[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

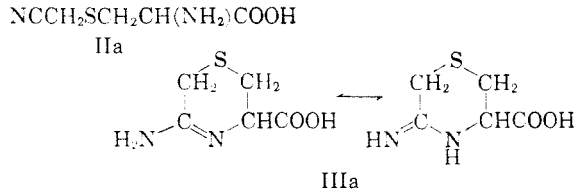
Potential Anticancer Agents.[1] XII. The Reaction of Chloroacetonitrile with L-Cysteine, DL-Homocysteine, and Cysteamine

LEON GOODMAN, L. O. ROSS, AND B. R. BAKER

Received June 23, 1958

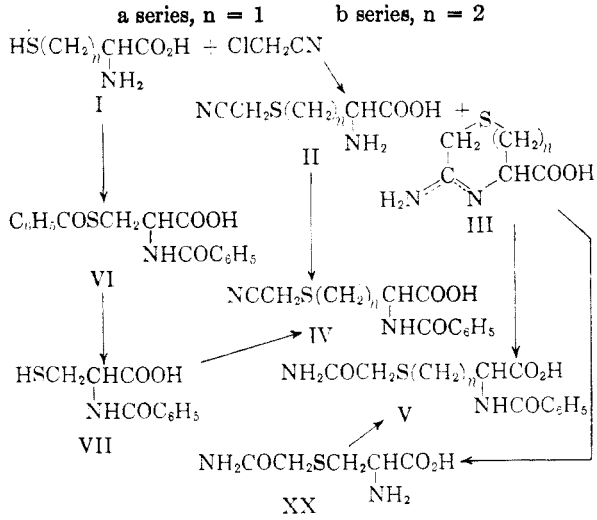
The reactions of chloroacetonitrile with L-cysteine and with DL-homocysteine give, as the major products, cyclic amidines rather than linear *S*-cyanomethyl compounds. The reaction of chloroacetonitrile with cysteamine leads to the linear *S*-cyanomethyl compound IX in basic solution and to the cyclic amidine XII (isolated as its picrate) in acid solution. Some observations on the similarly-constituted cyclic products, which may be formally represented as the *S*-cyano derivatives of L-cysteine, DL-homocysteine, and cysteamine, are presented.

In a previous paper of this series[2] it was reported that the reaction of chloroacetonitrile with L-cysteine (Ia) led to a product which showed no nitrile absorption in the infrared absorption spectrum and gave a weak yellow color with ninhydrin. These results gave strong indication that the product was not the expected α-amino acid, *S*-cyanomethyl-L-cysteine (IIa), but was probably the isomeric cyclic compound, L-3-amino-5,6-dihydro-2*H*-1,4-thiazine-5-carboxylic acid or a tautomer, either presumably existing as a zwitterion. The reactions of chloroacetonitrile with DL-



homocysteine (Ib) and with cysteamine (2-aminoethanethiol)(VIII) have now been investigated. Some observations on the structure of the products obtained from these reactions and of the related *S*-cyano analogs of L-cysteine, DL-homocysteine, and cysteamine are recorded.

A closer study of the reaction between chloroacetonitrile and L-cysteine (Ia) in the presence of base showed that the cyclic compound IIIa was the principal product obtained when the reaction mixture was acidified; evaporation of the filtrate left a residue which showed marked nitrile infrared absorption at 4.45 μ, strongly suggestive of the presence of IIa in the residue. This was confirmed by benzoylation of the residue under Schotten-Baumann conditions, which gave a 14% yield of *N*-benzoyl-*S*-cyanomethyl-L-cysteine (IVa). For comparison, compound IVa was prepared by the reaction of *N*-benzoyl-L-cysteine (VII) [prepared *in situ* from *N,S*-dibenzoyl-L-cysteine (VI)] with chloroacetonitrile. Compound IVa showed strong infrared nitrile absorption at 4.44 μ.



When DL-homocysteine [Ib) was allowed to react with chloroacetonitrile in the presence of aqueous base, the product formed upon acidification showed no nitrile absorption in the infrared and accordingly was assigned the seven-membered cyclic structure IIIb. Both IIIa and IIIb were basic compounds, as would be expected from their structures, and gave crystalline picrates. Compound IVb was prepared by benzoylation of the basic solution resulting from the reaction of Ib, chloroacetonitrile, and alkali. It showed nitrile infrared absorption at 4.44 μ.

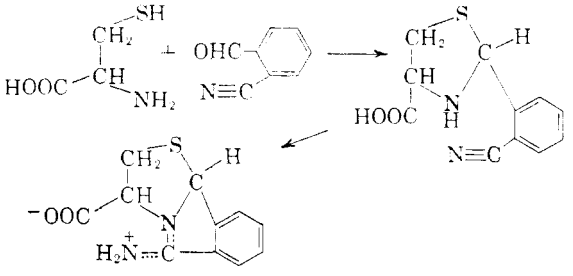
Benzoylation of IIIa and IIIb led to good yields of the corresponding *N*-benzoyl-*S*-carbamoylmethyl compounds Va and Vb. When stirred with aqueous base the cyclic compound IIIa was transformed to *S*-carbamoylmethyl-L-cysteine (XX), making it likely that this hydrolysis preceded the benzoylation in the conversion of IIIa to Va; presumably, the same is true for the transformation IIIb→Vb. Compound Va was prepared for comparison by benzoylation of *S*-carbamoylmethyl-L-cysteine (XX).

The cyclic structures written for IIIa and IIIb

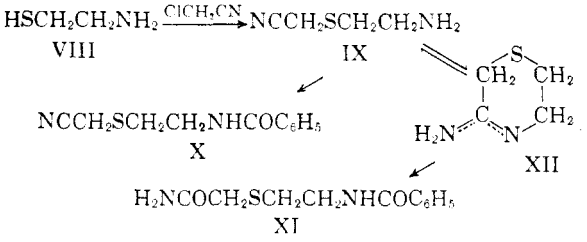
(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, and is in collaboration with the Sloan-Kettering Institute for Cancer Research. For the preceding paper of this series, *cf.* E. J. Reist, R. R. Spencer, and B. R. Baker, *J. Org. Chem.*, **23**, 1757 (1958).

(2) L. Goodman, L. O. Ross, and B. R. Baker, Paper V of this series, *J. Org. Chem.*, **23**, 1251 (1958).

are very similar to the "γ-lactamidines" structures written recently by Oliver, Dann, and Gates[3] for the products of the reaction of L-cysteine (and of DL-homocysteine and cysteamine) with *o*-cyanobenzaldehyde.

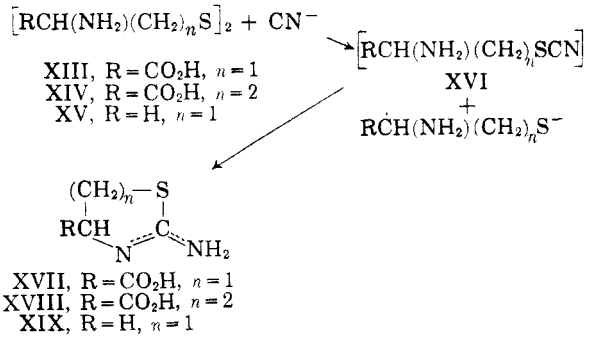


The reaction of cysteamine (VIII) with chloroacetonitrile was chosen as a reaction similar to those of the amino acids discussed above but which would not be subject to certain disadvantages resulting from the presence of a carboxyl group. Extraction



of the reaction product from the basic reaction mixture yielded a hygroscopic, unstable liquid which gave approximate analytical figures for *S*-cyanomethylcysteamine (IX) and showed a strong infrared nitrile band at 4.43 μ. Compound IX did not form an insoluble picrate in water. Benzoylation of the basic reaction mixture of VIII and chloroacetonitrile gave a good yield of *N*-benzoyl-*S*-cyanomethylcysteamine (X), accompanied by a small amount of *N*-benzoyl-*S*-carbamoylmethylcysteamine (XI), a possible indication of the presence of a small amount of the cyclic amidine XII in the basic reaction mixture. When the reaction mixture of chloroacetonitrile and VIII was made acid and then treated with aqueous picric acid, a 60% yield of a crystalline picrate was formed which had no infrared nitrile absorption and was clearly the picrate of the cyclic compound XII. This picrate also formed very slowly when the aqueous picric acid was added to the basic reaction mixture such that the final *p*H was still >7, a probable indication that a *p*H-dependent equilibrium between IX and XII exists and is displaced by the precipitation of the picrate of XII.

The compounds which can be envisioned as the *S*-cyano derivatives (XVI) of L-cysteine,[4-6] DL-homocysteine,[5] and cysteamine[6,7] have been studied by Schöberl and co-workers. These compounds are formed by the action of cyanide ion on the disulfides L-cystine (XIII), DL-homocystine (XIV), and cystamine (XV). The compounds exist



solely in the cyclic form as postulated by Schöberl; there was an absence of nitrile infrared absorption near 4.5 μ. All three compounds formed crystalline picrates, as would be predicted for the cyclic compounds. The picrates also showed no nitrile infrared absorption.

The infrared absorption spectra of IIIa, IIIb, XVII, and XVIII made it clear that these compounds existed as zwitterions; no carboxyl carbonyl absorption was present in the spectra of the compounds but such absorption near 5.80 μ was present in the spectra of the picrates of these compounds.

The ultraviolet spectra of IIIa and XVII were measured in 12*N* hydrochloric acid, in 0.1*N* hydrochloric acid, and in 0.1*N* sodium hydroxide. There were no absorption maxima beyond 227 mμ in any of the spectra. The spectrum of the thiazolinecarboxylic acid, formed by the cyclization of *N*-formylcysteine in strong acid solution and closely related to compounds XVII and XVIII, has recently been reported to give an absorption maximum at 268.5 mμ (ε 5180) in 12*N* hydrochloric acid.[8]

The benzoylation of L-cysteine (Ia) to *N*,*S*-dibenzoyl-L-cysteine (VI) in the presence of aqueous base has not been previously described in the literature. Compound VI previously has been prepared by treatment of L-2-phenyl-4-carboxyoxazoline with thiobenzoic acid.[9] The present convenient method makes it readily available as a source of *N*-benzoyl-L-cysteine (VII). It is possible that a small amount of racemization accompanies the direct benzoylation of L-cysteine, in view of the $[\alpha]_D^{30}$ −70.6° recorded as compared with Fry's[9] value of $[\alpha]_D^{21}$ −76°. When the benzoylation of L-cysteine was attempted in pyridine a different, unidentified product resulted.

(3) G. L. Oliver, J. R. Dann, and J. W. Gates, Jr., *J. Am. Chem. Soc.*, **80**, 702 (1958).
(4) A. Schöberl and R. Hamm, *Ber.*, **81**, 210 (1948).
(5) A. Schöberl and M. Kawohl, *Ber.*, **90**, 2077 (1957).
(6) A. Schöberl, M. Kawohl, and R. Hamm, *Ber.*, **84**, 571 (1951).
(7) S. Gabriel, *Ber.*, **22**, 1141 (1889).
(8) D. Cavallini, B. Mondove, and C. DeMarco, *Experientia*, **13**, 436 (1957).
(9) E. M. Fry, *J. Org. Chem.*, **15**, 438 (1950).

EXPERIMENTAL[10]

L-3-Amino-5,6-dihydro-2H-1,4-thiazine-5-carboxylic acid (IIIa) was obtained in 48% yield by the method described previously.[2] It had $[\alpha]_D^{26}$ −14.6° (1% in *N* HCl) and in the infrared it had λ_{max}^{KBr} (μ) 3.12 (NH), 5.98 (C=$\overset{+}{N}$H or C=N), 6.05–6.25 (probably C=N and CO_2^-, possibly NH_3^+), 6.70 (NH_3^+), 7.20 (CO_2^-).

Benzoylation of the mother liquors in the presence of potassium hydroxide, after separation of IIIa, led to a 14% yield of *N*-benzoyl-*S*-cyanomethyl-L-cysteine (IVa), which is described in detail below.

The picrate of IIIa was obtained by mixing hot solutions of 0.10 g. of IIIa in 10 ml. of water and 0.20 g. of picric acid in 15 ml. of water. The product precipitated slowly on chilling, m.p. 160–162°, resolidifying and decomposing at 195–197°; this was analyzed without further purification. In the infrared it had λ_{max}^{KBr} (μ) 3.16 (NH_2), 5.77 (carboxyl C=O), 6.00 (C=$\overset{+}{N}$H), 6.40, 7.47, and 7.57 (NO_2).

Anal. Calcd. for $C_{11}H_{11}N_5O_9S$: C, 33.9; H, 2.85; N, 18.0. Found: C, 34.0; H, 2.93; N, 18.1.

N-Benzoyl-S-carbamoylmethyl-L-cysteine (Va). (A) *By benzoylation of* IIIa. A mixture of 1.2 g. (7.6 mmoles) of IIIa, 1.3 g. (9.7 mmoles) of benzoyl chloride, 0.80 g. (20 mmoles) of sodium hydroxide, and 35 ml. of water was stirred at room temperature until the odor of benzoyl chloride could no longer be detected (about 1.5 hr.). The solution was adjusted to *p*H 2 with 6*N* hydrochloric acid and the resulting precipitate, after drying, was suspended in benzene. The insoluble portion (1.0 g., 48%), m.p. 186.5–188°, was washed thoroughly with benzene (15 ml.) and was recrystallized from 2:1 methanol–petroleum ether (62–70°), m.p. 187–188°, $[\alpha]_D^{31}$ −64.6° (1.00% in MeOH); λ_{max}^{KBr} (μ) 3.04 (NH), 5.87 (carboxyl C=O), 6.10 and 6.15 (amide carbonyls), 6.55 (amide NH), 7.82 and 8.11 (amide C—N), 13.90 (benzoyl).

Anal. Calcd. for $C_{12}H_{14}N_2O_4S$: C, 51.1; H, 5.00; N, 9.92. Found: C, 51.2; H, 5.02; N, 9.89, 9.92.

(B) *From S-carbamoylmethyl-L-cysteine* (XX). A mixture of 0.89 g. (5.0 mmoles) of *S*-carbamoylmethyl-L-cysteine,[2] 0.75 g. (5.3 mmoles) of benzoyl chloride, 0.38 g. (5.8 mmoles) of potassium hydroxide, and 10 ml. of water was treated as described in section A. The crude product, after treatment with benzene, weighed 1.15 g. (82%), m.p. 183–184°. After one recrystallization from methanol–petroleum ether (62–70°), it melted at 188–189°, $[\alpha]_D^{28}$ −67.6° (1.00% in MeOH), and gave no depression when mixed with the product from section A. Its infrared spectrum was identical with that of the preparation from IIIa.

Hydrolysis of IIIa *to S-carbamoylmethyl-L-cysteine* (XX). L-3-Amino-5,6-dihydro-2*H*-1,4-thiazine-5-carboxylic acid (IIIa) (0.89 g., 5.56 mmoles) was stirred for 1.5 hr. at room temperature with a solution of 0.35 g. (5.3 mmoles) of potassium hydroxide in 15 ml. of water. The solution was adjusted to *p*H 5 with 6*N* hydrochloric acid and chilled to give 0.35 g. (36%) of *S*-carbamoylmethyl-L-cysteine (XX), m.p. 175–185° dec. (lit.[2] m.p. 188–190° dec.). The infrared spectrum compared well with that of a previous sample of *S*-carbamoylmethyl-L-cysteine (XX).

N,S-Dibenzoyl-L-cysteine (VI). To a chilled mixture of 6.32 g. (40.0 mmoles) of L-cysteine (Ia) hydrochloride, 11.24 g. (80 mmoles) of benzoyl chloride, and 44 ml. of water was added, dropwise and with stirring, a solution of 10.56 g. (0.16 mole) of potassium hydroxide in 100 ml. of water. The mixture was stirred until all the benzoyl chloride had dissolved, yielding a solution with *p*H 7. Addition of 1.0 ml. of 6*N* hydrochloric acid resulted in a white precipitate which was filtered, dried, and suspended in 25 ml. of cold benzene to remove benzoic acid. The solid was filtered, washed with 20 ml. of cold benzene, and dried, yielding 5.0 g. (38%) of crude VI, m.p. 176–184° (prior softening). Recrystallization of 2.0 g. twice from methanol-water mixtures gave 1.0 g. of purified product, m.p. 163–165°, resolidifying and remelting at 182–184° (dec.). An analytical sample had m.p. 167–169°, resolidifying and remelting at 184–186°, $[\alpha]_D^{30}$ −70.6° (2.00% in 95% EtOH) [lit.[9] m.p. 179–182° (prior softening), $[\alpha]_D^{21}$ −76° (1.00% in 95% ethanol)]. In the infrared it had λ_{max}^{KBr} (μ) 3.05 (NH), 5.85 (carboxyl C=O), 6.03 (thioester C=O), 6.11 (amide C=O), 6.55 (amide NH), 7.07 and 7.62 (COOH), 13.90 and 14.50 (benzoyl).

Anal. Calcd. for $C_{17}H_{15}NO_4S$: C, 62.0; H, 4.59; N, 4.25. Found: C, 62.2; H, 4.78; N, 3.93, 4.06.

On a run of four times the size of the above, the yield of recrystallized product was 39 g. (76%).

N-Benzoyl-S-cyanomethyl-L-cysteine (IVa). (A) *From N,S-dibenzoyl-L-cysteine* (VI). A solution of 1.35 g. (4.1 mmoles) of VI in 9.5 ml. of 2*N* sodium hydroxide was stirred at room temperature for 10 min. Chloroacetonitrile (0.96 g., 13 mmoles) was added and the mixture was stirred until the chloroacetonitrile had dissolved. The solution was adjusted to *p*H 4 with 6*N* hydrochloric acid and the resulting white precipitate was suspended in 20 ml. of benzene, filtered, and washed with 20 ml. of benzene, leaving a residue, 0.70 g., of IVa, m.p. 107–111°, resolidifying and remelting at 145–150°. A further 0.15 g., m.p. 108–113°, resolidifying and remelting at 155–160°, was recovered by partial evaporation of the filtrate, giving a total crude yield of 79%. The combined products were recrystallized from ethyl acetate–petroleum ether (88–99°) to give pure IVa, m.p. 115–116°, resolidifying and remelting at 160–162°, $[\alpha]_D^{30}$ −72.8° (2.00% in 95% EtOH); λ_{max}^{KBr} (μ) 3.04 and 3.16 (NH), 4.44 (C≡N), 5.76 (carboxyl C=O), 6.10 (amide C=O), 6.57 (amide NH), 13.90 (benzoyl). A sample with m.p. 108–112°, resolidifying and remelting at 157–159°, was analyzed.

Anal. Calcd. for $C_{12}H_{12}N_2O_3S$: C, 54.2; H, 4.58; N, 10.6. Found: C, 54.5; H, 4.39; N, 10.5.

A second crystal modification which shows the single melting range, 164–166°, has also been isolated from some preparations of IVa.

(B) *From L-cysteine* (Ia). A mixture of 0.88 g. (5.0 mmoles) of L-cysteine hydrochloride (monohydrate), 1.5 ml. (24 mmoles) of chloroacetonitrile, 0.99 g. (15.0 mmoles) of potassium hydroxide, and 30 ml. of water was stirred at room temperature for 5 min. To the resulting solution was added 0.71 g. (5.0 mmoles) of benzoyl chloride and a solution of 0.33 g. (5.0 mmoles) of potassium hydroxide in 5 ml. of water and the mixture was stirred at room temperature until the odor of benzoyl chloride had disappeared (45 min.). The solution was adjusted to *p*H 2 with 6*N* hydrochloric acid, whereupon a white, crystalline solid and a brown oil, which slowly solidified, were precipitated. The white solid, which was separated before the oil had solidified, was washed thoroughly with benzene and yielded 0.30 g., m.p. 101–110°; the infrared spectrum showed this material to be *N*-benzoyl-*S*-cyanomethyl-L-cysteine (IVa) contaminated with some *N*-benzoyl-*S*-carbamoylmethyl-*L*-cysteine (Va). The brown solid was recrystallized from ethyl acetate–petroleum ether (62–70°) and gave 0.20 g. of product, m.p. 112–115°, whose infrared spectrum showed it to be quite pure IVa. The yield of IVa, based on both crops of product, was 38%.

L-3-Amino-2,5,6,7-tetrahydro-1,4-thiazepine-5-carboxylic acid (IIIb). A mixture of 0.272 g. (2.0 mmoles) of DL-homocysteine, 1.5 ml. (23.7 mmoles) of chloroacetonitrile, 0.144 g. (2.19 mmoles) of potassium hydroxide, and 34 ml. of water was stirred until all the chloroacetonitrile had dissolved (about 10 min.). The solution now had *p*H 9; by

(10) Melting points (obtained with the Fisher-Johns apparatus) and boiling points are uncorrected. Optical rotations were obtained using a Standard Polarimeter Model D attachment to the Beckman DU spectrophotometer, calibrated with standard sucrose solutions.[11]

(11) A. S. Keston, Abstracts of 127th Meeting, American Chemical Society, 18C (1955).

acidification with 6*N* hydrochloric acid the *p*H was adjusted to 3. After long standing at 0°, the solution deposited 0.160 g. (45.8%) of a white, crystalline solid, m.p. >300° (darkening near 240°). This was recrystallized from 10 ml. of hot water. In the infrared the solid had λ_{max}^{KBr} (μ) 3.12 (NH), 3.66 (N, weak), 5.93 (C=$\overset{+}{N}$H, probable), 6.08 (C=N or NH_2), 6.37 and 7.15 (CO_2^-), 6.60 (possibly NH_3^+).

Anal. Calcd. for $C_6H_{10}N_2O_2S$: C, 41.4; H, 5.78; N, 16.1. Found: C, 41.1; H, 5.81; N, 16.2.

In a preliminary run of one-half the above scale, a solution of 0.13 g. of picric acid in 10 ml. of hot water was added to the filtrate from the separation of IIIb. On chilling, a yellow precipitate, m.p. 225–232° (dec.), formed which was recrystallized from hot water, m.p. 235° (dec.). In the infrared it had λ_{max}^{KBr} (μ) 3.08 (NH), 5.79 (carboxyl C=O, weak), 5.98 (C=$\overset{+}{N}$H), 6.40 and 7.54 (NO_2).

Anal. Calcd. for $C_{12}H_{13}N_5O_9$: C, 35.7; H, 3.25; N, 17.14. Found: C, 35.9; H, 3.42; N, 17.3.

*N-Benzoyl-S-cyanomethyl-*DL*-homocysteine* (IVb). A mixture of 0.350 g. (2.57 mmoles) of DL-homocysteine, 1.1 ml. (17.4 mmoles) of chloroacetonitrile, and 0.660 g. (10.0 mmoles) of potassium hydroxide was stirred at room temperature for 10 min. until complete solution was attained. Benzoyl chloride (0.400 g., 2.84 mmoles) was added and the mixture was stirred for 90 min., at which time the odor of benzoyl chloride was no longer evident. The solution now had *p*H 9 and was adjusted to *p*H 2 with 6*N* hydrochloric acid. The resulting white precipitate was suspended in 20 ml. of benzene and the insoluble material was filtered and washed with 20 ml. of benzene, leaving 0.350 g. (49%), m.p. 130–138° (prior softening). This was recrystallized from a mixture of 9 ml. of methanol and 100 ml. of carbon tetrachloride, giving 260 mg. (36.8%), m.p. 135–140°. Two more similar recrystallizations gave the analytical sample, m.p. 138–140°. In the infrared it had λ_{max}^{KBr} (μ) 3.02 and 6.48 (NH), 4.43 (C≡N), 5.77 (carboxyl C=O), 6.05 (amide C=O), 13.82 and 14.42 (mono-substituted phenyl).

Anal. Calcd. for $C_{13}H_{14}N_2O_3S$: C, 56.1; H, 5.07; S, 11.5. Found: C, 55.9; H, 5.17; S, 11.5.

*N-Benzoyl-S-carbamoylmethyl-*DL*-homocysteine* (Vb). A mixture of 0.106 g. (0.61 mmole) of L-3-amino-2,5,6,7-tetrahydro-1,4-thiazepine-5-carboxylic acid (IIIb), 0.100 g. (0.71 mmole) of benzoyl chloride, 0.100 g. (1.54 mmoles) of potassium hydroxide, and 5 ml. of water was stirred at room temperature until the odor of benzoyl chloride was no longer evident (about 1 hr.). The resultant solution was acidified to *p*H 2 with 6*N* hydrochloric acid and, after long standing at 0°, the solution deposited 0.150 g. (83%), m.p. 160–162°. This was recrystallized from acetone–petroleum ether (62–70°) (1:1) to give the analytical sample, m.p. 164–166°; λ_{max}^{KBr} (μ) 2.99 (NH), 3.8–4.0 (carboxyl OH), 5.84 (carboxyl C=O), 6.08 (amide C=O), 6.52 (amide NH), 13.95, 14.15 (benzoyl).

Anal. Calcd. for $C_{13}H_{16}N_2O_4S$: C, 52.7; H, 5.44; S, 10.8. Found: C, 52.8; H, 5.74; S, 10.8.

S-Cyanomethylcysteamine (IX). A mixture of 0.57 g. (5.0 mmoles) of cysteamine (VIII) hydrochloride, 0.60 ml. (9.5 mmoles) of chloroacetonitrile, 0.67 g. (10 mmoles) of potassium hydroxide, and 10 ml. of water was stirred at room temperature until the chloroacetonitrile dissolved (5 min.); the solution then had *p*H 10. It was extracted with three 15-ml. portions of chloroform and the extracts were dried over magnesium sulfate. After being filtered, the extracts were evaporated *in vacuo*, finally at 2 mm., leaving 0.50 g. (86%) of nearly colorless hygroscopic oil. This oil was not quite pure and rapidly became colored on standing; it was submitted for analysis without further purification. In the infrared it had λ_{max}^{film} (μ) 2.98 and 5.98 (NH_2), 4.44 (C≡N), 7.05 (C—N).

Anal. Calcd. for $C_4H_8N_2S$: C, 41.3; H, 6.94. Found: C, 40.0; H, 6.58.

3-Amino-5,6-dihydro-2H-1,4-thiazine (XII) *picrate* was prepared when the same amounts of cysteamine (VIII) hydrochloride and chloroacetonitrile as described above were allowed to react with 0.30 g. (7.6 mmoles) of sodium hydroxide. The final solution was adjusted to *p*H 5 with 6*N* hydrochloric acid and was added to a hot solution of 1.28 g. (5 mmoles) of picric acid in 100 ml. of water. On chilling, 1.05 g. (60.6%) of picrate, m.p. 235–240° (dec.), precipitated. The analytical sample was obtained by recrystallization from hot water, m.p. 240–241° (dec.). In the infrared it had λ_{max}^{KBr} (μ) 2.98 (NH), 5.98 (C=$\overset{+}{N}$H), 6.10 ($N\overset{+}{H}_2$), 6.40 and 7.50 (NO_2).

Anal. Calcd. for $C_{10}H_{11}N_5O_7S$: C, 34.8; H, 3.21; N, 20.3. Found: C, 34.7; H, 3.23; N, 20.6.

When the strongly basic reaction mixture of cysteamine (VIII) and chloroacetonitrile was added to an aqueous picric acid solution such that the final solution had *p*H 9, there was no immediate precipitation. After 1 day about 10 mg. of XII picrate had precipitated. After 1 week 330 mg. (37%) of picrate, m.p. 233–235° (dec.), was isolated. It gave an infrared spectrum identical with that of the analytical sample.

N-Benzoyl-S-cyanomethylcysteamine (X). A mixture of 0.57 g. (5.0 mmoles) of cysteamine (VIII) hydrochloride, 0.75 ml. (12 mmoles) of chloroacetonitrile, 0.61 g. (9.3 mmoles) of potassium hydroxide, and 30 ml. of water was stirred at room temperature for 10 min. To the resultant solution was added 0.80 g. (5.7 mmoles) of benzoyl chloride and a solution of 0.40 g. (6.0 mmoles) of potassium hydroxide in 5 ml. of water. The mixture was stirred at room temperature until the odor of benzoyl chloride was no longer evident (about 90 min.). The final mixture contained a yellow oil; it had *p*H 6 and there was no visible change when the *p*H was adjusted to 2 with 6*N* hydrochloric acid. The oil was separated by extraction with 50 ml. of methylene chloride, the extract was dried over magnesium sulfate, filtered, and evaporated *in vacuo* to give 1.02 g. (93%) of a yellow oil which solidified on standing and whose infrared spectrum showed it to be mainly X. The solid was dissolved in hot benzene and a small amount of petroleum ether (62–70°) was added to the point of turbidity. On cooling, the solution deposited about 100 mg. of an unidentified white solid. The mother liquors from the unidentified solid were evaporated *in vacuo* and the residue was recrystallized twice from benzene–petroleum ether (62–70°) (1:2) to yield the analytical product m.p. 66–68°. In the infrared it had λ_{max}^{KBr} (μ) 3.00 (NH), 4.47 (C≡N), 6.10 (amide C=O), 6.54 (amide NH), 13.90 and 14.45 (benzoyl).

Anal. Calcd. for $C_{11}H_{12}N_2OS$: C, 60.0; H, 5.49; S, 14.6. Found: C, 60.2; H, 5.54; S, 14.3.

From another similar preparation of X, there was isolated a small amount of *N*-benzoyl-*S*-carbamoylmethylcysteamine (XI), m.p. 119–120°. In the infrared it had λ_{max}^{KBr} (μ) 3.02 (NH), 6.07 and 6.15 (amide carbonyls), 6.51 (amide NH), 13.94 and 14.42 (benzoyl).

Anal. Calcd. for $C_{11}H_{14}N_2O_2S$: C, 55.4; H, 5.92; S, 13.5. Found: C, 55.9; H, 5.93; S, 13.1.

L-*2-Amino-2-thiazoline-4-carboxylic acid* (XVII) was prepared according to the procedure of Schöberl and Hamm[4] and melted at 234–237° (dec.) after recrystallization from 50% aqueous ethanol. In the infrared it had λ_{max}^{KBr} (μ) 3.13–3.50 (NH_2 and CH), 5.92 (C=$\overset{+}{N}$H), 6.09–6.25 (NH_2, C=N, and CO_2^-), 7.12 (CO_2^-).

The picrate of XVII was prepared by mixing hot solutions of 0.10 g. of XVII in 10 ml. of water and of 0.20 g. of picric acid in 15 ml. of water. The quite soluble solid crystallized slowly on chilling, m.p. 158–168°, resolidifying and decomposing at 183–210°. The sample was analyzed without further purification. In the infrared it had λ_{max}^{KBr} (μ) 3.15 (NH_2), 5.75 (carboxyl C=O), 6.10 (C=$\overset{+}{N}$H or NH_3^+), 6.45 and 7.50 (NO_2). There was also a suggestion of NH_3^+ with a band at 6.70 μ.

Anal. Calcd. for $C_{10}H_9N_5O_9S$: C, 32.0; H, 2.42; N, 18.7. Found: C, 31.9; H, 2.57; N, 18.5.

L-2-Amino-5,6-dihydro-4H-1,3-thiazine-4-carboxylic acid (XVIII) was prepared according to the procedure of Schöberl and Kawohl[5] and had m.p. 257–259° dec. (lit.[5] m.p. 238–240° dec.) after recrystallization from 50% aqueous ethanol. In the infrared it had λ_{max}^{KBr} (μ) 3.05–3.40 (NH_2 and/or NH_3^+), 6.05–6.35 (C=N, NH_2 and/or NH_3^+, and CO_2^-), 7.23 (CO_2^-); there was no carboxyl carbonyl absorption near 5.9 μ.

The picrate of XVIII was prepared by mixing hot solutions of 0.10 g. of XVIII in 10 ml. of water and of 0.20 g. of picric acid in 15 ml. of water. The yellow solid, 0.20 g., m.p. 218–220° (dec.), slowly precipitated and was recrystallized from 20 ml. of hot water, m.p. 217–218° (dec.). In the infrared it had λ_{max}^{KBr} (μ) 2.98 and 3.05 (NH), 3.17 (NH_3^+), 5.79 (carboxyl C=O), 6.10–6.15 (aryl CH and possibly $C{=}\overset{+}{N}H$), 6.42 and 7.50 (NO_2).

Anal. Calcd. for $C_{11}H_{11}N_5O_9S$: C, 33.9; H, 2.85; N, 18.0. found: C, 34.2; H, 3.09; N, 18.2.

2-Amino-2-thiazoline (XIX) was prepared according to the procedure of Gabriel[7] and had m.p. 79–80° (lit.[7] m.p. 86°) after two recrystallizations from benzene–petroleum ether (62–70°) (1:2). In the infrared it had λ_{max}^{KBr} (μ) 2.83 and 3.26 (NH), 6.08 (C=N), 7.42 and 10.06 (strong bands of unknown assignment).

Anal. Calcd. for $C_3H_6N_2S$: C, 35.3; H, 5.92. Found: C, 35.4; H, 5.98.

The picrate of XIX was prepared by mixing ether solutions of XIX and of picric acid, m.p. 236–239° dec. After one recrystallization from hot water it had m.p. 241–243° dec. (lit.[6,7] m.p. 235° dec.). In the infrared it had λ_{max}^{KBr} (μ) 2.90 and 3.10 (NH), 6.11 ($C{=}\overset{+}{N}H$), 6.40 and 7.52–7.57 (NO_2). There was no NH_3^+ absorption around 3.25 μ.

Acknowledgment. The authors are indebted to Dr. Peter Lim for infrared interpretation.

MENLO PARK, CALIF.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

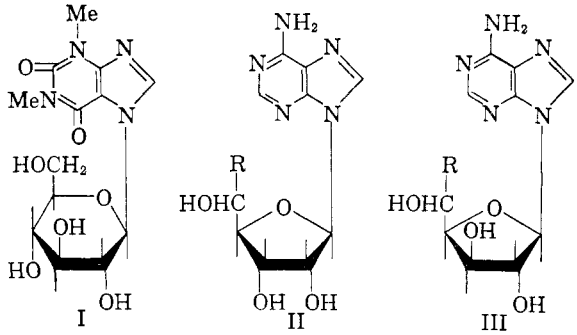
Potential Anticancer Agents.[1] XV. Synthesis of 9-β-D-Glucofuranosyladenine

ELMER J. REIST, ROLAND R. SPENCER, AND B. R. BAKER

Received July 21, 1958

The title compound (VIII) has been prepared by coupling chloromercuri-6-benzamidopurine with 2,3,5,6-tetra-*O*-benzoyl-D-glucofuranosyl chloride (VI) in 24% yield and with 2-*O*-acetyl-3-*O*-benzoyl-5,6-*O*-carbonyl-D-glucofuranosyl chloride (XVI) in 7% yield. A useful preparative method for the intermediate, 1,2-*O*-isopropylidene-D-glucofuranose 5,6-carbonate (XII), has been developed.

D-Glucose, the most common sugar existing in natural products, was the first sugar to be converted to a synthetic nucleoside when Fischer and Helferich,[2] in 1914, described the synthesis of 7-β-D-glucopyranosyltheophylline (I). Since that time, many nucleosides that contain the D-glucopyranose moiety[3] have been synthesized, due to the ready availability and stability of 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl bromide.[4] In addition, the pyranose ring structure is usually observed in natural materials containing D-glucose.[5] However, no nucleoside derived from the furanose form of D-glucose has been described in the literature.



The program under investigation in this laboratory on *C*-alkylpentofuranosyl nucleosides as possible anticancer agents has led to the synthesis of the two isomeric 5-*C*-methyl-D-ribosides (II, R = CH_3), 9-(6′-deoxy-β-D-allofuranosyl)adenine[6] and

(1) This program is under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, and is in collaboration with the Sloan-Kettering Institute for Cancer Research. For the preceding paper in this series, *cf.* L. Goodman, A. Benitez, C. D. Anderson, and B. R. Baker, *J. Am. Chem. Soc.*, in press.

(2) E. Fischer and B. Helferich, *Ber.*, **47**, 210 (1914).

(3) (a) B. R. Baker, J. P. Joseph, R. E. Schaub, and J. H. Williams, *J. Org. Chem.*, **19**, 1780 (1954); (b) J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951); (c) J. J. Fox, N. Yung, J. Davoll, and G. B. Brown, *J. Am. Chem. Soc.*, **78**, 2117 (1956); (d) D. W. Visser, I. Goodman, and K. Dittmer, *J. Am. Chem. Soc.*, **70**, 1926 (1948); (e) G. E. Hilbert and T. B. Johnson, *J. Am. Chem. Soc.*, **52**, 4489 (1930); (f) J. J. Fox, N. Yung, I. Wempen, and I. L. Doerr, *J. Am. Chem. Soc.*, **79**, 5060 (1957); (g) A. Holland, B. Lythgoe, and A. R. Todd, *J. Chem. Soc.*, 965 (1948).

(4) C. E. Redemann and C. Niemann, *Org. Syntheses*, **22**, 1 (1942) and references therein.

(5) W. W. Pigman and R. M. Goepp, Jr., *Chemistry of the Carbohydrates*, second printing, Academic Press, Inc., New York, N. Y., 1952.

(6) E. J. Reist, L. Goodman, R. R. Spencer, and B. R. Baker, Paper IV of this series, *J. Am. Chem. Soc.*, **80**, 3962 (1958).