compounds I ($ClPy^{\oplus}$), II (ClPyH), IV ($HOPy^{\oplus}$), 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.	$ClPy^{\oplus}$ I	ClPyH II	$HOPy^{\oplus}$ 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.

COLLABORATIVE RESEARCH, INC.
WALTHAM, MASSACHUSETTS

ORRIE M. FRIEDMAN
KURT POLLAK
EZRA KHEDOURI

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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).

determinations give values of 425 ± 21. There are three primary amino groups (Van Slyke), one N-methyl, and one C-methyl (Kuhn–Roth) in both III and IV.[7] C_1 and C_2 give positive Elson–Morgan and ninhydrin tests, and negative maltol, furfural, and Sakaguchi reactions. The two bases are very similar in all of their properties and are undoubtedly closely related in structure. Upon N-acetylation of III and IV, the parent triacetyl derivatives, I and II, are regenerated, respectively. That III and IV are not artifacts of the vigorous basic hydrolysis is shown by the identities of their paper chromatographic mobilities, rotations, infrared spectra, and elemental analyses with the same constants of gentamicin (the parent mixture). Acid hydrolyses of gentamicin, obtained either by reconstitution from III and IV or directly from the fermentation, affords mixtures with identical paper chromatographic patterns. From the acid hydrolyses of III, IV, and gentamicin, 2-deoxystreptamine is formed. The nature of the co-produced degradation products is under study, as are the total structures of III and IV.

The *in vitro* activity of gentamicin was determined by the twofold serial tube dilution method and results show this new antibiotic to be highly active against Gram positive and Gram negative bacteria. The acute toxicity (LD_{50}) of gentamicin in mice is 72 mg./kg. intravenously, 484 mg./kg. subcutaneously, 433 mg./kg. intraperitoneally, and greater than 9050 mg./kg. by oral administration. The therapeutic activity of gentamicin has been demonstrated by subcutaneous administration in mice infected intraperitoneally with *Klebsiella pneumoniae, Salmonella schottmuelleri, Pseudomonas aeruginosa, Diplococcus pneumoniae, and Staphylococcus aureus.*

TABLE II

ANTIBACTERIAL SPECTRUM OF GENTAMICIN

Microorganism	Minimal inhibitory concentrations.[a] (γ-base/ml.)
Aerobacter aerogenes	0.6
Alcaligenes fecalis	.6
Bacillus subtilis	.012
Escherichia coli	1.2
Klebsiella pneumoniae	0.14
Proteus mirabilis (5 strains)	.8–2.0
Proteus rettgeri	.8
Proteus vulgaris	4.8
Pseudomonas aeruginosa (4 strains)	0.08–0.20
Staphylococcus aureus (8 strains)	.028–0.30
Staphylococcus aureus (10 penicillin resistant strains)	.5
Salmonella schottmuelleri	1.2
Salmonella typhimurium	2.4
Streptococcus fecalis	9.6

[a] Gentamicin sulfate potency: 647 γ base/mg., Difco Antibiotic Medium No. 3 (Penassay Broth), Difco Laboratories, Detroit 1, Michigan.

Extensive toxicity studies with gentamicin demonstrate that the following doses can be administered chronically intramuscularly without demonstrable toxicity: 5.6 mg./kg. for at least 50 days in dogs; 12 mg./kg. for at least 40 days in cats; 40 mg./kg. for at least 40 days in rats. Considerably higher doses, 40 mg./kg. in dogs and 100 mg./kg. in rats, regularly produce renal tubular necrosis and vestibular function damage in 30 days. Pharmacological studies show that the antibiotic is almost completely excreted by glomerular filtration with some biliary excretion. Intramuscular administration in dogs gives blood levels for approximately 6 to 8 hr., and peak titers at 1 hr. are approximately 2.0 γ/ml. with a 1.0 mg./kg. dose. Fractionated urine samples show maximal antibiotic levels 50 times the peak blood levels, and 50 to almost 100% of the administered dose is excreted in 24 hr.

Intramuscular administration of single 0.2–3.2 mg./kg. doses to 10 normal volunteers showed onset and duration of blood levels and excretion patterns similar to the dog, though human blood levels, *e.g.* 4.0 γ/ml. at 1 mg./kg. dose, were double canine levels. The peak blood levels in volunteers given single doses of 0.4 mg./kg. intramuscularly averaged 1.6 γ/ml. and were above those levels necessary to inhibit the growth of most *Proteus* and all *Pseudomonas* strains tested as well as most other Gram negative bacteria and for penicillin sensitive and resistant *Staphylococcus*. The peak average antibiotic level in the urine in fractionated specimens at the 0.4 mg./kg. dose was 70 γ/ml. Single oral doses up to 1500 mg. of gentamicin in man result in approximately 0.2% absorption as detected in 24 hr. urines, with no measurable blood levels.

Gentamicin is being studied extensively in the clinic for the treatment of infections caused by Gram negative bacteria.

Acknowledgments. We gratefully acknowledge the assistance of Drs. M. Eggert, F. Fielder, S. Irwin, R. Taylor, and D. Williams of our Laboratories for the performance of some of the critical evaluative tests, and Dr. B. Calesnick, Hahnemann Medical College, for the human pharmacology.

BIOLOGICAL RESEARCH DIVISION
MARVIN J. WEINSTEIN
GEORGE M. LUEDEMANN
EDWIN M. ODEN
GERALD H. WAGMAN

CHEMICAL RESEARCH AND DEVELOPMENT DIVISION
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CARMINE T. CONIGLIO
WILLIAM CHARNEY
HERSHEL L. HERZOG

MEDICAL RESEARCH DIVISION
SCHERING CORPORATION
BLOOMFIELD, NEW JERSEY
JACK BLACK

RECEIVED MAY 17, 1963

(7) Nuclear magnetic resonance measurements in D_2O and deuteriopyridine solutions confirm the presence of both C-methyl and N-methyl in C_1, C_2, and their respective amides.

Effects of *d,l*-3-Phthalimidoglutarimide and N-Phthalyl-*d,l*-aspartimide on Rat Pregnancy

Sir:

Thalidomide-induced malformations have been reported in mice,[1,2] rats,[3–5] and rabbits.[1,2,6–9] It also

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(2) A. Giroud, M. Tuchmann-Duplessis, and L. Mercier-Parot, *Compt. Rend. Soc. Biol.*, **156**, 765 (1962).
(3) C. T. G. King and F. J. Kendrycк, *Lancet*, 1116 (1962-II).
(4) G. Bignami, D. Bovet, F. Bovet-Nitti, and V. Rosnati, *ibid.*, 1333 (1962-II).
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