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REVIEW ARTICLE

Some Antineoplastic Antibiotics

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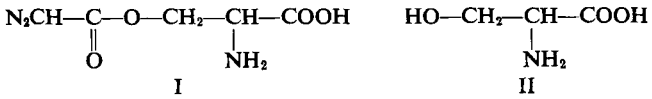
Keyphrases ☐ Antineoplastic antibiotics—review ☐ Antibiotics, antineoplastic—review ☐ Cancer chemotherapy—review ☐ Chemotherapy, cancer—review ☐ Azaserine and related compounds—use in cancer chemotherapy ☐ Actinomycins—use in cancer chemotherapy ☐ Mitomycins—use in cancer chemotherapy ☐ Streptonigrin—use in cancer chemotherapy ☐ Bleomycins—use in cancer chemotherapy ☐ Streptozotocin—use in cancer chemotherapy ☐ Mithramycin—use in cancer chemotherapy ☐ Anthramycin—use in cancer chemotherapy ☐ Daunorubicin—use in cancer chemotherapy ☐ Adriamycin—use in cancer chemotherapy

Cancer chemotherapy, at the present time, still deals very much with the treatment of symptoms rather than with the causes. Nevertheless, outstanding results have been attained with many drugs in the treatment of some neoplastic diseases such as acute lymphocytic leukemia, Burkitt's lymphoma, choriocarcinoma, Hodgkin's disease, squamous cell cancer, and Wilm's tumor. While oncologists may be on the threshold of a "cancer cure" relative to some types of disease, a general overview of the aspects of certain known antineoplastic agents would be appropriate for assessing the values and pitfalls of these agents. The information may contribute to the future design of better antineoplastic agents. It would also provide a useful guide for selecting proper agents for use in combination chemotherapy.

The present review focuses on the antineoplastic antibiotics, excluding nucleosides. Since the structures of these agents are varied and the general biological "rational approach" was not initially involved with the discovery of their activity, information gained from the study should be more objective than studies on more classical compounds in the categories of, for example, antimetabolites or alkylating agents. Each group of antibiotics is discussed whenever possible in the following order: structural aspects, antineoplastic activity in experimental animals, clinical aspects, toxicity, mode or mechanism of action, and metabolism.

AZASERINE, 6-DIAZO-5-OXO-L-NORLEUCINE, AND OTHER GLUTAMINE ANTAGONISTS

Azaserine (I) (*O*-diazoacetyl-L-serine)[1], a derivative of the amino acid L-serine (II) (1, 2), was originally isolated (3, 4) from the culture fluid of *Streptomyces fragilis* (5) and subsequently obtained by synthesis (6–8). The

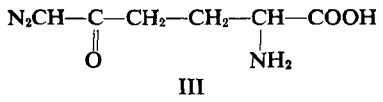


I II

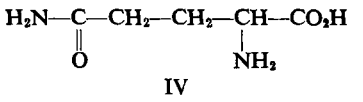
antibiotic crystallizes as light yellow-green needles, m.p. 157° dec. (8). It is very soluble in water and slightly soluble in methanol, ethanol, and acetone (1). Azaserine is relatively stable between pH 6 and 8. Biological activity is lost in strong acid (with evolution of N_2) and strong base. The antibiotic is stoichiometrically degraded *in vivo* to pyruvic acid and ammonia (9).

Antitumor activity of azaserine was first established on sarcoma 180 (10). It also inhibits growth of solid and ascites Ehrlich carcinoma (11–13) and adenocarcinoma EO771 (14). Clinically, azaserine gives brief remission to some children with acute leukemia (15) and causes temporary improvement in patients with Hodgkin's

[1] Sernyl, P 165.



III



IV

disease (15, 16). Beneficial results in other neoplastic diseases were reported but often were not consistent. Digestive tract disturbances (oral lesions, nausea, vomiting, epigastric pain, *etc.*), hematological changes (leukopenia but no megaloblastosis), some systemic intoxication (anorexia, weakness, apathy, jaundice, *etc.*), and lesions of the pancreas, liver, and kidneys are among the toxic symptoms observed (15–19). Azaserine resistance in a plasma cell neoplasm without any change in the active transport of the inhibitor has been noted (20).

Enhanced response is seen in some cases when this antibiotic is combined with 6-mercaptopurine for the treatment of acute leukemia in children (16). The synergistic effect of azaserine in combination with many other drugs has been studied in animals (21–25).

An isostere of azaserine, 6-diazo-5-oxo-L-norleucine (*III*), was isolated from the culture fluids of *Streptomyces* found in a sample of Peruvian soil (26, 27). Its structure was confirmed by synthesis (7). This antibiotic crystallizes as light yellow-green needles, m.p. 144–155° dec. It is very soluble in water and slightly soluble in methanol and ethanol. III is very sensitive to heat and pH changes.

The inhibitory effect of III on mouse sarcoma 180 was established at 0.1 mg./kg. (27), but severe loss in body weight and death were observed (28, 29). This antibiotic also possesses inhibitory activity against sarcoma MA-387, Ehrlich ascites carcinoma, Mecca solid lymphosarcoma (29), and Rous chicken tumor I (30). Antileukemic activity against leukemia L-1210, especially against several resistant strains (resistant to methotrexate, 6-mercaptopurine, *etc.*) has also been noted (31–33). Compound III is more active than azaserine in inhibiting mouse sarcomas but less effective in rat systems.

In clinical trials of III, some transient improvement was observed among patients with melanoma, lymphoma, and carcinoma (particularly choriocarcinoma) using doses of 0.3–1.1 mg./kg./day orally or 0.06–0.4 mg./kg. i.p. (34–38). Both azaserine and III are useful for the treatment of trophoblastic tumors; III can be given orally and can produce long remissions with slow-growing tumors. Clinical improvement is attributed to correction of hypercalcemia by III rather than to primary disease regression (39). Its side effects in man, such as oral toxicity, diarrhea, nausea and vomiting, and leukopenia, resemble those produced by azaserine (18, 35), except that lesions of the pancreas, liver, and kidneys are not observed. In animal studies, III is believed to be 50–100 times more toxic than azaserine with repeated doses. Unusual cumulative toxicity is also noted for III and to a much lesser extent for azaserine (18).

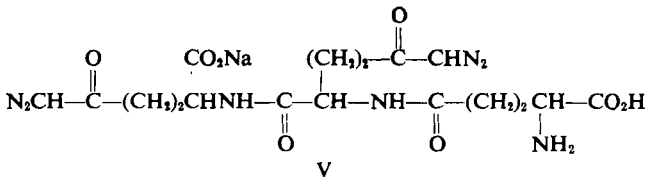
In combination therapy study, this antibiotic also enhances the effect of 6-mercaptopurine (21, 22).

Formate incorporation into both DNA and RNA purines are markedly inhibited by azaserine and III. Inhibitory effects of these antibiotics, which probably act as specific alkylating agents, are attributed mainly to their action on the enzyme system (formylglycinamide ribotide aminotransferase or phosphoribosylformylglycinamidine synthetase) responsible for conversion of formylglycinamide ribonucleotide (FGAR) to formylglycinamidine ribonucleotide (FGAM), one of the initial steps in *de novo* purine synthesis. The action is that of competitive inhibition of glutamine (IV). It is believed that azaserine or III combines with the enzyme through the binding sites utilized by glutamine (these sites normally would initiate the amination reaction of the amide NH_2 group of glutamine). The diazomethyl group of III irreversibly alkylates the neighboring sulfhydryl group of a cysteine residue in the active site of the enzyme, thereby preventing the enzyme from carrying out the aforementioned conversion (16, 39–57). This inactivation can be delayed by the presence of glutamine (50). Since III resembles glutamine structurally more closely than does azaserine, III is a more potent enzyme inhibitor than azaserine (40, 50, 58). Some other analogs, which are less closely related to glutamine, are much less effective enzyme inhibitors.

Since glutamine participates as a cofactor in several other steps in the pathways of purine biosynthesis (59), these antibiotics also interfere with other glutamine-involving conversions such as 5-phosphoribosyl-1-pyrophosphate (PRPP) to 5-phosphoribosylamine and xanthosine phosphate to guanosine phosphate (50, 60, 61). In addition, some enzyme systems involved in other metabolic pathways, with or without the involvement of glutamine [including the conversions of uridine nucleotides to cytidine nucleotides (62–64), nicotinic acid–adenine dinucleotide to diphosphopyridine nucleotide (65–67), xanthosine phosphate to guanosine phosphate (68), and FGAM to 5-aminoimidazole ribotide (50), and the formation of aminobenzoic acid (69) and anthranilic acid (70) from shikimic acid 5-phosphate] are also affected by azaserine and III. The anticancer activity of these antibiotics, which are actively concentrated in cancer cells by the amino acid transport mechanism in competition with glycine, glutamine, and tryptophan (71, 72), could well be due to these enzymatic blocks. It could also be the result of depletion of the adenine nucleotide pool caused by azaserine depression of thymidine kinase and thymidylic acid kinase levels in neoplastic cells (73).

Only the L-isomer of azaserine possesses biological effects in a variety of test systems. D-Azaserine is practically inert. On the other hand, the D-isomer of III was shown to have inhibitory activity, though to a lesser degree, against S-180. It has been suggested that in glutamine–NH_2 transfer reactions, the L- and D-isomers of III are interconvertible, whereas interconversion cannot be realized between the L- and D-azaserines (6, 8, 10, 50, 53, 54, 74–77).

Other antibiotics closely related to III have also been isolated. Alazopeptin, derived from a strain of *Streptomyces*, consists of two molecules of III linked by one molecule of alanine (78–80). It possesses inhibitory activity against some mouse neoplasms but is not effective against certain rat or hamster tumors (79). The effect



V

is presumably dependent on its suppression of purine synthesis. A group of antibiotics known as duazomycins (diazomycins) were isolated from *Streptomyces ambofaciens* (81), which consists of three types: A, B, and C (82). Duazomycin A is *N*-acetyl-III (83); the acetyl group can be removed *in vivo* (84). Duazomycin B (V) is better known as azotomycin, which consists of two molecules of III and one molecule of glutamic acid (82, 83). This antibiotic was reported to have some effect on solid tumors in man (85, 86). Azotomycin is hydrolyzed *in vivo* to yield glutamic acid and III (87, 88), which accounts for its antitumor activity (89). This antibiotic, which causes significant objective response against carcinoma of the colon and soft tissue sarcoma, is reportedly significantly less toxic than III (90). Azotomycin may prove to be therapeutically more effective by functioning as a transport for III and maintaining larger and higher blood and tissue concentrations (88). This antibiotic, either alone or in combinations with L-asparaginase, produces potent immunosuppression *in vivo*. As expected, complete reversal of inhibition is achieved by L-glutamine (91).

ACTINOMYCIN D

The actinomycins are a group of antibiotics possessing a common quinoid-containing phenoxazine chromophore (3-amino-1,8-dimethyl-2-phenoxazone-4,5-dicarboxylic acid) and differ in the amino acid components on two symmetrically substituted polypeptide side chains attached through the carboxylic groups (92–104). These side chains are necessary for biological activity. The aminoquinone portion of the molecule was claimed to give rise to free radicals which attack the SH groups of protein and possibly other targets (105). Among these antibiotics, actinomycin D (VI) (actinomycin C_1, dactinomycin, or meractinomycin) is the most widely studied against experimental tumors and the most often used clincally. Actinomycin D shows a powerful effect on mammary adenocarcinoma, melanoblastoma S-91, and glioblastoma. It is not too responsive against sarcoma 180 and lymphosarcoma in mice, and it is inactive toward leukemia L-1210 (106–111). Its LD_{50} in mice is 2 mg./kg. (112).

Clinically, actinomycin D causes tumor regression or decrease in size of metastasis of the following tumors: Wilm's tumor in children, breast carcinoma, choriocarcinoma, embryonal carcinoma, rhabdomyosarcoma, teratocarcinoma, Hodgkin's disease, lymphomas, melanoma, *etc.* (113–121). On a molar basis, the actinomycins are considered as some of the most active antineoplastic drugs (105). Actinomycin is most useful as an adjunct to surgery and radiation therapy in resectable tumors (122–125). The toxic effects of actinomycin D include nausea, vomiting, anorexia, ulcerations, diarrhea, and moderate depression of bone marrow. Liver and kidneys may also be affected. The anorexia and vomiting may be prevented by chlorpromazine or sedation.

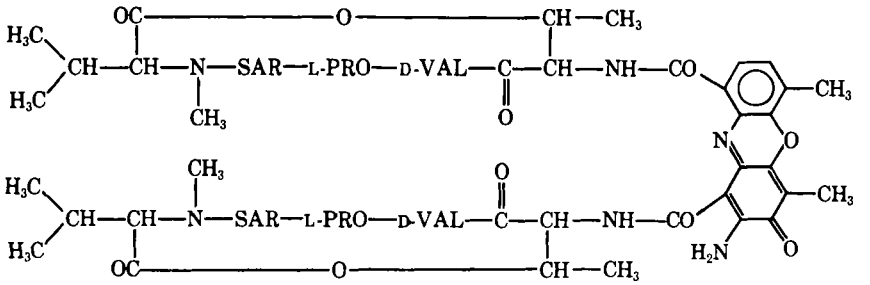
Actinomycin D and other actinomycins are believed to bind tightly, but reversibly, to the minor groove of DNA polymer. The resulting bound DNA can no longer serve as a template for the synthesis of RNA which, in turn, interferes with RNA (inhibits the RNA polymerase) and protein (blockage of the translation of t-RNA) syntheses (126–140). The site of binding is probably at the guanine of the guanine–cytosine pairs in DNA (141–148) with the amino group and the neighboring carbonyl group of actinomycin D. X-ray diffraction and molecular model studies of the interaction of actinomycin D with nucleic acids revealed that there is, on the average, one molecule of actinomycin D for every 18 DNA nucleotides (144). Actinomycin D inhibition of interferon formation (149–151), histone synthesis (152), glucose-6-phosphate dehydrogenase synthesis (153), gluconeogenic enzymes synthesis (154), and phospholipid synthesis (155) and marked alteration of GI amino acid metabolism in adrenalectomized rats (156), as well as other implications on gastric physiology (157), have also been reported.

Tumors do not readily develop resistance to actinomycin D (158). When resistance does occur, it is usually due to changes in actinomycin D permeability on account of alterations in membrane composition and conformation of cancer cells (159).

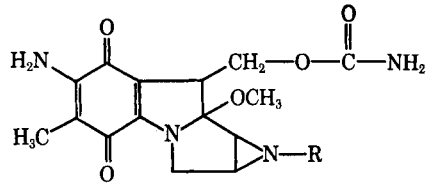
Multiple intraperitoneal injections of actinomycin D in rats can induce invasive, transplantable tumors (160).

MITOMYCIN C

Mitomycins A, B, and C and porfiromycin, isolated from *Streptomyces caespitosus* and several other species of *Streptomyces* (161–164), contain in common an



VI

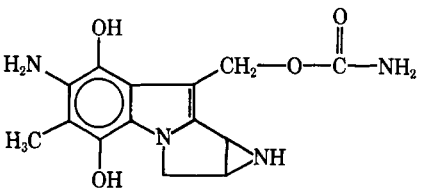


VII*a*: R = H
VII*b*: R = CH_3

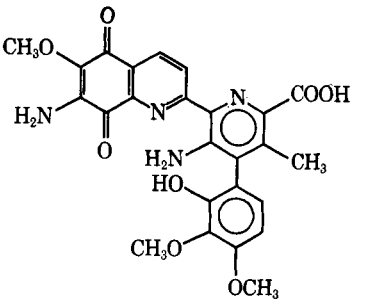
aziridine ring, a carbamate group, and an aminoquinone ring system (165–168). Although synthetic aziridines are quite well known, naturally occurring aziridines had never been found prior to the discovery of compounds of this type. Mitomycin C (VII*a*, mitomycin X), which is the carbamate of 6-amino-1,1*a*,2,8,8*a*,8*b*-hexahydro-8-hydroxymethyl-8*a*-methoxy-5-methylazirino[2′,3′:3,4]pyrrolo[1,2-*a*]indole-4,7-dione, is the most studied antibiotic in this series and has demonstrated marked activity against a variety of experimental animal tumors including the ascites variants of sarcomas, mammary adenocarcinomas, melanoma, rat hepatoma, Walker carcinosarcoma, and Jensen sarcoma (169–180). The LD_{50} of this deep blue-violet antibiotic for rats, dogs, and monkeys on parenteral administration varies from 1 to 2.5 mg./kg.; mice are less sensitive (181).

Mitomycin C has received extensive clinical trials in Japan and the United States (182–198). It produces objective remissions in carcinomas of the stomach, breast, lung, pancreas, *etc.* This antibiotic has a moderate effect in chronic myelogenic leukemia, Hodgkin's disease, malignant lymphomas, certain epithelial tumors, chorioepithelioma, and cancer of the urinary bladder. It also arrests growth in some patients with osteogenic sarcoma. The usual dose given is 1–2 mg./day for 20–40 days. At a dose of over 40 mg./kg., most patients develop toxicity, which is manifested by nausea, vomiting, anorexia, fever, severe depression of bone marrow function expressed by a decrease in the number of leukocytes and especially thrombocytes, and damage of the GI epithelium, liver, and kidney.

The presence of the aziridine ring in mitomycin C indicates that this antibiotic may act as a biological alkylating agent. This is supported by the fact that cross-resistance was observed between a mitomycin C-resistant lymphosarcoma to nitrogen mustard and ethylenimine derivatives and vice versa (199–203) and, like alkylating agents, it induces depolymerization of cellular DNA (177, 178, 204–207). The depolymerization of DNA is claimed to be due to activation of intracellular exonucleases by mitomycin C. In a culture of mammalian cells, there is a preferential suppression of DNA synthesis (208, 209), probably due to the inhibition of DNA precursors rather than to depolymeriza-



VII*c*



VIII*a*

tion of DNA (210). At high levels, mitomycin C may also suppress cellular RNA and protein synthesis. It interferes with the translation of RNA to protein (211). Mitomycin C causes enlargement of nucleoli and other changes resembling those induced by actinomycin D (212).

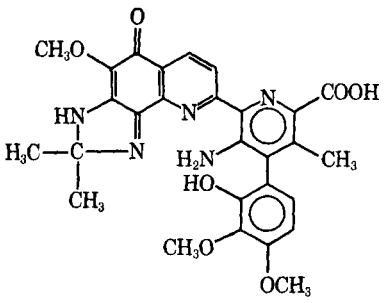
Mitomycin C is biologically inactive as such (213), and the active form is believed to be the reduced (which unmasks the alkylating aziridine ring) and aromatized form (VII*c*) (214). Since the reduced form is rather unstable, the presence of DNA during reduction is necessary for the crosslinking action to occur. The —CH_2—O— linkage of the carbamate side chain serves as the other reactive alkylating center, which crosslinks with guanine in DNA strands (148, 168, 210, 214, 216, 217). The activated mitomycin C resembles bifunctional alkylating agents, with a four-carbon chain linking the reactive centers. The reduced form of mitomycin C is attached to DNA *in vitro* in the ratio of one molecule to every 500 nucleotides (216).

For antitumor activity, the nitrogen atom in the aziridine ring must be unsubstituted. The corresponding *N*-methyl homolog, porfiromycin (VII*b*) (218–221), is practically without activity (222).

STREPTONIGRIN

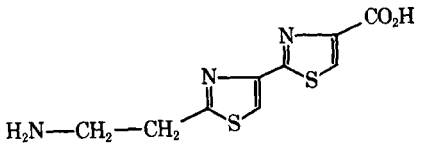
Isolated from the fermentation broth of *Streptomyces flocculus* (81, 223–225), this dark-brown, weakly acidic aminoquinone compound (VIII*a*) (226), which decomposes at 275°, is slightly soluble in water, lower alcohols, chloroform, or ethyl acetate and dissolves more readily in dioxane, dimethylformamide, pyridine, or aqueous sodium bicarbonate. Streptonigrin (bruneomycin) is a broad spectrum antitumor antibiotic (227–234). It possesses marked inhibitory activity against carcinoma 755, spontaneous mammary cancer in C_3H mice, and human sarcoma strains HS 1 and HEp 3. Streptonigrin also considerably inhibits the growth of adenocarcinoma EO771 and 1025, Wagner and Ridgway osteogenic sarcomas, Harding–Passey melanoma, Flexner–Jobling carcinoma, Walker carcinosarcoma 256, and Iglesias functional ovarian tumor at toxic levels. It is also highly active against HeLa cells in tissue culture. Animal toxicity studies showed that streptonigrin is tolerated in larger doses orally than parenterally (LD_{50} in dogs is 5 mcg./kg./day × 10 i.v. or 25 mcg./kg./day × 28 p.o.).

Clinically (235–245), streptonigrin has unequivocal activity against Hodgkin's disease, lymphomas, and related hematological malignancies. It can also produce



VIIIb

IX



X

objective response in chronic lymphatic leukemia and some solid tumors such as carcinomas of the breast, lung, head and neck, common bile duct, pancreas, and cervix. This antibiotic can conveniently be administered orally (and, as in the case of animal study, it is tolerated in larger doses orally than parenterally). Orally, dosage is 0.2–0.4 mg./day; intravenously, dosage is 5–7 mcg./kg./day for 6 days. Its toxic effect is manifested by nausea, vomiting, diarrhea, partial alopecia, thrombophlebitis, mental confusion, and occasional allergic reaction, but its most troublesome toxicity is in the delayed and sometimes prolonged severe bone marrow depression. The latter can be alleviated by dosage control or irradiation. The methyl ester of streptonigrin was also studied (246–254) and was claimed to be less toxic and to have a higher therapeutic index than its parent compound.

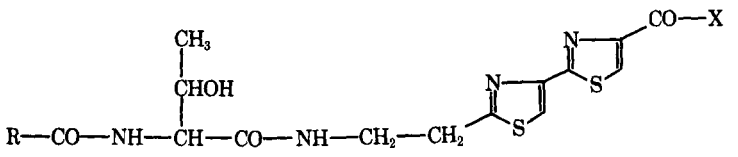
The aminoquinone moiety of streptonigrin, which is also present in actinomycin D and mitomycin C, plays an important role in the antineoplastic activity of these otherwise different structures (226). Streptonigrin, which induces lysogenic bacteria and has a preferential deleterious action on bacteria DNA synthesis and DNA-dependent RNA synthesis (254–257), interferes physically with DNA and causes its degradation when the antibiotic is chemically reduced in the presence of DNA (258–260). The extent of stably bound streptonigrin to DNA is estimated to be 1 mole per 2000 moles of deoxynucleotides. Reduction of streptonigrin is not required for binding. This antibiotic is bound preferentially to DNA during the S (DNA synthesis) stage of the cell cycle (261). This antibiotic also causes a decrease in ATP and protein synthesis of intact cells but does not inhibit ribosomal protein synthesis. Oxygen consumption was noted when blood cells from patients with lymphocytic or granulocytic leukemia were incubated with streptonigrin at 100 mcg./ml. At 50 mcg./ml., respiration in leukemic white blood cells was less affected, but anaerobic glycolysis was inhibited strongly and cellular ATP levels decreased markedly (251). Streptonigrin causes a nonstoichiometric disappearance of reduced glutathione from erythrocytes incubated without glucose. It was suggested that the marked effects of streptonigrin on cellular respiration and anaerobic glycolysis are primarily attributable to the catalytic oxidation of NADH and the resultant peroxide formation (262). Streptonigrin also causes extensive chromosomal breakage and rearrangements of cultured human leukocytes (232), and it alters the chromosomes of the mouse ovum during meiosis (263).

The importance of the aminoquinone moiety of streptonigrin can also be illustrated by the fact that closely related analogs, such as its isopropylidine derivative (VIII*b*), which no longer possess the free aminoquinone unit, have no effect *in vitro* on DNA or protein synthesis and have no other biological effects on human leukemia leukocytes (253, 264). Nevertheless, 7-amino-6-methoxyquinoline-5,8-dione (IX) (266), a compound containing the aminoquinone unit of streptonigrin, was found to be without activity against leukemia L-1210 in mice. In this regard, an N—O—O triangular feature was proposed as a common receptor–complement feature among some antileukemic compounds (267).

Streptonigrin, its methyl ester, and the isopropylidine derivative possess activity against the Rauscher murine leukemia virus *in vivo*. These compounds markedly prolong the survival of mice infected with this virus (268).

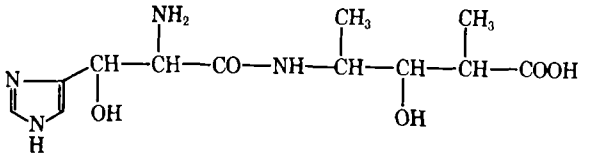
BLEOMYCINS

In connection with a study on phleomycins (269–278), a group of closely related sulfur-containing glycopeptide antibiotics, occurring in a partially saturated chelate with copper in cultures of *Streptomyces verticillus*, were isolated from a soil sample collected from a Japanese coal mine. These antibiotics were designated under the

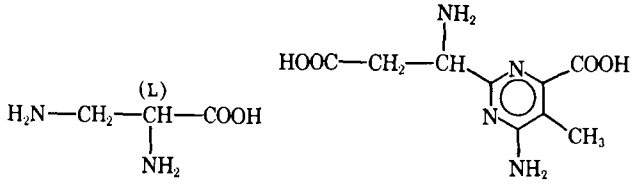


XI

bleomycin A_2: X = $NH-(CH_2)_3-\overset{+}{S}(CH_3)_2\ Hal^-$
bleomycin A_2': X = $NH-(CH_2)_4-NH_2$
bleomycin A_5: X = $NH-(CH_2)_3-NH-(CH_2)_4-NH_2$
bleomycin A_6: X = $NH-(CH_2)_3-NH-(CH_2)_4-NH-(CH_2)_3-NH_2$
bleomycin B_1: X = $NH-\underset{\displaystyle CO_2H}{\underset{|}{CH}}-(CH_2)_3-NH-\underset{\displaystyle NH}{\underset{\|}{C}}-NH_2$
bleomycin B_2: X = $NH-(CH_2)_4-NH-\underset{\displaystyle NH}{\underset{\|}{C}}-NH_2$



XII



XIII

XIV

collective name of bleomycin (279–310). The bleomycins, which possess antimicrobial activity against various bacteria and fungi including plant pathogens (286), consist of two main groups, bleomycins A and bleomycins B. Each group can be further separated into about six compounds of similar structure. The sulfur-containing chromophore 2′-(2-aminoethyl)-2,4′-bithiazole-4-carboxylic acid (X), which is the "core" of all bleomycins, was elucidated by X-ray analysis (290) and has since been confirmed by synthesis (311).

Complete structures of bleomycins have not yet been elucidated. Partial structures of bleomycins A_2, A_2', A_5, A_6, B_1, and B_2 are represented as XI. Among these bleomycins, the major component as well as the most important member is bleomycin A_2. Possessing a molecular weight of 1400, bleomycin A_2 contains, in addition to the aforementioned partial structure (composed of units of L-threonine, β-alanine, two cysteines, and 3-aminopropyldimethylsulfonium halide), two sugars (L-gulose and 3-*O*-carbamoyl-D-mannose, which are presumably linked to the remainder of the molecule by glycosidic bonds) and the peptide and amino acids illustrated by XII–XIV (287, 290, 291, 294, 297, 299)

The bleomycin A complex possesses good antitumor activity against the ascites type of Ehrlich carcinoma and sarcoma 180 in mice (281–285). The copper-free antibiotic is more active than the chelate (288). Clinically, bleomycin A_2 is useful in treatment of human epidermoid cancer, squamous cell carcinoma of the head and neck, lymphosarcoma, Hodgkin's disease, mycosis fungoides, Kaposi's sarcoma, and reticulosarcoma (300–304). It has been given by intraarterial, intravenous, intramuscular, and subcutaneous routes. The efficacy of bleomycin A_2 also has been proved in carcinoma of the thyroid and in brain tumors (glioma and meningioma) (294, 296, 298, 305). Common human warts have been found to respond to the local application of 2–3 mg. of bleomycin (306).

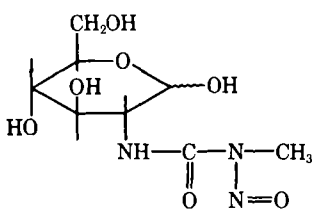
In contrast to the phleomycins, bleomycins exhibit very low renal toxicity. The most remarkable property of bleomycins is that they rarely cause leukopenia and thrombocytopenia in patients—leukopenia previously was considered as an unavoidable side effect of antineoplastic drugs. It does, however, show major toxic side effects in the skin. Furthermore, there is the frequent occurrence of pulmonary toxicity in the form of interstitial pneumonia, which in elderly patients or at high doses may progress to pulmonary fibrosis and death.

Since bleomycin contributes to the therapeutic effect without adding to bone marrow suppression, this antibiotic, when properly used, should be of particular interest in combination chemotherapy studies.

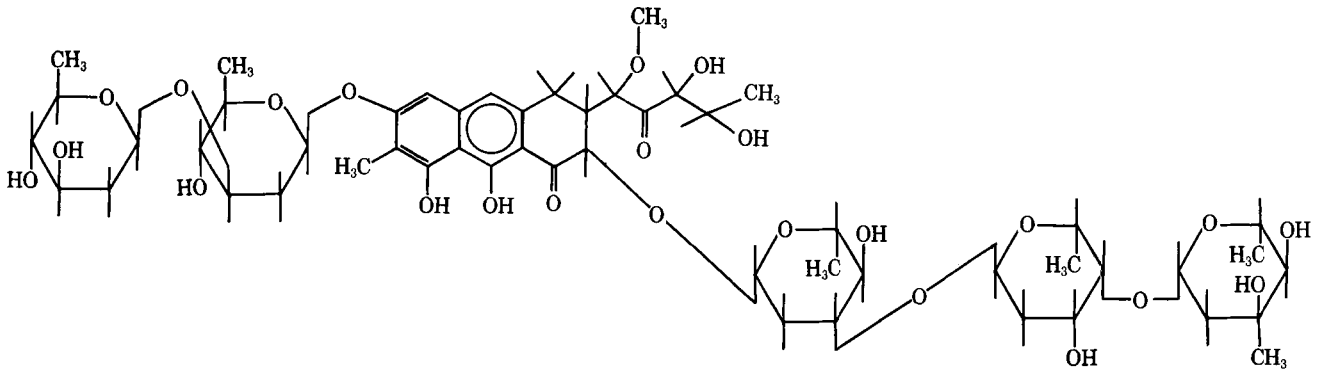
Bleomycin A_2 inhibits DNA synthesis in HeLa cells and *Escherichia coli* by interfering with the incorporation of thymidine into DNA. The nature of the interaction with DNA, however, is rather different from the action of many other anticancer agents. The unfractionated bleomycin complex, but not bleomycin A_2 or A_5, also inhibits protein synthesis in ribosomal systems by interfering with the formation of aminoacyl-s-RNA, particularly the arginyl- and lysyl-s-RNA (288).

In the presence of sulfhydryl compounds (cysteine, glutathione, *etc.*) or hydrogen peroxide, bleomycin A_2 binds to DNA and causes single-strand scission. The scission of DNA is believed to be the cause of the inhibition of thymidine incorporation into DNA (307), which results in interruption of mitotic cell division (288, 292, 308, 309). The effect is said to be similar to that of X-ray and UV irradiation; that is, cells are most sensitive in the late G_1 phase (pre-DNA synthesis), less sensitive in the late S phase (DNA synthesis), and least sensitive in the early G_1 phase (297, 310). However, the action of bleomycin is different from those of irradiation in that the antibiotic has virtually no effect on bone marrow (312). Furthermore, the action of the antibiotic is inhibited by addition of Cu^{+2}, Co^{+2}, Zn^{+2}, and ethylenediaminetetraacetic acid (313).

In *in vitro* experiments, bleomycin A_2 was found to react with DNA and cause a decrease in the melting temperature of the latter (292, 293). Bleomycin A_2 is the first antibiotic that causes a decrease in the melting temperature of DNA. Other antibiotics such as actinomycin D, anthramycin, daunorubicin, and phleomycin cause an increase in the melting temperature of DNA, thereby increasing the structural stability of DNA. These antibiotics inhibit the replication and transcription of DNA, whereas bleomycin A_2 inhibits DNA synthesis and mitotic cell division through the uncoiling of the double helix of DNA. Bleomycin A_2 preferentially interacts with synthetic polynucleotide poly d(GC)-poly d(CG) but not with poly d(AT)-poly d(TA). When the polynucleotides alone are exposed to pancreatic deoxyribonuclease, which produces single-strand scission in native DNA, there is no change in the melting temperature after this digestion. Thus, it indicates that the two effects of bleomycin—decrease in melting temperature and DNA strand scission—are independent. The effect of bleomycin A_2 on DNA polymerase reaction, the stimulation of degradation of DNA nuclease, and the



XV



XVI

inhibition of polynucleotide ligase also were observed (314).

The RNA's synthesized in the presence of bleomycins are smaller in size and seem to contain a larger ratio of pre-early RNA than those synthesized in the absence of these antibiotics (315). It was suggested that bleomycins inhibit RNA synthesis by binding to the DNA template and interfering with the elongation step of the RNA polymerase reaction. The RNA polymerase reaction was also reported to be inhibited by the antibiotics (316).

In a study of the effects of bleomycin on nuclear DNA in transplantable VX-2 carcinoma of rabbit, it was found that bleomycin prevents cells from entering visible mitosis but does not inhibit DNA synthesis. It was also claimed that DNA replication without cell cleavage results in a higher DNA content in a significant proportion of the cell population (317).

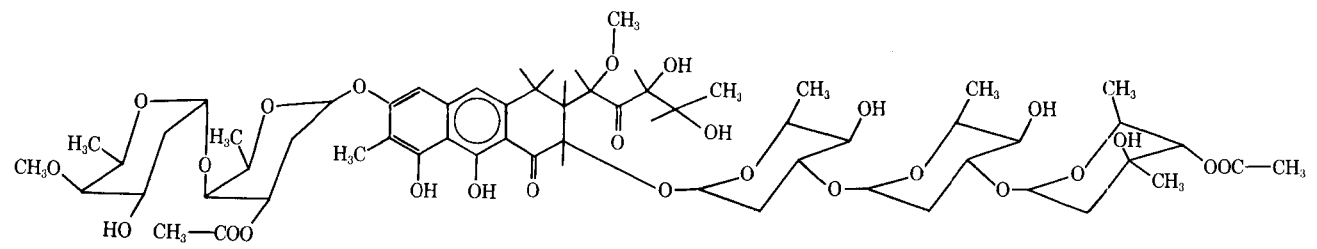
STREPTOZOTOCIN

Isolated from the fermentation broth of a strain of *Streptomyces achromogenes* var. 128 as ivory-colored crystals (318, 319) and subsequently synthesized (320), streptozotocin is a broad spectrum antibiotic effective in protecting mice infected with both Gram-positive and Gram-negative organisms by either the oral or intramuscular route (321–323). This antibiotic also possesses antitumor activity against sarcoma 180, Ehrlich carcinoma, Walker carcinosarcoma 256, and leukemias L-1210, P-388, and L-5178Y (324–326).

Chemically streptozotocin is an *N*-methyl-*N*-nitrosourea derivative—antitumor activity has been found in compounds of this type (327)—of D-glucosamine (328, 329). The antibiotic [XV, 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose] decomposes with evolution of gas at about 115°. The fact that various lots of isolated crystalline streptozotocin have shown wide variations in optical rotation, $[\alpha]_D^{28}$ +15–68°, indicates that this antibiotic is a mixture of α- and β-anomers with the C_1-hydroxyl unsubstituted. Streptozotocin is very soluble in water, soluble in lower alcohols, and relatively insoluble in less polar organic solvents.

Streptozotocin is a potent diabetogenic agent. Intravenous injection of streptozotocin in mice, rats, hamsters, dogs, and monkeys causes an initial hyperglycemia followed by hypoglycemia; the islets of Langerhans are disrupted, and the end result is a permanent tolbutamide-resistant diabetic state in these animals (325, 330–335). Studies of its toxicity in animals have shown that this drug has no undesired effect on bone marrow and the GI mucosa. Without loss of antitumor activity, the toxicity of streptozotocin diabetes can be alleviated by prior intraperitoneal injection of nicotinamide, the latter probably preventing the nicotinamide adenine dinucleotide depression caused by this antibiotic (332). Preliminary toxicologic studies in man showed that the maximum tolerated dose is higher than in animals (2.5 g./m.^{2} compared with 600–1000 mg./m.^{2} in dogs and monkeys). Nevertheless, patients given streptozotocin developed severe renal toxicity (335). Owing to its selective destruction of pancreatic islet β-cells, streptozotocin has been used clinically in palliative chemotherapy for the treatment of multiple-hormone-producing malignant islet cell carcinoma of the pancreas (metastatic insulinoma) and has caused symptomatic relief together with a decrease in the size of hepatic secondaries (336–340).

Streptozotocin has been shown to inhibit synthesis of DNA in *E. coli* without markedly inhibiting syntheses of RNA and protein (341). This antibiotic inhibits mammalian cell growth in all phases of the cell cycle (342). Since *in vivo* production of diazomethane or



XVII

other diazoalkanes by many synthetic *N*-alkyl-*N*-nitroso compounds is well documented (328), streptozotocin could act as a biological alkylating agent.

The *N*-nitrosoamine moiety of a number of compounds, which inhibits hepatic glucose-6-phosphatase activity, was indicated as possibly being carcinogenic (343–346). Streptozotocin was also reported to induce kidney tumors in rats (347–349). Glycogen deposits are found in the proximal tubules in children with glycogen-storage disease and in rats given streptozotocin (350). Various neoplastic lesions have also been observed in Chinese hamsters upon intraperitoneal administration of streptozotocin (351).

MITHRAMYCIN

Mithramycin (XVI), 2-[β-mycarosyl(1 → 4)α-oliosyl (1 → 3)β-olivosyl]-6-[β-olivosyl(1 → 3)β-olivosyl]-chromomycinone[2], is an antitumor antibiotic produced by submerged growth of *Streptomyces plicatus* (352) and was recently shown to be identical with antibiotic LA-7017 (353) and aureolic acid (354).

Mithramycin is a glycoside (m.p. 180–183°, $[\alpha]_D^{20}$ −51°, c 0.4 in EtOH). On acid hydrolysis, it yields chromomycinone, D-mycarose, D-olivose, and D-oliose in the ratio of 1:1:3:1. It is acidic [pKa 5.13 (355)] and very soluble in water. The antibiotic inhibits the growth of Gram-positive bacteria and HeLa cells *in vitro* and shows moderate activity against adenocarcinoma 755 in mice and human tumor type HS 1 in rats.

In addition to mithramycin, two structurally related (356) cancerostatic antibiotics, olivomycin (357) and chromomycin (XVII) (358), were isolated and studied (359, 360).

Mithramycin retards growth of heterotransplanted bladder cancer in the hamster-cheek pouch within 24 hr. (361). Studies on the effect of mithramycin on mouse glioma showed that 1.25 mg./kg./day on 4 consecutive days in Weeks 3, 6, and 9 gave the greatest degree of inhibition (362).

Mithramycin causes complete and partial remission in patients with embryonal carcinoma of the testis, melanoma, and lymphoma (363–368). The major clinical toxicity is hemorrhagic diathesis associated with a precipitous thrombocytopenia. Enhancement of antitumor activity was reported when both radiation therapy and mithramycin were used against glioma tumors in mice (369). However, recent evaluation of this antibiotic on experimental intracerebral glioma in mice showed no beneficial effect (370). This is probably due to a low drug concentration of the antibiotic in the brain (371) as a result of poor permeability through the blood–brain barrier (370). The optimal dosage is 50 mcg./kg./dose on alternate days, intravenously by push or infusion over several hours, for up to eight doses per course (372).

Mithramycin is a very toxic antibiotic. Administration of mithramycin intravenously at 0.1 mg./kg./day is lethal to dogs and monkeys. Some signs of toxicosis at lower doses are manifested by anorexia, emesis, listlessness, hypochoremia, azotemia, anemia, lymphopenia, melena, thrombocytopenia, and elevations of serum alkaline phosphotase (373), glutamic oxolactic transaminase, and glutamic pyruvic transaminase values. Gross necropsy findings are characterized by widespread hemorrhages and edema, pleural and peritoneal effusions, and compact rubbery livers. Nevertheless, normal liver cells exposed to mithramycin recover their capacity for RNA synthesis more rapidly than tumor cells. Generalized lymphoid tissue necrosis and widespread hemorrhages are also observed.

Mithramycin therapy in the treatment of testicular cancer (374, 375) is accompanied by a marked dose-related hemorrhagic diathesis in many patients. Vascular damage may be the major factor in initiating bleeding. Significant thrombocytopenia and an altered platelet function are also noticed (375). The drug toxicity can be markedly reduced by an alternate-day dosage regimen (374, 376).

Concentration of mithramycin in cell cultures of human brain tumor produces severe damage (377), as manifested by a change of nuclear size and shape and alterations in the composition and distribution of nuclear chromatin (377, 378).

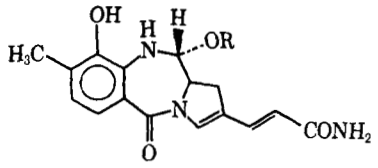
Distribution studies (371) with tritiated mithramycin in C_3H mice revealed that the highest concentrations are in the kidney and liver. Excretion of the antibiotic is rapid, and there is no evidence of metabolism of the carbohydrate moiety to CO_2 and H_2O through respiration. Although the concentration of mithramycin in the brain is rather low, its retention was found to be longer than that in other tissue.

Administration of mithramycin to the canine not only significantly decreases the RNA synthesis in the renal cortex but also causes a decrease in urine volume, sodium excretion, and *p*-aminohippurate clearance together with an increase in urinary potassium and plasma glucose (379). Mithramycin also has a hypocalcemic effect, leading to a decrease in urinary calcium excretion and in urinary hydroxyproline in patients with malignant disease (380).

Mithramycin inhibits RNA synthesis by inhibiting RNA polymerase through interaction with the DNA template (381). Differing from the mechanism of action of daunorubicin, which intercalates with base pairs in the DNA helix, mithramycin forms a stable complex with DNA by forming bridges between complementary strands of the helix, thereby stabilizing the secondary structure of DNA (382). Mg^{+2} ions are required for this binding (360). The binding causes a progressive decrease in the buoyant density of DNA in CsCl and Cs_2SO_4 density gradients at increasing antibiotic concentration with high guanine and cytosine content of DNA. Like actinomycin D, mithramycin does not directly affect the synthesis of DNA itself; however, this antibiotic does not cause stabilization of native DNA to thermal denaturation (382), which differs from the effect of actinomycins.

In *in vitro* and *in vivo* studies with mouse tumors (383, 384), HeLa cells (385), and *Bacillus subtilis* cell cultures (386), mithramycin markedly inhibited the incorporation of ^{32}P-phosphate (383) and ^{14}C-uracil (386) into RNA but had no effect on DNA or protein. This antibiotic reversibly inhibited RNA synthesis in

[2] Mithracin.



XVIII*a*: R = H
XVIII*b*: R = CH_3

chick embryo cells as well as the DNA-directed RNA synthesis of DNA viruses. The synthesis of virus RNA-dependent RNA polymerase was only slightly inhibited (387, 388).

ANTHRAMYCIN

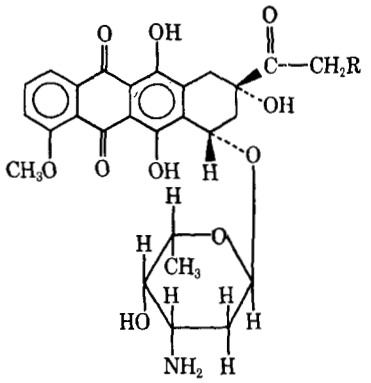
Anthramycin, the active constituent of refuin, is derived from a thermophilic actinomycete *Streptomyces refuineus* var. *thermotocrans* NRRL 3143 (389–391). Crude preparations contain approximately 0.5% of the antibiotic. It possesses antibacterial activity as well as antineoplastic properties against mouse tumors *in vivo* and human cancer cells *in vitro* (389, 392).

The chemical structure of anthramycin (XVIII*a*) was originally assigned on the basis of spectroscopic evidence (391, 393) and was confirmed by total synthesis (394). The generic name of this antibiotic was derived from the fact that it contains an anthranilic acid moiety.

On recrystallization from a mixture of methanol–water and acetonitrile, anthramycin was converted to its methyl ester (XVIII*b*) and anhydroanthramycin, respectively. These compounds are interchangeable in different media (391). In general, anthramycin and its derivatives are extremely sensitive to heat and are stable in solution only under essentially neutral conditions. The methyl ester of anthramycin (XVIII*b*), isolated as pale-yellow needles, is comparatively stable and well characterized. Compound XVIII*b*, therefore, is preferably used in biological study (392).

Compound XVIII*b* increases survival time of mice bearing various experimental leukemias (392). It is active against leukemias P-388 and L-1210, plasma cell tumor LCP (392), Ehrlich carcinoma (solid form), and sarcoma 180 (395). Anthramycin has been considered less toxic than a number of other neoplastic drugs (396). When given by slow intravenous infusion in doses up to 1 mg./day, a positive response in about 66% of a group of patients with advanced cancer was observed. It is more effective in breast carcinoma than in lung cancer and malignant melanoma.

Compound XVIII*b* strongly inhibits DNA synthesis in sarcoma 180 ascites and intestinal epithelium tumor cells (397). It also inhibits both RNA and DNA synthesis in mouse leukemic cells (392, 398), *E. coli*, HeLa cells (399), and Ehrlich ascite carcinoma cells (400). Unlike the actinomycins, anthramycin and related compounds do not inhibit RNA much more than DNA synthesis (398). Compound XVIII*b* reacts slowly with DNA (398), producing changes in the melting point and buoyant density of DNA, while undergoing changes in the absorbance of the UV spectra (398, 400, 401); thus the kinetics of the reaction can be easily followed. This antibiotic exhibits a high degree of preference for binding with the DNA helix (398, 400) which, in turn, prevents DNA from participating as a template in the biosynthesis of DNA and RNA (400). In fact, the DNA–XVIII*b* complex is so stable that it cannot be decomposed by many common physicochemical means. This suggests that covalent bonding exists in the complex (398, 402), or that there is strong electronic attraction between anthramycin and anionic phosphate groups of DNA (401), or that there is an interaction involving the seven-membered ring position of the antibiotic and DNA (401).



XIX*a*: R = H
XIX*b*: R = OH

Complexing with DNA could well be a primary cause of the *in vitro* and *in vivo* antitumor activity of anthramycin. Free purines and pyrimidines, their derivatives, s-RNA, and the rG:rC polymer are not able to complex with anthramycin (401, 402). Thus, the three-dimension structure of DNA, comprised of a specially oriented array of functional groups, accounts for the interaction with anthramycin. This antibiotic does react with heat-denatured DNA, but at a much slower rate. This again indicates that the secondary structure of DNA affects its binding to anthramycin. Structure–activity studies of anthramycin and related compounds (392, 401) seem compatible with the proposed receptor–complement triangulation requirement (267).

Anthramycin is a competitive inhibitor with respect to DNA for DNA-dependent RNA polymerase (399, 400) and DNAase enzymes (400). Cryst necrosis was reported to occur with XVIII*b* treatment subcutaneously in mice (403).

DAUNORUBICIN

The antibiotics daunomycin, isolated from cultures of *Streptomyces peucetius* var. *Carneus* (404–406), and rubidomycin, isolated from *Streptomyces coeruleorubidus* (407, 408), were found to be identical by comparison of their UV and IR absorption spectra and chromatographic behavior and by chemical studies (409). A generic name, daunorubicin, has since been adopted as the general term.

Daunorubicin (XIX*a*), usually obtained as its hydrochloride salt in the form of red needles [$C_{27}H_{29}NO_{10}\cdot$ HCl, m.p. 188–190° dec., $[\alpha]_D^{25}$ +253° (0.15 CH_3OH)], is an anthracycline glycoside which, when subjected to acid hydrolysis, yields daunomycinone (410), a pigmented aglycone of substituted anthraquinone, and

daunosamine (411), an amino sugar. Its structure and absolute configuration have been completely elucidated (412–415).

Daunorubicin is active against a variety of experimental animal tumors including sarcoma 180, Ehrlich ascites, Yoshida AH-130 hepatoma, and early and advanced stages of leukemia L-1210 in mice, and Walker carcinoma 256 and ascites myeloma in rats (416–419). Daunorubicin increases survival time of mice with leukemia L-1210 by 57%, with P-388 by 127%, and with P-388/57155 by 100% (420). Leukemic variant L-1210/C-95, which is resistant to 6-mercaptopurine, methotrexate, and cytoxan, is sensitive to this antibiotic. Its activity against L-1210 is comparable to that of 6-mercaptopurine but less effective than that of methotrexate or cytoxan. Clinically, this antibiotic exhibits antitumor effects in acute lymphoblastic leukemia (421–432), acute myeloblastic leukemia (426, 429, 433–437), metastatic neuroblastoma (423, 429, 430), lymphosarcoma (429), and rhabdomyosarcoma (423). Published clinical trials with daunorubicin in children with acute lymphoblastic leukemia recently were compared and reviewed (432). The antibiotic, which is effective when given intraperitoneally, intravenously, or subcutaneously but not orally (438), has been used over a narrow dose range. With 1 mg./kg. for 5 days, the remission rate is 40%; with 30–60 mg./m.^{2}/day for 5 days, the overall remission rate is 33% (432). The risk of toxicity is increased as the accumulated dose approaches 600 mg./m.^{2} or 22 mg./kg. General toxicity is hematological with severe leukopenia, thrombocytopenia, and bone marrow aplasia. Cardiac toxicity is also observed (439, 440).

The precise mechanism of action of daunorubicin has not yet been fully elucidated. In certain aspects, its action is similar to that of actinomycin D (441). When daunorubicin is added to a solution of DNA, the viscosity of the solution is increased, the sedimentation coefficient is decreased (382), and the bound DNA is more stable toward thermal denaturation (442). It, therefore, appears that daunorubicin complexes both strands of the DNA double helix (443); as a result, both DNA and RNA syntheses in a variety of biological systems are inhibited (381, 406, 441, 444–453). The antibiotic has a greater effect on DNA than on RNA synthesis in mammalian cells, although the extent of inhibition differs in different species. The binding sites of daunorubicin to DNA are quite similar to those of actinomycin D in that both antibiotics bind through the purine and pyrimidine bases. However, slight differences in exact binding sites for these antibiotics were noted (454). Daunorubicin-resistant cell lines also exhibit resistance to actinomycin D. The development of resistance is probably due to a decrease in permeability of the drug across the cell membrane (455).

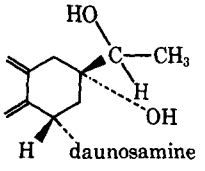
Daunorubicin induces ultrastructural nuclear and nucleolar lesions in rat embryo cells (456). Since this antibiotic forms a complex with DNA by intercalation between the base pairs, the lesion may represent a morphological expression for certain specific molecular action.

In a study of the action of daunorubicin on the metabolism of nucleic acids of normal and tumor cells cultivated *in vitro*, it was observed that this antibiotic particularly inhibits the fraction that possesses a fast turnover in tumor cells and a slow turnover in the normal ones (444). In synchronized mammalian cells (rat fibroblast L-cells), the late S phase of DNA synthesis is the most sensitive to inhibition by daunorubicin (450, 457). The RNA syntheses, which take place during the entire cell cycle and involve both nucleolar and extranucleolar structures, are strongly inhibited by this antibiotic (457, 458). Comparatively speaking, the extranucleolar RNA of HeLa cells is less sensitive to daunorubicin (441, 459) than the nucleolar RNA.

Daunorubicin decreases the mitotic index in synchronized cells, mouse fibroblast L-cells, and Burkitt lymphoma cells (460) by action on the interphase and on mitosis. The effect is generally proportional to the dose employed and the time of contact (461). Daunorubicin causes condensation of nucleoli, an increase in heterochromatic materials, chromosome breakage, and swollen cells (462, 463). The type of chromosome damage suggests that the antibiotic exerts its action on DNA in the premitotic phase (464, 465). From studies using protein and DNA inhibitors during chromosome aberration, it seems that protein synthesis is required for the reunion of daunorubicin-induced breaks, while DNA synthesis may not be a prior condition (466). Since daunorubicin has the unique ability to delay the onset of mitosis in cells that have already synthesized DNA (467), this characteristic should not be overlooked when the antibiotic is used in combination chemotherapy.

Replication of DNA virus in infected HeLa cells is markedly inhibited by daunorubicin. On the other hand, daunorubicin does not suppress the production of RNA virus. Apparently, this antibiotic either inhibits viral DNA replication or prevents m-RNA transcription (468).

The distribution of daunorubicin in tissue and in body fluid has been investigated (469–472). The antibiotic is readily transferred from blood to tissue and absorbed by various organs (473). Daunorubicin metabolism has been studied with rat tissue preparations (474, 475). It was found that this antibiotic is rapidly transformed into daunorubicinol as well as the aglycones of daunorubicin and daunorubicinol (475). The structure of daunorubicinol, which has also been isolated as a major metabolite of this antibiotic from human urine and enzymatic reactions (476), is the 13-hydroxy derivative of daunorubicin. A portion of its structure (XX) is shown here.



XX

The metabolite daunorubicinol still possesses antitumor activity (475); on the other hand, the aglycone daunomycinone displays no antineoplastic activity (267). The latter compound can also be obtained by acid hydrolysis of daunorubicin; the other hydrolysis

product, the amino sugar daunosamine, is also inactive.

Some *N*-acyl derivatives of daunorubicin were prepared and were claimed to be less toxic (477). Nevertheless, *N*-acyl analogs are in general less active (449). Chemical conversion of daunorubicin to another antibiotic, adriamycin (XIX*b*, *vide infra*), has also been accomplished (478, 479). The 14-hydroxy, 14-halogeno, and dihydro derivatives of daunorubicin, as well as its semicarbazone and thiosemicarbazone, have also been prepared and studied (480–484).

ADRIAMYCIN

Adriamycin (XIX*b*) (485) or 14-hydroxydaunorubicin was isolated as a hydrochloride salt [m.p. 205° dec., $[\alpha]_D^{20}$ +248° (0.1 CH_3OH)] from the culture of *Streptomyces peucetius* var. *caesius*, which was derived from *S. peucetius* by mutagenic treatment of the parent culture with *N*-nitroso-*N*-methylurethan (478). This antibiotic possesses the same anthraquinone chromophore and glycoside as daunorubicin (479) and, similar to the behavior of daunorubicin, mild acid hydrolysis of adriamycin yields the water-insoluble red aglycone adriamycinone and the water-soluble amino sugar daunosamine (479). Conversion of daunorubicin to adriamycin was achieved by hydrolysis of its *N*-protected 14-halogenated derivatives (480, 481).

Adriamycin markedly inhibits the growth of Ehrlich ascites carcinoma, an ascitic transplantable lymphosarcoma, solid sarcoma 180, and Oberling–Guerin–Guerin carcinoma in rodents; it also considerably increases the survival time of the treated animals (485). The antibiotic has a more favorable therapeutic index than daunorubicin in both animal tests (485) and clinical trials (486). Adriamycin exerts an inhibitory effect on cell production, which is particularly evident in the more actively proliferating tissues such as spleen, bone marrow, or germinal tissues (487).

In *in vitro* study, adriamycin was found to have immediate antimitotic and antimetabolic effects in HeLa cell cultures. The cell membrane is quickly penetrated by the antibiotic, which then binds to nuclear structures, particularly perinucleolar chromatin. The growth inhibition is proportional to the concentration and exposure time of the inhibitor applied. Both DNA and RNA syntheses are inhibited by adriamycin, as indicated by autoradiographic studies of ^3H-labeled thymidine and uridine uptake (488, 489).

In acute toxicity studies, the LD_{50} of the hydrochloride salt of adriamycin was found to be 20.8 mg./kg. i.v. in mice. In subacute toxicity studies in rabbits (1 mg./kg. i.v. every other day for 3 weeks), this antibiotic provoked a slight normochromic anemia with thrombocytopenia, without variation in prothrombin and coagulation times or in reticulocyte number (487).

Clinical trials of adriamycin against neoplastic diseases revealed that in 25 cases of lymphoblastic leukemia, nine complete and four incomplete remissions were obtained. Of 18 cases of acute myeloblastic leukemia, six complete and three incomplete remissions were achieved (490). Phase I and preliminary Phase II evaluation of this antibiotic were studied (486). Regression was noted mostly in acute lymphoblastic and chronic (myeloid and lymphocytic) leukemia as well as in lymphomas, neuroblastoma, embryonal rhabdomyosarcoma, ovarian teratoma, Wilm's tumor, Ewing sarcoma, and soft-tissue sarcoma (491). Adriamycin, in spite of its activity against a wide variety of solid tumors and leukemias resistant to most conventional therapy, seems to be more useful in inducing tumor regression than in maintenance therapy (486). This antibiotic is very toxic to bone marrow and renders the patients more susceptible to environmental pathogens. Its cardiac toxicity is definite but less frequent than the related antibiotics (490). Other toxicity consists primarily of stomatitis, mouth ulceration, alopecia, and irreversible marrow aplasia (486).

In a direct comparison of the antileukemic effect against L-1210 lymphoid leukemia and P-388 leukemia in mice (438), it was found that adriamycin is significantly more effective than daunorubicin in increasing median survival time and in producing long-term survivors. Both antibiotics are effective intraperitoneally and subcutaneously but not orally.

Adriamycin causes chromosome damage when used on human leukocytes in *in vitro* cultures. The distribution of exchange points along the length of the chromosome (or group of chromosomes) expresses the nonrandom effect of this antibiotic on the chromatin matter (492). Adriamycin displays a mutagenic effect on human lymphocytes cultured with phytohemagglutinin. The effect is believed to be responsible for the inhibition of blastogenesis, the cellular giantism, and hyperploidy (493).

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