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.

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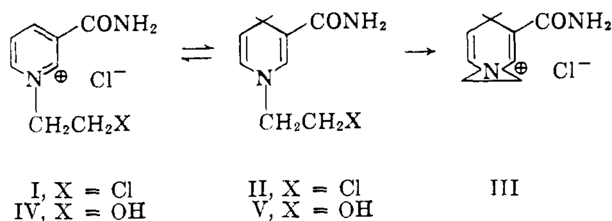
Communications to the Editor

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.



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).

compounds I (ClPy⊕), II (ClPyH), IV (HOPy⊕), and V (HOPyH) determined by assay with γ-(4-nitrobenzyl)-pyridine (NBP),[6] confirmed the expected pattern (Table I).

TABLE I

COMPARISON OF ALKYLATING ACTIVITY[a]

Concn. of cmpd. in. μg./ml.	ClPy⊕ I	ClPyH II	HOPy⊕ IV	HOPyH V
10	8	32	8	8
20	7	60	8	9
40	7	121	10	10

[a] Determined by NBP assay.[6] Values shown are for color density (Klett units) which is directly proportional to extent of alkylation. Samples were incubated with NBP at pH 4.6 for 1 hr.

Only the reduced chloropyridine (ClPyH, II) reacted as an alkylating agent and showed activity directly related to concentration; the other three compounds showed insignificant activity.

In tests for activity against the KB cell line in tissue culture[7] the two oxidized compounds I and IV showed no cytotoxicity ($ED_{50} > 100$ μg./ml.); the reduced dihydropyridine V showed borderline activity (ED_{50} 120 μg./ml.), whereas the reduced chloride II showed significant cytotoxicity (ED_{50} 26 μg./ml). The hydroxy compounds IV and V were included in this series for comparison and although the basis for the small amount of cytotoxicity produced by the dihydro compound V is not well understood, interestingly the corresponding dihydro chloride II was 4–5 times as cytotoxic. This difference in cytotoxicity most probably results from the capacity of the latter II to act as an alkylating agent. The pyridinium compound I, therefore, inactive as an alkylating agent is biologically inert, whereas the corresponding dihydro compound II is both chemically reactive and significantly cytotoxic.

If compounds of this type are indeed susceptible to the indicated reductive activation or oxidative detoxification *in vivo*, they are potentially selective in their action and represent a new class of agent with interesting potentialities for cancer chemotherapy.[8]

(6) O. M. Friedman and E. Boger, *Anal. Chem.*, **33**, 906 (1961). Technical assistance by Dr. S. Schichor in making these determinations is gratefully acknowledged.

(7) Determined in the laboratories of Arthur D. Little, Inc., Cambridge, Mass., in relation to the CCNSC Screening Program. We are indebted to Dr. P. Thayer and Mr. I. Wodinsky for these data.

(8) For references to previous work on other cytotoxic agents with latent activity, see O. M. Friedman, E. Boger, V. Grubliauskas, and H. Sommer, *J. Med. Chem.*, **6**, 50 (1963). For recent more general discussion of selective toxicity, see ref. 2, p. 148, and M. S. Burstone, "Enzyme Histochemistry and Its Application to the Study of Neoplasms," Academic Press, New York, N. Y., 1962, p. 563.

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RECEIVED MARCH 25, 1963

Gentamicin,[1] a New Antibiotic Complex from *Micromonospora*

Sir:

A new wide-spectrum, basic, water-soluble antibiotic mixture has been isolated from two previously undescribed species of microorganisms belonging to the genus *Micromonospora*. These species have been deposited with the Northern Utilization Research and Development Division, U. S. Department of Agriculture, Peoria, Illinois, where they have been assigned NRRL No. 2953 and NRRL No. 2985, respectively.[2]

When these organisms are grown in submerged culture in a yeast extract–cerelose medium they produce gentamicin and other basic antibiotics. Gentamicin consists of two closely related isomeric pseudo-oligosaccharides, referred to as C_1 and C_2, which have essentially identical polarities. The basic antibiotics are separated from the fermentation broth with the aid of a cation-exchange resin. Gentamicin then is separated from the co-produced antibiotics by selective precipitation of its dodecylbenzenesulfonate salts.[3] Recovery of the antibiotic from the dodecylbenzenesulfonates is accomplished with the aid of a strongly basic anion exchange resin. Gentamicin is distinguished from other antibiotics in the same chemical family[4] by its paper chromatographic behavior in a variety of solvent systems.

Acetylation of gentamicin with acetic anhydride in methanol affords the triacetylgentamicins C_1 and C_2 which are separated by partition chromatography on cellulose powder with the upper phase of a heptanol–pyridine–water system (6:4:3). Triacetyl C_1 (I) has m.p. 206–225° $[\alpha]^{25}D$ +143° (methanol) and analyzed for $C_{23-24}H_{40-42}N_4O_{10}$.[5] Triacetyl C_2 (II) has m.p. 206–222°; $[\alpha]^{25}D$ +151° (methanol) and analyzed for $C_{23-24}H_{40-42}N_4O_{10}$. Neither of these derivatives has important antibiotic properties.

TABLE I

COMPARATIVE PAPER CHROMATOGRAPHY OF GENTAMICIN

Antibiotic	Solvent system[a] and R_f value A	B	C	D
Gentamicin	0.59	0.26	0.10	0.30
Kanamycin	.30	.18	.25	.17
Neamine	.30	.23	.22	.0
Neomycin	.22	.12	.29	.0
Paromomycin	.33	.11	.38	.0
Streptomycin	.57	.40	.21	.06
Streptothricin	.36	.30	.27	.27

[a] Systems: A. Methanol–water (4:1 v./v.) plus 3% NaCl, using paper buffered with 0.95 *M* Na_2SO_4 plus 0.5 *M* $NaHSO_4$. B. Propanol–pyridine–acetic acid–water (15:10:3:12 v./v.). C. Propanol–water–acetic acid (50:40:5 v./v.). D. Aqueous phenol, 80%; Whatman No. 1 paper; ascending.

Hydrolysis of I and II with 1.2 *N* sodium hydroxide in water containing 10% methanol at reflux for 70 hr. affords, respectively, gentamicin C_1 (III), m.p. 94–100°, $[\alpha]^{25}D$ +158° (water), which analyzed for $C_{17-18}H_{34-36}N_4O_7$, and gentamicin C_2 (IV), m.p. 107–124°, $[\alpha]^{25}D$ +160° (water), which analyzed for $C_{17-18}H_{34-36}N_4O_7$. Titrations of C_1 and C_2 in 8 *M* LiCl[6] solution with 0.1 *N* hydrochloric acid give equivalent weights of 97 ± 2. Osmometric molecular weight

(1) Garymycin®.

(2) These organisms were among numerous cultures isolated by A. Woyciesjes, Syracuse, N. Y.

(3) *Cf.* D. A. Johnson and G. A. Hardcastle, U. S. Patent 2,967,177 (1961).

(4) *Cf.* K. L. Rinehart, "The Neomycins and Related Antibiotics," John Wiley and Sons, Inc., New York, N. Y., in press.

(5) Correct analytical values have been obtained for the new compounds.

(6) F. E. Critchfield and J. B. Johnson, *Anal. Chem.*, **30**, 1247 (1958).