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Structural Determinants Responsible for the Biological Activity of (−)-Emetine, (−)-Cryptopleurine, and (−)-Tylocrebrine: Structure–Activity Relationship among Related Compounds

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SUMMARY

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The structural basis for the cross-resistance and the common site of action of the benzoiosoquinoline alkaloids, (−)-emetine, (−)-tubulosine, (−)-cephaeline, and (−)-dehydroemetine, and of the phenanthroquinolizidine-type alkaloids, (−)-cryptopleurine, and the phenanthroindolizidine type, (−)-tylocrebrine, has been investigated by examining the cross-resistance of emetine-resistant mutants of Chinese hamster ovary cells to a large number of related compounds. On the basis of our results, we suggest that the aforementioned compounds possess common structural determinants which are responsible for their biological activity and that the requirement for biological activity is a planar molecule with two aromatic rings (rendered slightly electron richer, i.e., electronegative by methoxyl or hydroxyl groups) and the presence of a nucleophilic element such as nitrogen at a certain distance from the aromatic rings. The structure–activity relationship between the compounds indicates that the distance between the two aromatic rings, the angle between the nitrogen atom and the rings, and the electronegative character of the rings and planarity of the structure are critical features in determining the biological activity. Based on a comparison between the structures of compounds of the emetine type and those that are phenanthrene based, we have proposed the absolute sterochemistry of (−)-cryptopoeurine and (−)-tylocrebrine.

INTRODUCTION

In a recent publication (1) we showed that mutants of CHO cells independently selected for resistance to the protein synthesis inhibitors, (−)-emetine, (−)-tylocrebrine, and (−)-cryptopleurine, exhibit identical cross-resistance patterns to the three selective drugs, as well as to tubulosine and two other emetine derivatives, 2,3-dehydroemetine and cephaeline. The cross-resistance of mutants to these compounds was seen both in whole cells and in cell-free extracts, and the pattern was highly specific in the sense that no cross-resistance was seen to other inhibitors of protein synthesis such as cycloheximide, trichodermin, anisomycin, sparsomycin, and pactamycin. Genetic complementation analysis of these mutants indicated that they were allelic (1). In subsequent studies when second-step mutants resistant to very high concentrations of (−)-emetine were selected, cross-resistance to the other compounds also increased in parallel (2). These compounds belong to different families of alkaloids: (−)-Emetine, (−)-tubulosine, and (−)-cephaeline are benzoisoquinoline alkaloids; (−)-tylocrebrine is a phenanthroindolizidine; and (−)-cryptopleurine is a phenanthroquinolizidine. However, based on our results and a comparison of the various structures, we suggested that they possess common structural determinants responsible for their protein synthesis inhibitory activity (1, 2). Similar results have subsequently been reported from studies on yeast (3).

The aforementioned observations are of interest because of the wide range of biological activities that are shown by the compounds involved (see Refs. 4 and 5). Among the clinically useful activities, they possess antiameobic, antileukemic, antitumor, and antiviral properties (see Ref. 4). It is thus of importance to specify the common structural determinants of the compounds, so that modified analogs containing the structural features responsible for a specific activity can be synthesized and

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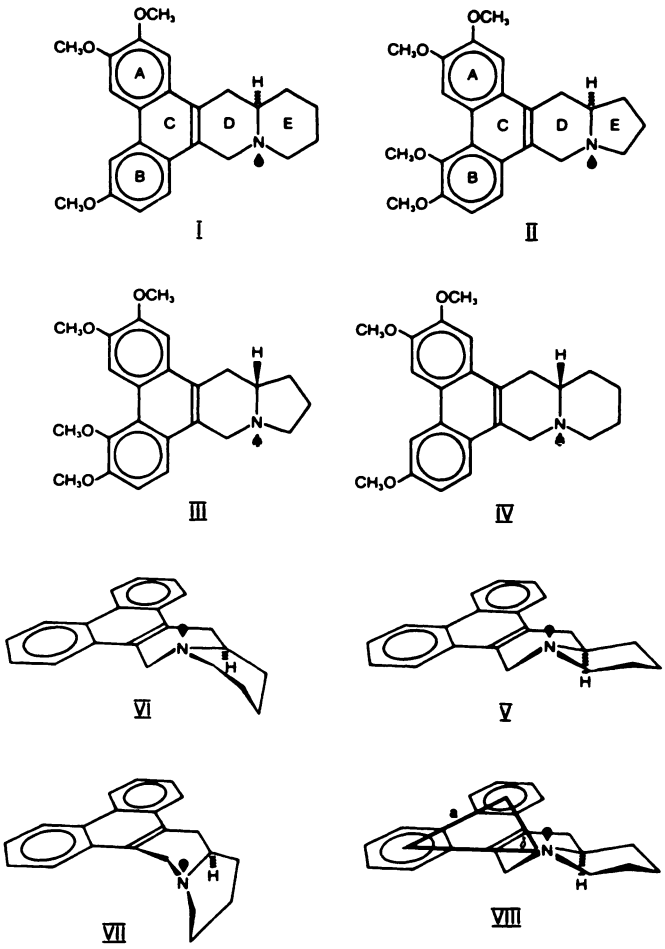
examined for biological and clinical effects. Therefore, in the present paper we have extended our studies on the cross-resistance of Emt^r mutants to a variety of compounds showing a partial structural similarity to emetine and to the phenanthroindolizidine and phenanthroquinolizidine groups of alkaloids. As a result of these and earlier studies, which are discussed in detail in the present communication, we are now able to specify more precisely the structural features of these drugs essential for biological activity. This provides a basis for assessing the structure–activity relationship between these and related compounds.

EXPERIMENTAL PROCEDURES

Chemicals. The sources of the compounds used in these studies are as follows (for general reference, see Refs. 6–8): corunnine and pontevedrine, Dr. L. Castedo, University of Santiago de Compostela, Spain; *O*-methylbulbocapnine and compound **XXXIII** (7, 8). Dr. Gutmann, Hoffman–LaRoche, Switzerland; 1,2-secoemetine dihydrochloride, Dr. R. W. Rees, Wyeth Laboratories, Philadelphia; compounds NSC 134754 and NSC 134756 (9), Dr. J. A. R. Mead, NIH, Bethesda, Md.; compounds **L** and **IL** (10), Dr. A. Buzas, University of Orleans, France; argemonine, norargemonine, and magnoflorine, Dr. J. Slavik, Purkyne University, Brno, Czechoslovakia; erybidine, Dr. H. Tanaka, Meijo University, Nagoya, Japan; liriodenine and uvariopsine, Dr. A. Cave, University of Paris, France; (−)-tylocrebrine ($[\alpha]_D^{20}$—92.0°, $CHCl_3$), Dr. N. Belcher, Pfizer Inc., Groton, Conn.; (+)-*O*-methyl psychotrine (11), Dr. J. Openshaw, The Wellcome Research Laboratory, Kent, U. K.; tubulosine (11), Dr. V. Deulofeu, Buenos Aires, Argentina; and thalicarpine (5), Dr. J. D. Douros, NIH, Bethesda, Md. Other compounds were obtained as follows: berberine, brucine, cephaeline, colchicine, emetine, hydrastine, papaverine, reserpine, and rotenone, Sigma Chemical Co., St. Louis, Mo.; allocryptopine, laudanosine, pavine, and tetrahydropalmatine, Aldrich Chemical Co.; capaurine, corydaline, cularine, dicentrine, ethaverine, glaucine, isocorydine, octaverine, phaenthine, reserpiline, tetrabenazine, and tubocurarine, ICN Pharmaceuticals Inc.; and cryptopleurine, Chemsea Manufacturing Co., Australia.

Cell culture and cell lines. The cell culture techniques employed in these studies have been described earlier (1, 2, 12–16). The parental-sensitive Chinese hamster ovary (CHO) cell line is a proline auxotroph (Pro$^-$). The two emetine-resistant (Emt^r) mutants, Emt^{rI}41 and Emt^{rII}63, which were employed in the cross-resistance studies have been investigated in detail earlier (1,2,12–16). Emt^{rI}41 is about 30- to 35-fold more resistant to emetine and was obtained from Pro$^-$ cells in a single step (1,2), whereas two selection steps were involved in obtaining Emt^{rII}63 (about 1500-fold resistant to emetine). We have shown earlier that the increased resistance of these mutants to emetine is due to an alteration in the 40 S ribosomal subunit (1,2). The altered ribosomal component has recently been identified as protein S20 (17).

Protocol for the resistance tests. The cross-resistance of these mutants to various compounds was measured either by complete dose–response curves or by plating 50



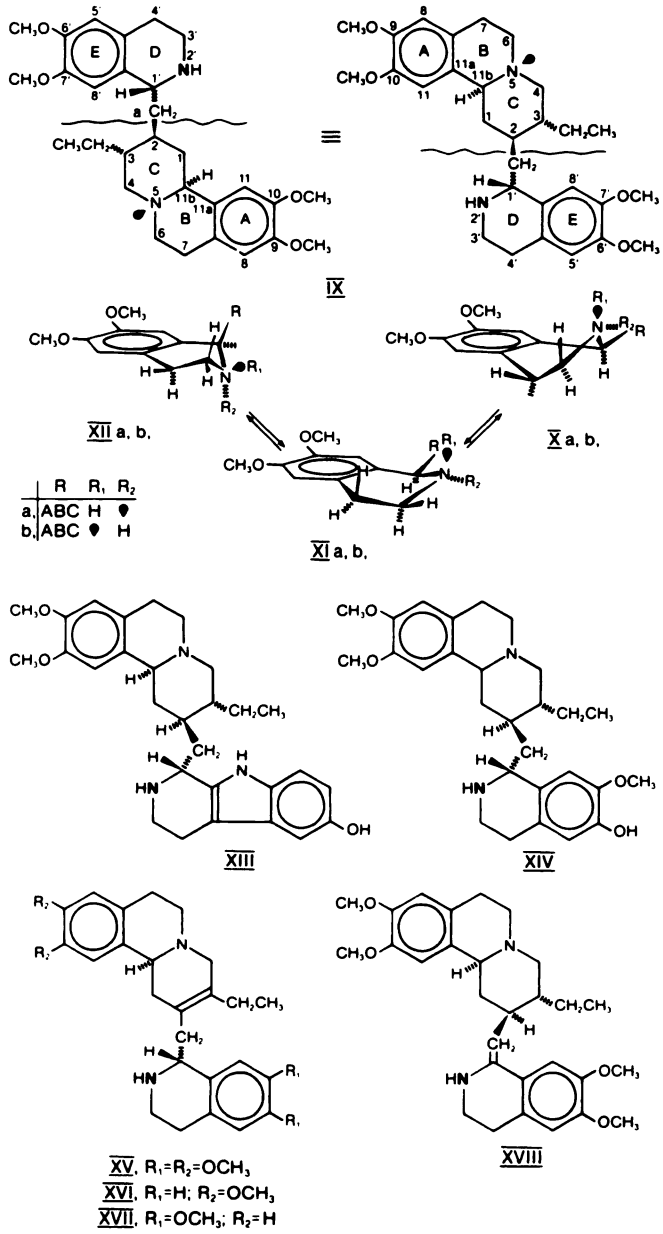
SCHEMES I–VIII

and 500 mutant and wild-type cells at various concentrations of the drugs in 24-well tissue culture trays (1,2). For complete dose–response curves, 1-ml volumes of the drug and the cells were added in that order to 60-mm plastic tissue culture dishes containing 4 ml of the medium supplemented with 10% fetal calf serum. For a 24-well dish test, the drug and cells were added in 0.5-ml volumes in that order to the individual wells. After 7 days of incubation at 37°C, the plates were stained with 1% methylene blue in 50% methanol and aggregates of 25 or more cells were counted as colonies in the determination of plating efficiencies (number of colonies observed per number of cells plated). The toxicity of a drug toward any cell line is given in terms of D_{10} values, which represent the concentration of drug which reduces the plating efficiency of the cell line to 10% of the value obtained in the absence of the drug.

Although in the present studies cross-resistance has been determined only *in vivo*, the results of our earlier studies show that the resistance *in vivo* for various compounds related to (−)-emetine correlates well with the resistance of protein synthesis in crude extracts (1, 2).

RESULTS AND DISCUSSION

Rationale of the cross-resistance studies. The work described here is based on the following basic premise: If any two given compounds owe their toxicity to the same



SCHEMES IX-XVIII

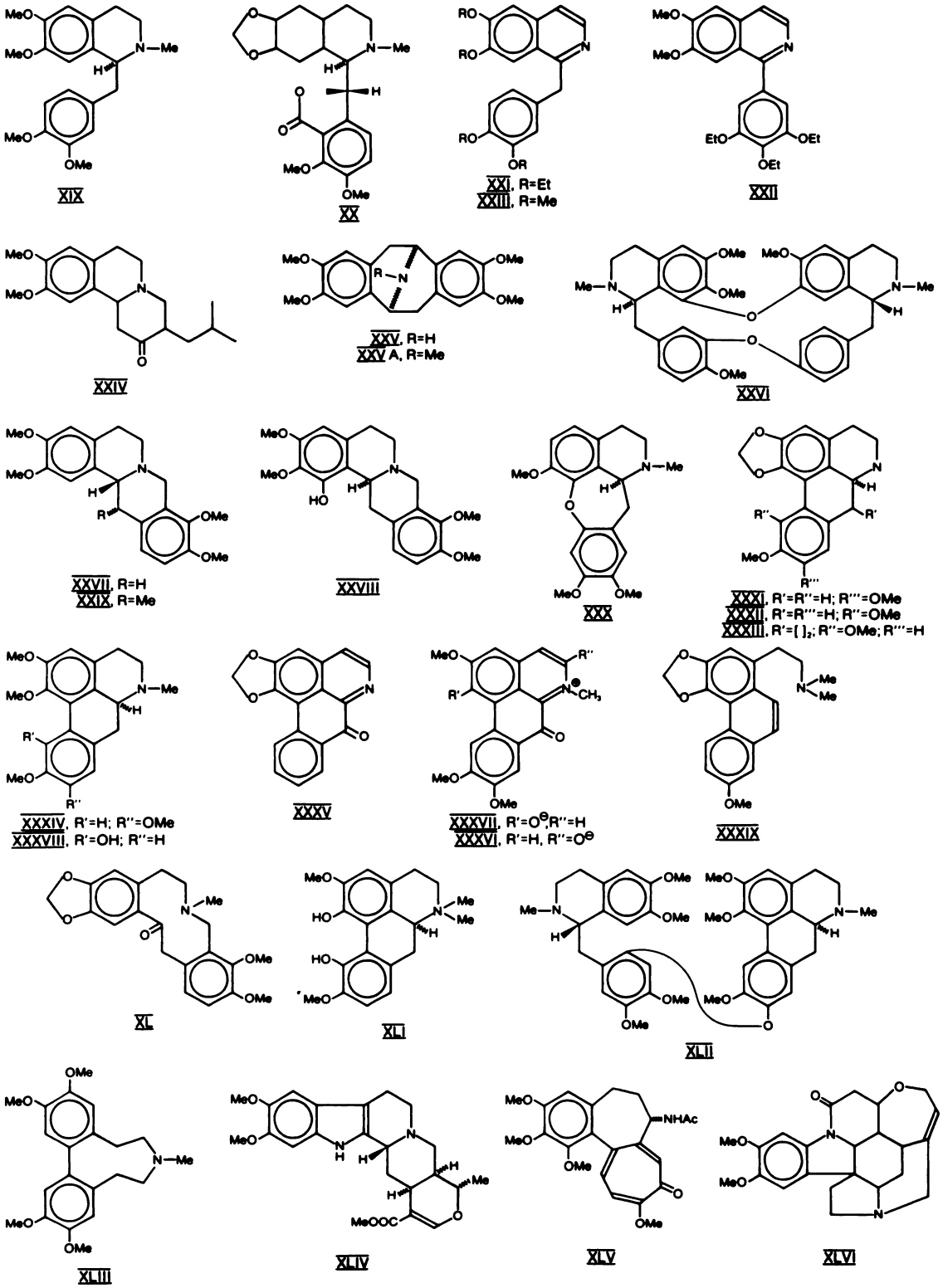
structural determinants, then cellular mutants which have become resistant to one compound due to an alteration in its target site should, at the same time, also exhibit cross-resistance to the other compound. At the same time, the failure of such mutants to exhibit cross-resistance to a given drug provides strong evidence that the compound lacks the appropriate structural determinants and that its toxicity may be due to other mechanisms (1, 2, 14). In the present studies cross-resistance has been examined against two different mutants, the single-step mutant Emt^{r}41 which is about 30- to 35-fold more resistant to (−)-emetine and the two-step mutant Emt^{rII}63 which is about 1500-fold resistant to (−)-emetine. Based on our earlier studies on cross-resistance (1, 2), it is expected that if a compound possesses structural determinants common to these compounds, then its degree of cross-resistance should be higher for the Emt^{rII} mutant as compared to the Emt^{rI}.

The structure of the presumptive common active site. Table 1 lists some of the compounds to which the Emt^{r} mutants exhibit cross-resistance. As expected, in every case, the Emt^{rII} mutant showed a much higher degree of cross-resistance as compared to the Emt^{rI} mutant. In view of the highly specific nature of this cross-resistance phenomenon (1, 2), we have assumed that analogous structural features which are responsible for the biological activity must be present in all these compounds. This assumption forms the basis of the examination of their structures for common structural determinants. Based on the results with emetine, tylocrebrine, and cryptopleurine, we will first propose a model for a common active site and then the structure–activity relationship between these and other compounds will be discussed in terms of this model.

The similarities between the two basic structural types of active compounds seen in Table 1, i.e., benzoisoquinoline and phenanthroquinolizidine and phenanthroindolizidine alkaloids, are not immediately apparent. Whereas emetine (and its congeners) possesses relative freedom of rotation around the connection between the two cyclic systems (2, 2a, 1′ bonds; cf. Fig. 1), structurally simpler phenanthrene-based compounds are more rigid (almost planar molecules). In view of the relative rigidity of the latter structures, they form the starting point of our structural investigations.

The latter type compounds, (−)-cryptopleurine (I) and (−)-tylocrebrine (II) differ in two minor aspects: (i) substitution in the aromatic part of the molecule and (ii) size of the ring E (five-membered vs six-membered). The relative stereochemistry of (−)-cryptopleurine is well established (18) and the transfusion of its D/E ring system was confirmed by the presence of Bohlmann bands in its infrared spectrum (19). So the cryptopleurine molecule is best portrayed by formula I (or its mirror image IV). The planar character of the phenthrene moiety in cryptopleurine is determined by the rigid benzene rings, and only rings D and E are nonplanar and more flexible. The half-chair conformation of ring D and the chair conformation of ring E (as in V) rather than the two boat ring E conformations (VI and VIII) are expected to be preferred (19). The distances, as measured on Dreiding models, between the center of the ring A and the nitrogen atom N (~5.1 Å) and ring B and N (~6.5 Å) are, however, affected only negligibly by the change from one conformation to another, and the space relationship of nitrogen and the plane of aromatic rings is identical in these various conformations. (−)-Tylocrebrine (II) strongly resembles (−)-cryptopleurine (I); the only difference is the smaller (five-membered) ring E and an extra methoxyl group. The smaller size of the ring, however, does not change the overall shape of the molecule in any significant manner.

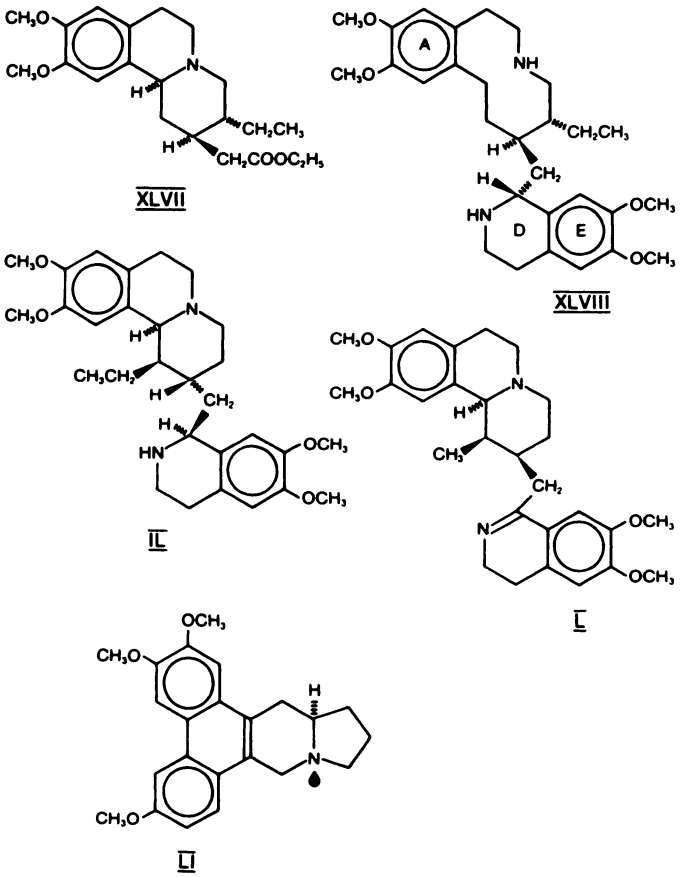
The presence of the methoxyl groups in the molecules is an important common structural feature of cryptopleurine and tylocrebrine. Because of their electron-releasing nature, these groups confer a slightly electron richer, i.e., electronegative, character on the relatively easily polar-



SCHEMES XIX-XLVI

izable aromatic rings, thus underlining the dipolar character of this part of the molecule. Based on these facts, the most likely active structure involves two aromatic rings, rendered slightly electronegative by methoxyl (or hydroxyl) substituents, and the nitrogen atom, forming a sort of triangle as in **VIII** and interacting with receptors as portrayed in Fig. 2.

The relative and absolute configuration of the less rigid molecule of (−)-emetine (**IX**) is also known, and the only uncertainty that remains involves the piperidine nitrogen



SCHEMES XLVII-LI

atom (ring D) and the possible equilibrium between forms like **X–XII**. While form **XI**$_a$ is somewhat preferred (20, 21), the low barrier separating these forms makes an interconversion of the conformers easy. Rings A, B, and C form one nearly planar system, and the 2,2a equatorial bond connecting rings C and D extends above this plane at a ~30° angle; the D–E ring system is similarly nearly planar, while the 1′,2a bond again extends above this plane at a ~30° angle. Both ring systems can assume various relative positions, and the stabilizing effects of the environment may determine which form the molecule eventually adopts.

The two ring systems can be perpendicular to each other, they can assume angular forms, or they can lie in the same imaginary plane, while the distance between the aromatic rings will vary from the minimum determined by atomic space requirements to about 7–8 Å (Fig. 1). All emetine derivatives are expected to exist in various degrees in any of these forms, depending on a combination of external factors and structural features (steric and solvation effects). This situation repeats itself in (−)-tubulosine **XIII** and (−)-cephaeline **XIV**, to a certain extent in $\Delta^{2,3}$ derivatives of (−)-emetine as 2,3-dehydroemetine (**XV**) and its desmethoxy derivatives **XVI** and **XVII**, and to a certain extent also in the $\Delta^{1',2a}$ derivatives, where it is, however, considerably more difficult to achieve planarity: (+)-*O*-methylpsychotrine (**XVIII**).

The planar forms assumed by emetine and its derivatives closely resemble the shape of phenanthrene-based molecules discussed previously. If a triangle is drawn through both aromatic rings and N-2′ in emetine (**IX**; cf. Fig. 1) (or through corresponding structural elements in **I** or **II**), the distance between N and side *a* remains the same in both types, although the angle δ is larger in **IX** than in **I** or **II**.

Based on these considerations, a hypothetical model of a simplified molecule containing the structural determinants responsible for the biological activity of emetine and its derivatives can be proposed. It should be a flat molecule with two aromatic rings rendered slightly electronegative (by methoxy or hydroxy groups), and the free electron pair of nitrogen (which can easily become a strongly electropositive center) should be at a certain distance from the aromatic part of the molecule. This would correspond to a relatively small receptor site binding nitrogen, while the aromatic ring (or rings) would be anchored on a comparatively large flat area such as the surface of the ribosome. This flat area should be substantially larger than the size of an aromatic ring.

Structure–activity relationships among various compounds based on the preceding model. The hypothetical model proposed can be tested by an examination of the relative activity of various compounds in which some of the structural features considered essential have been modified. We have found that the compounds were active in the case of modifications affecting these structural features only negligibly, but the degree of activity varied.

TABLE 1

Cross-resistance and relative activity of compounds related to emetine

Compounds	Structure	Relative degree of resistance for mutant lines[a]			Activity relative to emetine[b]
		Pro$^-$ (D_{10} M)	Emt^{rI} 41	Emt^{rII} 63	
(−)-Cryptopleurine	**I**	1.0 (2.1×10^{-9})	4.8	200.0	1400
(−)-Tylocrebrine	**II**	1.0 (7.5×10^{-9})	6.0	330.0	400
(−)-Cephaeline	**XIV**	1.0 (2.2×10^{-8})	23.0	480.0	140
(−)-Emetine	**IX**	1.0 (3.0×10^{-8})	33.0	1500.0	100
(−)-2,3-Dehydroemetine	**XV**	1.0 (4.0×10^{-8})	25.0	120.0	80
(−)-Tubulosine	**XIII**	1.0 (1.4×10^{-7})	17.0	450.0	22
(±)-NSC134754	**XVI**	1.0 (1×10^{-6})	15.0	55.0	3.3
(±)-NSC134756	**XVII**	1.0 (1×10^{-6})	15.0	50.0	3.3

[a] The relative degrees of resistance were obtained from the ratios of the D_{10} values for the mutant lines as compared to the parental-sensitive line (Pro$^-$).

[b] The activity relative to emetine was calculated from the minimal molar concentration of the compound as compared to emetine which produced similar growth inhibition of the sensitive cells, i.e., as the ratio of the D_{10} values for the Pro$^-$ line.

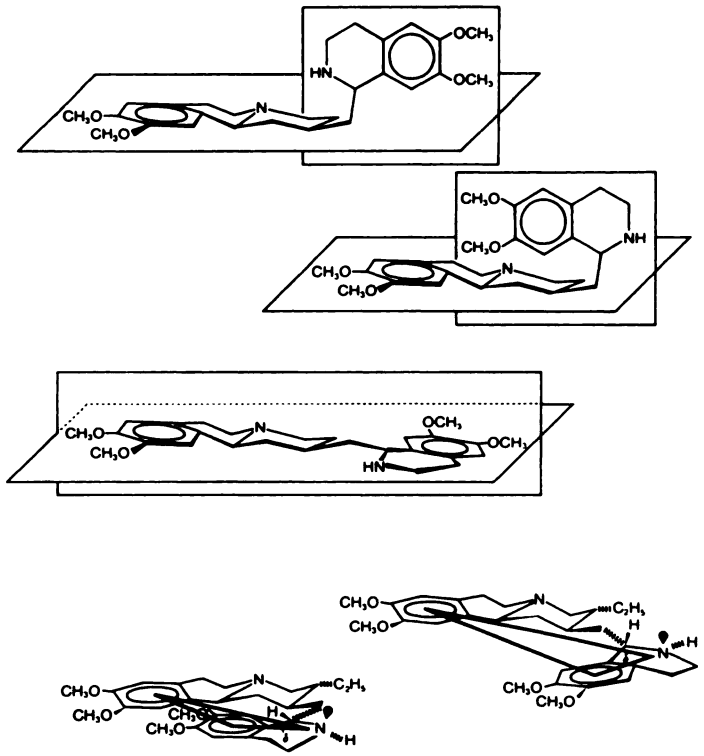


FIG. 1. *Some possible structural rearrangements of the emetine molecule*

The two ring systems are perpendicular to each other in the top two drawings and lie in the same plane in others. The planar forms assumed by emetine show a marked resemblance to phenanthrene-based molecules, cryptopleurine (I) and tylocrebrine (II) (cf. the lower drawings in this figure with VII and VIII).

For example, compounds which were found to be active in our system can be arranged in the order of diminishing activity as follows (cf. Table 1): cryptopleurine I > tylocrebrine II > cephaeline XIV > emetine IX > 2,3-dehydroemetine XV > tubulosine XIII > NSC 134754 XVI > NSC 134756 XVII.

If we assume that the flat electrodeficient surface is the anchoring site for these compounds, then the more rigidly planar cryptopleurine (I) and tylocrebrine (II) should be more active than emetine (IX) and its congeners (XIII–XV), which is what we have observed. Similarly, the additional OMe in tylocrebrine (II) may force the two aromatic rings out of the ideal plane slightly, and this explains the lower activity of tylocrebrine (II) as compared to cryptopleurine (I). The 2,3 double bond in 2,3-dehydroemetine (XV) restricts the freedom of assuming "angular" conformations while making ring C somewhat flatter, but the system can still become as planar as emetine itself, so the activity remains almost unchanged. Tubulosine (XIII), however, deviates from planarity, which is probably the reason for a slightly diminished activity. The effect of electron-releasing substituents in the aromatic rings can be followed in cephaeline (XIV) and compounds XVI and XVII. Cephaeline (XIV) is slightly more active than emetine (IX) or 2,3-dehydroemetine (XV) because one OMe becomes OH, a better electron-releasing group, while the complete removal of both OMe groups in either ring A (XVII) or ring E (XVI) greatly reduces the activity, thus suggesting that both aromatic rings are required for effective binding.

To confirm the necessity of the two aromatic rings (of the phenantherene type) as well as the distance a and the angle δ in the active site (see triangle in VIII), the cross-resistance of several compounds which differed in these latter aspects was examined. This group of compounds included cularine (XXX), dicentrine (XXXI), *O*-methylbulbocapnine (XXXII) and its dimer XXXIII, glaucine (XXXIV), liriodenine (XXXV), pontevedrine (XXXVI), corunnine (XXXVII), isocorydine (XXXVIII), uvariopsine (XXXIX), allocryptopine (XI), magnoflorine (XLI), (+)-thalicarpine (XLII), and erybidine (CXLIII). As expected, the results were negative for all of these compounds (cf. Table 2).

Similarly, no cross-resistance (cf. Table 2) was exhibited by compounds which differed even more in the overall structure, such as reserpiline (XLIV), colchicine (XLV), and brucine (XLVI). This argues against the simplistic model that a benzoisoquinoline (like the A,B,C ring system of emetine) may be the active moiety (1). The present model was tested further by examination of the activity of various representatives of both the isoquinoline and the benzoisoquinoline classes of compounds (cf. Table 2): *isoquinoline*—laudanosine (XIX), hydrastine (XX), ethaverine (XXI), octaverine (XXII), papaverine (XXIII), tetrabenazine (XXIV), pavine (XXV), aregmonine (XXVI), and phaenthine (XXVI); *benzoisoquinoline*—tetrahydropalmatine (XXVIII), capaurine (XXVIII), and corydaline (XXIX). None of these compounds, as expected from the model, exhibited any cross-resistance to the Emt^r mutants. An additional indication that the benzoisoquinoline system itself is not sufficient for protein synthesis inhibitory activity was the inactivity in the cross-resistance test of compound XLVII, although it has all the necessary features of the more complex half of the emetine molecule.

That the planar geometry of the molecule of emetine as a whole plays a role in assuming the "active" shape was demonstrated by the complete loss of cross-resistance activity by 1,2-secoemetine (XLVIII), in which the B,C rings become a medium-sized 10-membered ring whose geometry totally differs from that of the parent bicyclic system. Therefore, again the isoquinoline moiety (rings D,E) of emetine is not a sufficient structure for activity (cf. the preceding).

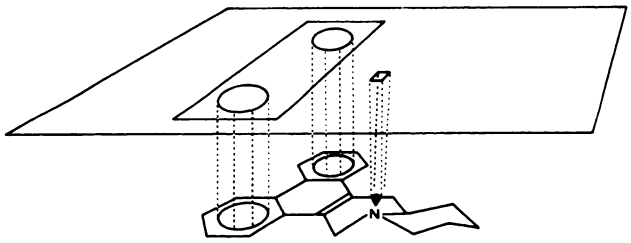


FIG. 2. *A model for the binding of emetine-type agonist to the hypothetical receptor site*

The two aromatic rings (in the same plane) and the nitrogen atom with its free electron at a certain distance from the aromatic rings, which constitute the active determinants in these molecules, are both involved in interaction with the receptor site.

TABLE 2

Toxicity and cross-resistance data on other structurally similar compounds

Compounds	Structure	Relative degree of resistance of cell lines[a]		
		Pro$^-$ (D_{10} M)	Emt^{rI} 41	Emt^{rII} 63
(a) Isoquinolines				
(−)-Argemonine	XXVA	1.0 (1×10^{-4})	1.0	1.0
Ethaverine	XXI	1.0 (2×10^{-5})	1.0	1.0
(+)-Hydrastine	XX	1.0 (>1×10^{-5})	1.0	1.0
(+)-Laudanosine	XIX	1.0 (>2×10^{-4})	1.0	1.0
Octaverine	XXII	1.0 (2.5×10^{-4})	0.8	0.8
Papaverine	XXIII	1.0 (5×10^{-5})	0.7	1.0
(−)-Pavine	XXV	1.0 (>5×10^{-4})	1.0	1.0
(−)-Phaenthine	XXVI	1.0 (1×10^{-5})	1.0	1.0
Tetrabenazine	XXIV	1.0 (2×10^{-4})	1.0	1.0
(b) Benzoisoquinolines				
(−)-Capaurine	XXVIII	1.0 (1×10^{-4})	1.0	1.0
(+)-Corydaline	XXIX	1.0 (1×10^{-4})	1.0	1.0
(+)-Tetrahydropalmatine	XXVII	1.0 (2×10^{-4})	1.0	1.0
(c) Other compounds				
Allocryptopine	XL	1.0 (1×10^{-4})	1.0	1.0
(−)-Brucine	XLVI	1.0 (>1×10^{-5})	1.0	1.0
(−)-Colchicine	XLV	1.0 (2×10^{-7})	1.0	1.0
Corunnine	XXXVII	1.0 (>1×10^{-4})	1.0	1.0
(+)-Cularine	XXX	1.0 (2×10^{-4})	1.0	1.0
(+)-Dicentrine	XXXI	1.0 (3×10^{-5})	1.0	1.0
Erybidine	XLIII	1.0 (3×10^{-4})	1.0	1.0
(+)-Glaucine	XXIV	1.0 (1×10^{-4})	1.0	1.0
(+)-Isocorydine	XXXVIII	1.0 (1×10^{-4})	1.0	1.0
(+)-*O*-Methylbulbocapnine	XXXII	1.0 (1×10^{-4})	1.0	1.0
(+)-*O*-Methylpsychotrine	XVIII	1.0 (5×10^{-5})	0.5	1.0
Compound XXXIII	XXXIII	1.0 (2×10^{-5})	1.0	1.0
Liriodenine	XXXV	1.0 (5×10^{-6})	1.0	1.0
(+)-Magnofluorine	XLI	1.0 (2×10^{-5})	1.0	1.0
Pontevedrine	XXXVI	1.0 (1×10^{-5})	1.0	1.0
(−)-Reserpiline	XLIV	1.0 (3×10^{-4})	1.0	1.0
Uvariopsine	XXXIX	1.0 (3×10^{-6})	1.0	1.0
(+)-Thalicarpine	XLII	1.0 (5×10^{-5})	1.0	1.0
1,2-Secoemetine	XLVIII	1.0 (2×10^{-5})	1.0	1.0
(−)-3-Ethyl-1,3,4,5,6,11b-hexahydro-9,10-dimethoxy-2H-benzo[*a*]quinolizine-2-acetic acid, ethyl ether	XLVII	1.0 (1×10^{-3})	1.0	1.0
(±)-Compound L	L	1.0 (1×10^{-4})	1.0	1.0
(±)-Compound LI	LI	1.0 (5×10^{-5})	1.0	1.0

[a] The relative degrees of resistance were obtained from the ratios of the D_{10} values for the mutant lines as compared to the parental-sensitive line.

The importance of both aromatic rings of emetine being in one plane was further documented by compound LI, whose inactivity could be accounted for by the unnatural configuration of the 2,2a bond making it impossible for the molecule to become planar.

Emetine and its congeners contain two nitrogen atoms in their molecules; however, only the isoquinoline ring nitrogen of IX and XII–XV corresponds to the sole nitrogen in I and II spacewise, so this N atom should be the one which is essential for the activity. This view is confirmed by the behavior of a derivative L. While significant loss of activity would not be expected based on the change in the overall shape of the molecule, the activity is completely lost when the tetrahedral nitrogen becomes planar and its electronic properties are changed accordingly (e.g., lower basicity). On the other hand, in (+)-*O*-methylpsychotrine (XVIII; 22), the molecule cannot assume the required planar form because of the 1′,2a double bond which also places the isoquinoline ring nitrogen outside the required area.

To visualize these arguments, a model of the emetine-type agonist receptor site can be constructed as shown in Fig. 2. It shows the flat area large enough to accommodate both aromatic rings, and if we assume that a metal atom is responsible for the chelation involving methoxylic or hydroxylic oxygen of the agonist, then this would provide one (or several) binding point. Alternatively, we can envisage a sort of metallocene-like bonding between the aromatic rings on one side and the presumed metal. This would provide the necessary stabilization of the drug–receptor complex, at the same time steering the nitrogen atom toward the active site. As the nitrogen itself can easily become involved in protonation (and formation of chelates as well), it may be associated with binding to an electron rich, i.e., electronegative, moiety of the receptor—as, e.g., a carboxylic group.

The present study allows one to draw some conclusions concerning the stereochemistry of tylocrebrine and cryptopleurine, as has already been mentioned. As the cross-resistance phenomenon is extremely sensitive to the spa-

tial relationships, and even small deviations from the required arrangements result in the loss of activity, we believe that the same degree of activity in very closely related compounds implies identical chiralities of the chiral centers of the compounds in question. Thus, the relative configuration of (−)-cryptopleurine is well established, while that of (−)-tylocrebrine is supported mainly by the structural analogy with (−)-cryptopleurine and thermodynamic considerations. The cross-resistance of these two compounds confirms not only that their relative stereochemistry should be identical, but that their absolute chiralities should be identical too. Moreover, provided that the present model of the structural determinant is correct, these absolute chiralities should correspond to that of (−)-emetine and its derivatives as portrayed in formulas X and IX. This aspect is presently the subject of further investigation, and a chemical proof of the absolute stereochemistry of (−)-cryptopleurine as well as (−)-tylocrebrine would confirm the present model of the structural determinant.[1]

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[1] The absolute chiralities of I and II were proposed in a recent publication (24) to be I [(−)-cryptopleurine] and III [(−)-tylocrebrine], i.e., opposite, and this was reflected in the opposite signs of their respective Cotton effects (circular dichroism curves: $[\theta]_{233}$ + 15,000 for I and $[\theta]_{255}$ − 4370 for III). Our sample of tylocrebrine surprisingly has shown the same sign of the Cotton effect as (−)-cryptopleurine I, $[\theta]_{232}$ + 25,000 for I and $[\theta]_{232}$ + 5300 for II, measured with a Jouan Dichrographe II instrument in 95% ethanol [$C_I = 0.63 \times 10^{-3}$; $C_{II} = 0.2 \times 10^{-3}$], suggesting that chiral perturbations of the chromophore resulting in the Cotton effect should be of the same character and therefore the absolute stereochemistry of both compounds should be the same, i.e., as in I and II. An identical absolute configuration was found in antofine (23) (LI) by its degradation to D-proline. This apparent controversy is under investigation at the present time in our laboratories and the results will be published elsewhere shortly.

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