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Research paper

Heuman indices of hydrophobicity of bile acids and their comparison with a newly developed and conventional molecular descriptors

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ABSTRACT

Bile salts (BSs), in addition to their physiological role in the digestion of lipids in vertebrates, are also of significant importance in biomedical investigations. For predicting biological–pharmacological activity and physico-chemical properties of BSs it is important to develop such molecular descriptors that adequately describe the structural characteristics of the steroid skeleton. The present study encompassed the following bile acids (BAs): cholic, chenodeoxycholic, deoxycholic, hyodeoxycholic, ursodeoxycholic, hyocholic, and ursocholic acid, as well as oxo derivatives of certain BAs. For all of them, Heuman hydrophobicity indices (HI_{BA}) (RP-HPLC parameters) were determined, and a detailed conformational analysis of the steroid skeleton showed that HI_{BA} has the discrimination power for BAs based on the size of the hydrophobic surface on the β side and the lateral L7 and L12 sides of the steroid skeleton. Also, HI_{BA} discerns the regiochemical characteristics of OH and oxo groups. Based on a survey of the structural factors of the steroid skeleton that influence the HI_{BA} values of the tested BAs, we constructed a new molecular descriptor, CHI_{BA}, with the characteristics of 2D and 3D topological descriptors. In respect of the structure of the steroid skeleton, the descriptor CHI_{BA} behaves as a reversed-phase chromatographic descriptor of BAs.

1. Introduction

Bile acids (BAs), which are synthesized in the liver of vertebrates, are hydroxy derivatives of 5β-cholanoic acid whose steroid skeleton forms a concave (α) and a convex (β) side of the molecule (Fig. 1). Bile salts (BSs) are amphiphilic compounds that belong to a special group of surface active molecules – biplanar amphiphiles. Namely, in contrast to classic amphiphiles with polar (hydrophilic) head and nonpolar (hydrophobic) tail, BSs molecules have two different surfaces (sides): the hydrophobic β side with angular methyl groups, and the hydrophilic α side with OH groups. In an aqueous solution, the carboxylic group of the side chain is also α-oriented. The ratio of the hydrophobic to the hydrophilic surface area of BSs determines a number of their properties: formation of micelles (critical micelle concentration), power of solubilization of the hydrophobic probe molecule, binding to the hydrophobic parts of biomolecules (phospholipids, albumin, ionic channels, receptors, etc.), membranotoxicity–membranolytic activity (membrane saturation, membrane solubilization, hemolytic potential, apoptosis, etc.) [1–7].

For the description of the degree of hydrophobicity of ionized and non-ionized BAs Heuman introduced the relative capacity factor (k_r) which represents the ratio of the capacity factors obtained by reversed-phase high performance liquid chromatography (RP-HPLC) of a BA (BS) and the capacity factor of taurocholate (TC) [8]. The introduction of the relative capacity factor eliminates the individual characteristics of the column (expressed as the ratio of the volumes of the stationary and mobile phases) [9]. Hence, the difference between the standard free energy of partition of BA (BS) between the hydrophobic stationary and the mobile phase (ΔG°_{BA}) and the standard free energy of partition of TC between these phases (ΔG°_{TC}) is in a direct relation with the relative capacity factor [8]:

$$\Delta G^{\circ}_{BA} - \Delta G^{\circ}_{TC} = -RT \ln k_r(\mathrm{BA}). \quad (1)$$

Heuman defined arbitrarily the hydrophobic index (HI_{BA}) for a BA (BS) as the ratio:

$$HI_{BA} = \ln k_r(\mathrm{BA}) / \ln k_r(\mathrm{TLC}), \quad (2)$$

where k_r (TLC) represents the relative capacity factor for taurolithocholate (TLC). Based on (2), it follows that for taurocholate $HI_{BA} = 0$, whereas for taurolithocholate $HI_{BA} = 1$. Those BAs or BSs that are more hydrophilic than cholic acid have negative

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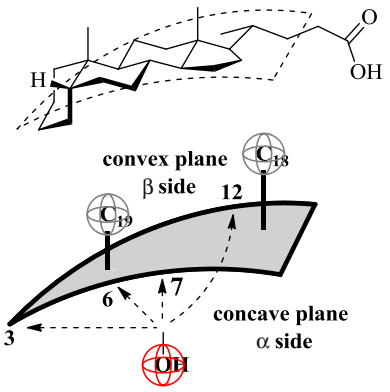

Fig. 1. Steroid skeleton of 5β-cholanoic acid and usual positions of OH groups.

values of the hydrophobic index, while the more hydrophobic ones have positive values, i.e. between 0 and 1 (if taurolithocholate is the most hydrophobic BS). Combining Eqs. (1) and (2) it is easy to see that there is a linear dependence between HI_{BA} and ΔG°_{BA}

$$\Delta G^{\circ}_{BA} = \Delta G^{\circ}_{TC} - RT \ln k_r(\mathrm{TLC}) HI_{BA}. \quad (3)$$

Using the principle of independent chemical reactions, Heuman et al. [8,10] showed that the process of binding of a particular type of BAs or BSs from their diluted solutions to the hydrophobic stationary phase (RP-HPLC) can be considered as independent events. Hence, the free energy of binding of one mole of a mixture of BAs or BSs can be presented as a sum of the products of the mole ratios x_{BA} and ΔG°_{BA}, i.e. $\Delta G = \sum x_{BA} \Delta G^{\circ}_{BA}$. Therefore, taking into account also Eq. (3), the total hydrophobicity of a given mixture of BAs (BSs) can be expressed as $HI_{\mathrm{mixture}} = \sum x_{BA} HI_{BA}$.

The application of *in silico* molecular descriptors of hydrophobicity [11] such as log *P* or *C*log *P* or QSA(P)R model of hydrophobicity of BAs in which topological descriptors act as independent variables, leads to relatively large fitting errors. Namely, many topological descriptors do not discriminate the positions/orientations of OH (oxo) groups in the steroid skeleton of BAs, i.e. they do not discern between the equatorial–axial positions and the α–β orientations of these groups [12]. Hence, the descriptors of hydrophobicity–hydrophilicity that are based on the chromatographic parameters are of great significance [13–15].

The objective of this work was to determine the Heuman indices of hydrophobicity (HI_{BA}) as chromatographic quantities for oxo derivatives of BAs (because of their reduced membranolytic properties, they are subject of intensive biomedical–biopharmaceutical investigations [2]), both of non-ionized and ionized forms (only the latter are being important from the pharmaceutical aspect) (Fig. 2). Also, the aim was to check whether HI_{BA} describes adequately the regiochemical characteristics of OH and oxo groups of the steroid skeleton of BAs. Hence, the work encompassed a detailed stereochemical–conformational analysis of the steroid skeleton in the light of the obtained HI_{BA} values. In this paper, to study the steroid skeleton of bile acids, was first used access via molecular graphs, which allows to defined referent system for the regiochemical distinction of steroids OH and oxo groups.

On the basis of the established structural effects of the steroid skeleton on the Heuman hydrophobicity index, a new topological descriptor of hydrophobicity of the steroid ring system of BAs was also developed, to enable the discrimination between the different positions (orientations) of OH (oxo) groups.

2. Experimental

2.1. Bile acids: synthesis of oxo derivatives of cholic, deoxycholic and chenodeoxycholic acids

Cholic, deoxycholic, chenodeoxycholic and hyodeoxycholic acids (Alfa Aesar, Germany, 99%) were used as the starting compounds for the synthesis of their oxo derivatives.

3α-Hydroxy-12-oxo-5β-cholanoic acid (12-oxolitholic acid, **12-OL**) and 3α,7α-dihydroxy-12-oxo-5β-cholanoic acid (12-oxochenodeoxycholic acid, **12-OCD**) were prepared according to the procedure of Miljković et al. [16], while 3α,12α-dihydroxy-7-oxo-5β-cholanoic acid (7-oxodeoxycholic acid, **7-OD**) and 3α-hydroxy-7-oxo-5β-cholanoic acid (7-oxolitholic acid, **7-OL**) were obtained according to Tullar [17]. The starting compound for obtaining 12α-hydroxy-3,7-dioxo-5β-cholanoic acid (**3,7-DOHiC**) was methyl cholate, selectively oxidized in one-pot reaction according to Kuwada et al. [18]. 3,7,12-Trioxo-5β-cholanoic acid (**3,7,12-TOC**) and 3,7-dioxo-5β-cholanoic acid (**3,7-DOC**) were obtained according to Fieser and Rajagopalan [19]. 7α-Hydroxy-3,12-dioxo-5β-cholanoic acid (**3,12-DOHiC**) was synthesized according to Tserng [20], the starting compound being 12-oxochenodeoxycholic acid (**12-OCD**). Bile acid derivative of 3,6-dioxo-5β-cholanoic acid (**3,6-DOC**) was obtained according to Windaus [3]. Ursodeoxycholic acid was purchased from Alfa Aesar, Germany, 99%. Ursocholic and 3α-hydroxy-3-oxo-5β-cholanoic acid (6-oxolithocholic acid, **6-OL**) were purchased from Sigma, New Zealand, 99%. Taurolithocholic acid sodium salt was purchased from Sigma, USA, 99.8%.

2.2. Reversed-phase HPLC method

The HPLC system Agilent 1100 Series, equipped with degasser, binary pump, automatic injector and DAD detector, with the software system for data processing AgilentChemStation was used, and the analyses were performed on a reversed-phase C-18 column: Eclipse Plus C18 (250 mm × 3 mm, 5 μm, 250 Å) column (Zorbax SD). Since the applied column is stable in acidic media, the experiments were carried out without a pre-column. The composition of the mobile phase and the buffer systems were identical to those in the Heuman experiment [8]. Solutions of BAs and their derivatives in methanol were prepared in the concentration of 1 mg/ml. All separations were performed isocratically at a flow rate of 1 ml/min and the column temperature rise from 25 °C. The detection was performed at 210 and 283 nm for BA oxo derivatives.

The HPLC capacity factor (k) was calculated from the eluted peak retention time (t):

$$k = \frac{t_x - t_0}{t_0}$$

where t_x and t_0 are the retention times of the BA and the unretained solvent front respectively.

2.3. Data treatment

The data were treated using the Statistica 8.0 package. The 3D models (energetically most favorable) of partial conformations of BAs were generated according to the MM2 protocol (ChemBio3D Ultra 11.0). Molecular descriptors were obtained using the program packages Alchemy and Dragon.

3. Results

Table 1 lists the values for the Heuman indices of hydrophobicity HI_{BA} of ionized and non-ionized forms of BAs. A comparison of the