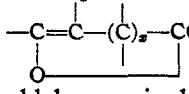
[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF WINTHROP CHEMICAL COMPANY, INC.]

The Mechanism of Action of Antibiotics. The Reaction of Unsaturated Lactones with Cysteine and Related Compounds

BY CHESTER J. CAVALLITO AND THEODORE H. HASKELL

A common property of many antibiotics which transcends chemical structural relationships is that of being inactivated by means of cysteine and certain related substances. It was suggested that possibly the fundamental mode of action of certain classes of antibiotics involves their ability to interfere with the normal function of sulfhydryl groups in bacterial metabolism.[1] Recently, Hauschka, Toennies and Swain[2] have observed a similar behavior with $\Delta^{\alpha,\beta}$-hexeno-δ-lactone and Geiger and Conn[3] have explained the antibacterial action of clavacin, penicillic acid and a number of α,β-unsaturated ketones on a similar basis. The latter investigators observed the disappearance of cysteine sulfhydryl groups in the presence of the antibacterial agents and, although no reaction products were isolated, there is evidence available in the literature showing that unsaturated ketones and acids are capable of adding RSH across the double bond,[4-7] the RS group, as a rule, becoming attached to the carbon farthest from the ketone or carboxyl group even when the double bond is not α,β- to the carbonyl group.[8]

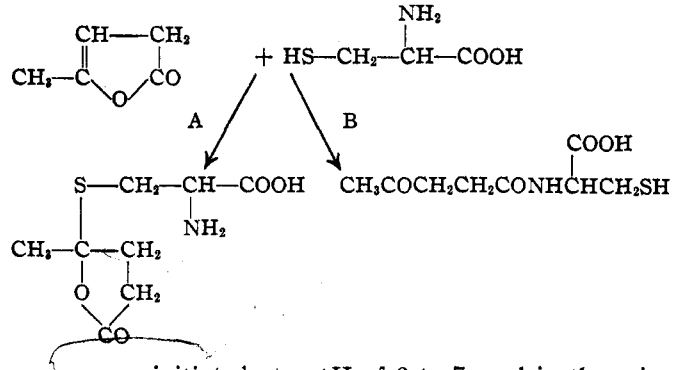
Although one might explain the inactivation by means of cysteine of penicillic acid, clavacin, anemonin (or protoanemonin),[1,9,10] the antibacterial agent $C_{15}H_{20}O_5$ from *Arctium minus*[11] and of $\Delta^{\alpha,\beta}$-hexeno-δ-lactone as involving only addition of RSH to a double bond, it cannot be ignored that all of these antibiotics are also lactones and the function of this group has not been investigated.

The lactone groups in clavacin and in $C_{15}H_{20}O_5$ appear to be essential for antibiotic activity, since treatment with alkalies destroys[11] or reduces[12] the activity. Extracts of Ranunculi also lose their antibacterial activity (protoanemonin) when treated with mild alkalies. Such behavior might be expected of compounds of the type  in which the lactone ring would be required for maintenance of the enolized unsaturated structure which could add RSH. In order to cast more light on this subject, the reaction of certain lactones with aminothiols was investigated.

The $\Delta^{\alpha,\beta}$- and $\Delta^{\beta,\gamma}$-angelicalactones were used as type compounds in the reaction with cysteine in aqueous media. The $\Delta^{\alpha,\beta}$-lactone yielded a very water soluble amphoteric reaction product and no cystine could be obtained by air oxidation of the reaction mixture. The $\Delta^{\beta,\gamma}$-lactone reacted with cysteine, yielding a crystalline product, $C_8H_{11}O_3NS(I)$, which contained one carboxyl group, one >C=C< unsaturated bond, no free —SH group, no lactone and no primary amino group. Cysteine methyl ester and β-aminoethanethiol gave similar products (with no free carboxyl group). Molecular weight determinations indicate that the over-all reaction involved one molecule each of cysteine (or aminothiol) and lactone with loss of one molecule of water.

Homocysteine reacted with $\Delta^{\beta,\gamma}$-angelicalactone to give a product in the same manner as did cysteine, indicating that the distance between amino and thiol groups in the carbon chain was not a limiting factor in this reaction.

The reaction of the lactones and aminothiols



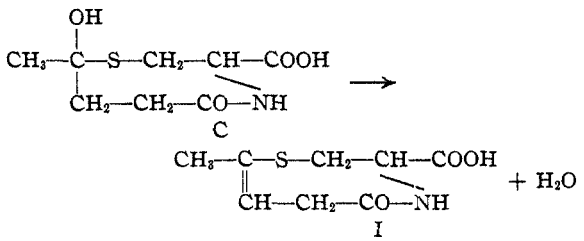
was initiated at a *p*H of 6 to 7, and in those instances in which a reaction took place, the *p*H was observed to fall rapidly. This could be explained by an opening of the lactone ring or by the disappearance of free amino groups during the reaction.

Cysteine did not react with γ-valerolactone, and alanine and S-methyl cysteine were unreactive toward $\Delta^{\beta,\gamma}$-angelicalactone. This indicated that the first step in the above reaction involved addition of the cysteine through the sulfhydryl group to the lactone double bond as in A rather than reaction of the lactone with the amino group of cysteine as in B.

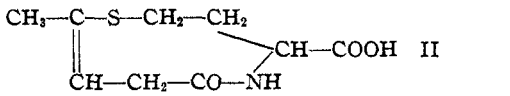
Reaction product A would then contain a basic

(1) Cavallito and Bailey, *Science* **100**, 390 (1944); also *J. Bact.*, **50**, in press (1945).
(2) Hauschka, Toennies and Swain, *Science*, **101**, 383 (1945).
(3) Geiger and Conn, THIS JOURNAL, **67**, 112 (1945).
(4) Posner, *Ber.*, **35**, 799 (1902); **37**, 502 (1904).
(5) Nicolet, THIS JOURNAL, **57**, 1098 (1935); **53**, 3066 (1931).
(6) Ruhemann, *J. Chem. Soc.*, **87**, 461 (1905).
(7) Morgan and Friedmann, *Biochem. J.*, **32**, 733 (1938).
(8) Schjanberg, *Ber.*, **74B**, 1751 (1941).
(9) Asahina and Fujita, *Acta Phytochim. (Japan)*, **1**, 1 (1922).
(10) Schmidt, *Z. Immunitäts.*, **102**, 233 (1942).
(11) Cavallito, Bailey and Kirchner, THIS JOURNAL, **67**, 948 (1945).
(12) Raistrick, *et al.*, *Lancet*, **245**, 625 (1943).

amino group and a lactone within the same molecule. Such a system could be expected to undergo an intramolecular reaction to yield C which upon loss of water could yield product I.



The compounds obtained from cysteine methyl ester and β-aminoethanethiol appear to be of this same type. The reaction product from homocysteine would be II by a similar reaction.



If the RS- group had added to the β- rather than γ-carbon of the lactone, the isomer of C so formed would not be expected to lose water under the experimental conditions.

The reaction product of cysteine and the $\Delta^{\alpha,\beta}$-angelicalactone could not be isolated in the pure state free of salts. The disappearance of cysteine and lactone with drop in *p*H and formation of an amphoteric solid indicates that the cysteine may have added as expected across the double bond and that the resulting lactone hydrolyzed without reacting with the amino group.

The effect of substitution or replacement of hydrogens in various positions of the $\Delta^{\alpha,\beta}$- and $\Delta^{\beta,\gamma}$-butenolactones on ease of reaction with cysteine was investigated. Whenever possible, the reaction products were isolated. The signs of reaction were drop in *p*H, disappearance of cysteine (non-recovery of cystine) and of lactone. When large quantities of cystine and unchanged lactone were recovered after several days reaction time, the reaction was presumed to be extremely slow or not to go. Both $\Delta^{\beta,\gamma}$-angelicalactone and α,α-dimethyl-$\Delta^{\beta,\gamma}$-angelicalactone reacted with cysteine, however α,α,β-trimethyl-$\Delta^{\beta,\gamma}$-angelicalactone, α,α,β-trimethyl-$\Delta^{\beta,\gamma}$-butenolide and β-methyl-$\Delta^{\beta,\gamma}$-angelicalactone (this lactone isomerizes very readily to the $\Delta^{\alpha,\beta}$-isomer) showed no measurable reaction with cysteine. Whereas $\Delta^{\alpha,\beta}$-angelicalactone reacted with cysteine, β-methyl-$\Delta^{\alpha,\beta}$-angelicalactone could be recovered quantitatively unchanged. This shows that in the $\Delta^{\beta,\gamma}$-series, substitution in the α- or γ-positions does not prevent the reaction, whereas β-substitution does. Similarly, β-substitution prevents reaction in the $\Delta^{\alpha,\beta}$-lactone. This may be of significance when attempting to predict antibiotic activity of unsaturated lactones.

Related compounds which were investigated were $\Delta^{\gamma,\delta}$-δ-pentenolactone which reacted with cysteine to give an amphoteric product, and levulinic acid, coumarin, dehydracetic acid and α-methyltetronic acid, none of which reacted with cysteine.

Experimental

Preparation of Lactones.—The lactones were prepared for the most part according to literature procedures. The γ-valerolactone,[13] $\Delta^{\alpha,\beta}$- and $\Delta^{\beta,\gamma}$-angelicalactones,[14] $\Delta^{\gamma,\delta}$-δ-pentenolactone[15] and α-methyltetronic acid[16] preparations were not changed. For the α,α-dimethyl-$\Delta^{\beta,\gamma}$-angelicalactone,[17] α,α,β-trimethyl-$\Delta^{\beta,\gamma}$-butenolide,[18] α,α,β-trimethyl-$\Delta^{\beta,\gamma}$-angelicalactone,[19,20] β-methyl-$\Delta^{\alpha,\beta}$- and β-methyl-$\Delta^{\beta,\gamma}$-angelicalactones[21] the literature procedures were followed except as modified below.

α,α-Dimethyl-$\Delta^{\beta,\gamma}$-angelicalactone.—The mesitonic acid[22,23] used in preparing the lactone was obtained as follows: A solution of 131 g. of potassium cyanide in 270 cc. of warm water was added to 98 g. of mesityl oxide dissolved in 700 cc. of hot ethanol. The mixture was heated on a steam-bath for twenty minutes, then cooled and a solution of 93 g. of ferrous sulfate in 180 cc. of water was added with shaking. The solution was warmed on a steam-bath for ten minutes, filtered, and the residue washed with hot ethanol. The ethanol was removed *in vacuo*, the residue was cooled and to it was added three times its volume of concentrated hydrochloric acid. The mixture was refrigerated overnight and then evaporated on a steam-bath to a thick dark sirup. The residue was extracted with ether, the ether was evaporated and the gummy residue dried *in vacuo* in a desiccator over sodium hydroxide. This was then distilled, yielding 33.5 g. (23% yield) of white solid mesitonic acid, b. p. 100–110° at 0.7 mm. (mesitylic acid, 8.5 g., was also obtained from the residue in the distillation flask by removing gummy material with ether and recrystallizing the residue from water).

α,α,β-Trimethyl-$\Delta^{\beta,\gamma}$-butenolide.—Ethyl α,α-dimethyl-β-hydroxypivalate was prepared in 63% yield, b. p. 88–91° at 17–18 mm. To 50 g. of this ester in 63 cc. of dry benzene was added 33 g. of phosphorus pentoxide and the mixture was heated on a steam-bath for fifteen minutes with frequent shaking. About 30 cc. of benzene was distilled off and the remaining liquid was decanted from the sticky mass. Distillation of this liquid yielded 34 g. (76% yield) of ethyl dimethylisopropenylacetate, b. p. 160° at 760 mm., 62–63° at 23 mm.

To a solution of 18.5 g. of potassium hydroxide (0.33 mole) in 185 cc. of absolute methanol was added 34 g. (0.22 mole) of the above ester and the mixture was refluxed for thirty minutes. The methanol was removed *in vacuo* and water was added to the solid residue. The solution was acidified with cold concentrated hydrochloric acid and the oil formed was extracted with ether, dried over Drierite and distilled, yielding 13 g. (45% yield) of the free acid, b. p. 111–115° at 25 mm.

A solution of 15 g. (0.094 mole) of bromine in 50 cc. of carbon disulfide was added dropwise to 12 g. (0.094 mole) of dimethylisopropenylacetic acid in 50 cc. of carbon disulfide, the temperature being maintained at 0°. The carbon disulfide was removed *in vacuo* and the crystals formed were washed with cold Skellysolve B, yielding 14.3 g. (53% yield) of the dibromo addition compound. Distillation of 25.6 g. of this acid on a Woods metal bath yielded 3.6 g. (32% yield) of the trimethylbutenolide, b. p. 63–67° at 12 mm.

(13) Schuette and Thomas, THIS JOURNAL, **52**, 3010 (1930).
(14) Gilmour, *J. Chem. Soc.*, **105**, 74 (1914).
(15) Vorländer and Knötzsch, *Ann.*, **294**, 319 (1897).
(16) Wolff and Erbstein, *ibid.*, **288**, 16 (1895).
(17) Pinner, *Ber.*, **15**, 579 (1882).
(18) Courtot, *Bull. soc. chim.*, [3] **35**, 298, 969, 995 (1906).
(19) Jacobs and Scott, *J. Biol. Chem.*, **93**, 144 (1931).
(20) Bardhan, *J. Chem. Soc.*, **131**, 2616 (1928).
(21) Jacobs and Scott, *J. Biol. Chem.*, **93**, 146 (1931).
(22) M. Qudrat-i-Khuda and S. K. Ghosh, *J. Indian Chem. Soc.*, **16**, 290 (1939).
(23) Lapworth, *J. Chem. Soc.*, **85**, 1219 (1904).

β-Methyl Angelicalactones.—β-Methyllevulinic acid[24,25] (29 g.) was distilled at atmospheric pressure from a Woods metal bath at 240 to 265°, the product distilling at 232–237°. The distillate was dissolved in ether, washed with 1% sodium bicarbonate solution, then with water, and dried over Drierite. The ether was evaporated and the product distilled *in vacuo*, the $\Delta^{\beta,\gamma}$-lactone distilling at 84–89° at 13 mm. On redistilling this lactone at 13 mm.[26] most of it was converted to the $\Delta^{\alpha,\beta}$-isomer, b. p. 111–112° (13 mm.), yield 8 g.

α,α,β-Trimethyl-$\Delta^{\beta,\gamma}$-angelicalactone.—α,α,β-Trimethylpentenoic acid, b. p. 121–124° at 8 mm., was obtained in 63% yield from ethyl α,α,β-trimethyl-$\Delta^{\beta,\gamma}$-pentenoate, b. p. 100–102° at 45 mm. which in turn was obtained in 77% yield from ethyl β-hydroxy-α,α,β-trimethyl-*n*-valerate, b. p. 90–96° at 11–12 mm.

To 24 g. (0.17 mole) of the trimethylpentenoic acid in 50 cc. of carbon disulfide at 0° was added dropwise with stirring 9 cc. (0.17 mole) of bromine in 50 cc. of carbon disulfide. After standing for ninety minutes in the cold, the carbon disulfide was distilled off and the residue distilled on a Woods metal bath at 225–250°. An oil distilled at 210° and this was dissolved in ether, washed with 1% sodium bicarbonate solution, then with water and dried over Drierite. The ether was distilled off and the product collected which distilled at 78–80° at 13 mm.; yield 8 g. (34%).

Reaction of $\Delta^{\beta,\gamma}$-Angelicalactone with Cysteine.—A solution of 4.83 g. (0.03 mole) of *l*-cysteine hydrochloride in 15 cc. of water was adjusted to *p*H 6.5 by addition of 10% sodium hydroxide solution. To this solution was added 3.01 g. (0.03 mole) of the angelicalactone in 10 cc. of ethanol. The solution was kept overnight at room temperature under nitrogen, during which time the *p*H dropped to 3.2. The *p*H was adjusted to 7.0 and oxygen was bubbled through the solution for three hours, after which the *p*H was dropped to 3.9 by addition of hydrochloric acid solution. No cystine was precipitated. (From a control solution containing cysteine alone, 85% of the original cysteine was recovered as cystine.) Upon slow evaporation at room temperature, large crystals separated. The product was filtered off, washed with a few cc. of dilute hydrochloric acid solution and dried at 80°, yielding 1.7 g. of crystals, m. p. 191–194°. A second crop of 1.7 g. was obtained (total yield 57%) by acidifying the mother liquor to *p*H 1.5. One recrystallization from water or ethanol gave the pure compound as prisms, m. p. 194°; $[\alpha]^{25}D$ in water (5 mg. per cc.) was −193°. The compound was soluble in water or ethanol, sparingly soluble in ether, benzene and chloroform and insoluble in the Skellysolves.

Anal. Calcd. for $C_8H_{11}O_3NS$: C, 47.76; H, 5.51; N, 6.96; S, 15.92. Found: C, 47.92; H, 5.22; N, 6.85; S, 15.98.

The reaction product had a neutralization equivalent of 200 (calcd. 201) and excess alkali did not show the presence of a lactone group. Grote's reagent[27] gave a negative test for free -SH groups, and a formol titration and Van Slyke amino nitrogen determination showed the absence of free amino groups. An iodine number determination (Hanus) showed the presence of one double bond (200 mg. of compound absorbed 1.0 millimole of halogen) not conjugated to a carbonyl group. A Rast molecular weight determination with 40 mg. of compound and 272 mg. of camphor gave a depression of 16°, representing a molecular weight of 367. A cryoscopic determination with 552 mg. of compound and 30.767 g. of dioxane gave a depression of 0.37°, representing a molecular weight of 239 (calcd. 201). The camphor fusion appears to have involved a chemical reaction.

Reaction of $\Delta^{\beta,\gamma}$-Angelicalactone with Cysteine Methyl Ester.—A solution of 1.75 g. (0.01 mole) of *l*-cysteine methyl ester hydrochloride in 10 cc. of water was adjusted to *p*H 6.8, then mixed with a solution of 1.0 g. (0.01 mole) of the lactone in 5 cc. of ethanol. The mixture was kept under nitrogen at 40° overnight, during which time the *p*H dropped to 1.8. The solution was allowed to evaporate at room temperature and the gummy residue taken up in absolute ethanol. Sodium chloride was filtered off and the alcohol was evaporated. The residue was extracted with dry ether, filtered, the ether evaporated and the product recrystallized twice from Skellysolve B. White needles were obtained, m. p. 67–69°; yield 400 mg. (19%); $[\alpha]^{25}D$ in water (5 mg. per cc.) was −234°. The compound was soluble in water, ethanol, ether, benzene and chloroform and sparingly soluble in Skellysolve B.

Anal. Calcd. for $C_8H_{13}O_3NS$: C, 50.23; H, 6.09; N, 6.51. Found: C, 50.01; H, 6.12; N, 6.37.

The reaction product contained no free carboxyl, sulfhydryl or amino groups. An iodine number showed the presence of one double bond (1.0 millimole of halogen absorbed by 221 mg. compound). A Rast molecular weight determination with 17 mg. of compound and 170 mg. of camphor gave a depression of 19°, representing a molecular weight of 211 (calcd. 215).

Reaction of $\Delta^{\beta,\gamma}$-Angelicalactone with Homocysteine.—A solution of 1.38 g. (0.01 mole) of *dl*-homocysteine in 10 cc. of water was adjusted to *p*H 6.5 and 1.0 g. (0.01 mole) of the lactone in 5 cc. of ethanol was added. After twenty-four hours at 40° under nitrogen, the *p*H was 3.3. The *p*H was lowered to 2 and the solution was slowly evaporated. The gummy residue was extracted several times with ether and evaporation of the ether yielded 300 mg. of crystals (14% yield), m. p. 155–156°. The product was recrystallized from water as prisms, m. p. 157°.

Anal. Calcd. for $C_8H_{13}O_3NS$: C, 50.23; H, 6.09; N, 6.51. Found: C, 50.22; H, 6.20; N, 6.53.

The reaction product had a neutralization equivalent of 223 (calcd. 215). No free sulfhydryl or amino groups were present. An iodine number showed the presence of one double bond (1.0 millimole of halogen absorbed by 243 mg. of compound).

Reaction of $\Delta^{\beta,\gamma}$-Angelicalactone with β-Aminoethanethiol.[28]—A solution of 1.16 g. (0.01 mole) of β-aminoethanethiol in 10 cc. of water was added to 1.0 g. (0.01 mole) of $\Delta^{\beta,\gamma}$-angelicalactone in 10 cc. of ethanol. Sodium hydroxide solution was added to *p*H of 6.0 and, upon standing, a rapid drop in *p*H took place. The solution was kept at 40° overnight under nitrogen, after which the *p*H was 2.5. The solution was concentrated and extracted with ether. The ether was distilled off and the residue refrigerated for several days, during which time large prisms crystallized out. These were filtered off and recrystallized twice from Skellysolve B. The yield was variable and low, the product had a m. p. of 37°, contained no carboxyl, sulfhydryl or basic amino groups. *Anal.* Calcd. for $C_7H_{11}ONS$: C, 53.50; H, 7.06; N, 8.92. Found: C, 53.49; H, 6.57; N, 8.73.

Reaction of α,α-Dimethyl-$\Delta^{\beta,\gamma}$-angelicalactone with Cysteine.—A solution of 1.57 g. (0.01 mole) of *l*-cysteine hydrochloride in 10 cc. of water was adjusted to *p*H 7.0 and to it was added 1.26 g. (0.01 mole) of α,α-dimethyl-$\Delta^{\beta,\gamma}$-angelicalactone in 10 cc. of ethanol. The solution was stored overnight at 40° under nitrogen, during which time the *p*H dropped to 4.0. The *p*H was raised to 7.0, oxygen was bubbled in for two hours, and then the *p*H was lowered to 1.0. The solution was extracted with ether, the ether evaporated and the residue recrystallized from water. The yield of product, m. p. 134°, was 240 mg. (11%) and about 50% of the starting cysteine was recovered as cystine. When the reaction was allowed to go for three days, the *p*H dropped to 3.5, the yield of product was 540 mg. (24%) and 10% cystine was recovered.

The reaction product could be obtained from cold water as hexagonal plates, m. p. 102°. When the compound was recrystallized from boiling water the melting point was higher, and, when dried above 60°, the m. p. was 134°. The higher melting form could be reconverted to the lower by dissolving it in dilute alkali and precipitating by

(24) Blaise, *Bull. soc. chim.*, [3] **23**, 920 (1900).

(25) W. Steinkopf, Merckoll and Strauch, *Ann.*, **545**, 45 (1940).

(26) Pauly, Gilmour and Will, *ibid.*, **403**, 152 (1914).

(27) Grote, *J. Biol. Chem.*, **113**, 571 (1936).

(28) Gabriel and Leupold, *Ber.*, **31**, 2832 (1898).

acidification. The lower melting form appears to be an unstable hydrate.

The 134° m. p. product had no free sulfhydryl or basic amino group, the neutralization equivalent was 237 (calcd. 229) and $[\alpha]^{25}D$ in water (10 mg. per cc.) was −217°. The iodine number was erratic and very high (of the hydrate as well). This may have resulted from oxidation of the sulfur atom which may be less firmly bound in this compound than in the angelicalactone reaction product.

Anal. Calcd. for $C_{10}H_{15}O_3NS$: C, 52.40; H, 6.60; N, 6.11. Found: C, 52.79; H, 6.79; N, 6.00.

Discussion

In a number of the reactions described, the yields were quite low. This may be partly accounted for by the solubility of the reaction products and by efforts to obtain the compounds in a pure state at the expense of yield. The compounds isolated may not represent the sole reaction product in each instance, as the water solubility and amphoteric nature of some of the possible reaction products would make isolation of small quantities extremely difficult. The compounds isolated, however, do give evidence as to how some of the unsaturated lactone antibiotics might react with the sulfur amino acids present in enzyme proteins. The experimental conditions used for the reactions were within the *p*H and temperature ranges which might exist in bacterial environments.

If the antibiotic acitivity of certain unsaturated lactones is dependent upon the ability of these compounds to add, across the double bond, RSH groups essential for bacterial metabolism, the reaction may in some instances be modified by the lactone group. The α,β-unsaturated lactones may not require the lactone structure for activity; however, with some antibiotics, a double bond may exist only as long as the lactone group stabilizes an enol-aldehyde or enol-ketone structure. These lactones might react with the protein moiety of an enzyme by addition of free enzyme —SH groups to the double bond, followed by reaction of the lactone with free amino groups present in the vicinity.

Acknowledgment.—We wish to thank the Misses Alice Rainey and Patricia Curran for the microanalyses.

Summary

The reaction of cysteine and related aminothiol derivatives with several types of unsaturated lactones has been investigated. Reaction appears to proceed through addition of the thiol group to the double bond, followed in the case of $\Delta^{\beta,\gamma}$-butenolides by reaction of the lactone with the amino group and loss of water. A β-substitution in both the $\Delta^{\alpha,\beta}$- and $\Delta^{\beta,\gamma}$-butenolide series prevents reaction with cysteine.

The reactions may be of significance as indicating a possible mode of action of unsaturated lactone antibiotics with sulfhydryl and possibly amino groups of enzyme proteins.

RENSSELAER, N. Y. RECEIVED SEPTEMBER 6, 1945

[CONTRIBUTION FROM THE STAMFORD RESEARCH LABORATORIES OF THE AMERICAN CYANAMID COMPANY]

Some Amine Derivatives of Acrylonitrile

BY O. F. WIEDEMAN AND W. H. MONTGOMERY

The reaction of acrylonitrile with ammonia to yield a mixture of β-cyanoethylamines has been reported by several investigators.[1,2,3] The β-cyanoethylamines have been prepared in these Laboratories with similar results, and in addition the as yet undescribed tertiary amine has been isolated.

$CH_2{=}CHCN + NH_3 \longrightarrow$

$H_2NCH_2CH_2CN$, β-Cyanoethylamine (or β-aminopropionitrile)

$HN(CH_2CH_2CN)_2$, Di-(β-cyanoethyl)-amine

$N(CH_2CH_2CN)_3$, Tri-(β-cyanoethyl)-amine

Tri-(β-cyanoethyl)-amine is a crystalline solid melting at 59°. It is interesting that it is only sparingly soluble in water while the primary and secondary amines are completely miscible with water. On cooling a hot aqueous or alcoholic solution of the impure tri-(β-cyanoethyl)-amine, it frequently separates as an oil instead of as crystals. However, with care and slow cooling it may be recrystallized from either solvent.

The instability of the primary amine has been previously discussed.[2,3] Buc, *et al.*, reported that vacuum-distilled samples were still stable after several months at room temperature or two years at 5°. Similar observations have been made here. However, many samples remained unchanged in tightly stoppered bottles at room temperature for several months and then decomposed within twenty-four hours for no apparent reason, yielding an orange mass (presumably acrylonitrile polymer) and developing a considerable ammonia pressure. Several additives were tested as a stabilizing influence. No effective stabilizer was found but carbon dioxide apparently accelerated decomposition.

The secondary amine is quite stable, although some decomposition occurs during distillation at 140–160° at 2–5 mm.

Whitmore, *et al.*, also reported the hydrogenation of β-aminopropionitrile to yield trimethylenediamine. The corresponding secondary and

(1) Hoffmann and Jacobi, U. S. Patent 1,992,615.

(2) Whitmore, Mosher, Adams, Taylor, Chapin, Weisel, and Yanko, THIS JOURNAL, **66**, 725 (1944).

(3) Buc, Ford and Wise, *ibid.*, **67**, 92 (1945).