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Lipophilicity of vinpocetine and related compounds characterized by reversed-phase thin-layer chromatography

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Abstract

A reversed-phase thin-layer chromatographic method was developed and applied to quantitate the lipophilicity of sparingly water-soluble eburnane alkaloids of therapeutic interest. Our method development included calibration, optimization and validation procedures, using also sets of auxiliary compounds. The log P_{TLC} values of five relatively hydrophilic eburnanes were verified by stir-flask studies. The alkaloids were found to have lipophilicity values in the 2.9–4.8 log P_{TLC} range. Conclusions on structure–lipophilicity relationships were drawn in terms of ring anellation, character and length of side chain, conformational preferences and moiety–solvent interactions, also supported by molecular mechanics studies.

Keywords: Lipophilicity; Partition coefficients; Vinpocetine; Vincamine; Apovincaminic acid; Alkaloids

1. Introduction

Lipophilicity is a molecular property of solute–solvent interactions, characterized generally in terms of partition coefficients. Its applications include such apparently diverse fields as the design of drugs for targeted delivery and development of chromatographic separations. Lipophilicity-based pharmacokinetic research has recently gained wide recognition by international conferences and books devoted entirely to partition properties [1–3]. In fact, the optimisation of lipophilicity is the leading principle in development of drugs acting in the central nervous system, a highly lipoid medium. On the other hand, partition is the theoretical background of several liquid and planar chromatographic methods and high throughput selection techniques for fast assortment of new chemical entities [4].

In order to quantitate lipophilicity, the commonly accepted parameter is log *P*, the logarithm of the partition coefficient. It is the concentration ratio of a solute in a single electrical state, being in equilibrium between two immiscible solvents. Octanol is the most often used organic solvent, and the octanol–water partition coefficient is the prime descriptor of lipophilicity in quantitative structure–activity relationship (QSAR) studies [5]. When basic/acidic species are present in the solution, the observed ratio of concentrations becomes pH-dependent and is named the distribution coefficient (*D*) or apparent partition coefficient [6].

Vincamine, vinpocetine and related eburnane de-

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rivatives are valuable therapeutic agents mainly in cardiovascular and cerebral insufficiencies [7]. The pharmacological activity is certainly bound to the fused, five-membered eburnane ring system (Fig. 1). Vinpocetine has become a reference compound in the pharmacological research of cognitive deficits in the central nervous system caused by hypoxia and ischaemia [8]. The medicinal success of vincamine, vinpocetine, vinburnine, brovincane and related compounds has inspired continual synthetic efforts to produce further derivatives of *cis*-D/E ring anellation, i.e., 3α-H,16α-ethyl: (3*S*,16*S*) configuration and recently, those of *trans*-D/E ring anellation [9,10].

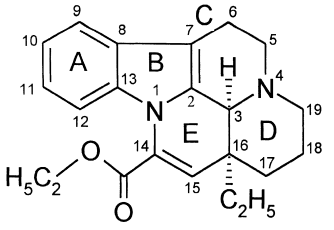
Despite the decades-long therapeutic use of eburnanes, and the well-known pharmacokinetic significance of lipophilicity and basicity, scarce physicochemical data appeared on any of these compounds. The obvious reason is the typically poor water-solubility of eburnanes arising from the rigid ring system and the relatively small number of polar groups. In our recent proton speciation study, however, the aqueous acid–base properties were elucidated indirectly by the extrapolation of protonation constants in methanol–water mixtures of gradually changing solvent composition [11]. The absorption of vinpocetine and apovincaminic acid was studied in rats by in situ loop experiments [12]. The same study included lipophilicity measurements of vinpocetine by gas chromatography at 37 °C reporting surprisingly low values as opposed to a skin permeation study [13] where the octanol–water partition coefficient, determined by high-performance liquid chromatography (HPLC) at 37 °C, was much higher (log P=3.56). Further literature data relate to the *o*-xylene–water and benzene–water partition coefficients of vinpocetine [14]. Nevertheless, no comprehensive study with comparable lipophilicity data appeared on this family of compounds.

Here we report the experimental log P determination of 10 eburnane alkaloids, including drugs and drug candidate molecules to interpret or predict their pharmacokinetic behaviour and structure–activity relationships as a function of molecular properties. The 10 compounds (Fig. 1) vary in the configuration of the C(3) carbon, saturation of the C(14)–C(15) bond, the character and chain-length of the substituent of the C(14)-carboxylic acid group. Vinpocetine and vincamine, so far the best-known molecules, have *cis*-D/E ring anellation, i.e., 3α-H,16α-ethyl: (3*S*,16*S*) configuration. Six of the compounds constitute *cis*- and *trans*-D/E anellation epimeric counterparts, and four of the compounds can be sorted into two groups of C(14)-configurational diastereomeric pairs.

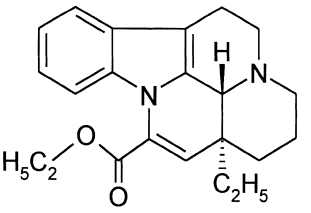
Several methods have been described for the direct determination of the distribution coefficient. The best-known ones are the shake-flask and stir-flask techniques, centrifugal partition chromatography, dual-phase potentiometry, cyclic voltammetry. Their advantages and limitations have recently been surveyed [3]. The alternative indirect methods are the chromatographic ones, reversed-phase (RP) HPLC and RP-thin-layer chromatography (TLC). The latter can be applied when a linear relationship can be proven between chromatographic capacity-factor data and directly measured log D values [15]. Such a linear relationship exists whenever the separation mechanism is partition of the analyte between the mobile and stationary phases. RP-TLC is rapid, easy to perform, requires small quantities of the samples and allows simultaneous analysis of several compounds. Furthermore, the test compounds need not be pure and a quantitative determination of their concentration (often posing analytical problems) is not necessary [16]. RP-TLC is especially suitable to investigate lipophilic substances (log $P>4$) with low water solubility.

Due to solubility problems we could not characterize the lipophilicity of all the 10 compounds with direct methods. Therefore we chose the RP-TLC method where simultaneous determination of the lipophilicity values assures the comparability of the results. The calibration set used to establish the actual linear relationship ($\log P = aR_M + b$) between the traditional log P and the chromatographic R_M data included five pyrido[1,2-*a*]pyrimidines [17,18], progesterone and chlorpromazine, compounds of three different structural categories (Fig. 2).

In order to move the highly lipophilic compounds from their starting point an organic co-solvent of sufficient eluting power had to be added to the mobile phase. The use of methanol was insufficient, therefore acetone was chosen as the co-solvent [19–



(3S,16S) ethyl *cis*-apovincaminate (1) (vinpocetine)



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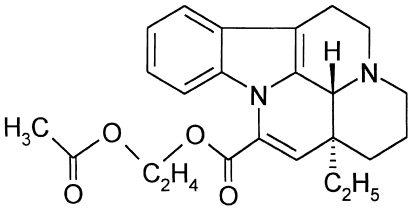

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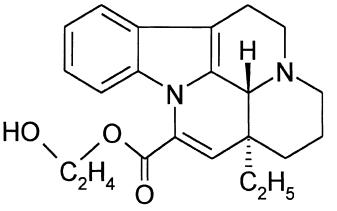

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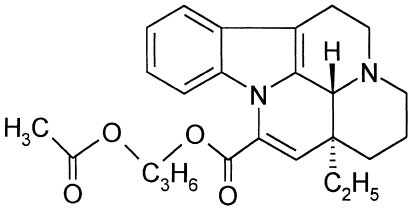
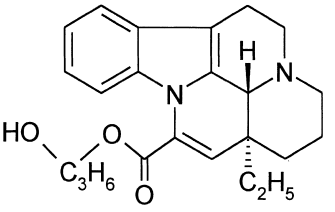
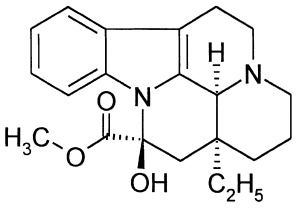


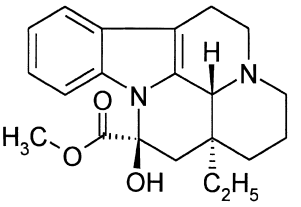
(3R,16S) (2-acetoxy)-propyl *trans*-apovincaminate (5)



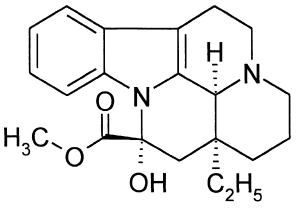
(3R,16S) (2-hydroxy)-propyl *trans*-apovincaminate (6)



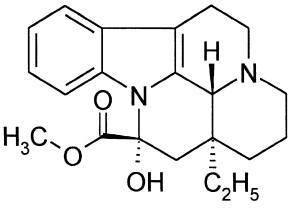
(3S,14S,16S) vincamine (7)



(3R,14S,16S) *trans*-vincamine (8)



(3S,14R,16S) epivincamine (9)



(3R,14R,16S) *trans*-epivincamine (10)

Fig. 1. Structures of vinpocetine and related compounds.

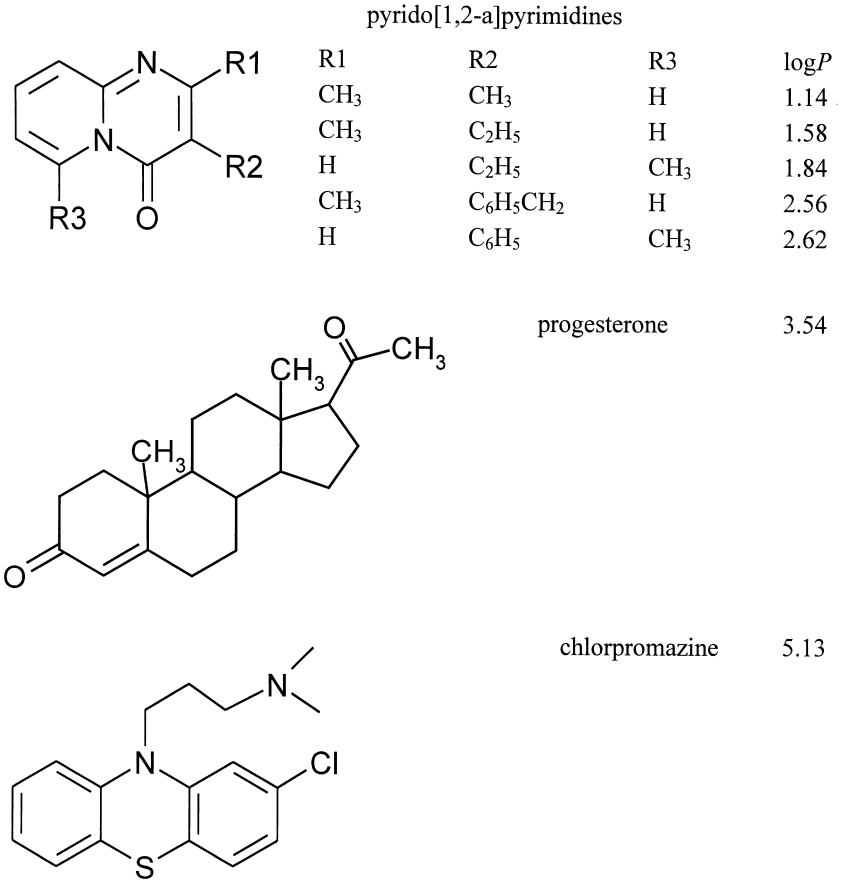


Fig. 2. Structures of the compounds in the calibration set and their log *P* values.

21]. Since the compounds are bases, the aqueous component of the mobile phase was 0.1 *M* NaOH to make the compounds migrate in their unprotonated form. For all the 17 compounds, R_M values have actually been obtained as extrapolated R_{M0} ones [19,22], due to the higher accuracy of the latter, as has previously been proven [15,23,24]. The extrapolated R_{M0} values correspond to an eluent of no organic co-solvent. Extrapolation for each compound resulted from seven TLC experiments of gradually changing mobile phase acetone content in the 35–65% (v/v) concentration range in 5% increments.

The accuracy of the method was tested by stir-flask log *P* determination on compounds with sufficient water-solubility for this traditional technique. Interpretation of the results was supported by molecular mechanics calculations.

2. Experimental

2.1. Materials

The 10 eburnane alkaloids were produced and purified by methods described earlier [9,10]. The synthesis of the five pyrido[1,2-*a*]pyrimidines was also described elsewhere [25]. Progesterone and chlorpromazine were of pharmacopoeial grade. Acetone and octanol were purchased from Fluka. All other reagents were of analytical grade. Freshly

boiled distilled water was used for the preparation of the solutions.

2.2. Partition coefficient measurements by the RP-TLC method

The TLC experiments were performed on 20 cm× 20 cm chromatography plates pre-coated with 0.25 mm layers of silanized silica gel $60F_{254}$ (Merck, Germany, article 5747). Before applying the spots the plates were dipped into a 0.1 *M* solution of NaOH in methanol to deprotonate the residual silanol groups. Then the plates were heated at 160 °C for 30 min. A 1-mg amount of the compounds was dissolved in 0.2 ml methanol and 2 μl of these solutions was spotted onto the plate in triplicate (Camag Nanomat, Switzerland). The paper-lined chromatography chamber (Desaga, Germany) was saturated with the actual mobile phase at 27 °C for at least 60 min before use. After development (150 mm) the plates were dried and the spots were detected by densitometry (λ=279 nm, Shimadzu, CS-9301PC).

2.3. Partition coefficient measurements by the stir-flask method

Stock solutions ($3\cdot10^{-3}$ *M*) of the drug molecules were prepared using octanol-saturated water. A 0.6-ml volume of these stock solutions was diluted to 6 ml with buffers containing citric acid, phosphoric acid and lysine. The pH was measured using a Radiometer pHC2406 combined pH electrode and a Radiometer pHM93 reference pH meter. After collecting samples from the aqueous phase for the subsequent UV-absorbance measurements, water-saturated octanol was added. Then the two phases were intensively stirred for 2 h in thermostated double-walled glass cells at constant temperature (25±0.1 °C). After separation of the equilibrated phases (by centrifugation at 500 *g* for 10 min) the concentration of the solute was determined in the aqueous phase by UV spectrophotometry (Perkin-Elmer Lambda 15) at several values above 250 nm around the λ_{max}. The distribution coefficients were calculated from the absorbance of the molecules in the aqueous phase before and after partitioning, respectively. The standard deviation of the measurements was within 0.1 log units.

2.4. Molecular mechanics calculations

The geometry of the conformers of *cis*-vinpocetine, *trans*-vinpocetine and the four diastereomers of vincamine was optimized by applying the MMFF94s force field. The charges were calculated by the MMFF94 method. The dielectric function was distance dependent, the dielectric constants were those of water (ε=78.5) and octanol (ε=9.9). An SGI media recorder was used to generate the three-dimensional models of *cis*- and *trans*-vinpocetine.

3. Results

The R_M values decreased linearly with increasing acetone content of the mobile phase. Table 1 shows the R_{M0} (intercept), *b* (slope), *s* (standard error) and *r* (correlation coefficient) values obtained for the eburnane alkaloids and compounds in the calibration set. The average standard deviation of R_{M0} values of different days was 0.049.

The following calibration equation was obtained using the five pyrido[1,2-*a*]pyrimidines in the calibration set:

$$\log P = 1.157R_{M0} + 0.4223 \quad s_{sl} = 0.0664,$$
$$s_{in} = 0.09346, s = 0.07285, n = 5, r = 0.9951$$

where s_{sl} and s_{in} are the standard deviations of the slope and the intercept, respectively; *s* is the standard error, *n* is the number of data points and *r* is the correlation coefficient. The standard error and correlation coefficient confirm that the separation mechanism is partition of the analyte between the mobile and stationary phases.

The log P_{TLC} and log $P_{shake\text{-}flask}$ values showed an excellent agreement for chlorpromazine (log P_{TLC} = 5.07, log $P_{shake\text{-}flask}$ = 5.13) and a sufficiently good one for progesterone (log P_{TLC} = 3.75, log $P_{shake\text{-}flask}$ = 3.54). These compounds were added therefore to the calibration set. The unified calibration equation is as follows:

Table 1
Extrapolated R_{M0} (intercept), b (slope), s (standard error) and r (correlation coefficient) values of eburnane alkaloids and compounds in the calibration set

Compound	R_{M0}	b	s	r
Ethyl *cis*-apovincaminate	3.325	−0.0496	0.04956	−0.9964
Ethyl *trans*-apovincaminate	3.817	−0.0563	0.04467	−0.9978
(2-Acetoxy)-ethyl *trans*-apovincaminate	3.562	−0.0543	0.05127	−0.9968
(2-Hydroxy)-ethyl *trans*-apovincaminate	2.795	−0.0454	0.04950	−0.9958
(3-Acetoxy)-propyl *trans*-apovincaminate	3.748	−0.0565	0.04861	−0.9974
(3-Hydroxy)-propyl *trans*-apovincaminate	3.024	−0.0486	0.05726	−0.9951
Vincamine	2.279	−0.0366	0.03580	−0.9966
trans-Vincamine	2.253	−0.0376	0.03542	−0.9968
Epivincamine	2.200	−0.0363	0.03723	−0.9963
trans-Epivincamine	2.505	−0.0399	0.03379	−0.9974
2,3-Dimethyl-pyrido[1,2-*a*]pyrimidine	0.689	−0.0213	0.03792	−0.9890
3-Ethyl-2-methyl-pyrido[1,2-*a*]pyrimidine	0.970	−0.0232	0.03395	−0.9924
3-Ethyl-6-methyl-pyrido[1,2-*a*]pyrimidine	1.152	−0.0241	0.03316	−0.9933
3-Benzyl-2-methyl-pyrido[1,2-*a*]pyrimidine	1.876	−0.0343	0.03941	−0.9953
6-Methyl-3-phenyl-pyrido[1,2-*a*]pyrimidine	1.909	−0.0338	0.03963	−0.9951
Progesterone	2.880	−0.0463	0.05398	−0.9952
Chlorpromazine	4.020	−0.0552	0.05694	−0.9962

$$\log P = 1.147R_{M0} + 0.4188 \quad s_{sl} = 0.03815,$$
$$s_{in} = 0.08451, s = 0.1102, n = 7, r = 0.9972 \qquad (2)$$

Eq. (2) was used for the determination of log P_{TLC} values of the eburnane alkaloids. Their values are listed in Table 2.

Reliability of the measured log P_{TLC} values is further justified by the log P values of vinpocetine, *trans*-vinpocetine and three vincamine epimers that could be partly examined by the stir-flask method. Due to the limited solubility of their neutral form, distribution coefficients could be determined in acidic solutions only, where the neutral form is the minor one. Nevertheless, with the knowledge of the K protonation constant [11] and the pH of the solution the log P values could be calculated for both the neutral and the cationic forms based on the $D = x_N P_N + x_C P_C$ relationship, where x is the molar fraction of the given species. When selecting the log D values for the calculation of the log P of the neutral (log P_N) and cationic (log P_C) form, values as alkaline and acidic as possible were chosen, respectively. Since partition of the cationic forms depends on the ionic environment in the solution [26–28], the calculated log P_C values are constants under conditions of a given buffer composition and ionic strength. The calculated log P_N and log P_C values, the relative concentration and the relative contribution of the neutral form are listed in Table 3. Partition coefficients of the neutral form exceed that of the cationic one by at least four orders of magnitude in each case. This is why the neutral form is the major contributor to the overall partition, even at low pH, where its relative, aqueous concentration is in the apparently negligible, 10^{-2}% range.

Table 2
Experimental log P values of eburnane alkaloids determined by RP-TLC

Compound	Log P_{TLC}
Ethyl *cis*-apovincaminate (vinpocetine)	4.23
Ethyl *trans*-apovincaminate (*trans*-vinpocetine)	4.80
(2-Acetoxy)-ethyl *trans*-apovincaminate	4.50
(2-Hydroxy)-ethyl *trans*-apovincaminate	3.62
(3-Acetoxy)-propyl *trans*-apovincaminate	4.72
(3-Hydroxy)-propyl *trans*-apovincaminate	3.89
Vincamine	3.03
trans-Vincamine	3.00
Epivincamine	2.94
trans-Epivincamine	3.29

Comparing the log P values of the five alkaloids where both stir-flask and RP-TLC determinations were carried out the average deviation is 3.4%, a fairly small value in light of the reported, typically 5–10% method disagreement between RP-TLC and traditional direct techniques [24]. The difference can

Table 3
Experimental log D values determined by the stir-flask method, calculated log P values of the neutral and cationic species, relative concentration and contribution of the neutral species

pH	Log D	Relative concentration (%)	Relative contribution (%)
Vinpocetine	Log K[a] = 7.69	Log P_N = 4.45	Log P_C = −0.249
2.29	−0.17	0.0004	16.6
3.29	0.43	0.0040	66.6
3.65	0.67	0.0091	82.0
4.22	0.99	0.0339	94.4
5.20	1.96	0.323	99.4
trans-Vinpocetine	Log K = 7.07	Log P_N = 4.75	Log P_C = 0.185
2.29	0.39	0.0017	37.9
2.78	0.92	0.0051	65.3
3.73	1.80	0.0457	94.4
4.26	2.18	0.155	98.3
5.21	2.88	1.36	99.8
Vincamine	Log K = 8.05	Log P_N = 2.92	Log P_C = −1.33
2.30	−1.32	0.0002	3.1
2.82	−1.11	0.0006	9.5
4.43	−0.62	0.0240	81.0
5.22	0.11	0.148	96.3
trans-Vincamine	Log K = 7.38	Log P_N = 2.96	Log P_C = −1.31
2.33	−1.24	0.0009	14.2
3.76	−0.49	0.0240	81.7
4.33	0.03	0.0890	94.3
5.32	0.87	0.863	99.4
6.19	1.74	6.07	99.9
Epivincamine	Log K = 7.96	Log P_N = 2.77	Log P_C = −1.29
2.28	−1.28	0.0002	2.3
3.69	−1.07	0.0054	38.1
4.29	−0.66	0.0214	71.1
5.02	−0.02	0.115	93.0
6.16	0.97	1.56	99.5

[a] Log K is the logarithm of the protonation constant determined in a previous work [11].

partly be accounted for by the inherent ambiguities in the log K values that had also been determined indirectly, because of the poor water-solubilities. Thus, the results of the stir-flask method justify the use of the RP-TLC method, which was feasible for all the 10 compounds. In the following discussion, conclusions will be drawn from the complete and self-consistent set of log P_{TLC} values.

4. Discussion

Most of the molecules are highly lipophilic as expected from the eburnane skeleton. Hydrophobicity of its aromatic indole moiety can be partly compensated by polar sites containing N or O atoms. Table 2 shows that the lipophilicity range of the 10 compounds covers two orders of magnitude.

The log P differences in Table 2 reflect either major effects, due to electronegativity changes in the molecular constitution, or minor, modulatory effects, because of structural isomerisms. The apovincaminic acid derivatives are more lipophilic than the vincamine ones, caused obviously by the absence of the polar hydroxyl group in position C(14). The chain length and the character of the substituent of the C(14)-carboxylic acid group also influences the lipophilicity. The addition of a methylene group

reduces the side chain polarity, thus increasing the lipophilicity. The terminating functional group of the side chain makes little difference in this phenomenon. In the case of the acetoxy-derivatives the difference between the log P_{TLC} of the propyl and ethyl side chain is 0.22 log units, whereas in the hydroxy-derivatives it is 0.27 log units. Hydroxyl groups are more polar than ester groups, thus the acetoxy-derivatives are more lipophilic than the hydroxy-derivatives, the difference is 0.88 log units in the propyl and 0.83 log units in the ethyl derivatives.

Concerning structural isomerism, the configurational differences in positions C(3) and C(14) make four covalently distinct molecules in the vincamine series, vincamine, *trans*-vincamine, epivincamine and *trans*-epivincamine. The barrier to nitrogen inversion at N(4) is high enough to preclude convex–concave pyramidal interconversions. In all of these molecules the orientation of the N(4) lone electron pair is the same as that of the C(16) ethyl group. Nevertheless, each of these four diastereomers exists in solution as a mixture of conformers, owing to the axial and equatorial changeability of C(14)-OH and further, subtle conformational variabilities in the ring system. The geometry of the conformers of these four diastereomers was optimized by molecular mechanics calculations both in the aqueous and the octanol phase. The recently reported, first experimental conformer-specific partition coefficients have shown that the most influential log *P*-modifying factor is the water-accessibility of the polar groups. If water-accessibility of a polar site is hampered by alkyl or aryl moieties, the resulting lipophilicity is high [29].

In light of these findings, log *P* values of the three pairs of compounds that differ in the configuration at C(3) (vinpocetine–*trans*-vinpocetine; vincamine–*trans*-vincamine; epivincamine–*trans*-epivincamine) can be interpreted as follows. Negligible difference occurs only between the lipophilicity of vincamine and *trans*-vincamine. However, the *trans*-epimers of vinpocetine and epivincamine are more lipophilic than the ones with *cis*-anellation, showing the respective 0.57 and 0.29 log units differences. Earlier studies also indicated that these epimers bear different physico–chemical properties and biological activities [9,11,30,31].

There are two major factors that contribute to the higher lipophilicity of the *trans*-epimers. Molecular mechanics calculations and nuclear magnetic resonance (NMR) experiments show that ring D in *cis*-epimers occupies a perpendicular position to the plane of the ring system, whereas it is parallel in the *trans*-epimers (Fig. 3). Thus the smaller hydrophobic surface area of *cis*-epimers makes them less lipophilic. Molecular mechanics calculations also show that orientation of the C(16) ethyl group is dependent on the D/E ring anellation. Namely, during rotation the ethyl group in the *trans*-epimers can approach more closely the basic N(4) nitrogen than in the case of the *cis*-epimers. Comparing the minimum energy conformers of the three epimer pairs the average difference between the methylene carbon of the ethyl group and the N(4) nitrogen is 0.148 Å both in water and in octanol. Thus, the ethyl

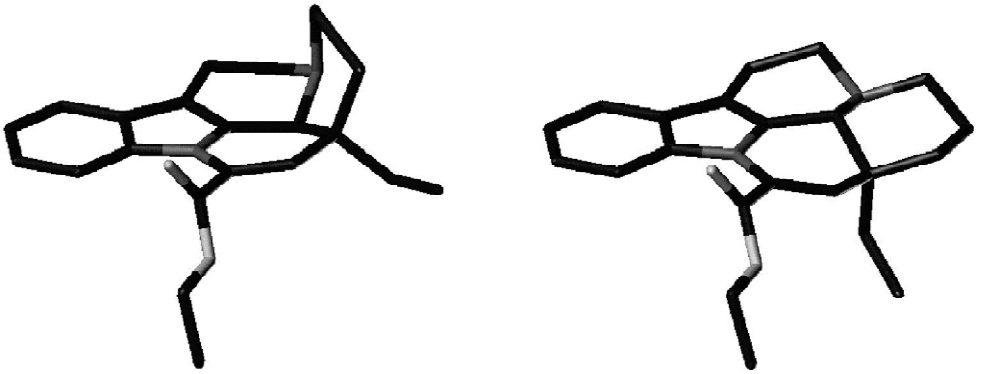


Fig. 3. Three-dimensional models of *cis*-vinpocetine (left) and *trans*-vinpocetine (right).

group can hamper the water accessibility of N(4) to a greater extent and renders the *trans*-epimers less hydrophilic.

Both RP-TLC (Table 2) and stir-flask (Table 3) measurements show that there is only a negligible lipophilicity difference between the two vincamine epimers. There must be an intramolecular factor that renders the *trans*-epimer of vincamine less lipophilic than expected. Molecular mechanics calculations showed that there are several low-energy conformers where the distances between the hydrogen bond donor C(14) hydroxyl group and the hydrogen bond acceptor sites of the molecule in its vicinity (the lone electron pairs of the ester group and the indole nitrogen) are different. The low lipophilicity of *trans*-vincamine shows that the intramolecular hydrogen bonds are stronger (the distance between the bridge atoms is shorter) in the *cis*-epimer than in *trans*-vincamine or the epimers of epivincamine [32]. The epimers of vinpocetine do not contain the C(14) hydroxyl group and therefore cannot form such intramolecular hydrogen bonds.

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