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Validation of a computational procedure for the calculation of the polar surface area (PSA) of organic compounds

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Polar molecular surface area (PSA) has been established as a valuable physicochemical parameter for the prediction of a number of properties related to the pharmacokinetic profile of drugs. Notable examples are the intestinal absorption [1–4], the Caco-2 permeability [3–7], the blood-brain barrier penetration [3, 8, 9], the corneal permeability [10] and the human jejunum permeability [3, 11]. The PSA of a molecule is defined as the area of its van der Waals surface that arises from all oxygen and nitrogen atoms plus the hydrogen atoms attached to them. As such, it is clearly related to the capacity of a compound to form hydrogen bonds, and accounts for 3D effects such as shielding or burial of polar groups by other parts of a molecule [2].

There are three main methods to calculate PSA: a) using a Boltzmann-weighted average value computed from an ensemble of low-energy conformers obtained by a detailed conformational search and termed the "dynamic" PSA (PSA_d) [1, 4-7, 12]; b) using a single low energy conformation of a molecule (PSA_s) [2, 5, 9]; c) by the summation of tabulated surface contributions of polar fragments, including also "slightly polar" fragments containing phosphorous and sulfur, and termed the topological PSA (TPSA) [3].

In the present work, we investigated the feasibility of calculating PSA by combining the 2D-drawing program ChemWindow [13] with the 3D molecular modelling freeware Molgen [14]. This standard procedure might provide an alternative to the available software packages, or the calculation via the internet (TPSA; http://www.daylight.com/meetings/emug00/Ertl/tpsa.html) which now compute this physicochemical property. An advantage of the proposed procedure could be that it uses readily available software, and does not require sophisticated hardware or interactive calculations.

The steps of the employed computational processes are as follows:

1) The 2D structure of the molecule is drawn (or appropriately imported) in the program ChemWindow and saved in the format of a .mol file.

2) The 2D structure is then imported in the program Molgen by selecting ChemWindow in the interface menu, where it is converted to a 3D structure.

3) The generated 3D structure is quickly optimised (MM2) to relieve any van der Waals, compression, bending, stretching and torsion strain. A single low energy conformation is, thus, generated, which is used in the next computational step without any alteration.

4) The values of the atomic radii are set to be the same as those previously used for calculating PSA_d [1, 4] as well as PSA_s [2] and TPSA [3], with the exception of hydrogen where the value of 1.17 [15] is used instead of the proposed three values (H, H attached to O and H attached to N). The oxygen and nitrogen atoms as well as the hydrogens attached to them are selected and their total contribution to the van der Waals area of the molecule is calculated (PSA_{CWM}).

In order to validate the above described computational procedure, we used the list of the twenty carefully selected compounds by Palm et al. [1], (which were also used by Clark [2] and Ertl et al. [3]). Their reported calculated PSA (PSA_d, PSA_s and TPSA) and ours (PSA_{CWM}) are shown in the Table. Furthermore, as there, a good linear correlation of their PSA_d values and the logarithms of Caco-2 permeability coefficients has been reported [7] for eight β-antagonists (Pc ranging from 1.02×10^{-6} to 242×10^{-6} cm/s), we included in the data set two additional compounds (H 95/71 and H 244/45, which were not present in the original list of twenty). The log ($Pc(10^{-6}$ cm/s)) of these eight b-antagonists is also shown in the Table.

From the tabulated data, the following simple regression equations are derived (eqs. 1–4).

$$PSA_{CWM} = 1.038\ (\pm 0.012) \times PSA_d \qquad (1)$$
$$n = 22, \quad r^2 = 0.989, \quad s = 5.827, \quad F = 1720.573$$

$$PSA_{CWM} = 0.969\ (\pm 0.011) \times PSA_s \qquad (2)$$
$$n = 20, \quad r^2 = 0.991, \quad s = 5.486, \quad F = 1937.320$$

$$PSA_{CWM} = 0.927\ (\pm 0.020) \times TPSA + 9.666\ (\pm 2.155) \qquad (3)$$
$$n = 20, \quad r^2 = 0.992, \quad s = 5.212, \quad F = 2148.564$$

$$\log (Pc(10^{-6}\ cm/s)) = -0.051\ (\pm 0.004) \times PSA_{CWM} + 4.761\ (\pm 0.260) \qquad (4)$$
$$n = 8, \quad r^2 = 0.970, \quad s = 0.166, \quad F = 191.137$$

Equations 1–3 show that there is a good correlation between the PSA values calculated with our procedure (PSA_{CWM}) and the reported PSA_d, PSA_s and TPSA. Furthermore, eq. 4 indicates that PSA_{CWM} values could be used in the estimation of the molecular permeability to the Caco-2 cell.

Although the data is limited, it is suggested that the computational procedure reported here could calculate PSA values in a quality similar to the previously reported ones,

Table: PSA values and logarithms of Caco-2 permeability coefficients for the compounds studied

Compound	[a,d]PSA_d	[b]PSA_s	[c]TPSA	PSA_{CWM}	[d]log ($Pc(10^{-6}$ cm/s))
Metoprolol	53.1	57.2	50.7	58.0	1.9633155
Nordiazepam	45.1	47.5	41.5	46.5	
Diazepam	33.0	34.5	32.7	34.3	
Oxprenolol	46.8	53.2	50.7	53.2	2.0777312
Phenazone	27.1	28.0	26.9	30.7	
Oxazepam	66.9	55.6	61.7	68.3	
Alprenolol	37.1	41.8	41.9	43.3	2.3838154
Practolol	73.4	77.2	70.6	78.2	0.5390761
Pindolol	56.5	60.9	57.3	62.7	1.7379873
Ciprofloxacin	78.7	80.1	74.6	84.9	
Metolazone	94.5	95.9	92.5	89.9	
Tranexamic acid	69.2	71.5	63.3	73.8	
Atenolol	90.9	93.3	84.6	92.3	0.0086001
Sulpiride	100.2	101.4	101.7	98.8	
Mannitol	116.6	129.6	121.4	127.2	
Foscarnet	115.3	117.3	94.8	106.8	
Sulfasalazine	141.9	148.6	141.3	134.0	
Olsalazine	141.0	147.0	139.8	146.8	
Lactulose	177.2	197.8	197.4	187.0	
Raffinose	242.1	266.8	268.7	257.6	
H 95/71	77.0			83.4	0.5740312
H 244/45	71.6			80.9	0.7803173

[a] From reference [1], [b] From reference [2], [c] From reference [3], [d] From reference [7]

which had been derived by three different methodologies. Finally, the detected variation between the PSA_{CWM} and the PSA_d, the PSA_s or the TPSA values is about ±10% which, for low molecular weight molecules, is not considered critical [1, 8] for making first estimates (e.g. BBB uptake).

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Alteration of membrane polarization by (−)-terpestacin, a biologically active fungal metabolite

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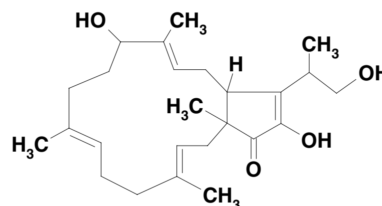
Previously we isolated (−)-terpestacin (**1**) from the fermentation broth of the hyphomycete *Ulocladium* sp. HKI 0226 [1] as a naturally occurring stereoisomer of (+)-terpestacin [2–4]. Compound **1** was found to inhibit syncytium formation by cells infected with respiratory syncytial virus (RSV) and to stimulate aerial mycelium formation by surface cultures of the fungus *Fusarium culmorum* JP15 [1]. Due to the interesting biological activities of (−)-terpestacin we investigated its effect on ion permeability of an artificial black-lipid bilayer membrane (BLM). (−)-Terpestacin (**1**) [1] and its stereoisomer [2] are lipophilic, moderately polar compounds which should well dissolve in biological membranes. Due to the presence of a β-hydroxyketone structure under physiological pH conditions the formation of a negatively charged ion can be suggested. Insertion of this anion into the membrane could increase the negative surface charge of the lipid bilayer due to the orientation of the polar negatively charged part of the molecule towards the water phase. In order to evaluate this suggestion we investigated the influence of **1** on an artificial membrane (BLM, see Experimental).



In concentrations up to 5 μg/ml of **1** given to one or two sides of the BLM made from soybean lecithin no change of membrane permeability was observed. This finding suggested that (−)-terpestacin (**1**) neither destabilized the membrane as a dipole-type tenside nor formed ion-penetrable pores or channels. In another series of experiments we added amphothericin B and ergosterol to the membrane. Under this condition ion-penetrable pores were formed in the BLM as was indicated by the decrease of electric resistance. Increasing concentrations of **1** (0.1–4 μg/ml) given to the membrane site with negative sign of the applied voltage caused a selective increase of potassium ion penetration across the membrane pores formed by amphotericin B and ergosterol. Thus we plotted the zero-current potentials which were necessary to stop the membrane current which appeared in presence of different KCl concentrations at both sides of the membrane. Thereby the KCl concentration was increased in the cis compartment and electrical potential of this compartment was measured in reference to the other compartment. Due to the higher penetrability of the potassium ions we had to apply a negative voltage to stop the current and increase in conductance caused by the flux of this ions (Fig). Amphotericin B is known to owe its therapeutical use as a systemic antifungal agent to the formation of ion-penetrable pores in presence of ergosterol [5, 6]. The constitut-