TABLE II

PICRATE AND METHIODIDE DERIVATIVES OF 12-DIALKYLAMINOALKYLBENZO[a]- AND -BENZO[b]PHENOXAZINE

Compound	Derivative	M.P.	Recrystn. Solvent	Molecular Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
I. $n = 2$ $R_1 = R_2 = CH_3$—	Methiodide	210°	95% Ethanol and ether	$C_{22}H_{25}IN_2O$	57.39	57.49 57.24	5.43	5.48 5.57	6.09	6.07 6.45
II. $n = 1$ $R_1 = R_2 = CH_3CH_2$—	Picrate	190°	Dioxane and ethanol	$C_{28}H_{27}N_5O_8$	59.89	59.96 59.95	4.81	4.93 5.09	12.44	12.28 12.78
II. $n = 2$ $R_1 = R_2 = CH_3$—	Picrate	234°	Propylene glycol and ethanol	$C_{27}H_{25}N_5O_8$	59.23	59.16 59.01	4.57	4.54 4.39	12.80	12.78 12.50
	Methiodide	242°	95% Ethanol and ether	$C_{22}H_{25}IN_2O$	57.39	56.95 57.20	5.43	5.61 5.53	6.09	5.88 5.93

tion and evaporation of the benzene yielded a green-yellow oil.

Anal. Calcd. for $C_{22}H_{22}Cl_2N_2O$: C, 65.86; H, 5.49; N, 6.98. Found: C, 65.95, 65.73; H, 5.69, 5.53; N, 6.85, 6.56.

The above oil was converted to the *hydrochloride* in ethanol-ether solvent. The over-all yield was 62% and the salt melted at 160°.

Anal. Calcd. for $C_{22}H_{23}Cl_3N_2O$: C, 60.34; H, 5.23; N, 6.40. Found: C, 60.48, 60.42; H, 5.16, 5.32; N, 6.33, 6.80.

12-{2-[Bis(2-chloroethyl)amino]ethyl}benzo[a]phenoxazine. (I, $n = 1$, $R_1 = R_2 = ClCH_2CH_2$—]. This preparation was conducted in accord with the procedure given above. The intermediate bis(hydroxyethyl)compound was not purified, but treated directly with phosphoryl chloride. The product was a solid, m.p. 68°.

Anal. Calcd. for $C_{22}H_{22}Cl_2N_2O$: C, 65.86; H, 5.49; N, 6.98. Found: C, 65.72, 65.67; H, 5.67, 5.75; N, 6.90, 6.97.

The *hydrochloride*, m.p. 140°, was formed in 64% over-all yield from 12-(2-chloroethyl)benzo[a]phenoxazine. The carbon analysis was somewhat high on this salt, although hydrogen and nitrogen values were satisfactory.

Anal. Calcd. for $C_{22}H_{23}Cl_3N_2O$: C, 60.34; H, 5.23; N, 6.40. Found: C, 61.30, 61.53; H, 5.37, 5.24; N, 6.45, 6.20.

Preparation of 12-dialkylaminoalkyl derivatives of benzo[a]- and benzo[b]phenoxazine. A solution of 1 equivalent of the benzophenoxazine in benzene was treated with 1.1 equivalents of *n*-butyllithium dissolved in hexane. After stirring under a nitrogen atmosphere for 30 min., 1 equivalent of the appropriate dialkylaminoalkyl chloride was added to the red-yellow slurry. The mixture was stirred and heated under reflux (nitrogen atmosphere) for 16 hr., during the early part of which a clear yellow solution was formed. Excess water was added and the benzene layer was extracted with several portions of 4% aqueous hydrochloric acid solution. The combined acid extracts were made basic with sodium hydroxide solution, and the precipitated base extracted with ether. After drying, the ether was evaporated and the residual oil was distilled.

The compounds prepared by this method are listed in Table I.

Table II lists some picrate and methiodide derivatives of the bases.

KNOXVILLE, TENN.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF MICHIGAN]

Synthesis of Potential Anticancer Agents. XI. Synthesis and Reactions of Derivatives of 6-Methyluracil-5-sulfonic Acid[1,2]

ROBERT C. ELDERFIELD AND RAJ N. PRASAD

Received April 14, 1961

On reaction with chlorosulfonic acid 6-methyluracil gives the 5-sulfonyl chloride. The sulfonic acid function in 6-methyluracil-5-sulfonic acid and its derivatives is remarkably susceptible to displacement as shown by a variety of reactions. A series of sulfonic acid esters and sulfonamides derived from 6-methyluracil-5-sulfonic acid has been prepared for evaluation as anticancer agents.

It is well known that alkylating agents such as ethylenimines, methanesulfonates, and sulfur or nitrogen mustards exert carcinostatic action against a number of transplanted animal tumors. However, lack of selectivity towards tumor cells and frequent high toxicity have often limited their use as practical chemotherapeutic agents in the management of human neoplastic disease. In this laboratory attempts have been made and are continuing to design agents which may not be active themselves but which possibly can be converted to active substances *in vivo*.[3] Such a goal can, at least in principle, be achieved by incorporating an alkylating function into a so-called "carrier molecule."

(1) The work here reported was done under Research Grant CY-2961 from the National Cancer Institute to The University of Michigan.

(2) For the preceding communication in this series, see R. C. Elderfield, M. Israel, J. H. Ross and J. A. Waters, *J. Org. Chem.*, **26**, 2827 (1961).

(3) See ref. 2 and earlier papers in this series.

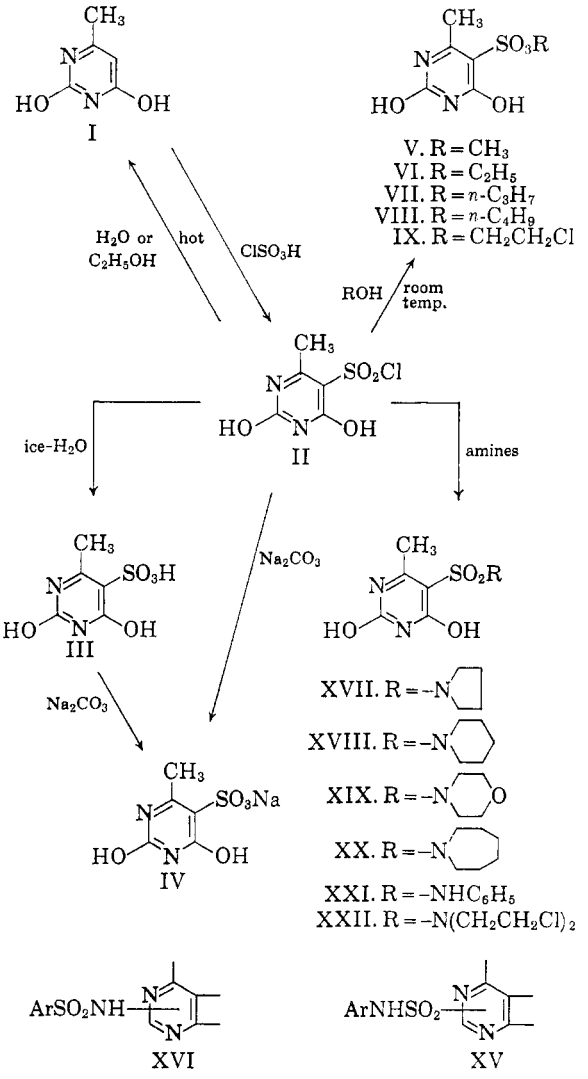
A similar approach has been made by a number of other workers.[4]

Investigation of derivatives of uracil as possible carcinostats has claimed the attention of several groups[5] and substances related to thymine[6] and orotic acid[7] have also been of interest.

The high order of activity against experimental animal tumors[8] shown by 2,4-bisaziridino-6-methyl-5-nitro-pyrimidine[9] as well as the reported activity of 5-bis(β-chlorethyl)amino-2,4-dihydroxy-6-methylpyrimidine (Dopan)[5c] suggested further investigation of derivatives of 6-methyluracil. Further, the observation of Kromov-Borisov and Karlinskya[10] that derivatives of pyrimidine-5-sulfonic acid display antileukemic activity prompted closer examination of these substances.

6-Methyluracil-5-sulfonyl chloride (II) was prepared in somewhat low yield by addition of 6-methyluracil (I) to previously heated freshly distilled chlorosulfonic acid as rather incompletely described for the preparation of uracil-5-sulfonyl chloride by Kromov-Borisov and Karlinskya.[10b] The preparation of uracil-5-sulfonyl chloride by refluxing uracil with chlorosulfonic acid has also been reported.[6] However, the material prepared in this manner failed to give satisfactory analytical data. Attempts to prepare II by the latter method were completely unsuccessful in our hands.

As indicated by Kromov-Borisov and Karlinskya[10b] for the uracil analog of II, the sulfonyl chloride (II) and the sulfonic acid arising from it are exceedingly labile. II slowly undergoes hydrolysis with ice water to yield the sulfonic acid (III). It reacts so readily with 0.01*N* sodium hydroxide that it can be titrated quantitatively in this manner. The sulfonic acid (III) was obtained as a very water soluble crystalline substance which formed a complex with one mole of dimethylformamide on recrystallization. Formation of a similar complex has also been noted with uracil-6-sulfonic acid.[11] III



was more conveniently obtained as its sparingly soluble sodium salt (IV) on reaction of II with cold aqueous sodium carbonate solution.

The sulfonic acid group in III is remarkably susceptible to displacement. When boiled with water or ethanol for a short time, the sulfonic acid function is eliminated from either II or III with regeneration of 4-methyluracil. However, when II was allowed to react with primary alcohols at room temperature, the analogous sulfonic acid esters (V–IX) were obtained. Secondary alcohols did not react with II under these conditions.

Reaction of II with hydrazine gave an almost quantitative yield of the sulfonhydrazide (X) which failed to give the azide (XI) on treatment with nitrous acid. XI was obtained by reaction of II with sodium azide.

Even more remarkable was the course of the reaction of X with aromatic aldehydes. When X was allowed to stand with salicylaldehyde in dilute methanol at room temperature for twenty minutes none of the expected hydrazone was obtained. Rather, a 95% yield of the known *o*-hydroxybenz-

(4)(a) P. Hebborn and J. F. Danielli, *Biochem. Pharmacol.*, 1, 19 (1958); (b) H. Arnold, F. Bourseaux, and N. Brock, *Nature*, 181, 931 (1958); (c) O. M. Friedman and A. M. Seligman, *J. Am. Chem. Soc.*, 76, 655, 658 (1954) inter alia.

(5)(a) J. Gut, J. Morávek, C. Párkányi, M. Prystač, J. Škoda, and F. Šorm, *Collection Czechoslov. Chem. Communs.*, 24, 3154 (1959); (b) D. A. Lyttle and H. G. Petering, *J. Am. Chem. Soc.*, 80, 6459 (1958); (c) L. F. Larianov, *Brit. J. Cancer*, 10, 26 (1956).

(6) R. H. Kerr, T. Enkoji, and T. J. Bardos, *J. Am. Chem. Soc.*, 78, 401 (1956).

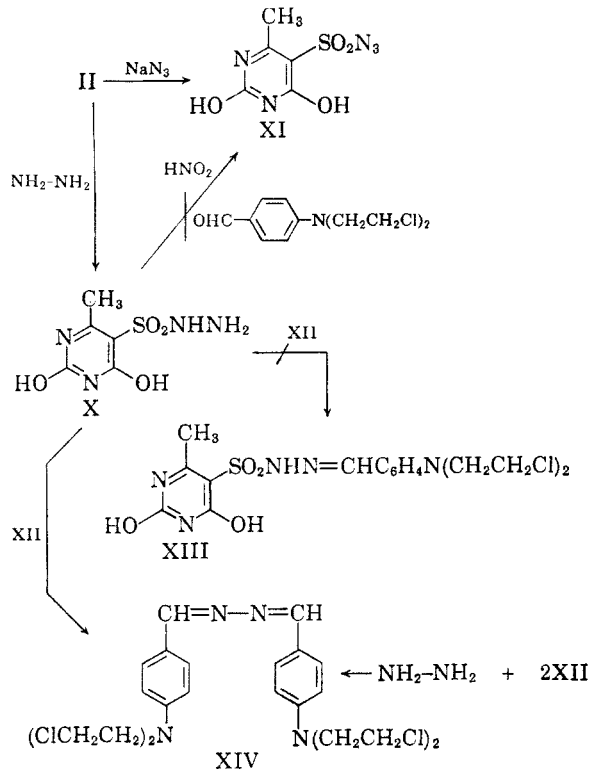
(7) A. B. Greenbaum, *J. Am. Chem. Soc.*, 76, 6052 (1954).

(8) Private communication from Dr. R. L. Jones, Jr., Jackson Memorial Hospital, University of Miami, Fla.

(9) R. C. Elderfield and R. N. Prasad, *J. Org. Chem.*, 25, 1583 (1960).

(10)(a) N. V. Kromov-Borisov and R. A. Karlinskya, *J. Gen. Chem. (U.S.S.R.)*, 24, 2212 (1954); (b) *J. Gen. Chem. U.S.S.R. Eng. Transl.*, 27, 2577 (1957).

(11) S. B. Greenbaum and W. L. Holmes, *J. Am. Chem. Soc.*, 76, 2899 (1954).



alazine[12] resulted. In a similar manner reaction of X with *p*-[*N*,*N*-bis(2-chloroethyl)amino]benzaldehyde (XII) did not lead to the hydrazone (XIII) but rather to the azine (XIV), the structure of which was confirmed by synthesis from XII and hydrazine. Apparently even under these extremely mild conditions the sulfonhydrazide group is cleaved with liberation of hydrazine and subsequent formation of the azines.

The successful application of a number of pyrimidinesulfonamides as chemotherapeutic agents against a variety of infections suggested an investigation of sulfonamides of the type of XV in which the sulfur is directly linked to the pyrimidine ring. It is curious that, in spite of the great interest in these sulfonamides, most of the substances hitherto described are of the type of XVI and comparatively little attention has been given to those of the type of XV.[13-15] It appears that the relative inaccessibility of the requisite sulfonyl chlorides may be responsible for this. With II now readily available, and in view of the reported inhibitory action of uracil-6-sulfonamide against a variety of bacterial systems,[13] we have accordingly prepared a series of 6-methyl-uracil-5-sulfonamides (XVII–XXII) for evaluation as tumor inhibitors.

(12) T. Curtius and R. Jay, *J. prakt. Chem.*, [2], **39**, 48 (1889).

(13) S. B. Greenbaum, *J. Am. Chem. Soc.*, **76**, 6052 (1954).

(14) G. R. Barker, N. G. Luthy, and M. M. Dhar, *J. Chem. Soc.*, 4206 (1954).

(15) W. T. Caldwell and G. E. Jaffe, *J. Am. Chem. Soc.*, **81**, 5166 (1959).

Attempts to prepare the unsubstituted sulfonamides by heating the sulfonazide (XI) with benzene in a sealed tube above 100° or in refluxing toluene[16] resulted generally in partial decomposition of the starting material although some was recovered under the milder reaction conditions.

The infrared spectra of the sulfonamides showed characteristic bands at 1010, 1100–1120, 1160–1170, and 1600–1660 cm.$^{-1}$ which were conspicuously absent in the spectrum of 6-methyluracil. The alkyl sulfonates showed fairly strong to weak bands at 790–810, 1000–1020, 1120–1130, 1220–1230, and 3160–3200 cm.$^{-1}$ The sulfonazide (XI) showed the characteristic azide absorption at 2160 cm.$^{-1}$ [17]

Results of evaluation of the compounds here described will be presented elsewhere.

EXPERIMENTAL[18,19]

6-Methyluracil-5-sulfonyl chloride (II). The procedure was patterned after the one given without great detail by Kromov-Borisov and Karlinskya for the chlorosulfonation of uracil.[10b] Completely dry 6-methyluracil (I) (63 g.) was added cautiously in small portions to 150 ml. of freshly distilled chlorosulfonic acid which had been previously heated to 110–115°. It is vital that the chlorosulfonic acid be distilled just prior to use. A vigorous reaction occurred at each addition and the rate of addition was controlled so that the temperature remained at 110–115°. When addition of I was complete (15–20 min.) the clear brown solution was allowed to cool to 80° and then poured in a thin stream over chopped ice with vigorous stirring. The white solid which separated was immediately collected, washed with a little ice water, sucked as dry as possible, triturated with a large volume of dry ether, collected, and immediately dried over phosphorus pentoxide in a vacuum desiccator. The yield of II, which was pure enough for subsequent operations, was 25 g. (22%). One recrystallization from boiling glacial acetic acid gave analytically pure II which melted at 255–260° dec. when placed in a bath preheated to 255°. When heated slowly the compound does not melt but slowly decomposes. Analytical data for this and other derivatives of the sulfonic acid are given in Table I.

6-Methyluracil-5-sulfonic acid (III). A mixture of 1.12 g. of II and 35 ml. of water was stirred for 4.5 hr. at room temperature. After filtering the solution was taken to dryness at room temperature leaving 1.2 g. of hygroscopic solid, m.p. 110–120° dec. A solution of the residue in 15 ml. of warm dimethylformamide was filtered into a mixture of 20 ml. of acetone and 300 ml. of anhydrous ether. Two additional crystallizations from the same solvent system gave material, m.p. 150–151°, which retained one mole of dimethylformamide as solvent of crystallization.

Sodium 6-methyluracil-5-sulfonate (IV). To a stirred solution of 2.12 g. of anhydrous sodium carbonate in 40 ml. of water was added 2.24 g. of II. Solution was complete within a few minutes and separation of the sodium salt of the sulfonic acid followed. After stirring for 4 hr., the salt was collected and washed successively with ice water, alcohol, and ether to give 0.7 g. (31%) of crystalline material, m.p. 250–260°. Rapid recrystallization from boiling water did not change the melting point.

(16) T. Curtius, *J. prakt. Chem.*, [2] **125**, 303 (1930).

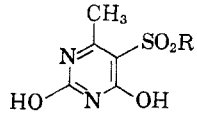
(17) E. Lieber, C. N. R. Rao, T. S. Chao, and C. W. Hoffman, *Anal. Chem.*, **29**, 916 (1957).

(18) All melting points are uncorrected for stem exposure.

(19) Microanalyses by Spang Microanalytical Laboratory, Ann Arbor, Mich.

TABLE I

5-SUBSTITUTED 6-METHYLURACILS



Formula No.	R	Formula	Yield, %	M.P.	Calcd. C, %	Calcd. H, %	Calcd. N, %	Found C, %	Found H, %	Found N, %
II	Cl	$C_5H_5ClN_2O_4S$	22	255–260 dec.	26.72	2.22	12.47	26.77	2.22	12.54
III	OH	$C_5H_6N_2O_5S$	70	150–151[a]	34.40	4.65	15.05	34.29	4.45	14.60
IV	ONa	$C_5H_5N_2O_5SNa$	31	250–260[b]	25.31	2.53	11.81	25.63	2.59	11.59
XXII	$N(CH_2CH_2Cl)_2$	$C_9H_{13}Cl_2N_3O_4S$	40	263–264 dec.	32.72	3.93	12.72	32.53	3.94	12.73
XVII		$C_9H_{13}N_3O_4S$	30	288 dec.	41.69	5.01	16.21	41.78	5.05	16.15
XVIII		$C_{10}H_{15}N\ O_4S$	55	260 dec.[c]	43.95	5.50	—	44.27	5.42	—
XIX		$C_9H_{13}N_3O_5S$	47	290 dec.[d]	39.27	4.70	15.27	39.44	4.83	15.14
XX		$C_{11}H_{17}N_3O_4S$	21	308 dec.[e]	45.99	5.92	14.63	46.22	6.04	14.70
XXI	NHC_6H_5	$C_{11}H_{11}N_3O_4S$	28	237–238[f]	46.97	3.91	14.59	46.87	3.97	14.82
V	OCH_3	$C_6H_8N_2O_5S$	81	204–205 dec.	32.73	3.63	12.72	32.55	3.43	12.44
VI	OC_2H_5	$C_7H_{10}N_2O_5S$	90	176 dec.[g]	34.56	4.52	11.52	34.99	4.54	11.49
VII	O-*n*-C_3H_7	$C_8H_{12}N_2O_5S$	40	188–190 dec.	38.71	4.83	11.29	38.93	5.06	11.05
VIII	O-*n*-C_4H_9	$C_9H_{14}N_2O_5S$	76	183–185 dec.	41.22	5.34	10.68	41.06	5.20	10.53
IX	OCH_2CH_2Cl	$C_7H_9ClN_2O_5S$	67	214–216 dec.	31.28	3.35	10.42	31.62	3.47	10.20
XI	N_3	$C_5H_5N_5O_4S$	47	193 dec.	25.97	2.16	30.30	26.04	2.20	30.23
X	$NHNH_2$	$C_5H_8N_4O_4S$	100	221–222 dec.[h]	25.21	4.20	23.53	25.60	4.24	23.80

[a] With one mole of dimethylformamide of crystallization. Analysis calculated on this basis. [b] With 0.5 mole of water of crystallization. Analysis calculated on this basis. [c] Recrystallized from boiling water; m.p. after drying at 60°/1 mm. over phosphorus pentoxide. [d] Recrystallized from water. Crude product triturated with ether to give crystalline material. [e] From dimethylformamide. [f] From methanol-benzene. [g] With 0.5 mole of water of crystallization. Analysis calculated on this basis. [h] With one mole of water of crystallization. Analysis calculated on this basis.

6-Methyluracil-5-sulfonic acid esters. These were prepared by the following general method. A mixture of 1.12 g. of II with the appropriate absolute alcohol was stirred at room temperature for 24 hr. After thorough chilling the crystalline precipitate was collected and washed successively with cold water and ether. The filter cake was dissolved in cold dilute potassium hydroxide, the solution was filtered and acidified with strong cooling with dilute hydrochloric acid. After refrigeration the esters were collected and, if necessary, purified again by recrystallization from acetone–petroleum ether (b.p. 70–90°).

6-Methyluracil-5-sulfonamides. In general these were prepared by a common procedure; deviations from which only will be noted. The following procedure is representative.

1-(6-Methyluracil-5-sulfonyl)pyrrolidine (XVII). A mixture of 2.24 g. of II and 20 ml. of pyrrolidine in 20 ml. of ether was slowly warmed with stirring during which the ether was allowed to evaporate. After the ether had been removed the residue was heated at 80° for 1 hr. The turbid solution was poured into ice water, filtered, and refrigerated. If no solid separated, the solution was concentrated almost to dryness under reduced pressure. The residue was triturated with a small amount of water, refrigerated overnight and the crystalline precipitate was collected. The product was recrystallized from ethanol-dimethylformamide.

Di-β-chloroethyl-6-methyluracil-5-sulfonamide (XXII). To a stirred suspension of 2.24 g. of II in 200 ml. of dry acetone chilled in an ice bath was slowly added a dried ether solution of di-β-chloroethylamine prepared from 5.4 g. of di-β-chloroethylamine hydrochloride. Addition was complete in 15 min. The mixture was stirred for an additional 30 min. at 0–5° and then for 24 hr. at room temperature. The separated solid (1.0 g., m.p. 190–194°) was collected. Concentration of the filtrate under reduced pressure gave an additional 4.3 g. of gelatinous material, m.p. 180–220°. The combined solid material was triturated with water, collected, and dissolved in 60 ml. of hot acetone. Filtration of the acetone solution into 110 ml. of boiling ethyl acetate and reduction of the volume of the solution to 60 ml. gave XXII as shiny plates. Further recrystallization from ethyl acetate gave analytically pure material, m.p. 263–264° dec.

6-Methyluracil-5-sulfonanilide (XXI). To a well stirred suspension of 1.4 g. of aniline in 10 ml. of 20% potassium hydroxide solution was slowly added 2.24 g. of II. After 30 min. the solution was filtered through decolorizing carbon and acidified to give 0.8 g. (28%) of XXI, m.p. 237–238° after recrystallization from methanol-benzene.

6-Methyluracil-5-sulfonazide (XI). To a well stirred solution of 2.6 g. of sodium azide in 30 ml. of water was added 2.24 g. of II. After stirring for 30 min., 10 ml. of methanol was added and stirring was continued for another 2 hr. The white precipitate (1.1 g.; 47%) was collected and washed successively with water, ethanol, and ether. Recrystallization from water and finally ethanol gave analytically pure material, m.p. 193° dec.

6-Methyluracil-5-sulfonhydrazide (X). A solution of 1 g. of 95% hydrazine hydrate in 50 ml. of methanol was added dropwise to a stirred ice cold solution of 2.24 g. of II in 20 ml. of methanol. The cooling bath was removed and the temperature slowly rose to 36°. After 1 hr., 60 ml. of methanol was added and stirring was continued for 48 hr. The crystalline precipitate (2.2 g.; 100%) was dissolved in 20 ml. of water and partially precipitated by addition of 100 ml. of methanol. After warming to redissolve the material the solution was filtered, 10–15 ml. of benzene was added and the solution was boiled down to half its volume. One more re-

crystallization from the same ternary mixture gave analytically pure material as long needles, m.p. 221–222° dec. when placed in a bath preheated to 220°.

Reaction of X *with salicylaldehyde.* A solution of 1.22 g. of salicylaldehyde in 20 ml. of methanol was added dropwise to a solution of 1.54 g. of X in 100 ml. of 50% methanol. Almost immediately a yellow precipitate separated. After stirring for 20 min., the crystalline material (1.6 g; 95%) was collected and recrystallized first from ethanol and then from benzene–petroleum ether to give shining yellow plates of salicylaldehyde azine, m.p. 220–222°. Curtius and Jay[12] report a m.p. of 205°.

Anal. Calcd. for $C_{14}H_{12}N_2O_2$: C, 70.00; H, 5.00. Found: C, 70.01; H, 5.23.

Reaction of X *with p-[N,N-bis(2-chloroethyl)amino]-benzaldehyde.* A solution of 1.1 g. of X in 30 ml. of water was added to a solution of 1.25 g. of XII[20] in 100 ml. of methanol. After stirring for 1 hr. at room temperature, 5 ml. of concd. hydrochloric acid was added to the mixture, the color of which changed to deep orange-red. After stirring for another hour, the solid was collected and washed thoroughly with water. The filter cake was extracted with ether in a Soxhlet extractor to give 0.8 g. of shiny yellow-orange plates of XIV, m.p. 165–166°. Further recrystallization first from chloroform and then from petroleum ether (b.p. 60–75°) gave analytically pure material with no change in the melting point.

Anal. Calcd. for $C_{22}H_{26}Cl_4N_4$: C, 54.10; H, 5.32; N, 11.47; Cl, 29.09. Found: C, 54.08; H, 5.27; N, 11.59; Cl, 28.93.

p-[N,N-Bis(2-chloroethyl)amino]benzaldehyde azine (XIV). To a cold solution of 1.25 g. of XII in 20 ml. of benzene was added 0.8 ml. of 95% hydrazine. After stirring for 2 hr. at room temperature, 10 ml. of pentane was added and the precipitate was collected. Recrystallization from petroleum ether (b.p. 90–100°) gave 0.5 g. of XIV, m.p. and mixture melting point with the substance prepared from X, 164–166°. The infrared spectra of XIV prepared by the two routes were identical.

Anal. Found: C, 54.16; H, 5.39; N, 11.32.

Acknowledgment. We acknowledge the valuable assistance of James M. Hudson in the preparation of certain intermediates.

ANN ARBOR, MICH.

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[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE]

The Oxidation and Acid Isomerization of Bacitracin A

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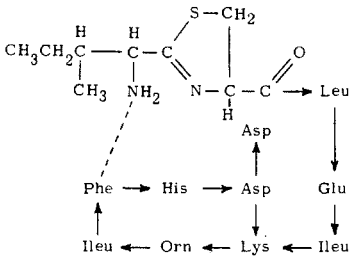
Received January 9, 1961

A study of the acid isomerization of bacitracin A has shown the reaction to follow first order reaction kinetics. The isomerization is reversible, and the two forms appear to be stereoisomers differing in antibiotic activity. Oxidation with performic acid has shown that the transformation results from an epimerization of the *N*-terminal isoleucine residue.

INTRODUCTION

The main structural features of bacitracin have been worked out by several investigators[1,2] and are best represented by Formula 1.



Bacitracin A

With this knowledge of the amino acid sequence and other structural considerations as a basis, a better understanding of the relationship between structure and antibiotic activity for this particular antibiotic polypeptide appeared possible.

During the study of some of the more subtle relationships connected with the structure and internal interactions of bacitracin A, we were struck, among other things, by the marked lability of this substance outside the *p*H range from 4.5 to 6.5.[3–5] The transformation leading to inactivation at *p*H's above 7.0 have been ascribed to deamination and autoxidation of the *N*-terminal amino thiazoline moiety.[6] More recently we have discovered[5] that the change which bacitracin undergoes in acidic media is related to the shift of the double bond in the thiazoline ring to the exocyclic position.

This latter reaction has been studied further and evidence has been obtained which indicates that the structure having a double bond exocyclic to the thiazoline ring is a transient intermediate which is responsible for epimerization at the alpha carbon of the *N*-terminal isoleucine.

We have also explored the possibility of separating the acid isomerized bacitracins by countercurrent distribution. These results are reported and

(1) W. Hausmann, J. R. Weisiger, and L. C. Craig, *J. Am. Chem. Soc.*, **77**, 723 (1955).

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