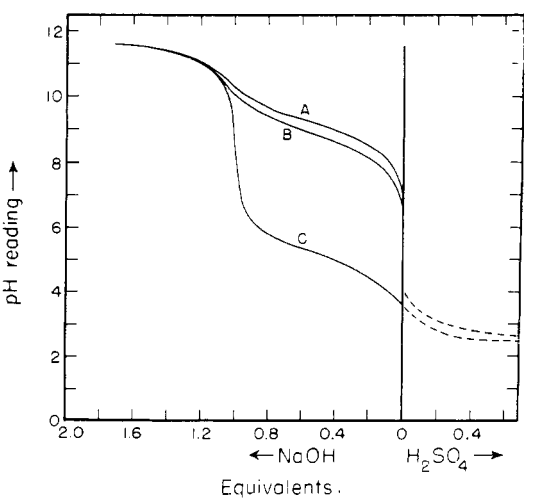


Fig. 3.—Titration curves of amine types and ammonia; A, ammonia; B, "γ-lactamidine" of 2-*o*-cyanophenylthiazolidine; C, 2-*p*-cyanophenylthiazolidine.

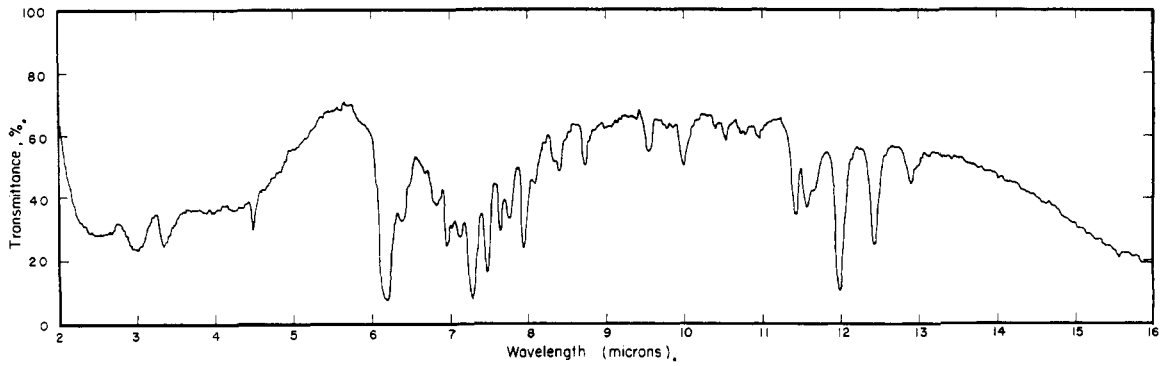


Fig. 4.—Infrared absorption spectrum of 2-*p*-cyanophenylthiazolidine-4-carboxylic acid (XXIII).

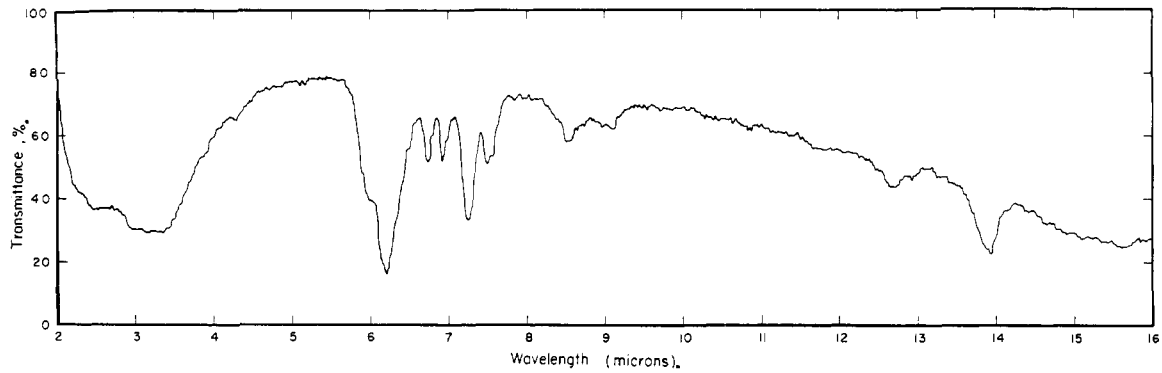
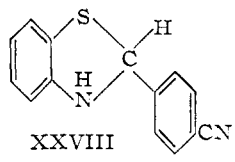


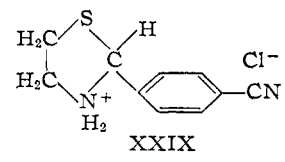
Fig. 5.—Infrared absorption spectrum of the "γ-lactamidine" (XXI).

amidinium group. It is noteworthy that the benzothiazoline XXV and the thiazolidine XXVI are formed more readily from the corresponding aminothiol hydrochlorides than under the buffered conditions ordinarily used in this type of reaction. In fact, XXV can be satisfactorily isolated only as the salt. The titration curve of the "γ-lactamidine" salt XXVI (B, Fig. 3) is displaced markedly from that of 2-*p*-cyanophenylthiazolidine XXIX (C, Fig. 3) and is comparable in basicity to ammonia (A, Fig. 3).

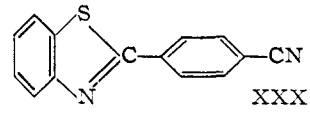
Since confirmation of the "γ-lactamidine" structure cannot be obtained by elemental analysis, the infrared absorption spectra of these compounds were compared with their analogs prepared from *p*-cyanobenzaldehyde to determine the presence or absence of the C≡N group. The absence of the C≡N band in the 4.2–4.5 μ region of the infrared spectra of the "γ-lactamidines" (XXI, XXV and XXVI) confirms the result of the titrations. The corresponding 2 - *p* - cyanophenylthiazolidine - 4-carboxylic acid (XXIII), 2-*p*-cyanophenylthiazolidine (XXIX) and 2-*p*-cyanophenylbenzothiazoline (XXVIII) all show the presence of the C≡N band at 4.47 μ. The infrared absorption spectra for XXIII, Fig. 4, and XXI, Fig. 5, demonstrate this behavior.



XXVIII



XXIX



XXX

Since benzothiazolines are known to oxidize readily to benzothiazoles, the identity of the benzothiazoline XXVIII was confirmed by the infrared spectrum which was different from the benzothiazole XXX prepared by ferric chloride oxidation of the benzothiazoline.[5]

Experimental

Reaction of Phthalaldehydic Acid and Cysteine: X.—A mixture of 3.0 g. (0.02 mole) of phthalaldehydic acid, 3.14 g. (0.02 mole) of cysteine hydrochloride and 1.6 ml. of pyridine in 60 ml. of ethanol and 20 ml. of water was heated on the steam-bath for 15 minutes and cooled. Addition of 75 ml. of water to the warmed solution, and then cooling, gave 3.4 g. of colorless needles. The melting point of this material was 161–162° but, upon cooling and remelting, was found to be 218–220°. After drying at 50° under vacuum for analysis, the melting point of the lactam X was 218–220°.

Anal. Calcd. for the lactam of 2-(*o*-carboxyphenyl)-4-carboxythiazolidine, $C_{11}H_9NSO_3$: C, 56.2; H, 3.83; N, 5.95; mol. wt., 235. Found: C, 56.7; H, 4.10; N, 6.3; neut. equiv., 235.

Reaction of Phthalaldehydic Acid and *o*-Aminobenzenethiol: XVa, XVb.—This reaction was carried out in essentially the same manner as that just described. A white crystalline material (a) was obtained which melted at 125–130°, then resolidified and melted at 164–168°. Recrystallization from alcohol gave a material (b) melting at 172–174°.

Anal. Calcd. for 2-(*o*-carboxyphenyl)-benzothiazoline, $C_{14}H_{11}NSO_2$: C, 65.4; H, 4.3. (A) Found: C, 65.7; H, 4.5. *Anal.* Calcd. for the lactam of 2-(*o*-carboxyphenyl)-benzothiazoline, $C_{14}H_9NSO$: C, 70.3; H, 3.8. (B) Found: C, 70.2; H, 3.9.

Reaction of Phthalaldehydic Acid and β-Mercaptoethylamine: XVI.—A mixture of 16.5 g. of phthalaldehydic acid (0.11 mole) in 25 ml. of alcohol and 7.7 g. (0.1 mole) of β-

(5) H. P. Lankelma and P. X. Sharnoff, THIS JOURNAL, **53**, 2654 (1931).

mercaptoethylamine in 25 ml. of water was refluxed for 10 minutes on the steam-bath. Upon cooling, the product separated as a yellowish oil. The mixture was diluted with water and extracted with ether. The crystals of lactam obtained on evaporation of the dried ether solution were recrystallized from alcohol–water, m.p. 97–100°. *Anal.* Calcd. for $C_{10}H_9ONS$: C, 62.8; H, 4.7; N, 7.3. Found: C, 62.7; H, 4.8; N, 7.2.

Reaction of Phthalaldehydic Acid with Homocysteine: XVII.—A mixture of 0.96 g. (0.01 mole) of phthalaldehydic acid and 1.35 g. (0.01 mole) of homocysteine (Nutritional Biochemicals, Inc.) in 10 ml. of water was warmed on the steam-bath for two minutes and left at room temperature for 15 minutes. A white precipitate weighing 1.1 g. was filtered off; melting point of the lactam, 230–231°.

Anal. Calcd. for the lactam of 2-(*o*-carboxyphenyl)-4-carboxytetrahydrothiazine, $C_{12}H_{11}NSO_3$: C, 57.8; H, 4.4. Found: C, 57.9; H, 4.0.

Reaction of Levulinic Acid and Cysteine: XI.—A mixture of 7.85 g. (0.05 mole) of cysteine hydrochloride, 5.8 g. (0.05 mole) of levulinic acid and 4.1 ml. of pyridine in 100 ml. of alcohol was heated on the steam-bath for 10 minutes and then cooled. A precipitate of cystine was filtered off and the mother liquor, on slow evaporation, gave 2.5 g. of long colorless needles, m.p. 189–192°; after drying, the lactam XI had m.p. 198–199°.

Anal. Calcd. for the lactam of 2-methyl-2(β-carboxyethyl)-4-carboxythiazolidine, $C_8H_{11}NSO_3$: C, 47.8; H, 5.47; N, 6.98; mol. wt., 201. Found: C, 48.2; H, 5.9; N, 7.3; neut. equiv., 205.

Reaction of Levulinic Acid and *o*-Aminobenzenethiol: XVIII.—A mixture of 25 g. (0.2 mole) of *o*-aminobenzenethiol and 23.2 g. (0.2 mole) of levulinic acid in 100 ml. of ethyl alcohol was heated on the steam-bath for 30 minutes and left at room temperature overnight. A white precipitate, m.p. 106–112°, formed on cooling. Recrystallization from alcohol resulted in a melting point of 122–123°.

Anal. Calcd. for 2-methyl-2(β-carboxyethyl)-benzothiazoline, $C_{11}H_{13}O_2NS$: C, 59.2; H, 5.9. Found: C, 59.3; H, 6.1.

Reaction of Ethyl Levulinate with *o*-Aminobenzenethiol: XIX.—A mixture of 25 g. (0.2 mole) of *o*-aminobenzenethiol and 28.8 g. (0.2 mole) of ethyl levulinate in 100 ml. of ethyl alcohol was heated on the steam-bath for 30 minutes and left at room temperature overnight. The solvent was distilled off on the steam-bath and the residual oil distilled *in vacuo* giving 24 g. of the benzothiazoline which distilled at 172–176° at 3 mm.

Anal. Calcd. for $C_{11}H_{11}NSO$: C, 64.4; H, 5.4; N, 6.8. Found: C, 64.0; H, 6.0; N, 6.4.

Reaction of Levulinic Acid and β-Mercaptoethylamine: XX.—A mixture of 12.8 g. of levulinic acid (0.11 mole) in 25 ml. of alcohol and 7.7 g. (0.1 mole) of β-mercaptoethylamine in 25 ml. of water were refluxed on the steam-bath for 15 minutes. The lactam, which separated as an oil, was extracted with ether from the diluted aqueous mixture. The ether solution was washed with sodium bicarbonate solution, dried, and distilled; 9 g., b.p. 86° (2 mm.).

Anal. Calcd. for $C_7H_{11}ONS$: C, 53.5; H, 7.0; N, 8.9. Found: C, 53.5; H, 6.9; N, 8.2.

Reaction of *o*-Cyanobenzaldehyde and Cysteine: XXI.—Solutions of 7.8 g. (0.05 mole) of cysteine hydrochloride in 20 ml. of water and of 6.5 g. of *o*-cyanobenzaldehyde were mixed and warmed on the steam-bath. Addition of 4 ml. of pyridine caused spontaneous refluxing of the warm solutions. The mixture was heated 2 minutes more, cooled, and filtered. The "γ-lactamidine" XXI (11.6 g., 99%) was recrystallized from aqueous alcohol and melted at 185–190° dec.

Anal. Calcd. for $C_{11}H_{10}O_2N_2S$: C, 56.4; H, 4.3; N, 12.0. Found: C, 55.9; H, 4.4; N, 11.8.

Reaction of *o*-Cyanobenzaldehyde and *o*-Aminobenzenethiol: XXV.—Into a solution of 2.50 g. (0.20 mole) of *o*-aminobenzenethiol dissolved in 30 ml. of absolute alcohol was passed an equivalent weight (0.73 g.) of hydrogen chloride gas. To this warm mixture, a solution, 2.62 g. of *o*-cyanobenzaldehyde in 5 ml. of absolute alcohol was added and the mixture was heated five minutes. The "γ-lactamidine" hydrochloride XXV separated almost immediately from the hot solution; yield 4.5 g. (83%), m.p. 192–193°. Recrystallization from aqueous alcohol resulted in a less pure product, probably due to hydrolysis to the free base which appears to decompose readily.

Anal. Calcd. for $C_{14}H_{11}N_2SCl$: C, 61.2; H, 4.0; N, 10.2. Found: C, 60.9; H, 4.3; N, 10.1.

Reaction of *o*-Cyanobenzaldehyde and β-Mercaptoethylamine: XXVI.—A mixture of one-hundredth mole each of β-mercaptoethylamine hydrochloride (1.15 g.) and *o*-cyanobenzaldehyde (1.31 g.), each dissolved separately in 5 ml. of hot absolute alcohol, was refluxed about five minutes on the steam-bath. The solution turned slightly pink, and the white crystalline "γ-lactamidine" hydrochloride XXVI separated on cooling. Recrystallized from absolute alcohol, the product (1.8 g., 80%) melted at 242–245° dec., with previous darkening.

Anal. Calcd. for $C_{10}H_{11}N_2SCl$: C, 53.0; H, 4.9; N, 12.4. Found: C, 53.2; H, 5.3; N, 11.8.

Reaction of *o*-Cyanobenzaldehyde with Homocysteine: XXVII.—A mixture of 1.35 g. (0.01 mole) of homocysteine and 1.11 g. (0.01 mole) of *o*-cyanobenzaldehyde in 10 ml. of ethyl alcohol and 5 ml. of water was heated for five minutes on the steam-bath and left at room temperature overnight. Two grams of the crystalline "γ-lactamidine" XXVII, m.p. 215–220° dec., was filtered off and washed with water. Recrystallization from alcohol raised the melting point to 228° dec.

Anal. Calcd. for $C_{12}H_{12}N_2SO_2 \cdot H_2O$: C, 54.2; H, 5.3; N, 10.1; S, 12.1. Found: C, 54.3; H, 5.5; N, 9.9; S, 12.5.

Reaction of *p*-Cyanobenzaldehyde with Cysteine: XXIII.—A solution of 5.24 g. (0.04 mole) of *p*-cyanobenzaldehyde dissolved in 40 ml. of alcohol and a solution of 6.28 g. (0.04 mole) of cysteine hydrochloride in 15 ml. of water were mixed and heated for five minutes on the steam-bath. The slightly yellowish thiazolidine XXIII which separated on cooling was filtered and recrystallized from 60% alcohol, yielding 7.35 g. (68.0%), m.p. 141° dec.

Anal. Calcd. for $C_{11}H_{10}O_2N_2S$: C, 56.4; H, 4.3; N, 12.0. Found: C, 56.2; H, 4.2; N, 12.2.

Reaction of *p*-Cyanobenzaldehyde with *o*-Aminobenzenethiol: XXVIII.—*p*-Cyanobenzaldehyde (2.62 g., 0.02 mole) and *o*-aminobenzenethiol (2.50 g., 0.02 mole) were dissolved separately in portions totaling 60 ml. of hot 50% alcohol. The solutions were mixed, and then heated on the steam-bath for ten minutes. On cooling overnight, 4.8 g. (100%) of the benzothiazoline XXVIII crystallized. Recrystallization from alcohol–water gave m.p. of 83–85°. There was considerable resolidification of the melt, probably due to oxidation to the benzothiazole. The infrared spectrum showed little, if any, of the *p*-cyanophenylbenzothiazole present as a contaminant.

Anal. Calcd. for $C_{14}H_{10}N_2S$: C, 70.6; H, 4.2; N, 11.7. Found: C, 70.9; H, 3.8; N, 11.7.

Reaction of *p*-Cyanobenzaldehyde with β-Mercaptoethylamine: XXIX.—A solution of 1.97 g. (0.015 mole) of *p*-cyanobenzaldehyde and 1.73 g. (0.015 mole) of β-mercaptoethylamine hydrochloride dissolved in 20 ml. of absolute alcohol was heated on the steam-bath for ten minutes. Crystallization of the thiazolidine hydrochloride XXIX took place rapidly on scratching the cooled solution. The same tendency to super-cool was noted on recrystallization from absolute alcohol; yield 1.8 g. (53%), m.p. 193–195°.

Anal. Calcd. for $C_{10}H_{11}N_2SCl$: C, 53.0; H, 4.9; N, 12.4. Found: C, 53.1; H, 4.8; N, 11.8.

Oxidation of 2-*p*-Cyanophenylbenzothiazoline: 2-*p*-Cyanophenylbenzothiazole (XXX).—To a solution of 0.43 g. (0.0018 mole) of 2-*p*-cyanophenylbenzothiazoline in 20 ml. of alcohol was added 0.92 ml. of a 2 *M* alcoholic ferric chloride solution. The mixture decolorized on heating to boiling. Just enough water was added to prevent crystallization from the hot solution. On cooling, the crystalline benzothiazole XXX was obtained, which, on recrystallization from alcohol–water, melted 170–171°. A mixture of the benzothiazoline and the benzothiazole depressed the melting point of the former to 78–80° before resolidification, melting being complete only at 168°.

Anal. Calcd. for $C_{14}H_8N_2S$: C, 71.2; H, 3.4; N, 11.9. Found: C, 70.9; H, 3.7; N, 11.9.

Starting Materials.—The *o*- and *p*-cyanobenzaldehyde were prepared by oxidation of the tolunitriles by chromic acid in the presence of acetic anhydride, according to the

method described in reference 6. The free base, β-mercaptoethylamine, was prepared by the method of Nathan and Bogert[7]; the hydrochloride used in later experiments was obtained from Evans Chemetics. Unless specified, chemicals were all Eastman Kodak Co. preparations.

Titration Curves.—The titration curves were obtained by dissolving the compound in alcohol, water or a mixture thereof and adjusting the solution to about 30% alcohol. The titrants were 0.1 *N* sodium hydroxide and 0.1 *N* sulfuric acid in water. Measurements were accomplished by using the Beckman model M *p*H meter with glass electrode and silver–silver chloride reference electrode. The curves are plotted in all cases with the neutral or uncharged molecule at the center. In Fig. 2, the *p*H values at 0.5 equivalent gave a rough estimation of the relative *pK*'s of the amines.

ROCHESTER, N. Y.

(6) "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 441.

(7) A. H. Nathan and M. T. Bogert, THIS JOURNAL, **63**, 236 (1941).

[CONTRIBUTION FROM THE DANIEL SIEFF RESEARCH INSTITUTE, THE WEIZMANN INSTITUTE OF SCIENCE]

The Constituents of *Ecballium elaterium* L. II. α-Elaterin[1,2]

BY DAVID LAVIE AND SHLOMO SZINAI

RECEIVED AUGUST 19, 1957

α-Elaterin, β-elaterin and elateridin have been found to have anti-tumor activity. The oxygen functions of α-elaterin have been studied, and the formation of ecballic acid, a degradation product of α-elaterin is explained by a benzilic acid type rearrangement. Pyrolysis of α-elaterin yielded a new crystalline degradation product, pyroelaterin.

Ecballium elaterium or squirting cucumber is a trailing plant growing in the Mediterranean region, belonging to the Cucurbitaceae. The fruit resemble small gherkins, but are thickly covered with bristly hairs. As these fruits ripen, the juice surrounding the seeds swells and a pressure is set up within the fruit until the joint between the stalk and the fruit ruptures and the fruit's contents and the seeds are shot out violently by the slightest touch. The juice yields a powerful cathartic drug called "elaterium" which has been used since ancient times for its medicinal properties[3] and was included in the British Pharmacopeia.

The fruit is collected when nearly ripe and the juice expressed after crushing or slicing. From the juice settles a deposit which is collected and dried. From this deposit or "elaterium," Morries and Hennell simultaneously reported the isolation of the crystalline elaterin in 1831.[4] The empirical formula of $C_{28}H_{38}O_7$ was proposed for this compound by Berg.[5] The same author showed that when elaterin is treated for a short time with alcoholic sodium hydroxide, one molecule of acetic acid is split off and an amorphous phenolic substance, elateridin, is formed. Under the prolonged action of boiling sodium hydroxide solution, elaterin is converted into a crystalline product, ecballic acid.[6] From this observation, and from the fact that elaterin was soluble in alcoholic alkaline solution and could be recovered unchanged by immediate acidification, a lactone was assumed to be present in the molecule.[7] Borsche and Diacont[6] also assumed that the prolonged treatment of elaterin with alkali would involve certain changes in the molecule which would not allow relactonization and would lead to the formation of an acid. From the acid hydrolysis of elaterin[5,8] an amorphous product was obtained, anhydro-elateridin. During the purification of crude elaterin, Power and Moore[9] isolated, in small quantities, a second compound, β-elaterin, to which they ascribed the physiological activity of the mixture.

In the course of a systematic screening of plant extracts in a search for substances with tumor necrotizing capacity, Belkin and Fitzgerald,[10a] found that *Ecballium elaterium* possesses marked activity. The neoplasm used in this investigation was Sarcoma 37 implanted intramuscularly into the right hind leg of hybrid CAF_1 mice. When α-elaterin was administered subcutaneously, it did not show any toxicity and had insignificant anti-tumor activity, while β-elaterin was active. When given intraperitoneally both α- and β-elaterin were found to be very active. Both produced tumor damage, the latter being more effective. Elateridin obtained by the hydrolysis of α-elaterin has been found to have also good activity.[10b]

This paper will deal with α-elaterin. The presence of a phenolic group in elaterin is indicated by the brown color which is obtained with ferric chloride, and its solubility in cold alcoholic alkaline solution. Further information regarding the nature of this group was obtained from the study of the ultraviolet spectrum of elaterin. It has a strong peak at 234 mμ (ε 11,700) and a shoulder at 267 mμ (ε 8,350). On addition of sodium hydroxide the shoulder disappears and a new peak is formed at 318 mμ (ε 5,000); this bathochromic shift is reversible with acid. Phenols are known to show shifts of about 50 mμ with alkali, the intensity increasing due to the formation of an enol ion. A decrease in the intensity, of about 40%, which accompanies the bathochromic shift is characteristic

(1) This investigation was supported (in part) by a research grant C-2810 PET from the National Cancer Institute of the National Institutes of Health, Public Health Service.

(2) Part I, D. Lavie, *Chemistry & Industry*, 466 (1956).

(3) V. Erspamer, *Riv. ital. essenze profumi piante offic. olii vegetali, saponi*, **28**, 264 (1946); *C. A.*, **41**, 2860 (1947).

(4) Morries, *J. Roy. Institute*, **1**, 352 (1831); Hennell, *Edinb. Med. Surg. J.*, **35**, 339 (1831).

(5) A. Berg, *Bull. soc. chim.*, [3] **35**, 435 (1906).

(6) W. Borsche and K. Diacont, *Ann.*, **528**, 39 (1937).

(7) C. W. Moore, *J. Chem. Soc.*, **97**, 1797 (1910).

(8) F. v. Hemmelmayr, *Ber.*, **39**, 3652 (1906); A. Berg, *Bull. soc. chim.*, [4] **7**, 385 (1910).

(9) F. B. Power and C. W. Moore, *J. Chem. Soc.*, **95**, 1985 (1909).

(10) (a) M. Belkin and D. B. Fitzgerald, *J. Nat. Cancer Inst.*, **13**, 139 (1952); (b) the bio-assays were made by M. Belkin and W. Hardy at the National Cancer Institute, Bethesda, Md., and will be published elsewhere.