some binding of DBA by such amino acids as leucine, isoleucine, and phenylalanine.

To test these expectations, the experiments described in the first paper were repeated with DBA instead of DEA, using glycine, alanine, leucine, isoleucine, phenylalanine, and serine as amino acids, serine being included in order to test the ability of the strongly hydrogen-bonding OH group to overcome the competition of the carbon tetrachloride. In the experiments with DEA, the OH group proved a potent binding factor in both serine and threonine.

As expected, none of these amino acids showed any binding of DBA in the pH range 8.5 to 10.2 where binding of DEA is quite pronounced.[1] There was, however, unmistakable binding of DBA by leucine in the pH range 7.0 to 7.5 where both DBA and leucine are predominantly cationic and where consequently, from the results obtained with DEA, one would predict no binding at all (Fig. 1). That binding does nevertheless occur can hardly be attributed to anything but van der Waals bonding between the butyl groups of DBA and the isobutyl group of leucine. As would be expected of van der Waals bonding, leucine binds DBA much more weakly than DEA, giving rise to a maximum binding coefficient [DBA–leucine complex]/([DBA][leucine]) = 2.2, against 9.7 for the DEA–leucine complex.

That isoleucine shows no binding at any pH may perhaps be attributed to steric hindrance: the branching in the alkyl chain near the polar end of the amino acid molecule interferes with the close approach of the DBA required for binding. The same may apply to phenylalanine.

If these considerations are valid, the experiments reported here may be said to provide a model for several known factors of drug–receptor interaction, namely, the role of van der Waals bonding between drug and receptor, the role of steric factors, and the importance of solubility effects. In cases for which the present instance can serve as a model, a lipophilic phase (carbon tetrachloride here, fat in the organism) can compete for the drug with the receptor, while the opposite should be true for receptors in body fats. It should be noted that these results also are a model of specificity. In the experimental arrangement used here, the amino acids could distinguish between two compounds as closely related in both structure and basicity as DEA and DBA, binding the former and rejecting the latter.

Thyroxine Analogs. X.[1] 3,5-Diamino-, 3,5-Dicyano-, and 3,5-Dicarboxy-DL-thyronines

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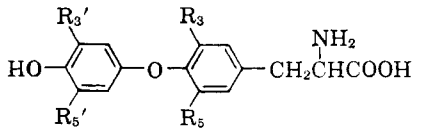
It has been established that analogs of the naturally occurring thyroid hormones in which the iodine atoms at positions 3 and 5 have been replaced by methyl groups (Ia)[3] and by halogens other than iodine (Ib)[4] retain significant thyroxine-like activity. Thyronine derivatives with a single iodine atom in the alanine bearing ring (Ic) show thyroxine antagonist properties[5,6]; 3,5-dinitrothyronines (Id) have been found to possess neither thyroxine-like nor thyroxine antagonist properties.[7]



Ia: $R_3 = R_5 = CH_3$, $R_3' = I$, $R_5' = H$
Ib: $R_3 = R_5 = Br$, $R_3' = I$, $R_5' = H$; $R_3 = R_3' = I$, $R_5 = R_5' = Cl$
Ic: $R_3 = R_3' = I$, $R_5 = R_5' = H$
Id: $R_3 = R_5 = NO_2$, $R_3' = R_5' = I$
Ie: $R_3 = R_5 = COOH$, $R_3' = R_5' = H$

In studies relative to substituent requirements in the alanine bearing ring of the thyronine nucleus, we have prepared additional 3,5-disubstituted analogs: 3,5-diamino-DL-thyronine (IV), 3,5-diamino-3′-methyl-DL-thyronine (VII), and 3,5-dicyano-DL-thyronine (XIII). The diamino analogs, IV and VII, were assayed for thyromimetic activity and IV was tested for antithyroxine effect. Barnes, *et al.*,[8] have reported the synthesis of 3,5-dicarboxy-L-thyronine. Since this material does not appear to have been evaluated biologically, we have prepared 3,5-dicarboxy-DL-thyronine (Ie), and have tested it both for thyroxine-like and for antithyroid effect.

Synthesis.—Chalmers, *et al.*,[9] have described the preparation of the L-isomer of the dinitrothyronine derivative II and the corresponding diamino derivative III. From DL-III, 3,5-diamino-DL-thyronine (IV) has been prepared by hydrolysis with hydriodic acid. Since a substituent such as iodine at position 3′ is required for maximum potency in the thyronine series, iodination of 3,5-diamino-DL-thyronine (IV) was attempted, using iodine in aqueous ethylamine[3] and iodine monochloride in aqueous hydrochloric acid. Neither an iodinated product nor reactant could be isolated due to rapid formation of dark polymeric material.

It has been shown[10,11] that 3,5-diiodothyronines bearing a 3′-methyl substituent possess a high order of thyromimetic potency. On this basis, it was felt that 3,5-diamino-3′-methyl-DL-thyronine (VII) should disclose any activity which might be present in the

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(2) In partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of California, 1962. Fellow of the American Foundation for Pharmaceutical Education, 1959–1962. Recipient of a Lunsford Richardson Pharmacy Award, 1962.

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(5) C. S. Pittman and S. B. Barker, *Am. J. Physiol.*, **197**, 1271 (1959).

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(7) H. A. Selenkow, C. A. Plamondon, J. G. Wiswell, and S. P. Asper, Jr., *Bull. Johns Hopkins Hosp.*, **102**, 94 (1958).

(8) J. H. Barnes, R. C. Cookson, G. T. Dickson, J. Elks, and V. D. Poole, *J. Chem. Soc.*, 1448 (1953).

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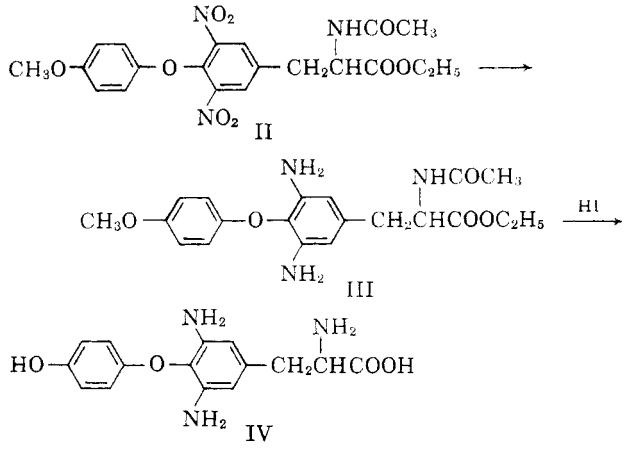
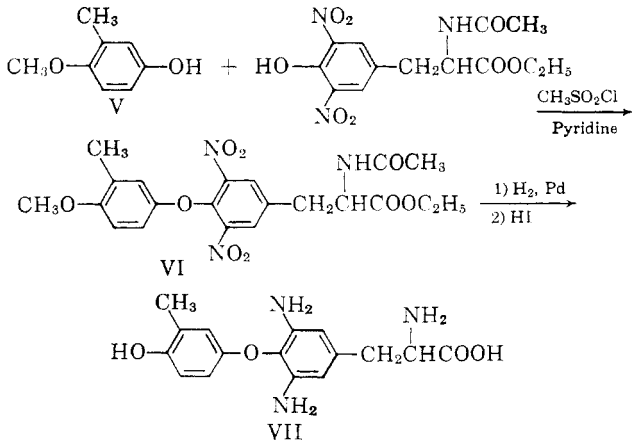
(10) C. S. Pittman, H. Shida, and S. B. Barker, *Endocrinology*, **68**, 248 (1961).

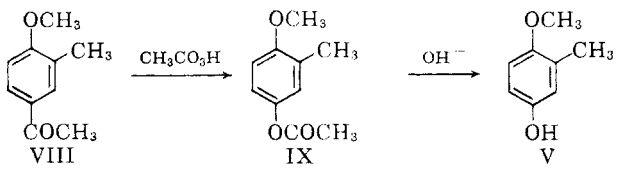
(11) E. C. Jorgensen, P. A. Lehman, C. Greenberg, and N. Zenker, *J. Biol. Chem.*, **237**, 3832 (1962).



diamino series, and the synthesis of this compound was carried out as shown below. Reported methods

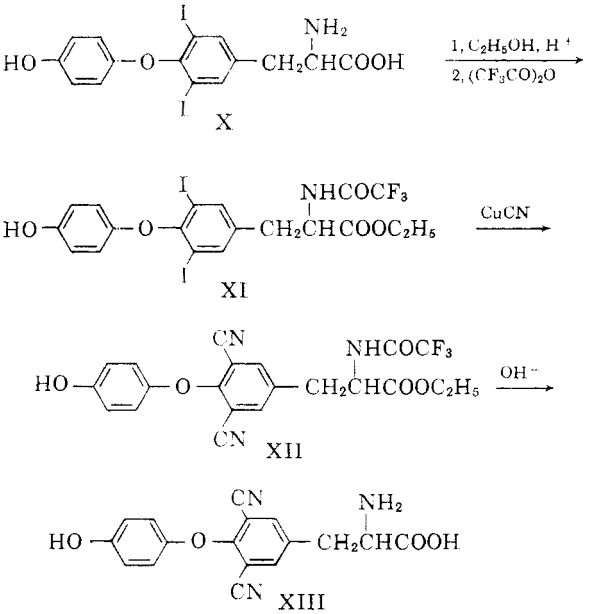


for the synthesis of 3-methyl-4-methoxyphenol (V), involving either decomposition of the diazonium salt obtained from the corresponding amine[12] or the Elbs persulfate oxidation,[13] were found to be unsatisfactory in our hands. 3-Methyl-4-methoxyacetophenone (VIII) was converted to 3-methyl-4-methoxyphenyl acetate (IX) by a variation of the procedure of von Wacek and Bezard.[14] The resulting acetate was hydrolyzed under alkaline conditions to yield 3-methyl-4-methoxyphenol (V). The required dinitrothyronine derivative VI was obtained from V and N-acetyl-3,5-dinitro-DL-tyrosine ethyl ester by the Meltzer[15] modification of the method of Barnes, *et al.*[8]; catalytic reduction in the presence of excess hydrochloric acid and hydrolysis of the crude product gave VII in 20% yield.



3,5-Dicyano-DL-thyronine (XIII) was prepared by replacement of the iodine groups of N-trifluoroacetyl 3,5-diiodo-DL-thyronine ethyl ester (XI) with cyano groups, followed by removal of the protective groups with mild alkaline hydrolysis.

Iodination of 3,5-dicyano-DL-thyronine (XIII) and of 3,5-dicarboxy-DL-thyronine (Ie) was attempted using iodine in aqueous ethylamine, but no pure material could be isolated.



Biological Results.—The thyronines Ie, IV, and VII were tested for thyroxine-like activity by the rat antigoiter procedure as described previously.[16] In addition, compounds Ie and IV were tested for antithyroid activity by concomitant administration of thyroxine and test compound to thiouracil-fed rats.[11] Results of these experiments are summarized in Table I, from which it can be seen that none of the compounds showed detectable thyroxine-like or antithyroid activity at the dosage levels employed. It has not yet been possible to obtain biological data on the dicyanothyronine (XIII).

Experimental

Melting points were taken in capillary tubes in an oil bath and are corrected. Microanalyses by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley. Ultraviolet spectra were determined on a Cary Model 11 recording spectrophotometer at a concentration of 10 mg./100 ml. in 0.05 *N* aqueous sodium hydroxide. Acid spectra were obtained by acidifying the alkaline solutions in the spectrophotometer cells with 5 drops of 6 *N* hydrochloric acid. Extinction coefficients for the acid solutions are therefore approximate.

3,5-Dicarboxy-DL-thyronine (Ie).—Obtained as described for the L-isomer by Barnes, *et al.*[8] Since difficulty was encountered in inducing this relatively water-soluble compound to precipitate from solution, the following purification procedure was devised. An aqueous solution of the crude dicarboxythyronine, obtained by hydriodic acid hydrolysis of 1.0 g. of N-acetyl-3-[3,5-dicyano-4-(*p*-methoxyphenoxy)phenyl]-DL-alanine ethyl ester, was chromatographed on 3 g. of Sephadex G-25. Fractions of eluent (water) equal to the volume of the column (15 ml.) were collected. The inorganic reaction by-product (NH_4I) appeared in the first fraction which contained no amino acid. All the amino acid was eluted in the second and third fractions; the second fraction deposited needles on standing at room temperature; yield, 300 mg. (35%), m.p. higher than 300°, lit.[8] higher than 360° for the L-isomer.

(12) E. Bamberger, *Ann.*, **390**, 175 (1912).
(13) W. Baker and N. C. Brown, *J. Chem. Soc.*, 2303 (1948).
(14) A. von Wacek and A. Bezard, *Ber.*, **74**, 845 (1941).
(15) R. I. Meltzer, D. M. Lustgarden, and A. Fischman, *J. Org. Chem.*, **22**, 1577 (1957).
(16) E. C. Jorgensen and P. Slade, *J. Med. Pharm. Chem.*, **5**, 729 (1962).

TABLE I

RAT ANTIGOITER ASSAY OF THYROXINE ANALOGS

Compound No.	Compound Name	Dosage[a] level	Thyroid Weight, mg./100 g. — Animals treated with — Untreated[b] animals	Thiouracil only	Thyroxine[c] 2 μg./100 g.	Thyroxine[c] 3 μg./100 g.	Thyroxine[c] 4.5 μg./100 g.	Compound	Potency, % L-thyroxine
Ie	3,5-Dicarboxy-DL-thyronine	100:1	6.57 ± 0.75[d]	20.24 ± 3.91	14.42 ± 3.48	10.33 ± 3.99	6.09 ± 2.95	27.04 ± 2.0	0
IV	3,5-Diamino-DL-thyronine	100:1	7.0 ± 1.20	22.5 ± 4.67	13.3 ± 4.96	11.6 ± 6.21	6.5 ± 1.36	23.8 ± 3.56	0
VII	3,5-Diamino-3′-methyl-DL-thyronine	100:1	7.55 ± 0.81	24.17 ± 6.28	19.53 ± 4.83	9.60 ± 3.66	6.38 ± 2.24	26.34 ± 4.90	0
Ie	3,5-Dicarboxy-DL-thyronine and 3.0 μg./100 g. L-thyroxine[c,e]	200:1	8.22 ± 1.62	29.8 ± 4.45	18.2 ± 5.21	10.8 ± 3.61	9.16 ± 3.86	11.2 ± 3.61	Antag.[f]
IV	3,5-Diamino-DL-thyronine and 3.0 μg./100 g. L-thyroxine[c,e]	100:1	6.57 ± 0.75	20.24 ± 3.91	14.42 ± 3.48	10.33 ± 3.99	6.09 ± 2.95	11.11 ± 2.07	Antag.[f]

[a] Molar ratio over the 3.0 μg./100 g. level of thyroxine. [b] This group received normal feed. All other animals received 0.3% thiouracil in their feed. [c] Sodium L-thyroxine pentahydrate. [d] All figures are means for 6 animals ± standard deviation. [e] Assay for antithyroid activity. [f] Reversal of thyroxine effect not significant.

Anal. Calcd. for $C_{17}H_{15}NO_8$: C, 56.51; H, 4.19. Found: C, 56.7; H, 4.4. Ultraviolet spectra: alkali, λ_{max} 344 mμ; ε 24,850; acid, λ_{max} 295 mμ; ε 13,880.

3,5-Diamino-DL-thyronine (IV).—The DL-dinitro ester II (10.0 g., 0.022 mole) prepared as described[9] for the L-isomer was dissolved in 100 ml. of methanol and shaken in the presence of hydrogen at an initial pressure of 3.15 kg./cm.^{2} and 1 g. of 10% palladium-on-carbon for 7 hr. The observed uptake of hydrogen was 103% of the calculated amount for reduction of two nitro groups. The catalyst was removed by filtration through Celite and the methanol was evaporated at reduced pressure. When attempts to crystallize the expected diamino ester failed, the black oil (8.5 g.) was dissolved in boiling acetic anhydride (10 ml.) and allowed to stand overnight. The resulting solution was treated at room temperature with 10 ml. of *N* sodium hydroxide and extracted with chloroform. The chloroform layer was washed with 5% hydrochloric acid and with water; after drying, the chloroform was evaporated, leaving 11.3 g. of a black residue. This was chromatographed on 100 g. of acid-washed alumina; fractions were eluted with 50% chloroform–benzene, chloroform, and methanol. On evaporation, the fraction eluted with chloroform deposited 9.0 g. of a black residue. This was dissolved in a minimum amount of ethanol; on cooling, 1.2 g. (12%) of crystalline N-acetyl-3-[3,5-diacetamido-4-(*p*-methoxyphenoxy)phenyl]-DL-alanine ethyl ester was deposited, m.p. 205–206°, lit.[9] for the L isomer, 226–227°. This acetamido derivative (1.0 g., 2.1 mmoles) was heated under reflux with 10 ml. of glacial acetic acid and 10 ml. of 47% hydriodic acid for 4 hr. The acids were removed by distillation *in vacuo*, and the yellow residue was dissolved in 5 ml. of water. The pH of the solution was adjusted to 5 with 40% aqueous sodium hydroxide, on refrigeration, 300 mg. (50%) of buff-colored needles was deposited, m.p. 287° dec.

Anal. Calcd. for $C_{15}H_{17}N_3O_4$: C, 59.50; H, 5.65. Found: C, 59.44; H, 5.89. Ultraviolet spectra: alkali, λ_{max} 302 mμ; ε 3150; acid, λ_{max} 287 mμ; ε 4480.

3-Methyl-4-methoxyphenyl Acetate (IX).—3-Methyl-4-methoxyacetophenone[17] (18 g., 0.117 mole) dissolved in 50 ml. of glacial acetic acid was added dropwise to 23 g. of 40% peracetic acid (0.12 mole) and 0.12 g. of *p*-toluenesulfonic acid dissolved in 50 ml. of glacial acetic acid. The addition was carried out below 20° and vigorous stirring was maintained. The mixture was allowed to stand overnight in the dark; water was then added and the acids were neutralized with solid sodium bicarbonate. The resulting mixture was extracted with ether, the ether extract was washed with water, dried, and the ether was evaporated. Distillation of the residue yielded a total of 10.5 g. (53%) of slightly yellow liquid, b.p. 104°(2 mm.), $n^{20}D$ 1.5159.

Anal. Calcd. for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.81; H, 6.60.

3-Methyl-4-methoxyphenol (V).—The preceding ester (5.2 g. of crude material obtained from 6.0 g. of VIII) was heated on the steam bath with 30 ml. of 10% aqueous sodium hydroxide for 1 hr. After cooling, the solution was washed with ether, acidified, and extracted with ether. The ether solution was dried, the ether evaporated, and the residue chromatographed on acid-washed alumina. The fraction eluted with ether (3 g.) was found to contain residual acetic acid as well as the phenol; this acetic acid was removed by extraction of an ethereal solution of the phenol with sodium bicarbonate solution. On drying and evaporation of the ether solution, a solid product was obtained, m.p. 43–44.5°. On recrystallization from benzene–ligroin, there was obtained in two crops 3.15 g. (58% over-all from VIII) of colorless needles, m.p. 45.5–46.5°, lit.[12] 46°.

N-Acetyl-3-[3,5-dinitro-4-(*m*-methyl-*p*-methoxyphenoxy)-phenyl]-DL-alanine Ethyl Ester (VI).—Prepared by the Meltzer[15] modification of the method of Barnes, *et al.*,[8] by the reaction of V with N-acetyl-3,5-dinitro-DL-tyrosine ethyl ester.[18] Yield of purified material: 63%, m.p. 82.5–83°.

Anal. Calcd. for $C_{21}H_{23}N_3O_9$: C, 54.66; H, 5.02. Found: C, 54.85; H, 5.26.

3,5-Diamino-3′-methyl-DL-thyronine (VII).—The preceding dinitro ester (VI, 3.8 g., 8.2 mmoles) was dissolved in 50 ml. of methanol containing 5 ml. of concd. hydrochloric acid and was shaken with hydrogen at an initial pressure of 2.8 kg./cm.^{2} in the presence of 0.2 g. of 10% palladium-on-carbon. The hydrogen uptake was complete in 30 min., after which the catalyst was removed by filtration through Celite and the solvent was evaporated. The crude product was heated under reflux with 20 ml. of glacial acetic acid and 35 ml. of 47% hydriodic acid for 16 hr. The acids were distilled *in vacuo*, and the residue was taken up in 5 ml. of water. The pH of the solution was adjusted to 5 with 40% aqueous sodium hydroxide, resulting in deposition of 450 mg. (20%) of buff-colored powder, m.p. 267–268° dec.

Anal. Calcd. for $C_{16}H_{19}N_3O_4$: C, 60.55; H, 6.04. Found: C, 60.50; H, 6.18. Ultraviolet spectra: alkali, λ_{max} 305 mμ; ε 3770; acid, λ_{max} 287 mμ; ε 5010.

N-Trifluoroacetyl-3,5-diiodo-DL-thyronine Ethyl Ester (XI).—3,5-Diiodo-DL-thyronine (X, 7.8 g.), obtained by hydrolysis from N-acetyl-3-[3,5-diiodo-4-(*p*-methoxyphenoxy)phenyl]-DL-alanine ethyl ester,[19] was suspended in 100 ml. of absolute ethanol and the mixture was saturated with dry hydrogen chloride at 10°. After 4 hr. the solvent was evaporated to 25 ml. and the solid hydrochloride (6.5 g.) was removed by filtration, dissolved in 50% ethanol, and was treated with 9.1 ml. of 0.5% aqueous sodium hydroxide. On partial evaporation of the alcohol, 4.0 g. (49%) of white solid, m.p. 228.5–229°, was obtained. This was suspended in 100 ml. of chloroform–ethyl acetate (1:1); to the suspension was added in portions 2.8 ml. of trifluoroacetic anhydride in 40 ml. of ethyl acetate. Vigorous stirring was maintained throughout the addition, which took about 0.5 hr. Solid material still remained in suspension, so 1.0 ml. of additional trifluoroacetic anhydride in ethyl acetate was added. The solution was washed with 5% aqueous sodium bicarbonate and with water, dried (Na_2SO_4), and reduced in volume to 25 ml. On addition of

(17) G. Stadnikoff and A. Baryschewa, *Ber.*, **61**, 1997 (1928).

(18) J. C. Clayton, G. F. H. Green, and B. A. Hems, *J. Chem. Soc.*, 2467 (1951).

(19) P. F. Bevilacqua, J. T. Plati, and W. Wenner, U. S. Patent 2,895,927 (July 21, 1959).

75 ml. of petroleum ether, 4.1 g. (92%) of white solid was obtained. The material had a double melting point, softening at 135–142°, resolidifying and melting at 168.5–169°.

Anal. Calcd. for $C_{19}H_{16}F_3I_2NO_5$: C, 35.00; H, 2.46. Found: C, 34.75; H, 2.91.

A similar preparation, that of N-trifluoroacetyl-3,5-diiodothyronine methyl ester, has been described.[20]

N-Trifluoroacetyl-3-[3,5-dicyano-4-(*p*-hydroxyphenoxy)-phenyl]-DL-alanine Ethyl Ester (XII).—The diiodo ester XI (2.0 g., 3.1 mmoles), cuprous cyanide (1.0 g., 11 mmoles), and pyridine (5 ml.) were heated under reflux for 6 hr. The reaction mixture was poured over ice, the yellow precipitate was collected and washed with water, and then stirred with 15 ml. of 2 *N* ammonium hydroxide and 10 ml. of ethyl acetate. After filtration, the ethyl acetate layer was washed with 2 *N* ammonium hydroxide, water, 2 *N* hydrochloric acid, and water. The solution was dried (Na_2SO_4) and the ethyl acetate evaporated, yielding 1.15 g. (85%) of a white solid, m.p. 199–201°.

(20) A. Taurog, *J. Am. Chem. Soc.*, **75**, 3473 (1953).

Anal. Calcd. for $C_{21}H_{16}F_3N_3O_5$: C, 56.38; H, 3.61. Found: C, 56.10; H, 3.71.

3,5-Dicyano-DL-thyronine (XIII).—The preceding dicyano ester (XII, 0.48 g., 1.1 mmoles) was dissolved in 4.5 ml. of 5% aqueous sodium hydroxide and allowed to stand overnight. The pH was adjusted to 5 with concd. hydrochloric acid. After overnight refrigeration, 360 mg. (64%) of buff-colored solid was obtained. This material was purified by one isoelectric precipitation at pH 5, m.p. 233–235° dec.

Anal. Calcd. for $C_{17}H_{13}N_3O_4 \cdot 2H_2O$: C, 56.82; H, 4.77. Found: C, 56.7; H, 4.7. Ultraviolet spectra: alkali, λ_{max} 296 mμ; ε 4570, acid, λ_{max} 283 mμ; ε 3250.

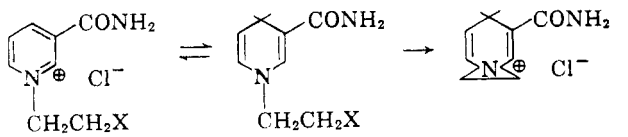
Acknowledgment.—This work was supported by grant-in-aid funds from the Smith Kline and French Laboratories, and funds from the University of California Academic Senate, for which we are most grateful.

Communications to the Editor

1-(β-Chloroethyl)-3-carbamylpyridinium Chloride. Prototype of a New Class of Latently Cytotoxic Potential Antitumor Agents[1]

Sir:

We report here the synthesis of 1-(β-chloroethyl)-3-carbamylpyridinium chloride (I) as a prototype of a new class of latently cytotoxic compounds, the action of which would be elicited by a process of reduction. In the pyridinium form I, this compound is unreactive as an alkylating agent; in the dihydro reduced form II, it is an active alkylating agent. The latter (II) can transform to a reactive species, an ethyleneimmonium intermediate (III) characteristic of the nitrogen mustards,[2] whereas I cannot. The process of reduction of I as well as the reverse process, oxidation of the reduced compound II, are analogous to processes known to occur in biological systems.



I, X = Cl; IV, X = OH — II, X = Cl; V, X = OH — III

Neoplastic cells possessing either high capacity to carry out reduction of I or limited capacity to bring about oxidation of II, relative to that of the most sensitive vital tissues such as bone marrow and intestinal epithelium, would be susceptible to attack by these agents. Administration of the oxidized form I to a host bearing tumors of the former type or administration of the reduced form II in the case of the latter could result in preferential localization of alkylating agent in the tumor. Enzyme studies relating to possible applications of these compounds in chemotherapy are now in progress.[3]

The chloroethyl pyridinium compound I was prepared from nicotinamide refluxed with ethylene chlorohydrin to afford 1-(β-hydroxyethyl)-3-carbamylpyridinium chloride (IV),[4] from which I, m.p. 183–185°, was obtained by chlorination with thionyl chloride. (*Anal.* Calcd. for $C_8H_{10}Cl_2N_2O$: C, 43.5; H, 4.53; N, 12.69; Cl, 32.25. Found: C, 43.64; H, 4.58; N, 12.58; Cl, 31.59; λ_{max} 266, ε 3300 in water). Reduction of the pyridinium compounds IV and I with excess sodium dithionite in aqueous sodium carbonate yielded 1-(β-hydroxyethyl)-3-carbamyl-1,4-dihydropyridine (V) and 1-(β-chloroethyl)-3-carbamyl-1,4-dihydropyridine (II), respectively. Compounds V, m.p. 119–121°, and II, m.p. 106–108°, were unstable and extremely difficult to obtain analytically pure. Both were characterized by single maximum absorption in the ultraviolet at 358 and 355 mμ, respectively, in the 360-mμ range characteristic of 1,4-dihydropyridines of this type.[5] The major by-product in the reduction was invariably a component with a single maximum absorption at about 295 mμ characteristic of compounds containing the β-amino-α,β-unsaturated carbonyl chromophore (presumably the 6-hydroxytetrahydropyridine) of the type that would result from hydration of the C-5 double bond.[5]

The structure of the dihydropyridines II and V were confirmed by reoxidation with silver nitrate to the pyridinium compounds I and IV (as the nitrates), characterized by the typical maximum in the ultraviolet (λ_{max} 266) and by the identity of their spectra otherwise with those of the nitrates from I and IV, respectively.

A comparison of the alkylating activity of the four

(1) Supported by a Cancer Chemotherapy National Service Centre research contract (SA43-62-170) from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(2) For a recent review, see: W. C. J. Ross, "Biological Alkylating Agents," Butterworth and Co. Ltd., London, 1962, p. 11.

(3) By Dr. K. Herrington in this Laboratory.

(4) H. G. Windmueller, C. J. Ackerman, H. Bakerman, and O. Michelson *J. Biol. Chem.*, **234**, 889 (1959).

(5) A. G. Anderson, Jr., and G. Berkelhammer, *J. Am. Chem. Soc.*, **80**, 926 (1958).