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Mechanism of Action of 2,2'-(Methylenediimino)bis-1,3,4-thiadiazole (NSC 143019), an Antitumor Agent

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SUMMARY

The mechanism of action of 2,2'-(methylenediimino)bis-1,3,4-thiadiazole (NSC 143019) was clarified by studies on its effects on monolayer cultures of growing cells of the mouse cell line BALB/3T3.

At concentrations below 50 μM, NSC 143019 specifically inhibited DNA and RNA syntheses without appreciably affecting protein synthesis. The syntheses of DNA and RNA were inhibited equally and concomitantly by the compound.

The inhibition was reversed by removal of the compound and was prevented competitively by an equimolar amount of nicotinamide. It was also reversed completely by guanosine (0.1 mM) or deoxyguanosine (0.1 mM) and was reversed partially by xanthosine (1 mM). Other nucleosides did not influence the inhibition.

The inhibition of DNA synthesis by NSC 143019 was not due to inhibition of RNA synthesis, and *vice versa*.

NSC 143019 inhibited the conversion of [8-^{14}C]hypoxanthine to acid-soluble and -insoluble guanine nucleotides but not to adenine nucleotides.

It was strongly suggested from these results that at concentrations of NSC 143019 below 50 μM the primary action of this compound might be due to the inhibition of GMP biosynthesis at the step of conversion of IMP to xanthosine 5'-phosphate.

INTRODUCTION

The antitumor activities of a number of 2-substituted thiadiazoles have been reported by many investigators (1, 3, 4, 7). There have been several *in vivo* studies (1, 3, 4, 7) aiming at the clarification of the mechanism of action of these compounds. An exchange reaction has been reported between thiadiazole compounds and the nicotinamide moiety of NAD^+ (1, 3), and the inhibitory action of these compounds has been shown to be antagonized by nicotinamide (1, 3, 4, 7). In addition, these compounds have been found to cause an increase in production of uric acid by some mechanism other than nucleic acid degradation (4). However, little is known about the exact site of inhibition by these compounds.

2,2'-(Methylenediimino)bis-1,3,4-thiadiazole (NSC 143019) was synthesized in the Agricultural Chemical Research Laboratories of this firm (Y. Okada, unpublished data) as one of a series of analogs of 1,3,4-thiadiazole. *In vivo* experiments showed that its antitumor and immunosuppressive actions were about 5 times higher than those of the parent compound, 2-amino-1,3,4-thiadiazole (NSC 4728), on a weight basis (5). That is, NSC 143019 was effective against L1210 leukemia, 6C3HED/OG lymphosarcoma, C1498 myeloid leukemia, Ehrlich carcinoma, Sarcoma 180, B16 melanoma, and X5563 myeloma at nontoxic doses in mice. This compound also significantly suppressed the production of serum antibody against sheep erythrocytes, and the graft-*versus*-host response in adult F_1 mice was affected by treatment with the drug. It was found that these activities, as well as the toxicity of the drug to the host, were prevented by simultaneous administration of an equimolar amount of nicotinamide (5). A teratogenic action of the compound and its reversal by nicotinic acid have also been reported (6).

The present report describes studies on the primary site of inhibition by the compound, using monolayer cultures of a mouse cell line (BALB/3T3).

MATERIALS AND METHODS

Cells. A clone of BALB/3T3 cells (a line from a BALB/C mouse embryo), A-31, was kindly given by Dr. T. Kakunaga of the Research Institute for Microbial Diseases, Osaka University, and was used in all experiments.

Medium. Eagle's minimum essential medium supplemented with 10% bovine serum was used.

Cell Culture and Determination of the Rates of DNA, RNA, and Protein Syntheses. Cells were suspended at a concentration of 3×10^4 cells/ml in the above medium, and 1-ml aliquots were seeded into glass Petri dishes (1.8 cm in diameter), each containing a coverslip (1.5 cm in diameter). Cells were cultured at 37° in a CO_2 incubator (5% CO_2:95% air). Test compounds were added to the cultures 24 hr after cell seeding, unless otherwise stated. One hr before cell harvest, 0.1 ml of [^{3}H]thymidine (5 Ci/mmole, 1 μCi/dish), [^{3}H]uridine (15 Ci/mmole, 1 μCu/dish), or [^{14}C]leucine (61 Ci/mmole, 1 μCi/dish) was added to each culture dish containing 1 ml of medium. In experiments on the incorporation of [^{14}C]leucine, the medium was replaced by medium containing one-tenth the normal concentration of leucine just before the addition of [^{14}C]leucine. Cells were pulse

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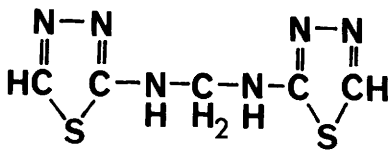
labeled for 1 hr. The acid-soluble fraction was washed out from the cells fixed on the coverslips, as described previously (9). The coverslips were dried and put into vials containing 10 ml of toluene scintillator solution and were counted in a Beckman LS 150 liquid scintillation spectrometer. The rates of incorporation of [^{3}H]thymidine, [^{3}H]uridine, and [^{14}C]leucine into the acid-insoluble fraction of cells per hr are denoted as the rates of DNA, RNA, and protein syntheses, respectively.

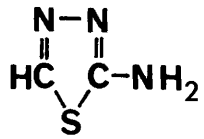
Cell Counting. Cells cultured on the coverslips as described above were counted with a Coulter counter (Z_B type equipped with an aperture tube with a diameter of 100 μm).

Incorporation of [8-^{14}C]Hypoxanthine into the Acid-soluble and Acid-insoluble Fractions of Growing BALB/3T3 Cells. Two samples of growing BALB/3T3 cells were taken from a single culture. One sample was treated with NSC 143019 (50 μM) for 16 hr, and the other was treated with 0.1% dimethyl sulfoxide, which was used to solubilize the test compound, for 16 hr, as a control. Then [8-^{14}C]hypoxanthine (9.66 mCi/mmole) was added to both cultures (0.1 μCi/ml). After labeling for 2 hr, the acid-soluble and -insoluble fractions were prepared, and the purine, pyrimidine, and their derivatives in these fractions were hydrolyzed to their bases, as described by Franklin and Cook (2). The purine bases were then separated by high-voltage electrophoresis on Toyo No. 51-A paper in a solution consisting of equal volumes of 1.5 M formic acid and 2 M acetic acid at pH 2.0. After electrophoresis for 30 min at 50 V/cm, the sheet was dried, and areas corresponding to spots of purine markers were cut out and their radioactivity was measured.

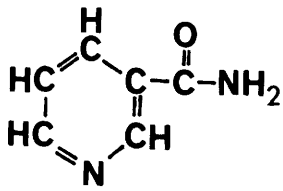
Chemicals. NSC 143019 and NSC 4728 (Chart 1) were synthesized by Dr. Y. Okada of the Agricultural Chemical Research Laboratories of this firm. These compounds were dissolved in dimethyl sulfoxide. After dilution and sterilization by passage through a membrane filter, these compounds were added at various concentrations to cultures containing a final concentration of 0.1% dimethyl sulfoxide. All control cultures contained 0.1% dimethyl sulfoxide. All bases and nucleosides were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan, except xanthosine, which was synthesized in the Chemical Research Laboratories in our Central Division. Radioisotopes were purchased from the Radiochemical Centre, Amersham, England, or from Daiichi Pure Chemical Company, Ltd., Osaka, Japan. Their purity was checked and, if necessary, they were purified further before use by column chromatography. Actinomycin D and cytosine arabinoside were purchased from Mann Research Laboratories, New York, N. Y., and Sigma Chemical Co., St. Louis, Mo., respectively. Other chemicals used were reagent grade.



NSC-143019



NSC-4728



Nicotinamide

Chart 1. Structures of NSC 143019, NSC 4728, and nicotinamide.

RESULTS

Inhibitory Actions of NSC 143019 on Syntheses of Macromolecules in Growing BALB/3T3 Cells

Effects of the Compound on DNA, RNA, and Protein Syntheses. NSC 143019 (50 μM) was added to growing BALB/3T3 cells, and 1, 3, 5, and 24 hr later, [^{3}H]thymidine, [^{3}H]uridine, or [^{14}C]leucine was added in the amount of 1 μCi/ml. After pulse labeling for 1 hr, the radioactivity incorporated into the acid-insoluble fraction was determined as described in "Materials and Methods." As shown in Chart 2, the rates of DNA and RNA syntheses began to fall 3 hr after addition of the compounds, and maximum inhibition of 80 to 90% was observed after 7 hr. In contrast, the rate of protein synthesis was not affected for at least 24 hr after addition of the compound.

Time and Concentration of NSC 143019 Required for Inhibition. As shown in Chart 2, the syntheses of DNA and RNA were inhibited concomitantly. The time required for 50% inhibition of nucleic acid syntheses after the addition of the compound was 4 to 5 hr. The effects of various concentrations of NSC 143019 on nucleic acid syntheses are shown in Chart 3. As in Chart 2, the syntheses of DNA and RNA were inhibited in parallel, depending on the concentration of the compound. The concentration required for 50% inhibition of nucleic acid syntheses was about 20 μM.

Reversal of the Inhibitory Action of NSC 143019 by Its Removal or by Addition of Nicotinamide. NSC 143019 (50 μM) alone or NSC 143019 (50 μM) plus nicotinamide (50 μM or 150 μM) was added to growing BALB/3T3 cells and, 16 hr later, the cells were pulse labeled with [^{3}H]thymidine or [^{3}H]uridine for 1 hr. The radioactivity incorporated into the acid-insoluble fractions was then determined as described in "Materials and Methods." In experiments on reversal of the inhibition, 16 hr after addition of 50 μM NSC 143019, the medium of some cultures

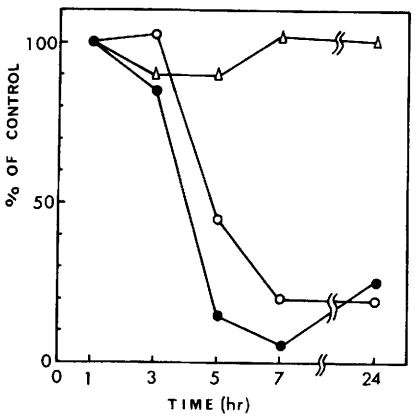


Chart 2. Rates of DNA, RNA, and protein syntheses in growing BALB/3T3 cells after addition of NSC 143019 (50 μM). NSC 143019 (50 μM) was added to growing BALB/3T3 cells and then at intervals the cells were pulse labeled with [^{3}H]thymidine (●), [^{3}H]uridine (○), or [^{14}C]leucine (△) for 1 hr. Radioactivity in the acid-insoluble fraction of the cells was counted as described in "Materials and Methods." *Abscissa*, hr after addition of NSC 143019; *ordinate*, radioactivity incorporated into the acid-insoluble fraction of growing BALB/3T3 cells as a percentage of that before addition of NSC 143019.

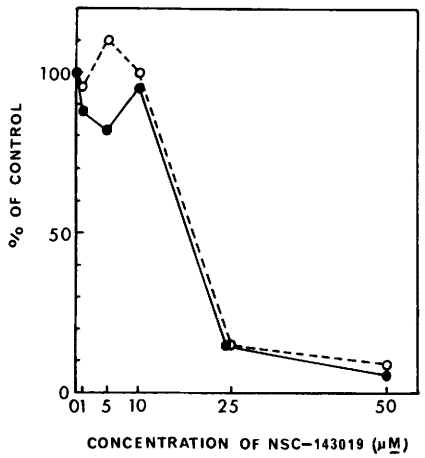


Chart 3. Effect of concentration of NSC 143019 on nucleic acid syntheses of growing BALB/3T3 cells. Growing BALB/3T3 cells were incubated with various concentrations of NSC 143019 for 16 hr and then pulse labeled with [^{3}H]thymidine (●) or with [^{3}H]uridine (○) for 1 hr and treated as described in Chart 2. *Abscissa*, concentration of NSC 143019 (μM); *ordinate*, radioactivity incorporated into the acid-insoluble fraction as a percentage of that before addition of the compound.

was replaced by medium without NSC 143019, while 50 or 150 μM nicotinamide was added to other cultures, and the rates of nucleic acid syntheses were examined 25 hr later. Results show that the inhibition of DNA and RNA syntheses were both nearly completely reversed by removal of the compound or by addition of an equimolar amount of nicotinamide or more (data not shown).

Relationship between the Inhibitions of DNA and RNA Syntheses by the Compound. Next, experiments were performed to see whether the inhibition of DNA synthesis was caused by inhibition of RNA synthesis or *vice versa* or whether both syntheses are inhibited by the compound through a common inhibitory mechanism. NSC 143019 (50 μM) was added to cultures of growing BALB/3T3 cells and, 16 hr later, nicotinamide (150 μM) was added with or without actinomycin D (0.1 μg/ml) or cytosine arabinoside (50 μg/ml). The rates of nucleic acid syntheses were measured 6 hr later. The results shown by *D* and *E* in Chart 4 are expressed as percentages of the control shown by *C* in Chart 4. Some cultures were not pretreated with NSC 143019, 0.1 μg actinomycin D per ml or 50 μg cytosine arabinoside per ml were added to these cultures. After 6 hr, the rates of nucleic acid syntheses were examined and are shown as percentages of the control without drugs by *d* and *e* in Chart 4. Comparisons of *C* with *c*, *D* with *d*, and *E* with *e* in Chart 4 show that continuous RNA synthesis may not be necessary for reversal of inhibition of DNA synthesis by NSC 143019, and *vice versa*. Thus this compound may inhibit the syntheses of DNA and RNA through a common inhibitory mechanism(s).

Analysis of the Primary Site of the Inhibitory Action

Mode of Reversal of the Inhibitory Action of NSC 143019 on DNA Synthesis by Nicotinamide. Next, experiments were carried out to see whether reversal of the inhibitory action of NSC 143019 by nicotinamide is competitive or noncompetitive. Two series of cultures were set up. In one series, growing cells were incubated with various concentrations of NSC 143019 for 16 hr. The extent of the inhibition by the compound is shown in Chart 5 as a *broken line* (data taken from Chart 3). In the other series, cells were incubated with

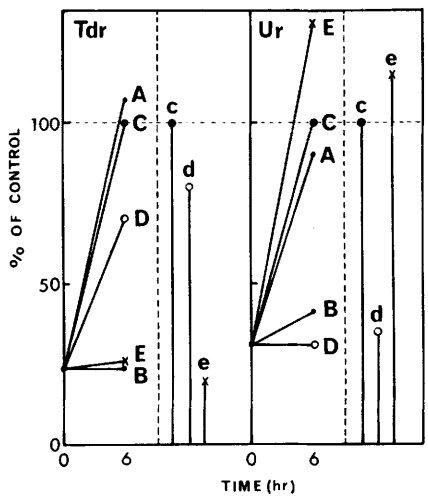


Chart 4. Effects of actinomycin D and cytosine arabinoside on reversal of the inhibitory action of NSC 143019 by nicotinamide. NSC 143019 (50 μM) was added to growing BALB/3T3 cells and, 16 hr later (at Time 0), the medium was changed to medium containing the following compounds. *A*, none; *B*, NSC 143019 (50 μM); *C*, NSC 143019 (50 μM) + nicotinamide (150 μM); *D*, C + actinomycin D (0.1 μg/ml); *E*, C + cytosine arabinoside (50 μg/ml). As control experiments, the medium of untreated, growing BALB/3T3 cells was changed to medium containing the following compounds: *c*, none; *d*, actinomycin D (0.1 μg/ml); *e*, cytosine arabinoside (50 μg/ml). The rates of nucleic acid syntheses were determined 6 hr after the medium change as described in Chart 2. The rates of nucleic acid syntheses in cells cultured in Medium C (for *A*, *B*, *C*, *D*, and *E*) or in Medium c (for *c*, *d*, and *e*) were taken as 100%. *Tdr*, incorporation of [^{3}H]thymidine; *Ur*, incorporation of [^{3}H]uridine.

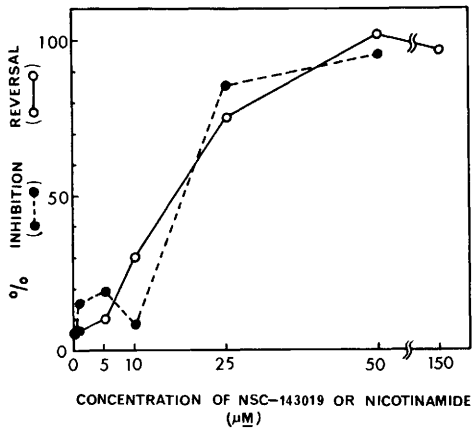


Chart 5. Inhibitory action of NSC 143019 on DNA synthesis and its reversal by nicotinamide. NSC 143019 (50 μM) was added to growing BALB/3T3 cells, and 16 hr later, various concentrations of nicotinamide were added. After incubation for 20 hr more, the rate of DNA synthesis was determined as described in Chart 2 (——). Data on the inhibitory action of NSC 143019 were taken from Chart 3 (– – –).

50 μM NSC 143019 for 16 hr, and then various concentrations of nicotinamide were added and these cultures were incubated for 20 hr. The results with nicotinamide are shown in Chart 5 as a *continuous line*. The 2 curves in Chart 5 coincided well at all molar concentrations, showing that the actions of NSC 143019 and nicotinamide are competitive.

Reversal of the Growth-inhibitory Action of NSC 143019 by Nucleosides. From the above-described results, it seemed possible that NSC 143019 may inhibit some step(s) involved in supply of nucleotide precursors for the syntheses of DNA and RNA. To examine this, 50 μM NSC 143019 was added to growing BALB/3T3 cells and, 16 hr later, various combinations of nucleosides were added. After incubation for 3 days more, the cells of these cultures were counted. As shown in Chart 6, only guanosine (0.1 mM) or deoxyguanosine (0.1 mM) caused nearly complete reversal of the inhibitory action of NSC 143019. At high concentration (1 mM), xanthosine caused partial reversal, while other nucleosides had no effect on the inhibition.

Changes in Metabolic Activity for Biosynthesis of Purine Nucleotides by Addition of NSC 143019. From the above results it was concluded that the primary action NSC 143019 might be to inhibit some step(s) in GMP biosynthesis. It seemed possible that the exact site of inhibition might be the reaction catalyzed by IMP dehydrogenase (see "Discussion"). To confirm this [8-^{14}C]hypoxanthine was added to cultures that had been incubated with or without NSC 143019 (50 μM) for 16 hr. After the cells were labeled for 2 hr, as described in "Materials and Methods," the acid-soluble and acid-insoluble fractions were extracted, and the nucleosides and nucleotides in these fractions were hydrolyzed to their bases. The bases were separated by high-voltage paper electrophoresis as described in "Materials and Methods," and their radioactivities were counted. As shown in Table 1, conversion of [8-^{14}C]hypoxanthine to guanine and its derivatives, but not to adenine and its derivatives, was found to be greatly reduced in cultures treated with the compound.

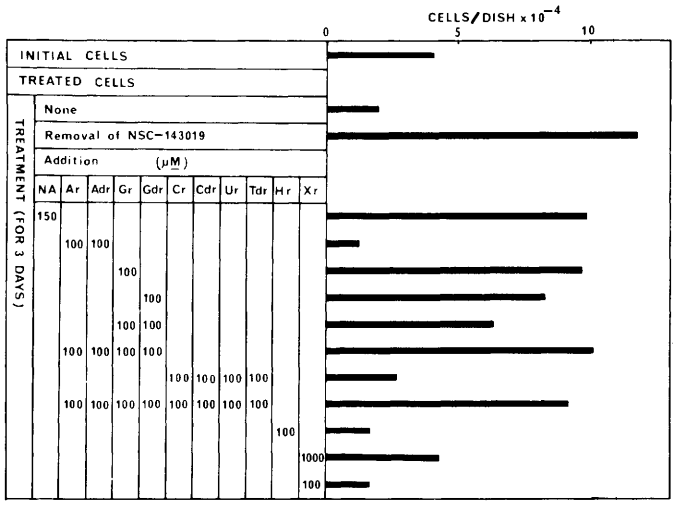


Chart 6. Reversal of the growth-inhibitory action of NSC 143019 by nucleosides. NSC 143019 was added to growing BALB/3T3 cells, and 16 hr later, some samples were taken to count cell numbers (*INITIAL CELLS* in the chart), while other samples were treated as shown in the chart (*TREATED CELLS*). Three days after these treatments, cells were counted. *NA*, nicotinamide; *Ar*, adenosine; *Adr*, deoxyadenosine; *Gr*, guanosine; *Gdr*, deoxyguanosine; *Cr*, cytidine; *Cdr*, deoxycytidine; *Ur*, uridine; *Tdr*, thymidine; *Hr*, hypoxanthine riboside; *Xr*, xanthosine.

Table 1

Effects of NSC 143019 on the incorporation of [8-^{14}C]hypoxanthine into acid-soluble and acid-insoluble fractions of growing BALB/3T3 cells

See "Materials and Methods" for experimental conditions. The total counts on the electrophoretogram are taken as 100%. Ratios of the values in the presence of NSC 143019 to those in its absence were calculated for each base and are expressed in parentheses as percentages.

Fraction	NSC 143019	Ade	Gua	HX[a]	X	Ade + Gua + HX + X
Acid-soluble	None	59.9 (100)	10.2 (100)	9.0 (100)	2.7 (100)	81.8
	50 μM	54.6 (91.2)	3.5 (34.3)	13.4 (148.8)	2.5 (92.2)	74.0
Acid-insoluble	None	34.2 (100)	47.4 (100)	1.3	1.4	84.3
	50 μM	61.5 (180.0)	22.8 (48.1)	1.8	1.2	87.3

[a] HX, hypoxanthine; X, xanthine.

Comparison of the Inhibitory Activities of NSC 143019 and NSC 4728

Concentrations Required for Inhibition. The inhibitory effects of various concentrations of NSC 143019 and its parent compound NSC 4728 (see Chart 1) were compared. As shown in Chart 7, the concentrations of NSC 143019 and NSC 4728 required for 50% inhibition of the syntheses of DNA and RNA were 20 and 300 μM, respectively. Thus, to inhibit nucleic acid syntheses, about 15 times more NSC 4728 than NSC 143019 is required on a molar basis, or about 7 times more on the basis of molar equivalents. Only 30% inhibition of protein synthesis was observed with 100 μM NSC 143019, and hardly any was observed with as much as 500 μM NSC 4728.

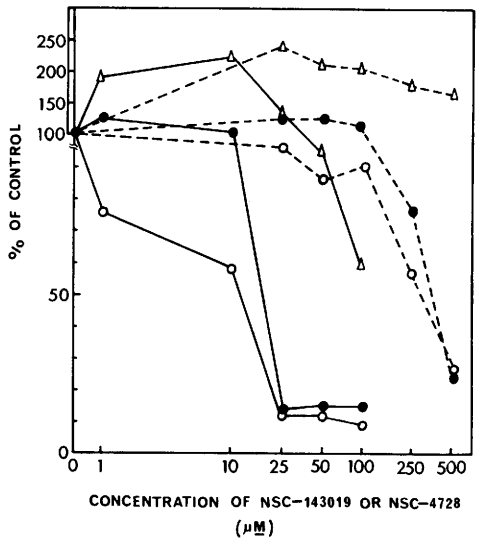


Chart 7. Effects of various concentrations of NSC 143019 and NSC 4728 on nucleic acid and protein syntheses of growing BALB/3T3 cells. At 16 hr after the addition of the compounds (——, NSC 143019; - - -, NSC 4728), the rates of DNA, RNA, and protein syntheses (incorporation of ●, [^{3}H]thymidine; O, [^{3}H]uridine; Δ, [^{14}C]leucine) were determined and are expressed as described in Chart 3.

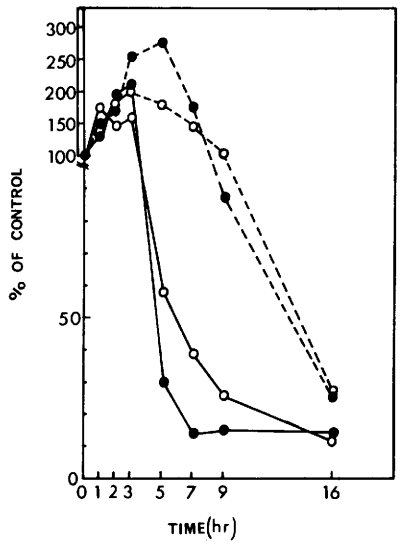


Chart 9. Rates of DNA and RNA syntheses in growing BALB/3T3 cells after addition of NSC 143019 (50 μM) and NSC 4728 (500 μM). At various times after the additions of the compounds (——, NSC 143019; - - -, NSC 4728), the rates of DNA and RNA synthesis (●, [^{3}H]thymidine; O, [^{3}H]uridine) were determined and are expressed as described in Chart 2.

Effect of Formaldehyde. To exclude the possibility that the inhibition was due to CH_2O alone or CH_2O with NSC 4728 (formed from NSC 143019) growing BALB/3T3 cells were treated with NSC 143019 (50 μM), CH_2O (50 μM), or CH_2O (50 μM) plus NSC 4728 (100 μM), and 16 hr later their rate of DNA synthesis was determined. As shown in Chart 8, 90% inhibition of DNA synthesis was observed with 50 μM NSC 143019, whereas CH_2O (50 μM) alone or with NSC 4728 (100 μM) had little inhibitory effect. The strong inhibitory effect of a higher concentration (250 μM) of CH_2O seemed to be a nonspecific, toxic effect.

Times Required for Inhibition by NSC 143019 and NSC 4728. As shown in Chart 9, about 4 and 13 hr were required for 50% inhibition of nucleic acid syntheses by NSC 143019 (50 μM) and NSC 4728 (500 μM), respectively.

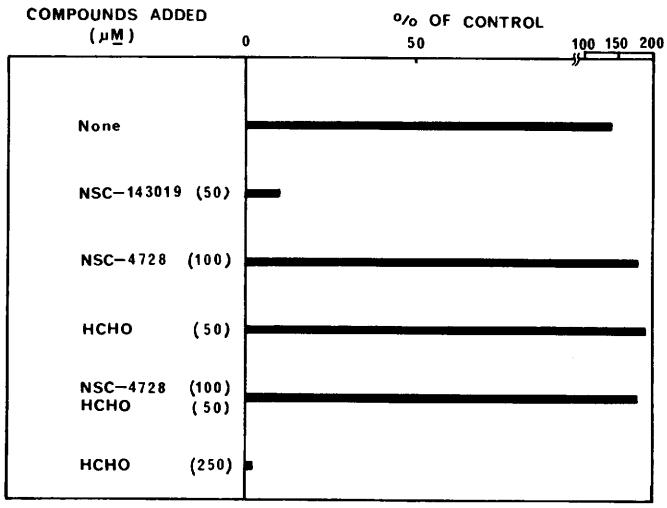


Chart 8. Effect of formaldehyde alone or formaldehyde with NSC 4728 on DNA synthesis of growing BALB/3T3 cells. The compounds indicated were added to growing BALB/3T3 cells, and 16 hr later the rate of DNA synthesis of the cells was determined and is expressed as in Chart 2.

DISCUSSION

Primary Site of Inhibition by NSC 143019. A number of *in vivo* studies have shown that the antitumor and immunosuppressive effects of thiadiazole compounds are antagonized by nicotinamide (1, 3–7). It has also been found that the nicotinamide moiety of NAD can be replaced enzymatically by thiadiazole (1, 3). From these findings, it at first seemed likely that the primary action of these compounds was to inhibit respiration. However, it was unexpectedly found that, at concentrations below 50 μM, NSC 143019 specifically inhibited nucleic acid syntheses. On the other hand, it has been reported from *in vivo* studies that some thiadiazole compounds cause increased uric acid production, which is not related to tissue destruction (4), suggesting the inhibition of biosyntheses of some nucleotide precursors. Further analyses in this work strongly suggest that the primary site of inhibition by the compound might be the reaction catalyzed by IMP dehydrogenase, although it would be much better to delineate a definite conclusion by experiments at the enzyme (IMP dehydrogenase) level.

There are 7 reported enzyme reactions in the pathways of purine and pyrimidine nucleotides biosynthesis that require NAD^+ or $NADP^+$ as a coenzyme; *i.e.*, phosphoribosylglycinamide formyltransferase (EC 2.1.2.2), phosphoribosylaminoimidazole carboxamide formyltransferase (EC 2.1.2.3), IMP dehydrogenase (EC 1.2.1.14), GMP reductase (EC 1.6.6.8), nucleoside diphosphate reductase (EC 1.6.99), dihydroorotate dehydrogenase (EC 1.3.3.1), and thymidylate synthetase (EC 2.1.1). From results on reversal of the inhibition of NSC 143019, it seems probable that this compound inhibits IMP dehydrogenase.

The slight reversal of the inhibition by xanthosine (Chart

6) may be due to the weak ability of the cells to convert this nucleoside into the corresponding nucleotide.

The results shown in Table 1 were obtained after hydrolysis of the purine and its derivatives to their bases. If the reaction from IMP to xanthylic acid is inhibited by NSC 143019, then the following bypath might operate: hypoxanthine → xanthine → xanthylic acid → GMP. It has been reported that catabolic reactions in metabolic pathways of purine nucleotides are repressed in growing cells (10). However, as NSC 143019 inhibits growth, some minor fraction of hypoxanthine may be converted to GMP via this bypath in which the 1st reaction is catabolic. This seems to be the cause of the residual radioactivity observed in the guanine residue in the acid-soluble fraction of cells treated with NSC 143019 (Table 1). The total incorporation of radioactivity into the acid-insoluble fraction was strongly suppressed by NSC 143019. Under these conditions, it seems likely that [8-^{14}C]hypoxanthine is partially converted to guanine nucleotide via the bypath described above and then rapidly integrated into nucleic acid.

Effective Structure (Metabolite) of NSC 143019 for Inhibition. An exchange reaction has been reported between the nicotinamide moiety of NAD^+ and thiadiazole compounds (1, 3). Moreover, the inhibition by NSC 143019 was reversed competitively by nicotinamide (Chart 5). Thus it seemed likely that a metabolic product of NSC 143019 (Chart 10, M*) may act in the cells as an analog of NAD^+. 2-Amino-1,3,4-thiadiazole (NSC 4728) or 2-hydroxymethylamino-1,3,4-thiadiazole are possible active intermediate metabolites. The nicotinamide moiety of NAD^+ will be replaced by M* by either reaction (*b*) or (*c*) in Chart 10, thus forming M* AD^+ (M*-adenine dinucleotide) as an analog of NAD^+.

Experiments showed that the inhibitory action of NSC 143019 was about 15 times that of NSC 4728 on a molar basis and that its effect was about 3 times more rapid than that of NSC 4728 (Charts 7 and 9). These results coincide well with the *in vivo* results reported by Matsumoto *et al.* (5). Our results show that, when added extracellularly, NSC 143019 is more inhibitory than NSC 4728, but they do not exclude the possibility that NSC 4728 is the effective inhibitor intracellularly. The difference in the activities of these compounds seems to be due to differences in their permeabilities, because NSC 143019 is more lipophilic than NSC 4728. Indeed, it seems likely that NSC 143019 enters the cells more effectively than NSC 4728 and is then converted to the latter. 2-Hydroxymethylamino-1,3,4-thiadiazole is chemically labile and is easily converted to NSC 4728 and CH_2O. However, there is no direct evidence that it is not an active inhibitor in the cells, although CH_2O (50 μM) alone or CH_2O (50 μM) with NSC 4728 (100 μM) had no effect when added extracellularly.

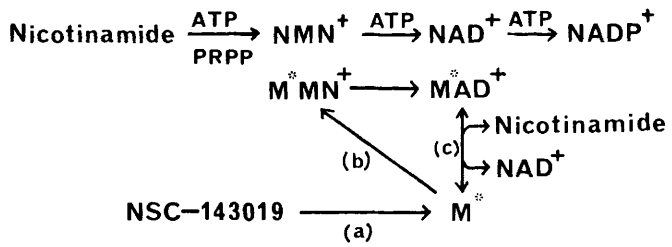


Chart 10. Presumptive pathways for potentiation of the inhibitory action of NSC 143019. *M**, a metabolite of NSC 143019 that can replace the nicotinamide moiety of NAD^+; *NMN*, nicotinamide mononucleotide; *PRPP*, 5-phosphoribosyl 1-pyrophosphate.

Effect of NSC 143019 on the Cell Cycle, Growth of Oncogenic Viruses, and Changes Induced by These Oncogenic Viruses. Growth of oncogenic viruses (bovine adenovirus type 3 and murine sarcoma virus) and cellular changes induced by these viruses (*e.g.*, induction of cellular DNA synthesis, mitosis, and focus formation) (9) were inhibited by NSC 143019 in parallel with inhibition of nucleic acid synthesis in the host cells (data not shown). Moreover, when G_1-arrested cells were transferred synchronously to the S phase by the serum factor (9), no specific point of the cell cycle was found to be sensitive for the inhibitory action of this compound (data not shown).

The antiviral agent, virazole, has recently been found to be converted to the 5'-phosphate in cells and to inhibit IMP dehydrogenase (8). It is interesting that, in this work, NSC 143019 was found to inhibit the same reaction. NSC 143019 seems to act as an analog of NAD^+ while Virazole-5'-phosphate is thought to act as an analog of IMP.

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