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Specificity of Deoxyribonucleic Acid Intercalating Compounds in the Control of Phenylalanine Ammonia Lyase and Pisatin Levels[1]

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ABSTRACT

Compounds with planar triple ring systems such as acridine orange, 9-amino acridine, 9-amino-1,2,3,4-tetrahydroacridine (tacrine), 6,9-diamino-2-ethoxyacridine lactate monohydrate (DE-acridine), 6-chloro-9-(3′-diethylamino-2′-hydroxypropylamino)-2-methoxyacridine·2 HCl (CDM-acridine), quinacrine, 6-chloro-9-(4′-diethylamino-1′-methylbutylamino)-2-methoxy-1,10-diazaanthracene (CDM 1,10-diazaanthracene), thionine, azure A, methylene blue, and pyronine Y when applied to excised pea pods were potent inducers of phenylalanine ammonia lyase or of pisatin, or of both. Compounds with an array of structural variation around the planar three-ring system were tested for their ability to induce these responses in pea tissue. In general, dimethylamino, diethylamino, or amino substitutions at position 2 and 6 or an amino (with or without an aliphatic side chain) substitution at position 9 of the three-ring system augmented induction potential. Methyl green, methylene blue, 2,7-diaminofluorene, nile blue, neutral red, pyrogallol red, ethidium bromide, nogalamycin, quinine, chloroquine, spermine, 8-azaguanine, gliotoxin, chromomycin A_3, actinomycin D, and mitomycin C were also potent inducers. The inhibition of phenylalanine ammonia lyase induction by the application of actinomycin D (300 micrograms per milliliter) or 6-methylpurine (1 milligram per milliliter) within 1 hour after inducer application indicated that newly synthesized RNA is necessary for induction. Phenylalanine ammonia lyase induction was also inhibited by cycloheximide (150 micrograms per milliliter).

Various chemicals with the potential to intercalate into DNA molecules have been used extensively to study DNA structure and mutagenic, antiviral, antimitotic, anticarcinogenic, antimalarial activity (1, 30, 37, 48). We recently reported that the genes responsible for the production of the isoflavonoid pisatin are expressed when DNA intercalating compounds such as ethidium bromide, actinomycin D, or acridine orange are administered in optimal concentrations to excised pea pods. The observation that certain intercalating compounds are potent inducers[3] (41) of pisatin production, has enticed us to test related compounds and further elucidate the chemical characteristics which mediate induction in this system. We have also assayed the induction of phenylalanine ammonia lyase (EC 4.3.1.5), a key enzyme in the biosynthesis of pisatin.

The present report compares 9-aminoacridine, acridine orange, ethidium bromide, methyl green, thionine, crystal violet, pyronine Y, and nile blue with various other structurally related compounds as inducers of pisatin and PAL.[4]

MATERIALS AND METHODS

Materials. Several compounds were generous gifts from the following scientists: nogalamycin, Gunther S. Fonken, Upjohn Co.; actinomycin D, Walter B. Gall, Merck Sharp and Dohme; myracil D, Erich Hirschberg, Columbia University; 5-diazouracil, Harry B. Wood, Jr., Cancer Chemotherapy National Service Center. Sources of the remaining compounds were as follows: quinine dihydrate, nile blue sulfate, pyrogallol red, 2,7-diaminofluorene, acridine, acridane, gallocyanine, tacrine, cresyl fast violet, neutral violet, 9-amino acridine·HCl, CDM 1,10-diazaanthracene, DE-acridine, CDM acridine, 10-thioxanthenone, Aldrich Chemical Co.; pyronine Y, acridine red, lacmoid, brilliant cresyl blue, crystal violet, azure A and B, thionine, Allied Chemical and Dye Corp.; methylene blue, neutral red, National Aniline and Chemical Co.; methylene green, auramine O, Hartman-Leddon Co.; ethidium bromide, 8-azaguanine, spermine, chromomycin A_3, acridine orange, Calbiochem; quinacrine·HCl, Nutritional Biochemical Corp.; chloroquine, cordycepin, Sigma Chemical Co.; Michler's ketone, K and K Laboratories, Inc.; proflavine·2HCl, Mann Research Laboratories; malachite green, methyl green, basic fuchsin, fast green, Fisher Scientific Co.; brilliant green, methyl blue, Matheson Coleman and Bell Co.

Methods. Experiments quantitating pisatin production and PAL activity utilized 1 g of fresh immature pea pods (1–2 cm long, harvested while still enclosed in the blossom). The general procedure, which involved splitting the pods and administering 1.5 ml of the aqueous solutions of the inducer compounds to the exposed pea endocarp, has been described in detail previously (41).

Standardized methods for the isolation and quantitation of pisatin (41) and for assaying PAL activity (20) have been described.

The compounds tested as inducers of pisatin or PAL were dissolved in water, and the solution was adjusted to pH 6.0 to 7.0, which approximates the pH of excised pea tissue. For

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[3] The term "inducer" in this paper will refer to an agent which can cause an increase in extractable phenylalanine ammonia lyase and pisatin.

[4] Abbreviation: PAL: phenylalanine ammonia lyase.

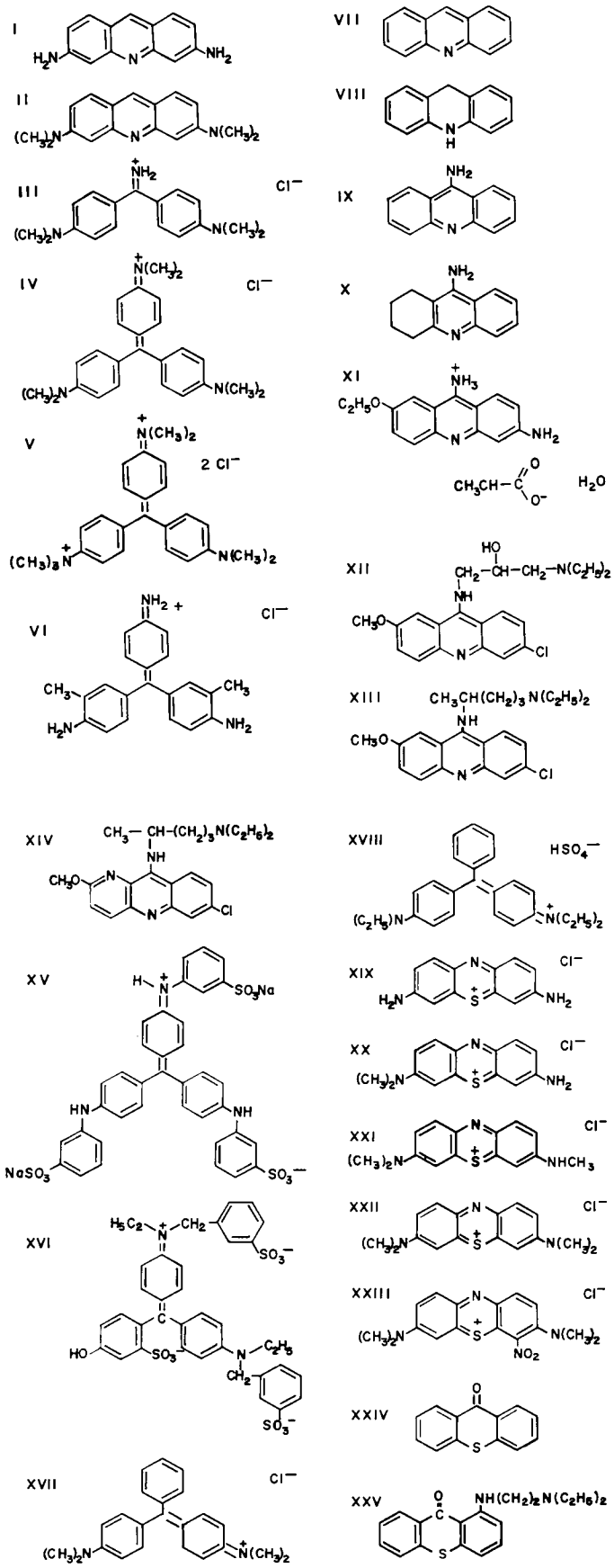

FIG. 1

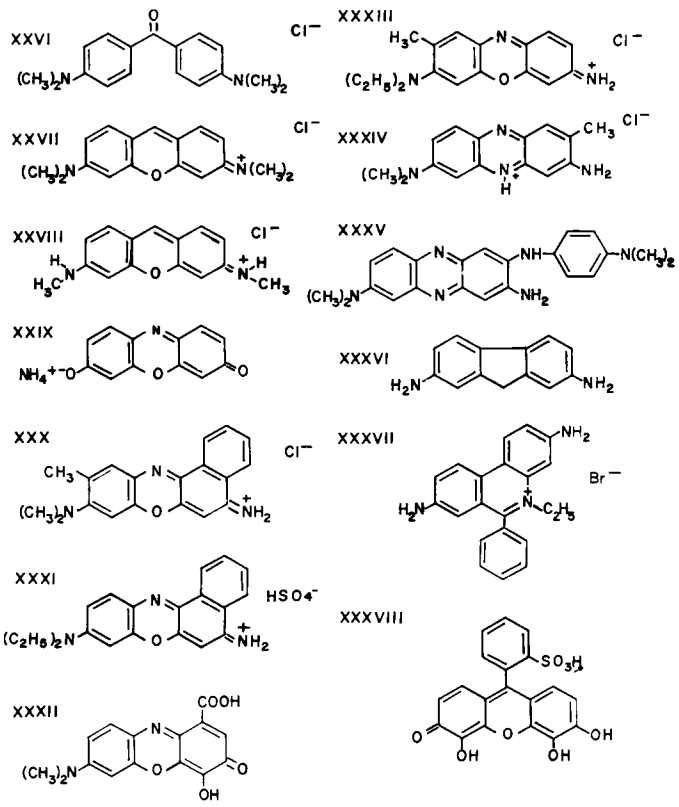

FIG. 1. Structures of compounds listed in Table I.

certain compounds not water-soluble in this pH range, the pH was adjusted to increase solubility as noted in the footnotes of the tables.

RESULTS

Inducer Specificity. It has been reported previously (41) that pea genes required for pisatin synthesis can be activated by certain DNA intercalating compounds. Figure 1 presents the structural formulas of compounds with various three-ring systems which are compared as inducers of PAL and pisatin in Table I. These compounds have been selected to demonstrate the chemical specificity required to increase PAL activity and pisatin production in pea tissue. The compounds included in this study represent a small fraction of compounds which were assayed.[5]

The unsubstituted acridine molecule, a DNA intercalating compound (48), is unable to induce PAL or pisatin, whereas proflavine, 2,7-diaminoacridine (0.5 mg/ml), proved to be a moderately effective inducer of pisatin but not PAL. As the proflavin molecule is structurally modified (compounds I–IV) with the methylation of the amino groups, the PAL and pisatin induction potential increases. The omission of the nitrogen at position 10 and the various changes at position 9 did not negate induction potential.

Many but not all of the inducers trigger the induction of both

[5] Other compounds tested which were not inducers include most of the plant hormones, animal steroid hormones, an array of secondary plant products, phenolic compounds, and other phytotoxic substances. This paper contains only the data obtained from the concentration of a given inducer which was optimal over the 1 to 1000 μg/ml concentration range tested.

Table I. *Induction of PAL and Pisatin Synthesis by Potential DNA Intercalating Compounds*

Inducer	Concn of Inducer[1]	PAL Activity[2]	Concn of Inducer[1]	Pisatin Synthesis[3]
	mg/ml	*% of control*	*mg/ml*	
I Proflavine[4]	0.05	146	0.5	++
II Acridine orange	1.0	659	1.0	+++
III Auramine O	1.0	363	1.0	++
IV Crystal violet	1.0	233	0.2	+++
V Methyl green	1.0	424	1.0	++
VI Basic fuchsin	1.0	260	1.0	++
VII Acridine[4]	1.0	49		0
VIII Acridane[4, 5]	1.0	54		0
IX 9-Amino acridine	1.0	660	1.0	++++
X Tacrine[4]	1.0	1023	1.0	+
XI DE acridine	1.0	970	1.0	++++
XII CDM acridine	1.0	817	1.0	++++
XIII Quinacrine	1.0	1209	1.0	++++
XIV CDM 1,10-diazaanthracene	1.0	775	1.0	++++
XV Methyl blue	1.0	220	1.0	++
XVI Fast green	1.0	97	1.0	+
XVII Malachite green	1.0	140	1.0	++
XVIII Brilliant green	1.0	267	1.0	++
XIX Thionine	1.0	667	1.0	+++
XX Azure A	1.0	517	1.0	++
XXI Azure B	1.0	241	1.0	+
XXII Methylene blue	1.0	648		0
XXIII Methylene green	1.0	547	0.5	+++
XXIV 10-Thioxanthenone[5]	1.0	48		0
XXV Myracil D	1.0	129	1.0	++
XXVI Michler's ketone[4]	1.0	171		0
XXVII Pyronine Y	0.2	506	0.2	++++
XXVIII Acridine red	1.0	205	1.0	++
XXIX Lacmoid	1.0	57		0
XXX Cresyl fast violet	1.0	84		0
XXXI Nile blue	1.0	1387	0.5	++++
XXXII Gallocyanine	1.0	133		0
XXXIII Brilliant cresyl blue	1.0	312		0
XXXIV Neutral red	1.0	563	1.0	+++
XXXV Neutral violet	1.0	132	1.0	++
XXXVI 2,7-Diaminofluorene[4]	1.0	743	1.0	++++
XXXVII Ethidium bromide	0.1	367	0.1	++++
XXXVIII Pyrogallol red	1.0	683	1.0	++++

[1] The concentration indicated in this table for each compound is the optimal stimulating concentration within the range 1×10^{-3} mg/ml to 1 mg/ml.

[2] PAL activity was assayed as micromoles of cinnamic acid per g tissue per hr at 39 C. The control tissue averaged 110 mμmoles.

[3] All compounds could not be compared as pisatin inducers within a single experiment. Therefore, all optimal inducer concentrations were repeated in at least three experiments. Values for pisatin extracted were within the following ranges: 0 = no detectable pisatin to 10 μg; + = 10 to 40 μg; ++ = 40 to 80 μg; +++ = 60 to 100 μg; ++++ = over 100 μg. Pisatin is expressed in micrograms produced per g pod after a 42-hr incubation period in the dark at 22 C. Water-induced controls produced <10 μg.

[4] The pH of solutions of these compounds was varied from the standard 6.0 to 7.0 range to improve solubility as follows: 2,7-diaminofluorene, pH 4.0; acridine, acridane, and Michler's ketone, pH 3.0; tacrine, pH 3.5. Proflavine was soluble at pH 3.0 and 6.0; however, it only induced at pH 6.0.

[5] Compounds with low water solubility.

PAL and pisatin (Table I). The constitutive level of PAL in pods is low but apparently does not limit the induction of pisatin (*i.e.*, when induced with proflavine, fast green, malachite green, myracil D, or neutral violet).

Basic fuchsin, a mixture of structure VI and the two homologue compounds, rosaniline and pararosaniline, is a moderately effective inducer of PAL and pisatin.

9-Amino acridine (IX) and its tetrahydro derviative, tacrine (X), are excellent inducers of PAL. DE-acridine (XI) differing from 9-amino acridine by an ethoxy group at carbon 2 and an amino group at carbon 6 is comparable to 9-amino acridine in induction potential. An alkylation of the 9-amino group (with aliphatic chains carrying terminal diethylamino groups) plus methoxy and chlorine substitutions at carbons 2 and 6, respectively, on the acridine skeleton (CDM-acridine, XII) enhance induction potential. CMD-1,10-diazaanthracene (XIV) is structurally identical with the potent inducer, quinacrine, except for the substitution of a ring CH group with nitrogen. This modification only slightly reduces the PAL induction potential but does not influence pisatin induction.

The structural variations of compounds XV to XVIII represent even greater diversion from the structure of acridine orange (II), and thus there is a deterioration in induction potential.

Thionine (XIX) differs from the acridine, proflavine (I), by substitution of a nitrogen for the carbon at position 9 and of a sulfur for nitrogen at position 10. Thionine is a potent inducer and proflavine is a lower level inducer. The extent of methylation of amino groups of thionine derivatives (as was the case with proflavine derivatives) affects the induction potential (compounds XX through XXIII).

Pyronine Y (XXVII) contains oxygen at position 10 in place of the nitrogen of the acridine molecules. The induction potential and the methylamino substitution of pyronine Y are similar to that of acridine orange (II). A reduction in the methylation of the amino groups of pyronine Y reduces the induction potential (*i.e.*, acridine red, compound XXVIII).

Of the compounds assayed in Table I, the greatest contrast in induction potential was observed between the homologues, nile blue and cresyl fast violet. Nile blue (XXXI) is a very potent inducer, whereas cresyl fast violet (XXX) does not induce. The difference in inducer specificity in this case was determined by a single methyl group.

Various other compounds (Table II) with the potential to intercalate into DNA or to alkylate or substitute for bases in DNA can induce increased levels of PAL activity. Some of these compounds previously have been shown to be inducers of pisatin synthesis (20, 41). Berberine, a plant alkaloid which can intercalate (28) into DNA, is also a potent inducer of pisatin; however, it only slightly induces PAL.

Cellular Uptake. The cellular uptake of many, but not all, of the compounds in Table I have been studied in detail (41). We have observed with the light microscope the cellular uptake of these compounds, which are highly colored dyes, by sectioning the pod tissue. No statement can be made at this time concerning the cellular uptake of compounds such as 9-amino acridine which are not detectable in pea tissue with a light microscope.

Enzyme Activation. The more potent inducers were combined with PAL extracted from noninduced tissue to determine if enzyme activity *per se* was increased. Adding inducer compounds to the reaction mixture did not increase the activity of the extracted enzyme.

Induced increases in PAL activity are subject to inhibition by inhibitors of RNA and protein synthesis (Table III). Actinomycin is an intercalating compound with a high affinity for DNA (23) and has been used as a standard inducer for this pea

Table II. *PAL Induced by Antibiotics, Antimalarial Drugs, Spermine, and 8-Azaguanine*

Inducer	Concn. of Inducer[1]	PAL Activity[2]
	mg/ml	*% of control*
Mitomycin C	0.1	1160
Actinomycin D	0.01	1059
Chromomycin A[3]	0.05	1439
Nogalamycin	0.25	911
Tetracycline	0.1	177
Gliotoxin	0.2	880
Quinacrine	1.0	639
Quinine	1.0	1444
Chloroquine	1.0	627
Spermine	1.0	610
8-Azaguanine	1.0	500[3]

[1] A 1.5-ml quantity of each inducer solution at the indicated concentration was applied per g of pea pods. Enzyme was extracted after 18 hr.

[2] Average PAL activity of control (H_2O-induced tissue) is 110 mμmoles of cinnamic acid per g pea pod per hr at 37 C.

[3] Optimal induction by 8-azaguanine occurs 48 hr after application of inducer. This value represents PAL activity after 48 hr.

system. At low concentrations (10 μg/ml), actinomycin D induces PAL to levels 10-fold those of the water-induced control. However, at higher concentrations (300 μg/ml), actinomycin substantially inhibits increases in PAL activity if applied within 1 hr after the inducer. Apparently some synthesis of new messenger RNA occurs within 1 hr after application of the inducer.

The base analogue, 6-methylpurine, is an inhibitor of RNA synthesis in plants (10, 27). Increased PAL synthesis (Table IV) is completely inhibited by 6-methylpurine when applied up to 1 hr subsequent to inducer application. The induced PAL and pisatin syntheses are also inhibited by the base analogues, 5-diazouracil and cordycepin. Cycloheximide, an inhibitor of protein synthesis, effectively inhibits increases in PAL activity until 6 hr after application of the inducer.

DISCUSSION

It has been proposed (41, 18) that increased PAL and pisatin synthesis occurs in pea tissue as a result of gene activation and that such activations can be induced by an array of compounds having the potential to change the conformation of double-stranded DNA. These conformational changes hypothetically incited by an inducer are believed to occur when the inducer compound intercalates between base pairs, alkylates DNA moieties, or substitutes for bases in the DNA molecule.

Since all of the inducers of pisatin or PAL described thus far have the potential to change the conformation of DNA, we prefer the explanation that control of this gene activation occurs at the transcription level (*i.e.*, the DNA becomes more transcribable either by dissociating a repressor component [41] or assuming a more desirable conformation for transcription).

Two other explanations have been forwarded for the stimulatory effects initiated with DNA intercalating compounds such as actinomycin D. It has been suggested that actinomycin D may specifically inhibit repressor formation by complexing with regulatory genes (36) and thus derepress the corresponding structural genes (25). Secondly, the "superinduction" (44) of enzymes such as tyrosine aminotransferase by actinomycin D in the presence of steroid inducers is thought to be controlled at the level of translation.

This paper presents data to characterize further this induction phenomenon in relation to the models presented above. It also evaluates as inducers of PAL and pisatin additional compounds having the potential to intercalate into DNA.

It was of interest to determine more conclusively how dependent PAL and pisatin inductions are on RNA synthesis. It has been established that high levels of actinomycin D inhibit pisatin formation up to 1 hr after the application of inducer, even though RNA synthesis in pea tissue is not completely inhibited at the time (41). Since 6-methylpurine is reportedly (27) a potent inhibitor of all RNA synthesis in plant tissue, it was evaluated along with cordycepin (17) and 5-diazouracil (22) as an inhibitor of induction. All three compounds were effective inhibitors of PAL and pisatin induction when applied with the inducer. The ineffectiveness of 6-methylpurine (Table IV) as an inhibitor of PAL induction when applied 3 hr after the inducer suggests that a portion of the RNA re-

Table III. *Effect of Inhibiting Protein and RNA Synthesis at Varying Time Intervals after Induction on PAL Production*

Interval after Application of Inducer[1]	PAL Activity			
	A[2]	B[2]	C[2]	D[2]
hr	*μmoles cinnamic acid per g tissue per hr at 37 C*			
0	116	167	103	225
1			121	293
2			213	579
3	138	236		
4			311	
6	116	437	323	
12	130	1,013	634	
18	167	1,232		

[1] Inducer consisted of 1.5 ml of actinomycin D solution (10 μg/ml) per g immature pea pods.

[2] Columns A, B, C, and D represent the following experiments: A = level of PAL activity in excised pea pods in the absence of inducer; B = PAL activity assayed at the indicated interval following inducer application; C = PAL activity assayed 18 hr after inducer application with cycloheximide (150 μg/ml) added at the indicated interval; D = PAL activity assayed 18 hr after inducer application with actinomycin D (300 μg/ml) added at the indicated interval.

Table IV. *Effect of 6-Methylpurine on Actinomycin D-induced Synthesis of PAL*

Inducer	Time of 6-Methylpurine Application[1]	PAL Activity[2]
	hr	*% of water-induced control*
H_2O		100
Actinomycin D (0.01 mg/ml)		1076
Actinomycin D (0.01 mg/ml)	−1	52
Actinomycin D (0.01 mg/ml)	0	61
Actinomycin D (0.01 mg/ml)	+1	109
Actinomycin D (0.01 mg/ml)	+3	676
6-Methylpurine (1.0 mg/ml)		68

[1] The concentration of 6-methylpurine used to inhibit was 1.0 mg/ml.

[2] Average PAL activity of H_2O-treated control is 110 mμmoles of cinnamic acid per g pea pod per hr at 37 C. After inducer application the pea tissue was incubated in the dark for 18 hr at 22 C.

quired for induction is synthesized shortly after application of inducer. The induction of PAL was severely inhibited by actinomycin D (300 μg/ml) up to 2 hr after inducer application and by cycloheximide (150 μg/ml) up to 3 hr after inducer application. These results and the previous observation (41) that there is a net increase in the rate of both RNA and protein synthesis after application of inducer alone reiterate that enhanced formation of pisatin and PAL require the synthesis of new RNA and protein.

Many of the compounds assayed in this study are either known intercalators of DNA or have structural features compatible with intercalation. The results from Table I indicate that planar three-ring structures (*i.e.*, acridine orange, 9-amino acridine, quinacrine, thionine, and pyronine Y) are desirable but not obligatory features of the inducers of PAL and pisatin. Some compounds known to intercalate into DNA are not inducers. For example, the intercalator, acridine, does not function as an inducer.

Dimethylamino, diethylamino, or amino substitutions at the 3 and 6 positions of the three-ring skeleton of acridine appear to be beneficial to the induction potential. The induction potential is also high in compounds with various alkylamino substitutions at position 9. A planar three-ring molecule, 9-amino acridine, and its partially hydrogenated, nonplanar analogue, tacrine, both induce PAL and pisatin. However, tacrine is much less effective in inducing the latter response.

The ability of certain intercalators to combine with nucleic acids *in vivo* is well known (1). Lerman (30, 31) suggested that the planar triple ring system of proflavine becomes intercalated between adjacent base pairs of the double helix. The extension of the DNA molecule when complexed with proflavine has been demonstrated (7), and evidence has also been provided (12) for the actual uncoiling of supercoiled DNA when ethidium bromide is intercalated.

The inducing compounds with basic side chains attached to the ring system are also capable of ionic binding to phosphate groups in one or both strands of DNA. For example, the acridine ring of quinacrine is intercalated between the base pairs of double helical DNA (31), and the aliphatic diamine side chain apparently bridges complementary DNA strands across the minor groove by ionic attraction to phosphate groups (33). Quinacrine (11), chloroquine (2), and spermine (43) (a tetraamine structurally related to the aliphatic side chain of the two drugs) interact with and stabilize the double helix (predominantly by ionic attraction). All of the compounds mentioned above are effective inducers of PAL and pisatin productions (Tables I and II). These compounds (11) and actinomycin D (23) are inhibitors of DNA-dependent RNA polymerase *in vitro*; however, as mentioned above, induction of PAL and pisatin by actinomycin D in pea tissue is accompanied (in the initial hour) by an increased rate of RNA synthesis (41).

The acridine compounds known to be mutagens function by intercalating with DNA *in vivo* (13, 34, 38). The specificity of PAL and pisatin inducers remains to be ascribed. The pisatin inducers, actinomycin D, chromomycin A_3, and mitomycin C, have in common their preferential binding to guanine-rich DNA (48). However, nogalamycin (5), also a potent inducer of PAL (Table II), is believed to bind to dA or dT moieties of DNA. Recent studies with actinomycin D indicates that intercalation depends on a specific sequence of bases (49) in addition to the presence of the guanine base (24, 47). The establishment of base-specific binding of intercalators such as ethidium bromide has met with little success. Ethidium bromide does preferentially bind to double-stranded polynucleotides (6, 29). Interestingly, ethidium bromide and quinacrine each have chromosome-specific attachment patterns when viewed microfluorometrically in pea cells (8, 9).

The penetration and accumulation of certain intercalators into intact cells has been analyzed. An autoradiographic study using the intercalator, actinomycin D-^{3}H (Hadwiger, unpublished), has shown that the pea endocarp cell takes up a small fraction of these molecules in the 1st hr, indicating a low level of membrane permeability. Once inside the cell the effectiveness of a given number of DNA intercalating molecules may depend in part on their specificity and relative affinity for DNA. For example, only one molecule of actinomycin D (which has a high affinity for DNA [48]) bound to the operator of the *lac* locus in *Escherichia coli* is sufficient to inhibit the binding of repressor (37).

At present we cannot eliminate the possibility suggested by Pollock (36) that inducers act indirectly by selectively inhibiting the transcription of a repressor component. This explanation would require each of these inducers to possess similar specificities.

It appears that the DNA intercalating compounds which induce PAL and pisatin are not acting at the translation level. If the action of these inducers were to fit the model described by Tompkins *et al.* (44), there must be preformed mRNA and the compounds must inhibit the synthesis of a post-transcriptional repressor. Since synthesis of new RNA is required for induction, preformed mRNA apparently does not exist. If DNA intercalating compounds inhibit synthesis of a "post-transcriptional repressor," the ineffectiveness of high concentrations of compounds like actinomycin D (which would have even more potential to inhibit post-transcriptional repressor synthesis) in inducing pisatin or PAL would remain unexplained. Also the specificity required for the inhibition of repressor formation would necessarily have to relate to all classes of pisatin and PAL inducers (*i.e.*, polypeptides, alkylating agents, base analogues, intercalating compounds and heavy metals).

Thus, the compounds reported as inducers of PAL and pisatin in this paper include an extensive list of known or probable DNA intercalators. The effectiveness of these inducers appears to depend on both structural specificity and concentration.

Components other than these complex ring systems which have been reported as inducers of the pisatin pathway include heavy metals (35, 40), polypeptides (20, 14), and polyamines. Recently both heavy metals (15, 26, 50) and polypeptides (20, 42) have been shown to influence DNA conformation, which is consistent with our suggestion that sites on the pea DNA are susceptible to change by a variety of components.

Although reports (16, 48) on the modes of action of many of these inducers (antibiotics, antimalarial drugs, dyes, etc.) have centered on inhibition (of growth, nucleic acid synthesis, protein synthesis, polymerase activity, etc.), we believe that the stimulatory effects of lower concentrations of these components have been largely overlooked. Further, when stimulatory effects of these components have been observed, explanations are usually based on negating effects of these compounds (44). Gene activation may be involved in some of the numerous actions *in vivo* of these substances. For example, actinomycin D augments hormone action (44), increases antibody production (3), increases interferon synthesis (32), and inhibits tumors (4, 45).

Thus, certain intercalating compounds, in addition to their role as antibiotics, mutagens, and antimalarial drugs, may have the potential in certain concentrations to activate genes. Indeed, since the effective concentrations of some antibiotics and drugs within the localized area of action are often difficult

to resolve (21, 39, 46), the primary action (or characteristic side effects of these compounds) may stem from responses activated in host or pathogen rather than exclusively from the biocidal effects on the pathogen (19).

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