with stirring. The aqueous phase was extracted ($CHCl_3$, three 10-ml portions) and the combined $CHCl_3$ phases were thoroughly washed (H_2O) to remove *p*-nitrophenol, dried ($MgSO_4$), and evaporated. The residual gum was triturated first with PhMe, then with Et_2O, to give a solid which was recrystallized from EtOH to give 1.5 g (45%) of colorless needles, mp 153.5–155°. *Anal.* ($C_{15}H_{11}N_5O_4 \cdot 0.5H_2O$) C, H, N.

Dipotassium 6-[DL-α-(5-Tetrazolyl)phenylacetamido]penicillanate (IV).—To a cold (0–5°) solution of 1.62 g (7.5 mmoles) of 6-aminopenicillanic acid and 1.515 g (15 mmoles) of Et_3N in 25 ml of dry CH_2Cl_2 was added with stirring 2.439 g (7.5 mmoles) of *p*-nitrophenyl DL-α-(5-tetrazolyl)phenylacetate. The mixture was stirred for 2 hr at 0–5° and for 17 hr at room temperature and was then added to 25 ml of ice–water with vigorous stirring. The aqueous phase was extracted ($CHCl_3$, two 20-ml portions) and acidified to pH 4.5 and reextracted (EtOAc, three 20-ml portions) to remove *p*-nitrophenol. The pH was then lowered to 2.0 with 42% H_3PO_4 and the penicillin was extracted into EtOAc (three 20-ml portions). After the extracts had been washed (H_2O) and dried ($MgSO_4$), 15 mmoles of a 50% solution of potassium 2-ethylhexanoate (KEH) in *n*-BuOH was added and the solvent was evaporated to half-volume under reduced pressure at 35°. The product precipitated on the walls of the flask from which it was recovered by trituration with dry Et_2O, filtration, and drying over P_2O_5 *in vacuo*. This yielded 2.5 g of hygroscopic material for which a satisfactory analysis could not be obtained; the purity of the penicillin was estimated from ir and nmr spectra to be 90%.

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Tetracyclic Phenothiazines. II.[1a] Antibacterial Evaluation of 3-Keto-3H-pyrido[3,2,1-*kl*]phenothiazine-2-carboxylate[1b,c]

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Extension of our efforts to prepare tetracyclic phenothiazine derivatives of potential medicinal interest led us to synthesize 3-keto-3H-pyrido[3,2,1-*kl*]phenothiazine-2-carboxylate (I). A structural relationship can be seen between I and the quinolone antibacterial agents, nalidixic acid[2] and oxolinic acid,[3] which are particularly effective against gram-negative organisms. The synthesis of nalidixic acid analogs is of particular interest as a result of the discovery that nalidixic acid selectively inhibits DNA synthesis in growing bacteria.[4]

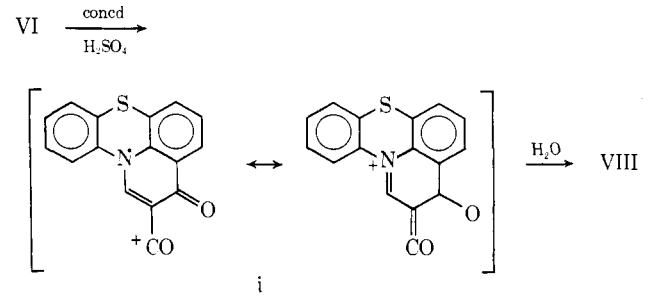
The reactions employed for the preparation of I (isolated as the salt, VIII) are outlined in Scheme I. The tetracyclic ketone II (prepared by procedures previously reported[5]) was converted *via* the glyoxalate III to corresponding β-keto ester IV. Bromination of IV gave the pyridophenothiazinium salt V,[6] which was not isolated but was converted directly to its conjugate base, the unsaturated β-keto ester VI. It was later discovered that VI could be obtained in greater over-all yield by ketone cleavage of the unsaturated glyoxalate VII using NaOEt. Compound VII was obtained from III by bromination followed by neutralization with triethylamine.

The unsaturated β-keto ester VI proved to be surprisingly resistant to alkaline hydrolysis; treatment of VI with ethanolic KOH at reflux temperatures for 18 hr failed to yield I. Unchanged starting material was also obtained when 5% HCl or 72% H_2SO_4 were used as catalysts. Finally, the procedure employed for the hydrolysis of mesitoic ester[7] using concentrated H_2SO_4 was successfully carried out to give VIII, the conjugate acid of I.[8] Attempts to obtain I by ketone cleavage of VII with KOH at 25° gave an uncharacterizable residue. At 0° the glyoxylic acid IX was obtained.

Microbiological Testing.—Compound VIII was tested against a variety of microorganisms *in vitro* employing the method of turbidity measurement of growth in nutrient broth.[9] The results are shown in Table I. Inconclusive results were obtained when VIII was tested against *Streptococcus pyogenes*, *Shigella dysenteriae*, and *Neisseria gonorrhea* due to difficulty in forming uniform suspensions. Use of the paper disk–agar diffusion method[10] failed to show antibacterial activity of VIII, apparently as a result of poor diffusion of the compound in solid agar.

Experimental Section

Melting points were determined on a calibrated Fisher-Johns melting point apparatus. The microanalyses were performed by the Galbraith Laboratories, Knoxville, Tenn. The ir and uv spectra were obtained with a Beckman IR-8 spectrophotometer and Cary 15 spectrophotometer, respectively. Where analyses



i

The resistance of VI to hydrolysis in aqueous acid may be due to its conversion to the conjugate acid. The failure to obtain alkaline hydrolysis seems rather unusual, since esters of nalidixic acid and oxolinic acid and their analogs are readily hydrolyzed under the same conditions.

(1) (a) For paper I in the series, see A. R. Martin, G. G. Briggs, and T. J. Yale, *J. Pharm. Sci.*, **57**, 166 (1968). (b) This investigation was supported in part by the National Institute of Mental Health, National Institutes of Health, U. S. Public Health Service, Grant No. MH 12425. (c) Abstracted in part from the M.S. thesis of D. S. Huang, Washington State University, 1968.

(2) (a) G. Y. Lesher, E. J. Froelich, M. D. Gruett, J. H. Bailey, and R. P. Brundage, *J. Med. Pharm. Chem.*, **5**, 1063 (1962); (b) E. W. McChesney, E. J. Froelich, G. Y. Lesher, A. V. R. Crain, and D. Rosi, *Toxicol. Appl. Pharmacol.*, **6**, 292 (1964); (c) K. Shimizu, T. Harada, M. Hatakeyawa, O. Kumii, T. Zindachi, E. Yamada, and K. Shimada, *Chemotherapy* (Tokyo), **12**, 384 (1964).

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(4) W. H. Dietz, T. M. Cook, and W. A. Goss, *J. Bacteriol.*, **91**, 768 (1966).

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(6) This result is analogous to the bromine-induced dehydrogenations of II and of its sulfoxide previously reported.[1a]

(7) H. P. Treffers and L. P. Hammett, *J. Am. Chem. Soc.*, **69**, 1232 (1947).

(8) The susceptibility of VI to hydrolysis in concentrated H_2SO_4 can be explained on the basis of its conversion to the resonance-stabilized acylium ion i, which is converted to VIII when the reaction mixture is poured into water.

(9) (a) Society of American Bacteriologists, "Manual of Microbiological Methods," McGraw-Hill Book Co., N. Y., 1957, p 173; (b) transmission readings were measured at 500 mμ on a Beckman DB spectrophotometer.

(10) R. N. Goodman in "Antibiotics, Their Chemistry and Non-medical Uses," H. S. Goldberg, Ed., Van Nostrand Co., New York, N. Y., 1959, pp 322–448.

SCHEME I

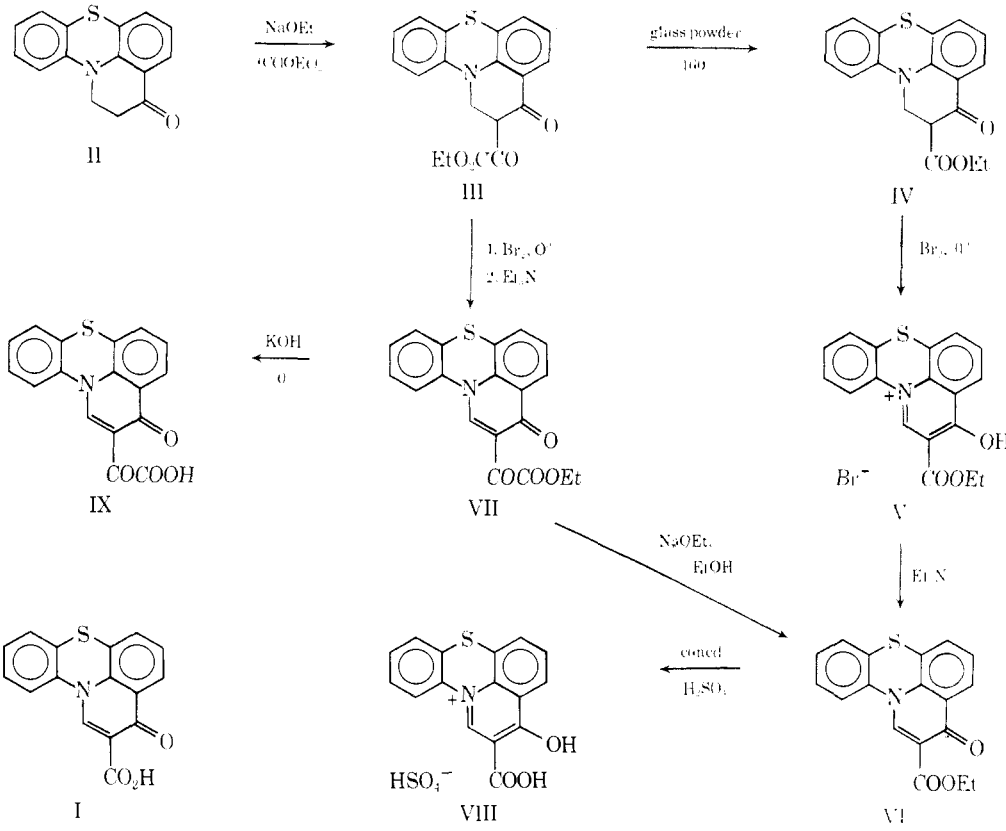

TABLE I

In Vitro ANTIBACTERIAL ACTIVITY[a] OF 2-CARBOXY-3-HYDROXY-3H-PYRIDO[3,2,1-*kl*]PHENOTHIAZINIUM BISULFATE (VIII)

Organism	MIC, μg/ml
Bacillus subtilis	300
Klebsiella pneumoniae	6.5
Corynebacterium diphtheriae	15
Staphylococcus aureus	20
Aerobacter acrogenes	12.5
Brucella abortus	100
Escherichia coli	150
Pasturella multocida	17.5
Salmonella anatum	8
Proteus vulgaris	270

are indicated, analytical results were within ±0.4% of the theoretical values.

Ethyl 1,2-Dihydro-3-keto-3H-pyrido[3,2,1-*kl*]phenothiazine-2-glyoxalate (III).—To 460 ml of anhydrous PhMe and 10.6 g (0.46 g-atom) of Na was added 560 ml of anhydrous EtOH. After the Na had dissolved, excess EtOH and PhMe were removed by distillation. To the white residue of NaOEt were added 58 g (0.23 mole) of 1,2-dihydro-3-keto-3H-pyrido[3,2,1-*kl*]phenothiazine (II)[5] and 62 ml of diethyl oxalate. The red solution was stirred for 1 hr, and a reddish precipitate separated out when the solution was cooled. Et_2O (20 ml) and 400 ml of 10% NaOH were added to dissolve the precipitate. The solution was extracted with C_6H_6 several times. The combined C_6H_6 extract was washed with 10% NaOH solution. The C_6H_6 was evaporated and the unreacted ketone was recovered. The combined 10% NaOH solution was acidified with 10% HCl to yield a reddish precipitate which, after filtering and recrystallizing from 95% ETOH, gave 50 g (62%) of reddish needles, mp 162°. *Anal.* ($C_{19}H_{13}NO_4S$) C, H, N, S.

Ethyl 1,2-Dihydro-3-keto-3H-pyrido[3,2,1-*kl*]phenothiazine-2-carboxylate (IV).—Compound III (15 g, 0.043 mole) and 15 g of powdered glass were mixed in a dry flask in a bath preheated to 170°. Gas evolution began to occur at 157°, then the temperature was allowed to increase to 162°. The mixture was heated for 2.5 hr. The reddish residue was extracted with $CHCl_3$ and the $CHCl_3$ solution was evaporated to dryness. The residue was dissolved in MeOH giving an orange gummy solid which was recrystallized several times from MeOH to yield 4 g (29%) of yellow crystals, mp 113°. *Anal.* ($C_{18}H_{15}NO_3S$) C, H, N, S.

Ethyl 3-Keto-3H-pyrido[3,2,1-*kl*]phenothiazine-2-glyoxalate (VII).—A solution of 10.3 g (0.03 mole) of III in 1200 ml of CCl_4 was cooled to 0°, and 20 ml of CCl_4 solution containing 4.9 g (0.031 mole) of Br_2 was added. A large quantity of the hydrobromide was formed almost immediately. A mixture of 15.5 g of the latter and 100 ml of Et_3N was allowed to stand 15 min. Et_3N was removed and the resulting solid was collected and washed (H_2O). The solid was recrystallized from 95% EtOH to give 9 g (85.5%) of yellow solid, mp 191–192°. *Anal.* ($C_{19}H_{13}NO_4S$) C, H, N, S.

3-Keto-3H-pyrido[3,2,1-*kl*]phenothiazine-2-glyoxalic Acid (IX).—A solution of 1 g (2.85 mmoles) of VII in 125 ml of 95% EtOH was cooled to 0° and 0.78 g (5.7 mmoles) of KOH in 5 ml of H_2O was added. The solution was cooled at 0° for 7 hr and was then allowed to stand at room temperature for 8 hr. It was poured into the ice water and acidified (10% HCl). The yellow precipitate was filtered and recrystallized from 95% EtOH to yield 0.6 g (65%), mp 251–253°. *Anal.* ($C_{17}H_9NO_4S$) C, H, N, S.

Ethyl 3-Keto-3H-pyrido[3,2,1-*kl*]phenothiazine-2-carboxylate (VI). Method A. Ketonic Cleavage of VII.—To a solution of 0.68 g (0.03 g-atom) of Na in 20 ml of absolute EtOH, cooled to 0°, was added 3.4 g (9.7 mmoles) of VII dissolved in 100 ml of dry DMF. The reaction mixture was cooled at 0° for 6 hr and then poured into the ice water. The solution was acidified and the yellow precipitate which formed was collected. Recrystallization of the precipitate from dioxane yielded 2 g (64%) of yellow crystals, mp 287°. *Anal.* ($C_{18}H_{13}NO_3S$) C, H, N, S.

Method B. Bromination of IV.—A solution of 2.0 g (6.2 mmoles) of IV in 300 ml of CCl_4 was cooled to 0° and 5 ml of CCl_4 containing 1.0 g (6.3 mmoles) of Br_2 was added. Almost immediately a brown solid was formed. A small sample of the latter, after recrystallization from dioxane, gave ir bands (KBr) at 3030 (OH) and 1692 cm^{-1} (ester carbonyl). The remaining material was stirred for 15 min with 10 ml of Et_3N, which was then removed. The resulting solid was collected on a filter and washed (H_2O). After recrystallization from dioxane 1.2 g (60%) of VI, mp 282–283°, was obtained. This substance was identical

with the product obtained from method A by comparison of uv and ir spectra.

2-Carboxy-3-hydroxy-3H-pyrido[3,2,1-*kl*]phenothiazinium Bisulfate (X).—A solution of 2.4 g (7.4 mmoles) of VI in 50 ml of concentrated H_2SO_4 was heated on water bath for 20 hr. The mixture was poured onto ice water and a yellow precipitate was formed. A crude yield of 2.9 g (99.8%) of brown solid was obobtained and recrystallized from *t*-BuOH and distilled H_2O. The first crop was recrystallized from a mixture of *t*-BuOH and deionized distilled H_2O to which a few drops of concentrated H_2SO_4 had been added. Brown crystals were obtained, mp 300°. *Anal.* ($C_{16}H_{11}NO_7S_2$) C, H, N, S.

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The Bacteriostatic Effectiveness of 1-Acyl-3-(3,4-dichlorophenyl)ureas

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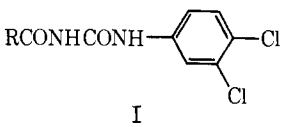
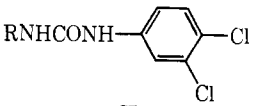
In continuation of our search for antibacterial compounds, a number of 1-acyl-3-(3,4-dichlorophenyl)ureas (I) were prepared. 1-Acylureas have been reported,[1]



I

but not as bacteriostats. A recent communication[2] from this laboratory described the synthesis and bacteriostatic properties of a similar series of compounds, the 1-alkyl-3-(3,4-dichlorophenyl)ureas (II). The activity was found to increase with chain length, reaching a maximum with the *n*-octyl derivative, then decreasing as the chain was lengthened further. A series of N-acylureas were prepared to determine whether these also are antibacterial and, if so, the chain length of R at which optimum activity occurs.



II

Synthesis of the desired materials involved treating 3,4-dichlorophenyl isocyanate with aliphatic amides, halogenated benzamides, and nicotinamide under anhydrous conditions. Relevant data, including minimum inhibitory concentrations (MIC's) in soap for *Staphylococcus aureus* ATCC 6538 are shown in Table I.

In the aliphatic series maximum activity (1–5 μg/ml) was observed when R was Pr, Bu, or 9-decenyl. In the series derived from benzamides, MIC's of 0.1–0.5 μg/ml were observed when R was 2,4-dichlorophenyl, and 1–5 μg/ml when R was 3,4-dichlorophenyl. Interestingly, in the carbanilide series, a structurally related group of compounds, maximum activity is observed with the 3,4-dichloro isomer and not the 2,4 derivative.[3]

Experimental Section

Since one of the reactants is 3,4-dichlorophenyl isocyanate, which readily forms 3,3′,4,4′-tetrachlorocarbanilide in the presence of water, the reaction must be carried out under extremely anhydrous conditions. The first few members of the series were prepared by first heating the amide in C_6H_6 for 2 hr and azeotroping moisture through a Dean–Stark trap, then adding 1 equiv of 3,4-dichlorophenyl isocyanate in dry *o*-dichlorobenzene. The mixture was heated to 130–140° and C_6H_6 was collected. Finally, the reaction was continued under reflux for 10 hr. At the end of this period, most of the solvent was distilled off and the residue was cooled and triturated with C_6H_6, causing a crude product to precipitate. The solid was filtered off and air dried. In most cases it was found to be a mixture of 3,3′,4,4′-tetrachlorocarbanilide and the desired material. Separation was achieved by boiling the mixture in C_6H_6, filtering while hot, and cooling the filtrate, whereupon the acylurea precipitated.

The method of choice, which avoids formation of tetrachlorocarbanilide, was to run the reaction in dry PhMe for 24 hr according to the procedure of Wiley.[1] Yields were generally in excess of 70% and ranged from 38 to 97%. The following preparation is representative.

TABLE I
1-ACYL-3-(3,4-DICHLOROPHENYL)UREAS
3,4-$Cl_2C_6H_3NHCONHCOR$

No.	R	Mp, °C[a]	Yield, %	MIC, μg/ml[b]	Formula[c]	Recrystn solvent
1	C_2H_5	173	78	20	$C_{10}H_{10}Cl_2N_2O$	EtOH
2	*n*-C_3H_7	155–156	38	1–5	$C_{11}H_{12}Cl_2N_2O_2$	PhH
3	*n*-C_4H_9	159	97	1–5	$C_{12}H_{14}Cl_2N_2O_2$	PhH
4	*n*-C_5H_{11}	130–131	86	20	$C_{13}H_{16}Cl_2N_2O_2$	PhH
5	*n*-C_7H_{15}	128–129	82	20	$C_{15}H_{20}Cl_2N_2O_2$	EtOH
6	*n*-C_8H_{17}	114–115	78	20	$C_{16}H_{22}Cl_2N_2O_2$	EtOH
7	*n*-$C_{11}H_{23}$	105–106	70	20	$C_{19}H_{28}Cl_2N_2O_2$	EtOH
8	9-Decenyl	100	69	5	$C_{18}H_{24}Cl_2N_2O_2$	EtOH
9	*n*-$C_{13}H_{27}$	99–100	52	20	$C_{18}H_{32}Cl_2N_2O_2$	EtOH
10	3-Pyridyl	272–273	94	20	$C_{13}H_9Cl_2N_3O_2$	*d*
11	*o*-ClC_6H_4	208	70	20	$C_{14}H_9Cl_3N_2O_2$	Me_2CO
12	*p*-ClC_6H_4	273	87	20	$C_{14}H_9Cl_3N_2O_2$	*d*
13	2,4-$Cl_2C_6H_3$	222	65	0.1–0.5	$C_{14}H_8Cl_4N_2O_2$	Me_2CO
14	3,4-$Cl_2C_6H_3$	235–236	96	1–5	$C_{14}H_8Cl_4N_2O_2$	Me_2CO

[a] All melting points were taken on a Fischer-Johns melting point apparatus and are uncorrected. [b] Minimum inhibitory concentration against *S. aureus* ATCC 6538. [c] All compounds were analyzed for C and H, and the results were within 0.4% of the theoretical values except for **13** where the C variance was 0.5%. [d] Triturated with Me_2CO.

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