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Molecular modeling study of tubulosine and other related ipecac alkaloids

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Summary

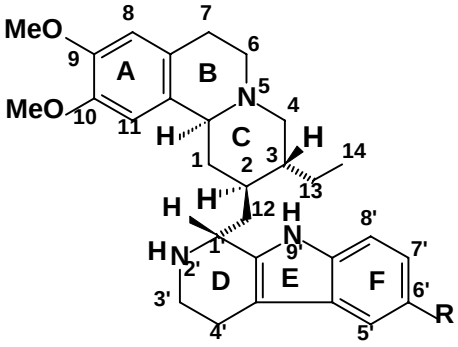
A molecular modeling study of two alkaloids, tubulosine and psychotrine, isolated from the sap of *Pogonopus speciosus*, and other related ipecac alkaloids, showed that these flexible alkaloids favor a nonplanar structure. The biologically active compounds had conformations with a similar angle between aromatic ring A, the nitrogen in ring B, and ring D. This angle was related to the biological activity reported for these compounds. Our results support the hypothesis of two different types of receptor interactions, one for the nonplanar compounds and another for the planar compounds.

Introduction

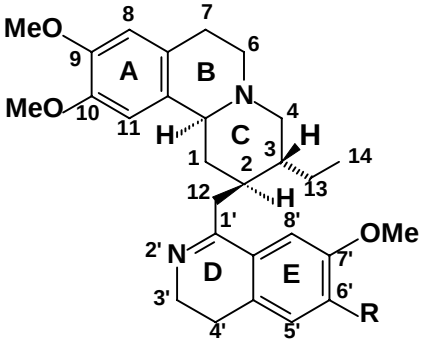
In a previous paper [1] we reported the isolation of two alkaloids, tubulosine and psychotrine, from the sap of *Pogonopus speciosus* K. Schum. (Rubiaceae) Tubulosine **1**, exhibited potent activities in our bioassays including brine shrimp lethality [2] the inhibition of crown gall tumors in potato discs [3] and 9PS, 9KB, human lung, human breast, and human colon cancer tumor cell cytotoxicities [1]. The activity of tubulosine **1** in rodent tumor models studied at the NCI was restricted to leukemic mice, with increase in life span (ILS) percentages of 30% in L1210 leukemia and 80% in P388 leukemia. Other active alkaloids studied were: *O*-methyltubulosine **2** (40–50% ILS in L1210 and 60–80% ILS in P388), emetine **6** (40–235% ILS in L1210 and 50–80% ILS in P388), and cephaeline **7** (30–60% ILS in L1210 and 50% ILS in P388). However, the related alkaloid, psychotrine **4**, did not show any of these activities [4]. Tubulosine **1** and emetine **6** were found to inhibit protein synthesis in mouse fibroblast (3T6) cells and chick embryo fibroblasts by blocking elongation factor-2-dependent translocation and elongation of peptide chains [4]. A study [5] of the structure-activity relationships involving cross-resistance patterns in two emetine-resistant mutants of Chinese hamster ovary cell variants to a large number of compounds showed that the ipecac alkaloids, tubulosine **1**, emetine **6**, cephaeline **7**, dehydroemetine **10**, the phenanthroquinolizidine-type alkaloid cryptoleurine **11**, the phenanthroquinazolidine-type alkaloid tylocrebrine **12**, and other related compounds possess protein inhibitory activity, while psychotrine **4**, *O*-methylpsychotrine **5**, isotubulosine **8**, isoemetine **9**, and secoemetine **13** lack this effect. The previous study concluded that the aforementioned compounds have the same site of action and that the critical structural requirement for biological activity is a relatively planar molecule with two aromatic rings. The x-ray crystallographic results in our previous report [1] indicated, however, that the structure of tubulosine **1** was not planar [1]. Furthermore, a reported molecular modeling study for tubulosine **1**, emetine **6**, and usambaresine **14** indicated a common, nonplanar conformation for these three alkaloids [6].

Materials and methods

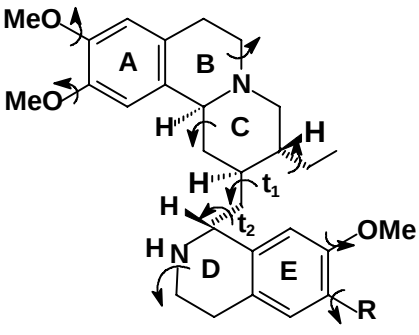
The compounds selected for this study are shown in Figure 1. All computations were performed on a CAChe (computer aided chemistry) workstation run-



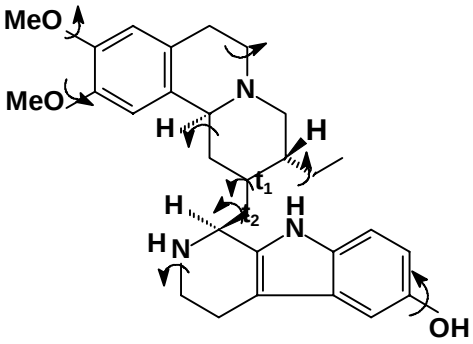
Tubulosine 1 R = OH
O-Methyltubulosine 2 R = OMe
Deoxytubulosine 3 R = H



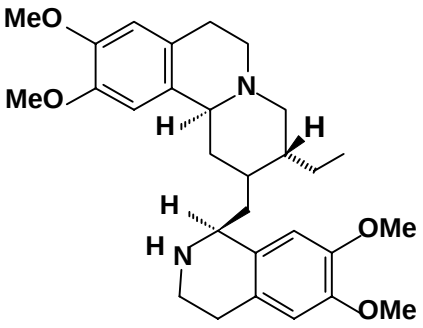
Psychotrine 4 R = OH
O-Methylpsychotrine 5 R = OMe



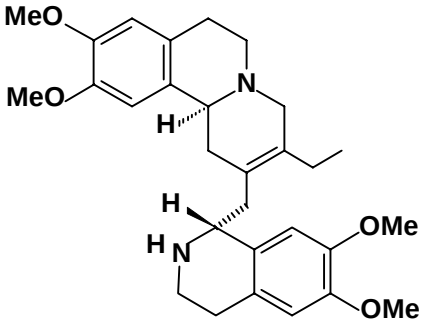
Emetine 6 R = OMe
Cephaeline 7 R = OH



Isotubulosine 8

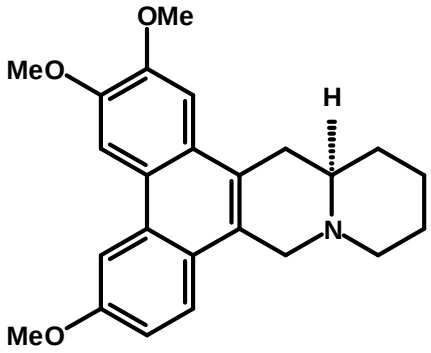


Isoemetine 9

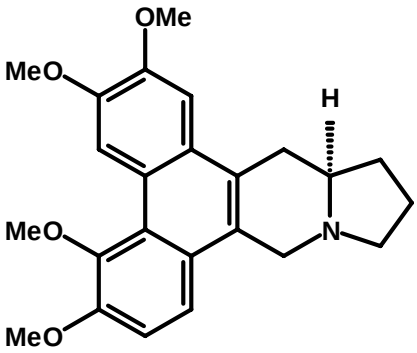


Dehydroemetine 10

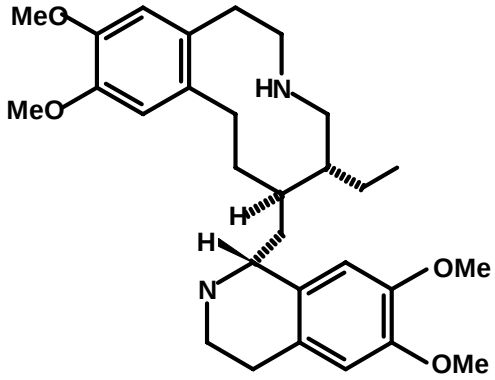
Figure 1. Structures of emetine and analogs.



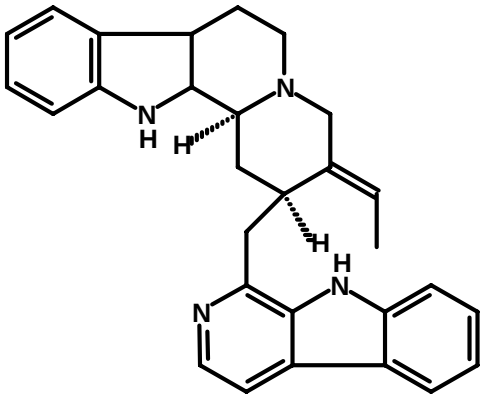
Cryptoleurine **11**



Tilocrebrine **12**



Secoemetine **13**



Usambaresine **14**

Figure 2. Structures of other emetine analogs.

ning CAChe Scientific proprietary software, version 3.5.1 [7].

Three-dimensional models of the different alkaloids were constructed using the molecular editor of the CAChe system. These structures served as starting points in an extensive conformational analysis of each compound used to define the possible requirements for binding to the receptor. We initially minimized each structure using Molecular Mechanics (MM) [8]. In order to obtain conformations around the global minimum of the compounds and to study the flexibility of the alicyclic ring(s), a sequential search was run (angles searched are marked in Figure 1). The sequential search results were analyzed and the conformations that differed by less than 5 kcal/mol from the lowest energy conformation were reminimized using MM [8]. The lowest energy conformation identified by this process was then used to run a Molecular Dynamics [9] simulation at 900 K for 100 ps with a time step of 1.0 fs, and equilibration period of 1.0 ps. Solvent molecules were not included in the calculations. The Trajectory file was analyzed and conformations less than 5 kcal/mol from the minimum energy conformation were chosen, then the Molecular Dynamics simulation was run at 300 K for 100 ps with a time step of 1.0 ps. The Trajectory file was

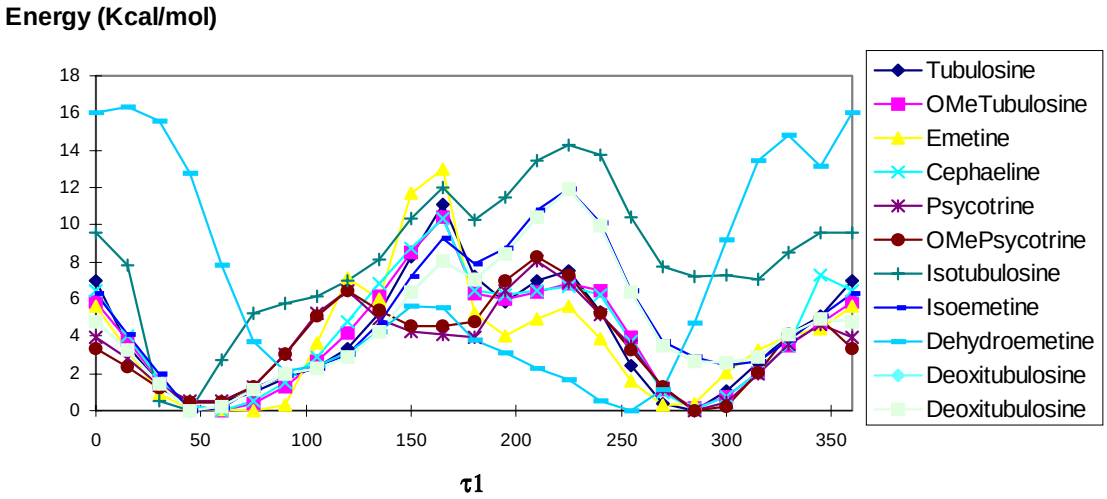


Figure 3. Torsion angle τ_1 versus energy of emetine type analogs.

analyzed and conformations less than 5 kcal/mol from the minimum energy conformation were chosen, these conformations were minimized using MM [8], and the conformation with the lowest energy was selected to perform the conformational analysis.

A conformational study was then made of the flexibility of this molecule around angles τ_1 and τ_2 (Figure 1) using MM [8]. The dihedral angles were investigated by performing geometry searches over the range 0 to 360°, at 15° resolution with minimization at each step by the block diagonal Newton Raphson (in some cases, searching an angle in both directions, once from 0 to 360° and once from 360° to 0°, to identify the lowest energy).

CAChe molecular mechanics uses Allinger's MM2 force field [8]. The molecular mechanics calculations were investigated in the CAChe system by minimizing the total molecular energy according to the molecular mechanics expression:

$$E_{\text{total}} = E_{\text{bonding}} + E_{\theta} + E_{\phi} + E_{\text{improp}} + E_{\text{elec}} + E_{\text{vdW}} + E_{\text{hb}},$$

where E_{bonding}, E_{θ}, E_{ϕ}, E_{improp}, E_{elec}, E_{vdW}, E_{hb} indicate bond lengths, bond angles, dihedral angles, improper torsions, electrostatic potential, van der Waals interactions and hydrogen bonding, respectively. Further, MM calculates energies at 0 K relative to a hypothetical 'perfect' geometry, rather than an absolute energy [10].

Finally the low-energy conformations of the studied analogs where superimposed using as a reference the common interesting atoms N-5 in alicyclic ring B, C9 in aromatic ring A, and N2′ in ring D.

Results and discussion

The alkaloids studied can be divided into two categories based on their structures; first, the emetine analogs: tubulosine **1**, *O*-methyl tubulosine **2**, deoxytubulosine **3**, psychotrine **4**, *O*-methylpsychotrine **5**, emetine **6**, cephaeline **7**, isotubulosine **8**, isoemetine **9** and dehydroemetine **10**, compounds with similar alicyclic rings A, B, and C (structures are given in Figure 1). The second set includes the secoemetine **13** (the alicyclic rings have been replaced by a macrocyclic ring) and the phenanthroquinolizidine analogs, cryptoleurine **11** and tylocrebrine **12**, that were included for comparative purposes (structures are given in Figure 2).

Since the analysis of the requirements for binding to the receptor is based on the structures found in the conformational search, this step must be carried out using a technique that assures as many minima as possible in an objective and efficient manner. For this reason we used for the first set (emetine analogs **1** to **10**) the combination of a sequential search using MM, followed by Molecular Dynamics at high and low temperature, followed again by minimization, which provides a rapid way to scan the conformational space.

In order to perform the conformational analysis for the emetine analogs, a sequential search was first performed taking into account the angles depicted in

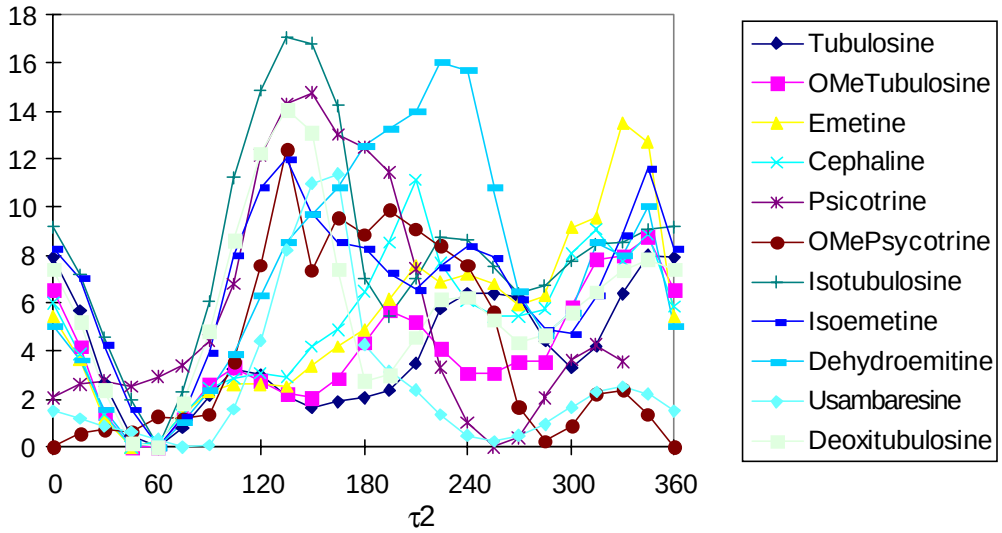


Figure 4. Torsion angle τ_2 versus energy of emetine type analogs.

Figure 1, over the range of −80 to 80° with minimization every 40° in the alicyclic rings, and over the range of −180° to 180° with minimization every 30° for the rest of the angles. Following identification of low energy structures for the different compounds, the lowest energy conformation chosen for each one of them was subjected to molecular dynamics simulation at high temperatures. Molecular dynamics is used to overcome energy barriers, a technique frequently used to explore systematically the conformational space of cyclic compounds [11–14]. The search for reasonable structures of large molecules benefits most from dynamics simulation because it is often impractical to compute energy maps of geometrical searches for large molecules.

The lowest energy conformation after running MM and MD calculations was used to perform the geometrical searches around the rotatable bonds τ_1 and τ_2 shown in Figure 1. These two angles measure the freedom between the block of rings A, B, and C and the rest of the molecule. The outcomes for τ_1 and τ_2 geometrical searches are given in Figures 3 and 4.

Sequential search and molecular dynamic simulations were performed for analogs **11** and **12** to obtain the lowest energy conformations.

Once the conformational search was completed, distances and angles common to the active analogs were identified, using low energy conformations of each compound (5 kcal/mol around the lowest energy conformation). These low energy conformations were in the same family. Some common interesting atoms in the emetine analogs include the N in alicyclic ring B, C9 in aromatic ring A, and N2′ in ring D. These atoms are possible points for interaction with the receptor region. None of the studied analogs favor planar minimum energy conformations. Thus, this study did not support the previously reported requirement of a planar structure for the activity of the ipecac alkaloids. That proposal, made by Gupta's group [5], was based on the comparison of the ipecac alkaloids, whose structures are more flexible, with cryptoleurine **11** and tylocrebrine **12**, whose structures are more rigid. Their main assumption was that cryptoleurine **11** and tylocrebrine **12** bind at the same site as the emetine type alkaloids because the cross-resistance patterns of these compounds were similar. It has been shown, however, that high affinity exists for {14a-^{3}H} cryptoleurine **11** with 80S and 40S ribosomes of yeast, but emetine **6** and tubulosine **1** had no such affinity [15]. This fact weakens the rationale of Gupta et al. [5] and indicates the possibility that emetine type alkaloids bind at a different site.

For the final analysis we measured distances between the key atoms C9, N2′ and N5, and angle C9-N5-N2′. The mean distances between the key atoms of at least five low energy conformations of each compound are shown related to the biological activity, in Tables 1 and 2. Active compounds have similar distances between the C9 and N2′ atoms (between 7 and 8.2 A). The inactive compounds, isotubulosine **8** and isoemetine **9**, also had distances in this range, but they have the 1′*S* configuration, and isomers must have the

Table 1. Mean values for distances (in Å) between relevant atoms and angle C9-N5-N2′, correlated with increase in life span (ILS) of leukemic mice studied at the National Cáncer Institute

Compound		Energy	C9–N2′	C9–N5	N5–N2′	C9–N5-N2′ angle	ILS in 1210 leukemia	ILS in P388 leukemia
Tubulosine	Mean	15.96	7.13	5.10	7.23	110.38	30%	80%
	Standard deviation	1.14	0.77	0.01	0.88	8.48		
OMeTubulosine	Mean	20.94	8.02	5.10	5.88	94.71	40–50%	60–80%
	Standard deviation	0.90	0.58	0.49	0.81	1.03		
Emetine	Mean	18.60	8.26	5.09	5.91	96.88	40–235%	50–80%
	Standard deviation	0.91	0.41	0.00	0.27	3.17		
Cephaeline	Mean	9.72	8.12	5.10	5.89	97.98	30–60%	50%
	Standard deviation	1.00	0.44	0.00	0.28	3.52		
Psychotrine	Mean	12.01	9.18	5.09	5.64	117.37	Inactive	Inactive
	Standard deviation	1.35	0.30	0.01	0.43	1.99		

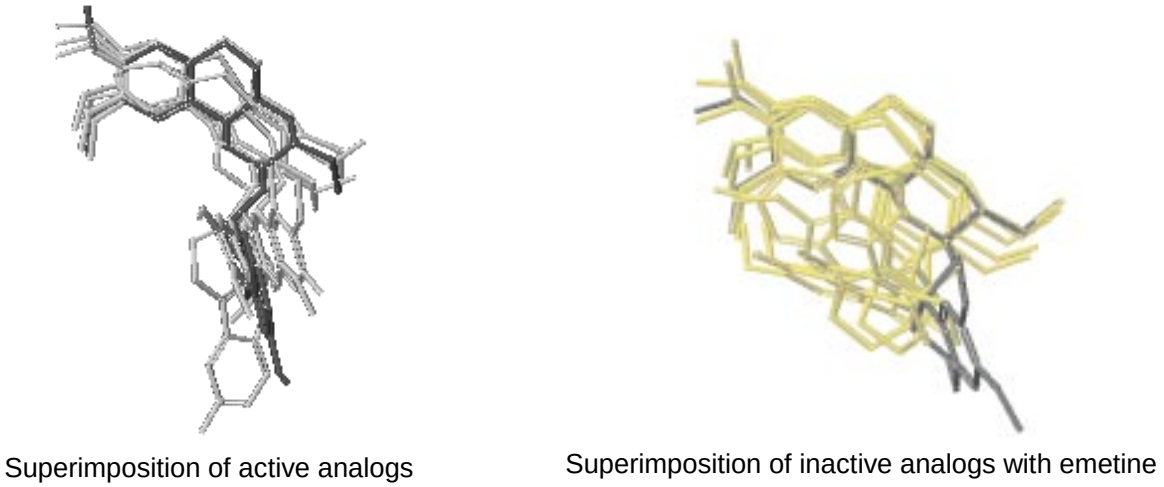


Figure 5. Superimposition of active analogs (in gray) with emetine (reference compound, in black) and superimposition of inactive analogs (in gray) with emetine (in black).

1′*R* configuration in order to show biological activity [4]. Active compounds have similar distances between the N5 and N2′ atoms (between 5.9 and 7.2). The angle formed between C9, N5 and N2′ also shows differences between the active and inactive emetine analogs as can be seen in Tables 1 and 2. Although the angular differences are not statistically significant when the standard deviations are taken into account, this angle has a range between 92.7–110° for the active analogs, and together with the atomic distances reported, makes possible that the active analogs occupied a common space when superimposed (Figure 5).

The structure of dehydroemetine has a C2–C3 double bond, upon superimposition of the active analogs (based in atoms C9, N5 and N2′), it was found that dehydroemetine superimposes in the space occupied by the active analogs. The superimposition of all the active analogs, including dehydroemetine is shown in Figure 5.

Table 2. Mean values for distances (in Å) between relevant atoms and angle C9-N5-N2′, correlated with the inhibition of protein synthesis reported by Suffness and Cordell [4]

Compound		Energy	C9–N2′	C9–N5	N5–N2′	C9–N5-N2′ angle	Inhibition of protein synthesis
Tubulosine	Mean	15.96	7.13	5.10	7.23	110.38	Active
	Standard deviation	1.14	0.77	0.01	0.88	8.48	
Emetine	Mean	18.60	8.26	5.09	5.91	96.88	Active
	Standard deviation	0.91	0.41	0.00	0.27	3.17	
Deoxitubulosine	Mean	15.33	7.58	5.09	5.84	92.73	Active
	Standard deviation	1.83	0.96	0.01	0.88	1.23	
Cephaeline	Mean	9.72	8.12	5.10	5.89	97.98	Active
	Standard deviation	1.00	0.44	0.00	0.28	3.52	
Isoemetine	Mean	16.11	7.67	5.10	5.54	89.65	Inactive
	Standard deviation	2.00	0.52	0.01	0.51	2.48	
Isotubulosine	Mean	11.45	7.59	5.13	5.67	89.14	Inactive
	Standard deviation	2.17	0.38	0.01	0.25	3.42	
OMePsychotrine	Mean	24.28	6.98	5.08	4.76	90.25	Inactive
	Standard deviation	1.57	0.24	0.01	0.46	1.69	

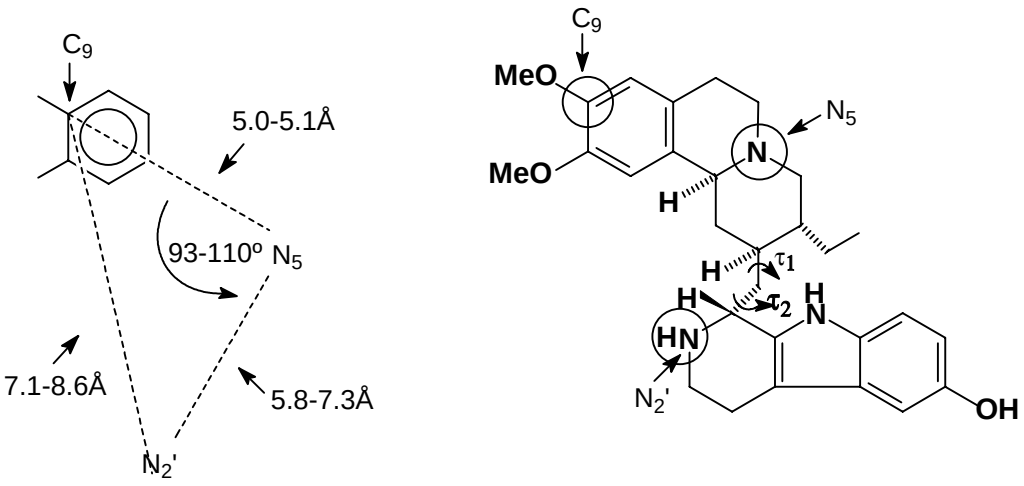


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Figure 6. Structural requirements for biological activity in emetine type alkaloids.

Finally, the active analogs were superimposed, using the atoms C9, N5 and N2′, with the inactive analogs, and it was found that a region between the two blocks of rings was occupied only in the inactive analogs. It was deduced that this region must be unoccupied in order to elicit biological activity. For example, the superimposition of all the active analogs including emetine, and the superimposition of the inactive analogs with emetine is shown in Figure 5.

Conclusions

The structural overlaps allowed the identification of a region that must be occupied in the receptor in order

to produce biological activity. Based on the molecular modeling and biological data, the following conclusions are proposed: (1) A planar structure may not be the critical feature for activity since all the ipecac alkaloids favor a nonplanar structure. (2) The site of action of the emetine analogs may be different from that of tylocrebrine and cryptoleurine since these two compounds do favor a planar structure and they do not have a good superimposition with the emetine tipe analogs. (3) The *R* configuration at C1′ is important for emetine type analogs activity, since all the studied *R* isomers are active and all the studied isomers with *S* configuration are inactive. (4) The proposed pharmacophore of the ipecac alkaloids could be outlined with the following general requirements: a distance between C9 and N2′ approximately 7–8.2 Å, a distance between C9 and N5 approximately 5.0–5.1 Å, a distance between N5-N2′ approximately 5.9–7.2 Å and an angle formed by C9-N5-N2′ in a range between 92.7–110°, schematically represented in Figure 6.

Acknowledgements

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