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COMPARISON OF INHIBITION OF REVERSE TRANSCRIPTASE AND ANTILEUKEMIC ACTIVITIES EXHIBITED BY PROTOBERBERINE AND BENZOPHENANTHRIDINE ALKALOIDS AND STRUCTURE–ACTIVITY RELATIONSHIPS*

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Key Word Index—Enzyme inhibition; reverse transcriptase; antileukemic activity; protoberberine alkaloids; benzophenanthridine alkaloids; structure–activity relationships.

Abstract—Protoberberine and benzophenanthridine alkaloids were screened for their inhibition of reverse transcriptase activity of RNA tumor viruses in the presence of poly rA. oligo dT, poly dA. oligo dT, poly rC. oligo dG, activated calf thymus DNA and 70S RNA template primers. The comparison of inhibition of reverse transcriptase and antileukemic activities revealed that most of the protoberberine and benzophenanthridine alkaloids tested compared well with their reported antileukemic activities. In general, it was observed that the higher T/C % value resulted in a stronger inhibition of reverse transcriptase activity. The inhibition of nucleic acid polymerases from calf thymus and bacteria and structure–activity relationships are also presented. The alkaloids with the benzophenanthridine ring system were found to display potent inhibition of reverse transcriptase and antileukemic activities.

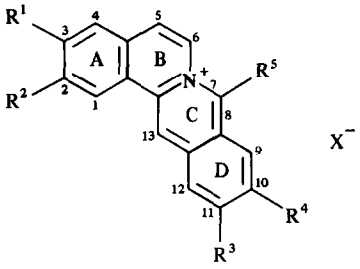
INTRODUCTION

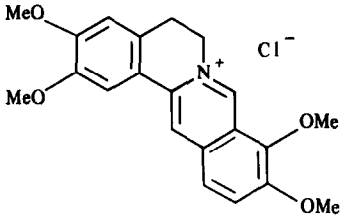
Among the isoquinoline alkaloids [1, 2], protoberberine and benzophenanthridine alkaloids possess a wide variety of pharmacological and biological activities. These activities include antimicrobial [3], antianthelmintic [4], antiarrhythmic [5], antihypertensive [6], abortifacient [7], uterine stimulant [7], antitumor [8] and antileukemic and antineoplastic properties [9, 10]. However, during the last decade, dehydroprotoberberine (coralyne and related alkaloids) and benzophenanthridine alkaloids (fagaronine, nitidine and related alkaloids) were reported to be effective against both L1210 lymphoid and P388 lymphocytic leukemias [11–14]. Additionally, these alkaloids inhibited enzymes such as horse liver alcohol dehydrogenase [15, 16], xanthine oxidase [17] and *O*-methyltransferase [18, 19] and transfer RNA methyltransferase [20]. In view of these enzyme inhibitions and antileukemic activities, some protoberberine and benzophenanthridine alkaloids were tested for inhibition of reverse transcriptase activity of RNA tumor viruses. It was observed that fagaronine, nitidine, palmatine and other related alkaloids were potent inhibitors of reverse transcriptase activity [21, 22]. Thus, the preliminary screening data [23, 24] prompted the examination of additional alkaloids, **1a–1f**, **2**, **5–7**, **9** and **10**, for inhibition of reverse transcriptase activity. The inhibitory effect of the alkaloids was then compared to its antileukemic activity. The results revealed that the potent inhibitors of reverse transcriptase activity compared well with the reported antileukemic activity of the alkaloids. Most moderate enzyme inhibitors also showed a good relationship with the antileukemic activity of alkaloids.

Reverse transcriptase (RNA-directed DNA polymerase) was first discovered by Baltimore, Temin and Mizutani in RNA tumor viruses (retroviruses) in 1970 [25, 26]. Since then, extensive work concerning the characterization, role and association of retroviruses with leukemia, sarcomas and lymphomas of several animals [27–29] including subhumans, gibbon apes [30] has been reported. In the past decade, human leukemia and lymphoma cells were reported to contain DNA polymerase similar to retroviruses [31]. However, the recent isolation of reverse transcriptase and retroviruses from human T-cell leukemias and lymphomas [32–36] suggests strong evidence of the implication of retroviruses in human cancer. Since reverse transcriptase is the key enzyme which leads to the transformation of susceptible cells [37, 38], the inhibitors of the enzyme and retroviruses play an important role in the prevention and chemotherapy of viral infection. The purpose of the present investigation was to search for additional inhibitors and to examine whether a correlation exists between antitumor (antileukemic) and inhibition of reverse transcriptase activities exhibited by coralyne isomers, their analogs and related alkaloids. Screening, comparison of inhibitory effect and structure–activity relationships based on an *in vitro* inhibition of reverse transcriptase

*Part VII in the series "Enzyme Inhibition". For Part VI see Sethi, M. L. (1983) *J. Pharm. Sci.* **72**, 538.

Abbreviations: Poly rA. oligo dT, polyriboadenylic acid. oligodeoxythymidylic acid; poly dA. oligo dT, polydeoxyadenylic acid. oligodeoxythymidylic acid; poly rC. oligo dG, polyribocytidylic acid. oligo deoxyguanylic acid; AMV, avian myeloblastosis virus; MuLV, murine leukemia virus; BSA, bovine serum albumin; EDTA, ethylenediaminetetraacetic acid tetrasodium salt.





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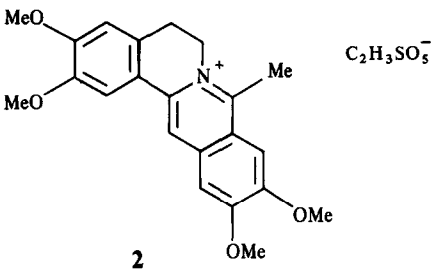
1a R^1, R^2, R^3, R^4 = OMe, R^5 = Me, $X = C_2H_3SO_5$
1b R^1, R^2, R^3, R^4 = OMe, $R^5 = C_2H_5$, $X = C_3H_5SO_5$
1c R^1, R^2, R^3, R^4 = OMe; $R^5 = C_3H_7$; $X = C_4H_7SO_5$
1d $R^1 + R^2 = OCH_2O$, R^3, R^4 = OMe; R^5 = Me, $X = C_2H_3SO_5$
1e R^1, R^2 = OMe; $R^3 + R^4 = OCH_2O$, R^5 = Me, $X = C_2H_3SO_5$
1f R^1, R^2, R^3 = OMe, R^4 = H, R^5 = Me, $X = C_2H_3SO_5$



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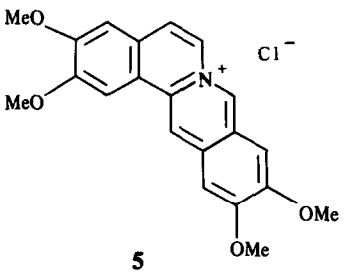

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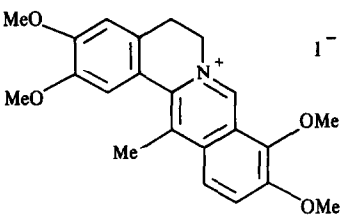

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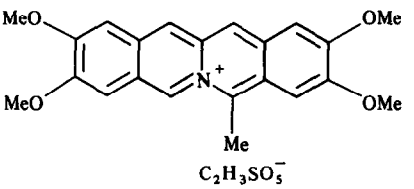

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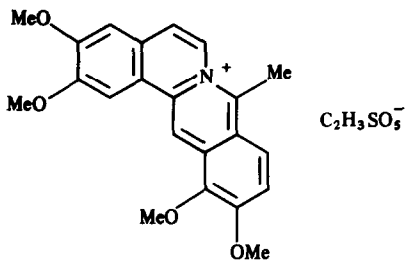
activity of RNA tumor viruses are discussed. In addition, inhibitions of calf thymus DNA and bacterial RNA polymerases by these alkaloids are presented.

RESULTS AND DISCUSSION

Screening of alkaloids for inhibitory effect

In the previous study of the inhibitors of reverse transcriptase activity by benzophenanthridine alkaloids, screening of these alkaloids led to the classification of strong, moderate and weak inhibitors of reverse transcriptase activity of RNA tumor viruses [23]. The results revealed that the potent antitumor or antileukemic benzophenanthridine alkaloids were strong inhibitors of reverse transcriptase activity. However, this kind of generalization could not be made about the protoberberine alkaloids [24]. In a continued effort to develop an *in vitro* test system for antitumor or antileukemic compounds, the present investigation was undertaken to examine some more protoberberine alkaloids for inhibition of reverse transcriptase activity and to compare and correlate antileukemic activity with the enzyme inhiition displayed by coralyne isomers and analogs.

To establish a relative sensitivity of nucleic acid polymerase inhibition by protoberberine and benzophenanthridine alkaloids, a comparison of inhibition of reverse transcriptase, calf thymus and bacterial enzymes was made as shown in Table 1. The results indicated that when the same concentration of alkaloids (80 μg/ml) in the standard assay methods was used, the per cent inhibition of the reverse transcriptase by the alkaloids was much higher than that of the corresponding calf thymus DNA and bacterial RNA polymerases. The latter enzymes were



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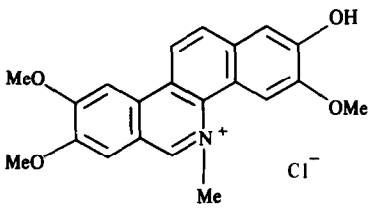

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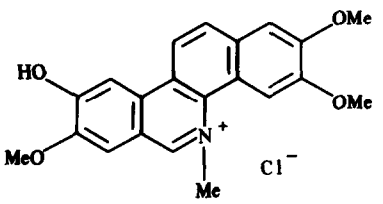

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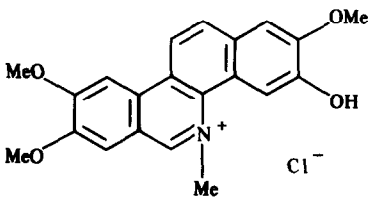

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Table 1. Inhibition of reverse transcriptase, calf thymus and bacterial nucleic acid polymerases by alkaloids*

Compound	% Inhibition of nucleic acid polymerases		
	Reverse transcriptase	Calf thymus DNA	Bacterial RNA
1a	100	25	15
1b	95	15	8
1c	40	10	5
1d	75	8	5
1e	78	8	5
1f	70	10	8
2	60	16	10
3	65	5	4
4	100	10	5
5	85	15	10
6	65	10	8
7	45	8	5
8	100	20	10
9	100	15	10
10	100	10	8

*See standard assay conditions in Experimental. The per cent inhibition was calculated based on average readings from three assays, deducting the background counts (400–500 cpm). The average per cent variation in the individual reading was approximately ±7%. Compounds (80 μg/ml) were dissolved in DMSO. The control assay mixtures contained an equivalent volume of DMSO without the compound. The per cent inhibition in the case of reverse transcriptase was an average value of AMV and MuLV enzyme inhibitions.

inhibited to the maximum extent of 25 and 15%, respectively, even with the potent inhibitors, **1a**, **1b**, **4**, **8** and **10** (see structures of alkaloids and Table 2). Thus, screening data indicated that nucleic acid polymerases from calf thymus DNA and bacteria were relatively less sensitive to inhibition by these alkaloids than the reverse transcriptase enzyme. Since inhibitions of nucleic acid polymerases by the alkaloids were dependent on the different templates employed in the assays, the comparative enzyme inhibitions by the alkaloids merely indicated a selective inhibition of reverse transcriptase activity under standard assay conditions. In earlier studies, coralyne chloride, a hexadehydroberbinium salt, and related alkaloids were reported to interact with the calf thymus DNA *in vitro* to form a stable complex [39]. This property could be responsible for the inhibition of calf thymus DNA polymerase. However, greater sensitivity of reverse transcriptase inhibition by alkaloids suggested that the biological activity (antileukemic or antitumor) of protoberberine and benzophenanthridine alkaloids may be due to the inhibition of reverse transcriptase activity.

The enzyme inhibitory curves exhibited by alkaloids **3**, **4** and **8** have been reported elsewhere [21, 24]. The inhibitory curves of AMV reverse transcriptase activity by the alkaloids **1a–1f** and **2**, **5–7** are shown in Figs. 1 and 2, respectively. Similar results were obtained on MuLV reverse transcriptase activity (data not shown). The slope of inhibitory curves was dependent on the type of inhibitors. The potent inhibitors generally showed a sharp decline of inhibitory curves when approximately 40 μg/ml alkaloid concentration was present in the assay mixture. The mode of inhibition of the enzyme by alkaloids, **1a–1f**, **2** and **5–7** was found to be similar to the results published [21, 22, 24] earlier for alkaloids **3**, **4** and **8**. Basically, the alkaloids inhibited the reverse transcriptase activity by interacting with the template primers (unpublished results).

Comparison of inhibition of reverse transcriptase and antileukemic activities

Table 2 shows a comparison of the inhibition of reverse transcriptase with the reported antileukemic activities of protoberberine and benzophenanthridine alkaloids.

Table 2. Comparison of inhibition of reverse transcriptase and antileukemic activities of alkaloids

Compound	Inhibition of reverse transcriptase activity ID_{50} (50% inhibition)* (μg/ml)	(μM)	Antileukemic activity† T/C %	Dose (mg/kg)	Ref. of antileukemic activity
1a	30	60	175	80	12
			176	100	
			170	160	
1b	35	72	215	50	11
			219	100	
			181	200	
1c	95	170	100	100	11
			110	200	
1d	50	103	134	25	11
			122	50	
1e	45	92	144	25	11
			121	100	
1f	45	92	186	33	11
			167	50	
			145	100	
2	80	158	104	25	11
			107	50	
			116	100	
3	60	124	105	3.13	40
			102	6.25	
			107	12.50	
4	30	70	Data not reported		8
5	45	111	143	40	11
			154	80	
			166	160	
6	60	115	180	20	12
			180	80	
			150	120	
7	70	139	136	10	12
			171	20	
			177	40	
8	6	16	190	25	10
			210	50	
			265	100	
9	80	209	108	50	13
			107	100	
			109	150	
10	12	32	151	75	13
			147	100	
			160	150	

*Fifty per cent inhibitions of AMV and MuLV enzymes were determined in the presence of poly rA. oligo dT and poly dA. oligo dT template primers. The mean ID_{50} values were calculated from three different assays representing at least three readings of each assay. The average variation in inhibition of reverse transcriptase activity in the presence of each template primer was $\pm 5\%$.

†The data on the antileukemic activity in both leukemias (L1210 and P388) for all the compounds are not reported in the literature. Therefore, the antileukemic activity of compounds presented here is against leukemia P388, except for compounds **1c–1e** and **2**, for which the antileukemic activity is against leukemia L1210. Furthermore, the T/C% value is shown only at two or three dose levels since the test animals either died or their survival rate was decreased in higher doses. The detailed information about the survival rate and the T/C% value at other dose levels may be referred to in the references cited.

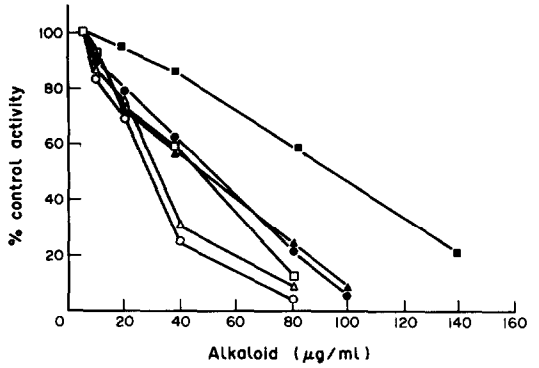

Fig. 1. Effect of alkaloids on AMV reverse transcriptase activity. In a standard assay mixture of 0.10 ml (Experimental) containing 5 μl of reverse transcriptase, different concentrations of alkaloids **1a** (○), **1b** (△), **1c** (■), **1d** (●), **1e** (▲) and **1f** (□) were added to the assay mixture for enzyme inhibition. The control enzyme activity (100%) after deducting the blank reading was 250 pmol/assay.

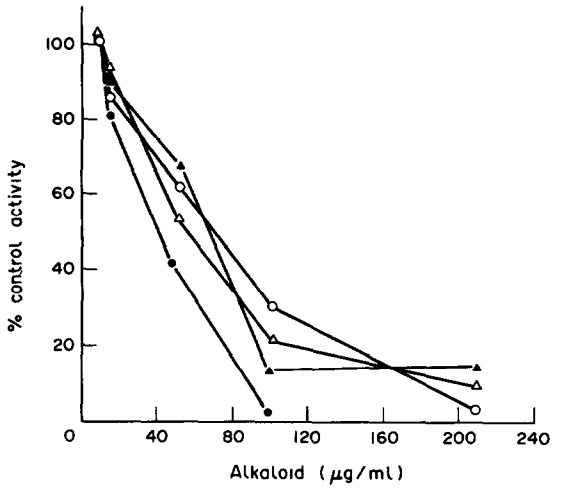

Fig. 2. Effect of alkaloids on AMV reverse transcriptase activity. In a standard assay mixture of 0.10 ml (Experimental) containing 5 μl of reverse transcriptase, different concentrations of alkaloids **2** (○), **5** (●), **6** (△) and **7** (▲) were added to the assay mixture for enzyme inhibition. The control enzyme activity (100%) after deducting the blank reading was 250 pmol/assay.

Antileukemic activity was reported by Zee-Cheng [11, 12] and other workers [10, 40] against leukemias P388 and L1210 in mice by the general screening method [41] published elsewhere. Based on the ID_{50} values (μg/ml or μM), alkaloids **1a**, **1b**, **4**, **8**, **10**; **1d–1f**, **3**, **5–7**; and **1c**, **2**, **9** were classified as strong, moderate and weak inhibitors of reverse transcriptase activity, respectively. Although determination of the ID_{50} (50% inhibition) values to classify strong (up to 35 μg/ml), moderate (35–70 μg/ml) and weak (above 70 μg/ml) inhibitors of reverse transcriptase activity is not an ideal method, these concentrations, in general, agreed with the compounds displaying potent or weak enzyme inhibition reported by other workers [42, 43].

Comparing the reverse transcriptase and antileukemic activities (Table 2), it was observed that the strong inhibitors **1a**, **1b**, **4**, **8** and **10** (ID_{50}, 6–35 μg/ml) of reverse transcriptase activity possessed T/C% of 147–265 whereas moderate inhibitors **1d–1f**, **3**, **5–7** (ID_{50}, 35–70 μg/ml) had T/C% 102–186. Compounds **1c** (ID_{50} 95 μg/ml) **2** and **9** (ID_{50} 80 μg/ml) were weak inhibitors of reverse transcriptase activity, thereby exhibiting no activity against leukemia L1210 or P388 systems (T/C% $\leq$ 116). Among the compounds **1a**, **1b**, **4**, **8** and **10**, which were strong inhibitors, compound **8** possessed the maximum inhibition of reverse transcriptase (ID_{50} 6 μg/ml) and antileukemic (T/C% 265) activities. A 50% enzyme inhibition by other potent inhibitors, **1a**, **1b** and **10**, also corresponded well with their antileukemic activities. These alkaloids, therefore, showed a good correlation of antileukemic and inhibition of reverse transcriptase activities. The moderate inhibitors **1d–1f** and **5–7** also indicated a good correlation of a 50% enzyme inhibition and antileukemic activity as shown in Table 2. Weak inhibitors **1c**, **2** and **9** were inactive antileukemic compounds. No quantitative data were attempted to compare the reverse transcriptase inhibition and the antileukemic activity of the compounds. The results simply indicated that the alkaloids having an ID_{50} value up to 70 μg/ml were active, and above this value, they were inactive antileukemic compounds. Thus, in general, potent inhibitors of reverse transcriptase activity possessed a good antileukemic activity. However, alkaloid **3** did not show a correlation between inhibition of reverse transcriptase activity and the antileukemic activity. This could be due to the fact that the antileukemic activity was determined in lower doses since higher doses were toxic to the animals [40]. Similarly, enzyme inhibition by alkaloid **4** could not be compared with its antileukemic activity due to the toxicity of the compound and because no data were available. Nevertheless, alkaloids **3** and **4** were reported to possess antitumor activity in experimental animals [8]. The lack of correlation of inhibition of reverse transcriptase and the antileukemic activities of the alkaloids **3** and **4** could be due to the other factors discussed under structure–activity relationships.

Structure–activity relationships

Alkaloid **1a**, an 8-methyldehydroprotoberberine salt, was reported to possess a 50% inhibition of reverse transcriptase activity in the range of 20–25 μg/ml [44]. However, our results indicated a 50% inhibition of enzyme at a slightly higher dose level (25–30 μg/ml, Table 2). In any case, alkaloid **1a** exhibited a good antileukemic activity against both leukemias L1210 and P388 in mice [11]. A 50% enzyme inhibition by alkaloid **1b** was almost equal to that of **1a**, but the antileukemic activity was increased slightly. The results indicated that the 8-ethylhomolog of **1a** did not change the antileukemic activity or the enzyme inhibition significantly.

The higher homolog (8-propylcoralyne, **1c**) was a weak inhibitor of reverse transcriptase activity. Correspondingly, this homolog was inactive against leukemia L1210 (T/C% $\leq$ 110, Table 2). The replacement of two methoxyl groups at either the 2,3 or 10,11-positions of **1a** by a methylenedioxy group (**1d**, **1e**) or the removal of one methoxyl group from position 10, as in compound **1f**, or a methyl group from position 8, as in compound **5**, decreased the reverse transcriptase inhibition slightly.

However, these compounds, **1d–1f** and **5**, were still active in P388 or L1210 leukemia. The results imply that the antileukemic activity or the inhibition of reverse transcriptase activity by **1a** was not significantly changed by the removal of a methoxyl or methyl group or by the substitution of an alkoxyl group at the positions mentioned above. As a matter of fact, the reverse transcriptase inhibition or antileukemic activity was retained as long as compound **1a** possessed an aromatic B-ring. This could be elucidated by comparing the antileukemic activity and inhibition of reverse transcriptase activity by **2** or its positional isomer **3** with **1a**. These compounds, which have a saturated ring B, were found to be antitumor active in experimental animals but inactive against leukemia L1210 or P388 in mice [8, 11, 40]. Correspondingly, inhibition of reverse transcriptase activity by **2** or **3** was significantly reduced (ID_{50} 60–80 μg/ml) as compared to **1a** (ID_{50} 30 μg/ml). The results therefore indicated that the aromatic rings, particularly the B-ring, appear to be an essential requirement in demonstrating the maximal enzyme inhibition or a significant antileukemic activity. However, there could be other possibilities which influence the inhibitory effect of the alkaloids on reverse transcrippase activity. For instance, (a) a charge on the nitrogen atom or a counter-ion effect due to the different salts used in the assay decreased or increased the solubility of the compounds, thereby affecting the enzyme inhibition; (b) the position of functional groups (hydroxyl, methyl and methoxyl) on the A–D rings may play an important role in the enzyme inhibition, which is evident by the comparative enzyme inhibition and structural differences of the compounds; (c) the skeletal arrangement of rings as in benzophenanthridine alkaloids **8** and **10** resulted in potent compounds as compared to protoberberine alkaloids **1a–1f**, **2–7**; and (d) compounds **8**, **10** with *N*-methylation (N–Me) and aromatic rings were potent enzyme inhibitors or antileukemic compounds as compared to *C*-methylation (C–Me) compounds **1a–1f**, **2–7**.

Additionally, a steric effect of the functional groups in rings A–D on inhibition of reverse transcriptase activity was observed by isomers of coralyne, **6** and **7**. These isomers exhibited comparable antileukemic activity to **1a** but the inhibition of reverse transcriptase activity was approximately half that of **1a**. This could be due to the steric effect of methoxyl groups and the N–O–O triangular pharmacophore in the structure. The steric effect of the functional groups and the N–O–O triangular feature were first observed by Zee-Cheng and co-workers in the structure and antileukemic activity relationship of coralyne isomers **6** and **7** and some other antileukemic compounds [11, 12, 45].

Among the benzophenanthridine alkaloids, alkaloid **8** was found to be the most active inhibitor of reverse transcriptase activity [21, 24]. Interestingly, the isomers of **8**, namely, **9** (ID_{50} 80 μg/ml) and **10** (ID_{50} 12 μg/ml) showed a significant difference in 50% enzyme inhibition as compared to **8** (ID_{50} 6 μg/ml, Table 2). Consequently, alkaloid **10** possessed an antileukemic activity and **9** was inactive. On the other hand, the methylation product of **8** (*O*-methylfagaronine) was active against reverse transcriptase activity [21] and both P388 and L1210 leukemias [13]. The results imply that the presence of a phenolic group at position 1 or 2 in **8** is important in retaining the antileukemic activity. The higher 50% inhibition of reverse transcriptase activity by **8** as compared to **1a–1f**, **2–7**, **9** and **10**, therefore, may be due to the structural specificity, the skeletal arrangement of the A–D rings and other possibilities discussed above.

In conclusion, the antileukemic activity exhibited by coralyne isomers and benzophenanthridine alkaloids indicated a good correlation between antileukemic and inhibition of reverse transcriptase activities. The results suggested that inhibition of reverse transcriptase activity may be a good test system in evaluating the antitumor (antileukemic) activity of the compounds. Further support to this conclusion stems from the fact that several antineoplastic agents such as actinomycin [46], chromomycin [47], olivomycin [47], Ara-CTP [48] and adriamycin [49] are good inhibitors of the reverse transcriptase activity. A structure–activity relationship is discussed to indicate a suitable placement of functional groups for the maximum inhibition of reverse transcriptase activity by the alkaloids. The results may be helpful in the synthesis of potent antitumor benzophenanthridine and protoberberine alkaloids.

EXPERIMENTAL

Material The purified AMV enzyme with spe. act. 37000 units/mg of protein was obtained through the courtesy of Dr. J. Beard (Life Science Research Labs, St. Petersburg, FL). The enzyme preparation contained 16400 units of reverse transcriptase/ml (one unit of enzyme activity is the incorporation of 1 nmol of deoxythymidine monophosphate into an acid-insoluble product in 10 min at 37°). It was purified and assayed by the method of ref [50]. The enzyme from MuLV propagated in JLS-V9 cell line had an activity of 4.00 nmol of thymidine monophosphate incorporation/30 min per ml and was obtained from Bionetic Laboratory Products, Kensington, MD. 70S RNA from MuLV (gift from Dr. Reitz, Litton Bionetics Inc., Bethesda, MD) was purified by the method of ref. [51]. The templates were purchased from P. L Biochemicals, and consisted of poly rA oligo dT, poly dA. oligo dT, poly rC. oligo dG, activated calf thymus DNA and bacterial RNA. Substrates, [^{3}H]TTP (sp. act. 14 Ci/nmol), [^{3}H]GTP (sp act. 14 Ci/nmol) and unlabelled nucleotides were obtained from Schwartz-Mann. All other reagents were of analytical grade.

The alkaloid samples tested were coralyne acetosulfate, **1a** (NSC 154890); 8-ethylcoralyne ethosulfate (homocoralyne), **1b** (NSC 156625); 8-propylcoralyne proposulfate, **1c** (NSC 157113); 2,3-methylenedioxy-10,11-dimethoxydibenzo[a, g]quinolizinium acetosulfate, **1d** (NSC 156627); 2,3-dimethoxy-10,11-methylenedioxydibenzo[a, g]quinolizinium acetosulfate, **1e** (NSC 169459); 10-demethoxycoralyne acetosulfate, **1f** (NSC 168212); 5,6-dihydrocoralyne acetosulfate, **2** (NSC 154889); 13-methylpalmatine iodide, **3**; palmatine chloride, **4** (NSC 209407); norcoralyne chloride, **5** (NSC 169688); stracoralyne acetosulfate, **6** (NSC 177526); isocoralyne acetosulfate, **7** (NSC 196541); fagaronine chloride, **8**; and isomers of fagaronine, **9**, **10**.

Methods All templates were dissolved in buffer containing 0 01 M tris (hydroxymethyl) aminomethane hydrochloride (Tris–HCl, pH 7.0), 0 01 mM EDTA and 0 10 M NaCl. Activated calf thymus DNA was prepared by incubating 4.50 mg DNA and 5 mg BSA in 10 ml buffer containing Tris–HCl, pH 7.0 (0 05 M) and 5 mM $MgCl_2$ with 0 01 mg of crystalline pancreatic deoxyribonuclease (Worthington) for 15 min at 37°. It was cooled at 4°, further incubated at 70° for 5 min and stored at 4°. Appropriate concns of alkaloid solns were dissolved in DMSO (dimethyl sulfoxide). Reverse transcriptase assays were performed according to the method of ref. [21]. The procedure essentially consisted of the addition of alkaloids in the enzyme

assay mixture (100 μl) containing buffer salts (5.00 μmol Tris–HCl, pH 7.3, 8.00 μmol KCl, 0.10 μmol $MnCl_2$), dithiothreitol (0.50 μmol), BSA (20.00 μg), substrate, enzyme and template primer (2.0 μg). The inhibitory results were calculated by determining the remaining activity and expressed as per cent of control. The control experiments were carried out with an equivalent vol. of DMSO but no alkaloid.

RNA polymerase assays were carried out by adding compound soln in assay mixture (100 μl) containing 5 μmol Tris–HCl (pH 8.0), 25 nmol each of adenosine, cytosine and guanosine triphosphates, 2.5 nmol [^{3}H]UTP (250 cpm/pmol), 5 μg phosphocreatine kinase, 0.4 μmol creatine phosphate, 25 μg BSA, 0.2 pmol $MnCl_2$, 0.2 μmol $MgCl_2$, 0.1 μmol NaF, 0.4 μmol dithiothreitol, 10% (v/v) glycerol, 15 μg denatured calf thymus DNA and bacterial RNA polymerase (*E. coli*). The reaction mixture was incubated at 37° for 30 min. After incubation, enzyme reaction was stopped by cooling at 4° and by the addition of 25 μl 0.1 M EDTA. 100 μl of reaction mixture was spotted uniformly onto a 2.5 cm circular DE-81 Whatman filter paper and washed batchwise by swirling three times in 10 ml 5% Na_2HPO_4 per filter paper, followed by two washings, each of H_2O and 95% EtOH. Filter papers were dried and radioactivity was determined in toluene-base scintillation fluid.

DNA polymerase assays were performed by adding compound solns in assay mixture (100 μl) containing 100 μmol of sodium glycinate (pH 8.8), 10 nmol each of deoxyadenosine, deoxycytosine and deoxyguanosine triphosphates, 2 nmol [^{3}H]TTP (250 cpm/pmol), 1 μmol $MgCl_2$, 0.1 μmol NaF, 10% (v/v) glycerol, 15 μg activated DNA and calf thymus DNA polymerase (α). The reaction mixture was incubated for 30 min. After incubation, enzyme reaction was stopped and the method completed as described for RNA polymerase assay.

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