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Influence of Thiols on Inhibition of Ribonucleic Acid Synthesis *in Vitro* by a 1-Nitro-9-aminoalkylacridine Derivative, C-283

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SUMMARY

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1-Nitro-9,3-*N,N*-dimethylaminopropylaminoacridine (C-283) is of pharmacological interest as a potent inhibitor of tumor growth and RNA synthesis *in vivo*. Its effect on RNA synthesis directed by *Escherichia coli* RNA polymerase is strongly influenced by sulfhydryl compounds. Its maximal inhibitory effect requires simultaneous mixing of a sulfhydryl compound, such as mercaptoethanol, dithiothreitol, or α-thioglycerol, with DNA and drug at pH 8.0 prior to addition of the enzyme. Very slight inhibition was observed with the nitroacridine in the absence of a thiol. The influence of sulfhydryl compounds has been ascribed to reaction of the template with the 1-nitro group of the dye. Among the DNA-interacting dyes so far investigated, only 1-nitro analogues of C-283 enhance inhibition in the presence of sulfhydryl compounds. There are indications that the metabolite rather than the nitroacridine itself is responsible for the inhibitory effect *in vivo*.

INTRODUCTION

1-Nitro-9,3-*N,N*-dimethylaminopropylaminoacridine (C-283) inhibits tumor growth *in vivo* (1) and RNA synthesis in regenerating rat liver and cell culture (2). The ultrastructural changes induced by the compound in cancer cells (3) and its binding to DNA have been characterized (4). Clinical application of the dye is under investigation (5). The inhibitory effect of C-283[1] (Fig. 1) on RNA synthesis *in vitro* has been shown to require 2-mercaptoethanol (6). Mercaptoethanol reacts with the dye in the absence of DNA, giving a product of low inhibitory potency. On the other hand, our initial findings indicated that addition of DNA, mercaptoethanol, and the nitroacridine results in greater inhibition than produced in reaction mixtures without thiols (6).

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[1] The abbreviations used are: C-283, 1-nitro-9,3-*N,N*-dimethylaminopropylaminoacridine; C-205, 1-nitro-9,3-*N,N*-dimethylaminobutylaminoacridine; C-663, 1-amino-9,3-*N,N*-dimethylaminopropylaminoacridine; C-137, 9,3-*N,N*-dimethylaminopropylaminoacridine; C-492, 1-nitro-10-*N,N*-dimethylaminopropylo-9-acridone.

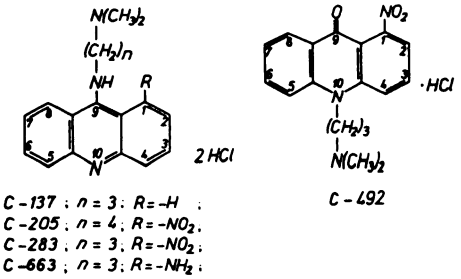

FIG. 1. *Formulae of acridines studied*

As mercaptoethanol or other sulfhydryl compounds are standard components of the DNA-dependent RNA polymerase (ribonucleoside triphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) system *in vitro*, further studies of this phenomenon seemed of interest. To elucidate the mechanism of the mercaptoethanol effect, the influence of two other sulfhydryl compounds, α-thioglycerol and dithiothreitol, on the drug, its analogues, and their inhibition of the RNA polymerase reaction has been investigated.

The aim of our experiments was to answer the following questions. (a) Does the influence of mercaptoethanol on the inhibitory effect of C-283 depend on the chemical structure of the dye or does it reflect a more general phenomenon of sensitization of the template by sulfhydryl compounds (6)? (b) What is the influence of other thiols on RNA polymerase inhibition? These questions were also of interest in view of the report from another laboratory that the effect of the nitroacridine in the cell can be exerted by its metabolite rather than by the drug itself (7).

MATERIALS AND METHODS

C-283, its analogues C-137, C-205, and C-663 (1, 8), and a 1-nitroacridone derivative, C-492 (9) were kindly provided by Dr. J. Konopa, Institute of Chemistry and Organic Technology, High Technical School, Gdańsk, Poland. Actinomycin D was obtained from Calbiochem. Ethidium bromide was a gift of Professor P. Chambon, Strasbourg.

[^{14}C]ATP and [^{14}C]GTP (Radiochemical Centre, England) nonradioactive nucleoside triphosphates (P-L Biochemicals, United States, and Waldhof, Germany), calf thymus DNA (Worthington), 2-mercaptoethanol (Loba-Chemie, Austria), α-thioglycerol (Calbiochem), dithiothreitol (Koch-Light, England), Omnifluor (NEN Chemie, Germany), DEAE-cellulose (Cellex-D, 0.7 mEq/g, Calbiochem) and Sephadex G-200 (Pharmacia, Sweden) were used. Other analytical grade chemicals were purchased by Cefarm, Poland.

DNA-dependent RNA polymerase was extracted from *Escherichia coli* strain MRE 600 and treated with streptomycin sulfate (10). The salting-out extraction step and DEAE-cellulose chromatography were performed according to Burgess (11). Further purification was performed by Sephadex G-200 filtration with buffer containing 0.5 M KCl (6). Enzyme preparations with a specific activity of 150–300 units/mg of protein (11) were obtained and stored in dry ice. During 6 months of storage losses of activity of about 50% were observed.

E. coli RNA polymerase ("holoenzyme") and calf thymus DNA were used for the assay according to Burgess (11) with the following modifications: the KCl concentration was lowered to 0.05 M and, if not specified otherwise, 12 mM mercaptoethanol or 2 mM dithiothreitol was used. DNA concentrations are indicated in the legends to figures and tables. ^{14}C-Labeled substrates with specific activities of 500–1000 cpm/nmole were used in most of our experiments. After incubation for 10 min at 37° bovine serum albumin and trichloracetic acid–pyrophosphate were added to the samples, and the precipitates were filtered, washed, and counted as described previously (6). Radioactivity was estimated with a Wallac liquid scintillation counter, model 81000.

DNA was determined spectrophotometrically, assuming $A^{1\,cm}_{259} = 20.0$ for 1 mg/ml. To purify DNA complexed with dyes, the dye-DNA mixtures were extracted with isobutyl alcohol and dialyzed according to Mandel and Chambon (12). The DNA concentration following extraction of dyes was checked by total phosphorus determinations (13).

The infrared spectra of C-283 and its

insoluble product isolated following the reaction with mercaptoethanol were determined with a Unicam SP 1200 instrument. The samples were measured in KBr pressed discs as described by Sixma and Wynberg (14).

RESULTS

The effect of C-283 on the transcription process *in vitro* is influenced markedly by mercaptoethanol (Table 1). When C-283 is incubated with mercaptoethanol prior to addition of DNA and nucleoside triphosphates, the inhibitory effect is abolished and some stimulation of RNA synthesis is observed. On the other hand, DNA has a protective effect against the action of mercaptoethanol. Significant inhibition is observed with all the components when incubated for 1 hr prior to addition of enzyme (Table 1). Moreover, prior incubation of C-283 with mercaptoethanol, DNA, and nucleoside triphosphates increases the inhibition. Addition of mercaptoethanol with the enzyme produces a smaller inhibitory effect, and only slight inhibition, or even stimulation, is noted in the absence of the thiol. A certain thiol influence also occurs with the 1-nitroacridone. C-492. No such dependency is seen with other acridines lacking a nitrous group (C-137, C-663) or with ethidium bromide. In fact, inhibition of RNA synthesis by these compounds does not depend on the preliminary incubation conditions (Table 1).

Similar phenomena have been noted for the nitroacridine with other sulfhydryl compounds (Table 2). The inhibition of DNA-dependent RNA synthesis following incubation of C-283 with DNA, nucleoside triphosphates, and dithiothreitol is higher than in the presence of mercaptoethanol or thioglycerol at corresponding drug concentrations. Similar enhancement of the inhibitory effects in the presence of dithiothreitol occurs with C-492 and C-205, an analogue of C-283 that has a 9-aminobutyl instead of a 9-aminopropyl group (Table 2).

As previously noticed (6) and as indicated by the results presented here (Tables 1 and 2), the inhibitory effect of the nitroacridine depends on time of simultaneous interaction of the dye with DNA and a thiol. Significant inhibition has been noted following prior incubation of the nitroacridine with thiols and DNA for 1 hr, and a slight further increase in inhibition occurs if the preliminary incubation is extended to 3 hr (Fig. 2). Inhibition of RNA synthesis by an analogue lacking a nitro group (C-137) is practically independent of incu-

TABLE 1

Influence of mercaptoethanol on inhibitory effects of C-283, its analogues, and ethidium bromide

The inhibitors, DNA (40 μg/assay), mercaptoethanol and nucleoside triphosphates (NTP) were added to the other incubation components (Tris-HCl buffer, pH 8.0, KCl, $MgCl_2$, albumin, and EDTA) as indicated and incubated in the dark at 20° for 1 hr before addition of the enzyme, or were added just before the enzyme (zero time). *E. coli* RNA polymerase in 0.1 ml was added to the incubation mixtures, and the samples (final volume 0.25 ml) were incubated for 10 min at 37°. Between 1 and 3 units (4–15 μg) of RNA polymerase were used, with identical amounts added in the same experiment. Further details are indicated under MATERIALS AND METHODS. The results are expressed as a percentage of control experiments (without an inhibitor). Where averages of two independent experiments are given, ± range values are shown.

Components added and incubated for 1 hr	Components added at zero time	C-137, 0.025 mM	C-283, 0.1 mM	C-492, 0.2 mM	C-663, 0.075 mM	Ethidium bromide, 0.04 mM
		%	%	%	%	%
DNA, NTP, inhibitor	Mercaptoethanol, enzyme	68 ± 4	76 ± 2	59	51 ± 2	39
DNA, NTP, inhibitor	Enzyme (no mercaptoethanol	71 ± 2	91 ± 1	66	48 ± 1	43
Mercaptoethanol, DNA, NTP	Inhibitor, enzyme	73 ± 3	84 ± 2	86	49 ± 1	
Mercaptoethanol, inhibitor	DNA, NTP, enzyme	73 ± 3	115 ± 3	83	51 ± 2	40
Mercaptoethanol, DNA, NTP, inhibitor	Enzyme	74 ± 1	64 ± 5	58	50 ± 4	41

TABLE 2

Influence of α-thioglycerol and dithiothreitol on inhibitory effects of C-283, C-205, C-492 and C-137

Each assay mixture contained 14 μg of polymerase preparation, 40 μg of DNA, and 12 mM thioglycerol or 2 mM dithiothreitol. Other details are given under MATERIALS AND METHODS and in Table 1.

Components mixed 1 hr before assay	Components added at zero time	Expt. I: α-Thioglycerol			Expt. II: Dithiothreitol			Expt. III: Dithiothreitol		
		Control	C-137, 0.05 mM	C-283, 0.1 mM	Control	C-137, 0.05 mM	C-283, 0.1 mM	Control	C-205, 0.1 mM	C-492, 0.1 mM
		nmoles [^{14}C]ATP	*% control*		*nmoles [^{14}C]ATP*	*% control*		*nmoles [^{14}C]ATP*	*% control*	
DNA, NTP, inhibitor	Thiol, enzyme	1.73	36	71	1.80	43	42			
DNA, NTP, inhibitor	Enzyme (no thiol)	1.73	38	98	1.71	38	101	1.57	98	74
Thiol, NTP, inhibitor	DNA, enzyme	1.73	44	126	1.84	43	126			
Thiol, NTP, DNA, inhibitor	Enzyme	1.88	38	39	1.92	44	15	1.92	23	43

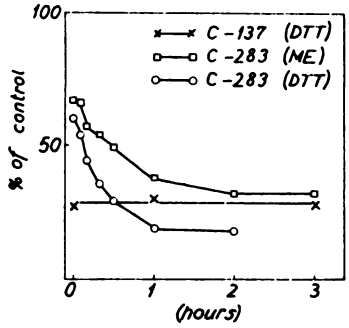

FIG. 2. *Influence of prior incubation time on inhibitory effect of acridines*

The acridines (C-283, 0.2 mM, or C-137, 0.04 mM) were added to incubation mixtures containing DNA (40 μg/assay), nucleoside triphosphates, mercaptoethanol (ME) or dithiothreitol (DTT), and other components except the enzyme. The samples were allowed to stand for the times indicated. At zero time the enzyme was added, and RNA synthesis was assayed as described in MATERIALS AND METHODS and Table 1.

bation time. It has been found in a separate series of experiments that the inhibitory effect of C-283 does not depend on the time of exposure of the template to thiols. The inhibitory effect does not appreciably change if DNA is mixed with mercaptoethanol or dithiothreitol, kept for several hours and C-283 added 1 hour before the enzyme.[2]

[2] Unpublished observations.

The reaction of C-283 with thiols described above prompted us to examine the possibility of the drug directly reacting with sulfhydryl groups of the enzyme. No increase in inhibition was found when the enzyme was incubated for 30 min at 37° with the drug in the absence of the template, substrates, and sulfhydryl compounds (results not shown).

The simultaneous interaction of the nitroacridine, DNA, and thiols has been shown to be essential for inhibition. This observation suggested covalent binding between the nitroacridine and DNA in the presence of thiols. To explore this possibility, DNA was exposed to the drug and dithiothreitol for 2 hr. Then the unfixed dye was washed out with isobutyl alcohol and the DNA was dialyzed (12) and reexamined for template activity. In control experiments either untreated DNA or DNA following incubation with ethidium bromide, C-137, and actinomycin was treated as above. Nitroacridine-treated DNA shows much lower template activity than the DNA repurified from complexes formed with the other dyes (Table 3). These experiments strongly suggest that covalent binding of the drug has to be taken into consideration.

It was observed in further experiments that C-283 can be removed efficiently by

isobutyl alcohol extraction and dialysis if the nitroacridine-DNA complex is formed in the absence of a thiol (Table 4). Prior treatment of DNA with only mercaptoethanol or with C-283 without thiol does not result in inhibition (Table 4).

As an unusual dependence on sulfhydryl compounds was observed, it was of interest to examine the changes in inhibition at different template concentrations. A double-reciprocal plot of the velocity of RNA synthesis vs. DNA concentration (15) is shown in Fig. 3. An increase in DNA concentration resulted in a decrease in the inhibitory effect of the dye. Over a large range of DNA concentrations the nitroacridine shows competitive inhibition, as found for proflavine by Hurwitz *et al.* (16), for other acridines by Nicholson and Peacocke (17), and for ethidium bromide by Waring (18). In some experiments no changes of the inhibition at high DNA concentrations were observed. This observation corresponded with inhibition of RNA synthesis by an excess of the template in control assays (results not shown). Substituting native calf thymus DNA for denatured DNA did not markedly change the inhibition, and essentially the same results were found for phage T4 DNA.[2]

The effects of different concentrations of C-283, its analogues, and ethidium bromide are presented in Fig. 4. Inhibition of RNA synthesis by the nitroacridine in the presence of dithiothreitol is at least as great as that observed using ethidium bromide or the other acridines studied. As already pointed out, very little inhibition by C-283 was found when the sulfhydryl compounds were omitted from incubation mixtures (Fig. 4). The effect of C-137 and C-663 on RNA synthesis *in vivo* was not

TABLE 3

Effect on template activity of the treatment of DNA with C-283 and other DNA-interacting drugs in the presence of dithiothreitol and mercaptoethanol

Samples of DNA (600 μg/ml) with either 0.3 mM C-137, 0.25 mM C-283, 0.25 mM ethidium bromide, or 0.08 mM actinomycin D in 40 mM Tris-HCl buffer (pH 8.0) containing 0.15 M KCl, 0.2 mM EDTA, and either 10 mM dithiothreitol or 20 mM mercaptoethanol were incubated for 2 hr in the dark at room temperature. Then the samples were extracted with isobutyl alcohol and dialyzed as indicated under MATERIALS AND METHODS. The DNA concentration was checked by total phosphorus determinations in the dialysates. The template activities of these DNA samples and DNA with inhibitors added 1 hr before the addition of the enzyme were assayed at 2 mM dithiothreitol or 12 mM mercaptoethanol as described in the legend to Fig. 3. The dye concentrations were chosen at DNA to inhibitor ratios similar to those for complex formation before extraction. DNA concentrations were 30 μg (experiment I) and 20 μg (experiment II), and enzyme concentration was 11 μg and 6.4 μg per assay respectively (vol. 0.25 ml). In experiment II thiol-treated DNA was used in controls and in assays with inhibitors added 1 hr before the enzyme.

DNA first treated with	Activity		Inhibitor added 1 hr before enzyme	Activity	
	nmoles [^{14}C]ATP	*% control*		*nmoles [^{14}C]ATP*	*% control*
Experiment I					
Dithiothreitol (control)	1.35		None (control)	1.45	
+C-137		96	C-137, 0.056 mM		61
+C-283		10	C-283, 0.05 mM		19
+Ethidium bromide		99	Ethidium bromide, 0.05 mM		15
Experiment II					
Dithiothreitol (control)	0.81		None (control)	0.81	
+Actinomycin		78	Actinomycin, 0.011 mM		5
+C-283		7	C-283, 0.035 mM		19
+Ethidium bromide		86	Ethidium bromide, 0.035 mM		7
Mercaptoethanol (control)	0.60		None (control)	0.60	
+Actinomycin		97	Actinomycin, 0.011 mM		3
+C-283		35	C-283, 0.035 mM		43
+Ethidium bromide		104	Ethidium bromide, 0.035 mM		8

TABLE 4

Effect of prior treatment of DNA on template activity

Samples of DNA were treated as specified below and extracted with isobutyl alcohol, and template activity was assayed. DNA and enzyme concentrations were, respectively, 30 and 11 μg in experiment I, 20 and 8.1 μg in experiment II, and 20 and 6.3 μg in experiment III. Thiols and C-283, when present, were added together with DNA 1 hr before enzyme. For further details, see the legends to Fig. 3 and Table 3.

DNA treated with	Components added 1 hr before enzyme			[^{14}C]ATP incorporated
	C-283	Mercapto-ethanol	Dithio-threitol	
	mM	*mM*	*mM*	*nmoles*
Experiment I				
Mercaptoethanol	0	0	0	0.74
	0.05	0	0	0.75
Experiment II				
C-283 (no thiol)	0	0	0	1.13
	0	0	2	1.33
Experiment III				
No treatment	0	12	0	0.96
C-283 (no thiol)	0	12	0	0.88
Mercaptoethanol	0	12	0	0.96
Mercaptoethanol + C-283	0	12	0	0.28
Mercaptoethanol + ethidium bromide	0	12	0	0.90

studied, but their effect on tumor growth is insignificant (8).

The presence of thiols in our incubation mixtures is essential for the inhibitory effect of C-283. A reaction of the nitro group at position 1 of the acridine ring seems to be responsible for the enhancement of inhibition. It has been found that C-283 in Tris-HCl, pH 8.0, reacts with mercaptoethanol or thioglycerol to give products of different optical properties. The reaction seems to be rather complex. A new absorption maximum at 580 nm, in addition to the characteristic one for C-283 at 400 nm, was observed (Fig. 5). A similar phenomenon has been observed with other 1-nitroacridines (C-205 and C-492) while no reaction has been shown with C-137, C-663, and the other derivatives lacking the nitro group so far studied.[2] Prolongation of nitroacridine exposure to mercaptoethanol at pH 8.0 results in precipitation of a water-insoluble product(s). This product(s) was isolated and examined by infrared spectrophotometry. Characteristic bands for the nitro group of C-283 at 1580 cm^{-1} and 1300–1400 cm^{-1} (Fig. 6A) are hardly detectable in the product (Fig. 6B). Other differences between the curves can be seen. A new large band for hydroxyl group at about 1040 cm^{-1} appears (Fig. 6B).

DISCUSSION

1-Nitro-9,3-*N*,*N*-dimethylaminopropylaminoacridine has been shown to inhibit DNA-dependent RNA polymerase. Effects of acridine derivatives as well as other DNA-interacting compounds have been investigated (16–20). An unusual feature of the inhibitory effect of C-283 is its dependency on sulfhydryl compounds. Two findings characterize this dependence: abolition of the inhibition if the nitroacridine is previously incubated without DNA, and amplification of the drug action when a reaction of the dye and thiol occurs in the presence of DNA. The reaction can be detected by changes of the absorption characteristics of C-283 under conditions of pH and ionic strength similar to those for RNA synthesis *in vitro*. Although products of the reactions with various sulfhydryl compounds are characterized by different absorption curves, qualitatively the same results have been obtained in our study of their effects on RNA synthesis.

One aim of this study has been to distin-

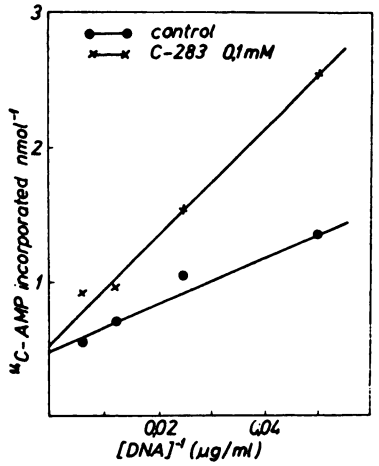

FIG. 3. *Inhibition of RNA synthesis by C-283 at various DNA concentrations*

The samples were prepared by simultaneous mixing of DNA, nucleoside triphosphates, mercaptoethanol, C-283, and other components except the enzyme (volume, 0.15 ml) and incubated for 1 hr at 20°. The control assays were prepared similarly, but without acridine. The reaction was initiated by addition of the enzyme (30 µg/assay) and terminated after 10 min. C-283 and DNA concentrations were varied as shown. Other details are described under MATERIALS AND METHODS.

guish between "sensitization" of the template to the drug action by sulfhydryl compounds and chemical modification of the inhibitor which results in the transient appearance of a more active product binding to DNA.

The first hypothesis was based on similar observations concerning the DNA-bleomycin interaction (21, 22). The data presented here argue against the "sensitization" hypothesis. The inhibition of RNA synthesis by the other acridines (C-137, C-663) and ethidium bromide (Tables 1 and 2; Fig. 2) is thiol-independent. The sulfhydryl-dependent inhibition increases with time, approaching the maximum 1 hr after incubation of the drug and DNA with mercaptoethanol (Fig. 2; see also ref. 6). As mentioned above, the inhibitory effect did not increase if C-283 was added to the incubation mixture following prolongated exposure mercaptoethanol or dithiothreitol. Moreover, when DNA was treated with mercaptoethanol without C-283, purified under the conditions specified in the

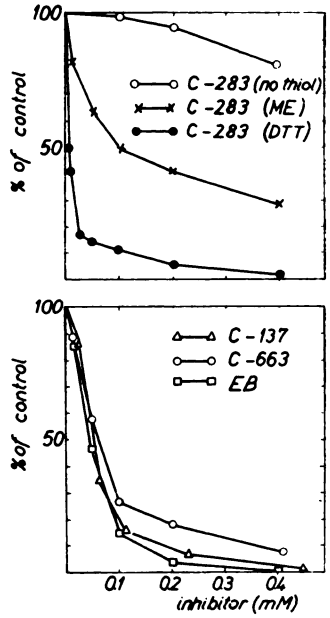

FIG. 4. *Effect of acridines and ethidium bromide on RNA synthesis*

The controls and samples containing the inhibitor were prepared as described in the legend to Fig. 3; 5–30 µg of enzyme preparations and 40 µg of DNA per assay were used. RNA synthesis was measured as [^{14}C]AMP incorporation. The upper panel shows inhibition by C-283 in the presence of mercaptoethanol (ME) or dithiothreitol (DTT) or without added thiol. The effects of C-137, C-663, and ethidium bromide (EB) (lower panel) were measured at 12 mM mercaptoethanol.

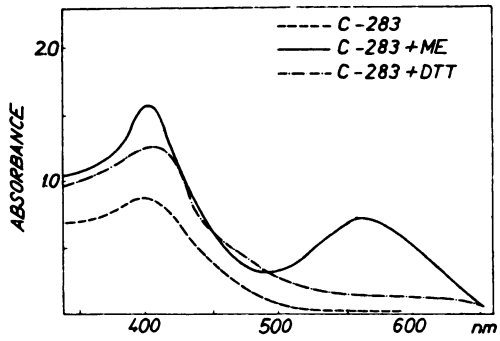

FIG. 5. *Absorption spectra of C-283 following exposure to thiols*

C-283 (0.1 mM) in Tris-HCl (40 mM), pH 8.0, containing either mercaptoethanol (ME) (10 mM), dithiothreitol (DTT) (1 mM), or no thiol was incubated for 1 hr at 20° in the dark. Absorbance was measured using 1-cm glass cuvettes and a Unicam SP 500 spectrophotometer.

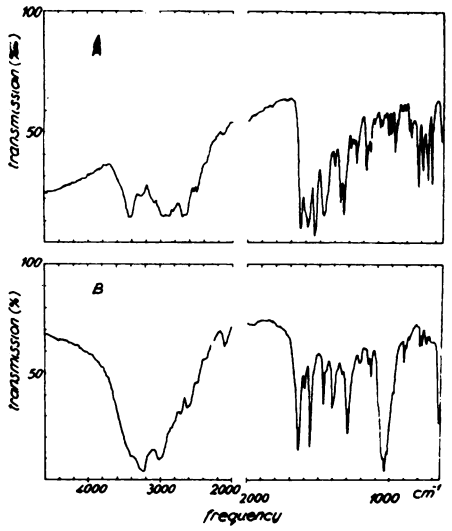

FIG. 6. *Infrared spectra of C-283 and its product(s) isolated following exposure to mercaptoethanol*

C-283 (0.4 mM) in 20 mM Tris-HCl buffer, pH 8.0, containing 40 mM mercaptoethanol (total volume, 10 ml) was incubated for 24 hr at room temperature in the dark. The insoluble precipitate was centrifuged, washed with water, and dried under reduced pressure. Infrared spectra of C-283 (A) and the product (B) formed were measured as described under MATERIALS AND METHODS.

legend to Table 3, and re-examined for template activity in the presence of C-283 but without thiols, no inhibition was found (Table 4). Therefore the sulfhydryl dependency is not caused by a thiol interaction with the template itself.

The second hypothesis better explains the present data. In the course of the nitroacridine-thiol reaction a by-product responsible for the inhibitory effect may be transiently formed. Binding of this hypothetical by-product to the template would be strong and very efficient for the inhibition of RNA synthesis. This binding would protect the nitroacridine derivative against further modification, which occurs in the absence of DNA and gives an inactive compound.

The binding seems to be covalent. The drug cannot be removed from the template by isobutyl alcohol extraction and dialysis, a procedure which efficiently restores full or nearly full template activity to DNA previously complexed with actinomycin, ethidium bromide, or C-137 (Table 3).

The nature of binding of the dye to DNA remains unknown. Similar thiol-dependent inhibition was found for two other 1-nitro derivatives (C-205 and C-492, Tables 1 and 2) but not for analogues of C-283 lacking the nitro group (C-137, C-663) or ethidium bromide. Therefore a reduction of the nitro group of C-283 appears to be involved. That reduction of the 1-nitro group is necessary for tight binding of C-283 to the template and inhibition under our conditions is indicated by the reactivity of 1-nitroacridines with mercaptoethanol. As shown in Fig. 6B, the final product(s) of the reaction does not give transitions characteristic of the nitro group. It does not mean that the same product is also responsible for the inhibitory effect. Incubation of the drug with thiols in the absence of DNA results in an inactive product (Tables 1 and 2). Moreover, we could not find any essential changes either the ultraviolet or visible spectra of DNA treated with C-283 in the presence of dithiothreitol or mercaptoethanol and repurified as described above (results not shown). These observations suggest that the amount of drug bound is rather low. Therefore the observations concerning the nitroacridine changes influenced by thiols in the absence of template reflect their reactivity rather than indicating the nature of the product responsible for the inhibition. The reactivity has, however, been correlated with the enhancement of inhibition by sulfhydryl compounds.

A possibility of covalent binding of C-283 to DNA is supported by the results of our study on its effect on the initiation and elongation steps of RNA synthesis *in vitro*. Unlike proflavine (23) or ethidium bromide (20), the nitroacridine affects both chain length and the number of polynucleotides initiated.[3]

The present experiments do not explain why a low inhibitory effect of C-283 was observed in the absence of sulfhydryl compounds (Tables 1 and 2; Fig. 4). Further

[3] Manuscript in preparation.

experiments to account for this point have been undertaken.

It is of interest to consider whether the phenomenon observed by us mimics a situation which can occur *in vivo*. Konopa and Kołdej (7) have suggested that a metabolite of C-283, rather than the drug itself, and possibly its covalent binding to DNA are responsible for the effect *in vivo*. This could explain an obvious difference between the large effect of C-283 on RNA synthesis *in vivo* or in cell cultures (2) and the lack of inhibition under our experimental conditions when the unmodified dye was used (i.e., sulfhydryl compounds were omitted from the RNA polymerase assays).

Further research needs to be undertaken to elucidate the nature of the chemical reaction of C-283 with thiols under our experimental conditions.

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