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Research paper

Modification of the apparent lipophilicity of steroidal drugs with gamma-cyclodextrin

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Abstract

The interaction between 17 steroidal drugs and gamma-cyclo-dextrin (gamma-CD) was determined by charge-transfer chromatography and the relative strength of interaction was calculated. The relationship between the strength of interaction and the physico-chemical parameters of steroidal drugs was elucidated with principal component analysis. Gamma-CD interacted with each steroidal drug decreasing the apparent hydrophobicity of the guest molecules. Calculations indicated that the interaction between the drugs and gamma-CD is of mixed character: steric, hydrophobic and electronic forces are involved in the complex formation. The marked influence of gamma-CD on the apparent hydrophobicity of drugs suggests that this interaction may modify the biological properties (absorption, uptake, half-life etc.) of drug-gamma-CD complexes resulting in modified efficacy.

Keywords: Gamma-cyclodextrin; Steroidal drugs; Principal component analysis

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides built up from 6–8 glucopyranose units. Due to their ring structures CDs have the capacity to form inclusion complexes with a wide variety of organic, even with inorganic compounds [1,2]. The formation of various drug-CD inclusion complexes has been extensively studied. Thus, the formation of the inclusion complexes of taxol [3] and other anticancer drugs [4], colchinine [5], non-steroidal anti-inflammatory drugs [6], oligonucleotides [7] etc. has been reported. The physicochemical and pharmacological characteristics of drug-CD inclusion complexes deviate considerably from those of uncomplexed drug molecules. Due to this modification, the formation of inclusion complexes improves the nasal bioavailability of luteinizing hormone-releasing hormone agonist, buserelin, in rats [8], enhances absorption [9], modifies the solubility of a wide variety of drugs [10–12], protects against gentamicin nephrotoxicity in the rat [13], decreases the ocular irritation of pilocarpine prodrug [14], increases hydrocortisone penetration [15], improves acitrecin delivery through hairless mouse skin [16], modifies hydrocortisone release [17] and increases the stability of the guest molecule [18]. The impact of the various interactive forces on the strength of the inclusion complexes has been vigorously discussed. It is assumed that hydrophobic forces [19] as well as polar and steric factors [20] play a considerable role in the complex formation. However, it has been found that dipole-dipole, ion-dipole, van der Waals and hydrogen bonding interactions may also influence the host-guest interaction [21].

Various chromatographic techniques can be successfully used for the study of molecular interactions [22]. The advantages of the chromatographic techniques are that they use a low quantity of compounds and the interacting molecules need not be very pure, because the impurities are separated during the chromatographic process. These tech-

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niques have also been previously used for the study of the complex formation of steroid hormones with cyclodextrins [23,24].

The objectives of this work were to study the interaction of steroidal drugs with gamma-cyclodextrin (gamma-CD) by means of charge transfer chromatography, to compare their inclusion forming capacity and to elucidate the role of molecular parameters in the inclusion-complex formation.

2. Materials and methods

Polygram UV_{254} (Macherey-Nagel, Dürren, Germany) plates were impregnated by overnight predevelopment in *n*-hexane-paraffin oil 95:5 (v/v). *N*-hexane and paraffin oil were of analytical and pharmacopeial grades, respectively. The chemical structures of steroidal drugs are shown in Fig. 1. Drugs were the gift of Professor Sándor Görög, Gedeon Richter, Budapest, Hungary and were used without further purification. The drugs were separately dissolved in methanol (analytical grade) at a concentration of 3 mg/ml and 2 μl of the solutions were plotted on the plates. Water-methanol mixtures were used as eluents, the methanol concentration ranging from 0 to 80 vol.%. As the object was to study the complex formation between the solutes and gamma-CD and not the study of the effect of gamma-CD on the separation of solutes, they were separately spotted on the plates. In this way, the competition between the steroidal drugs for the binding sites of gamma-CD was excluded. Methanol was chosen as the organic solvent miscible with water because it forms only weak inclusion complexes with cyclodextrins [25,26]. The application of this wide range of methanol concentrations was motivated by the highly different hydrophobicity of steroidal drugs. Gamma-cyclodextrin was purchased from CYCLOLAB (Budapest, Hungary) and used as received. It was added to the eluents in the concentration range of 0–15 mg/ml concentrations. Developments were carried out in sandwich chambers (22 × 22 × 3 cm) at room

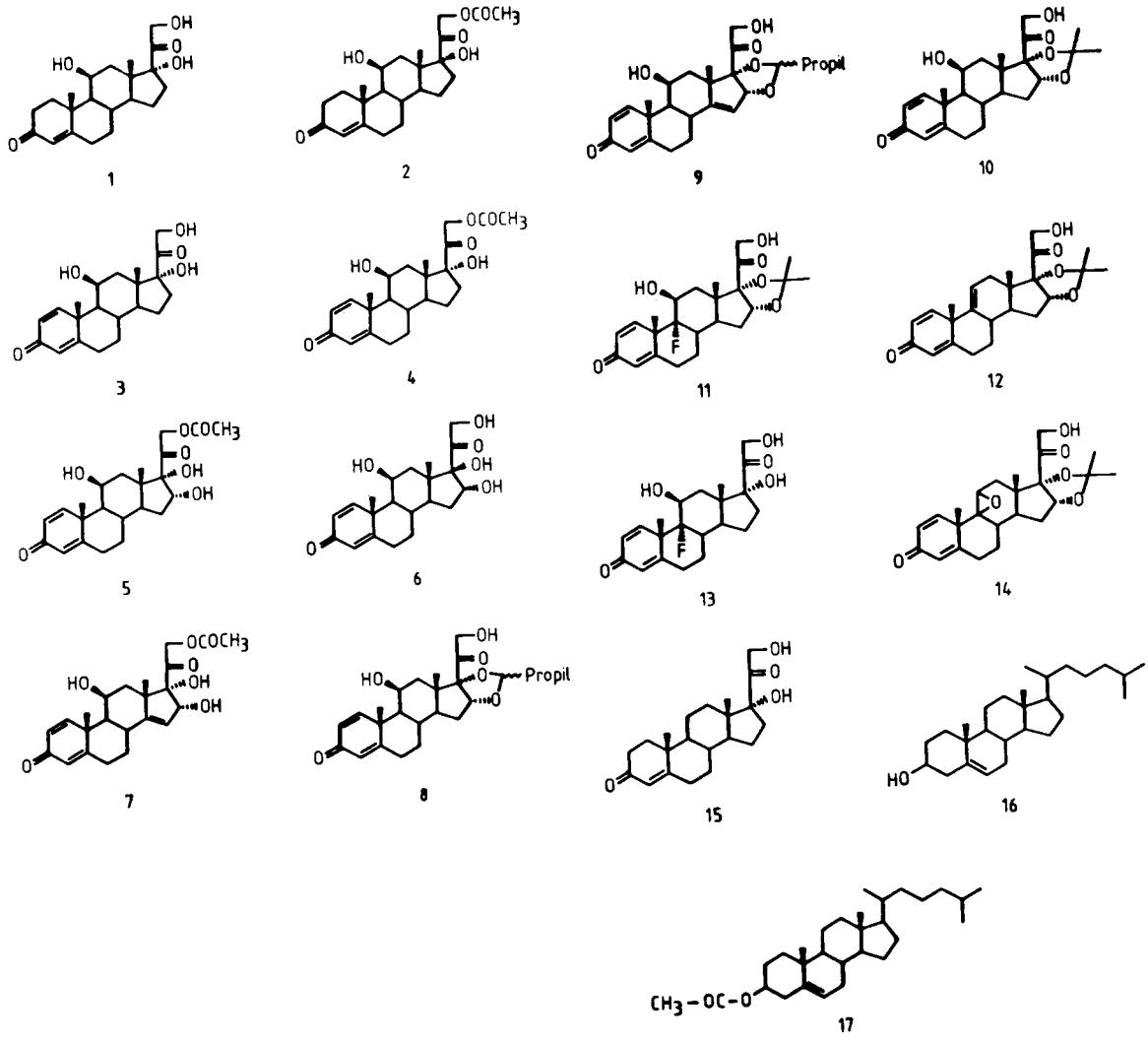

Fig. 1. Chemical structure of steroidal drugs.

temperature, the distance of development being about 16 cm. After development, the plates were dried at 105°C and the spots of steroidal drugs were revealed by their UV spectra and by iodine vapour. Each experiment was run in quadruplicate. As the repoducibility of thin-layer chromatography is lower than that of high-performance liquid chromatography, the use of replications higher than three was necessary to obtain reliable results.

The R_M value characterizing the molecular hydrophobicity in reversed-phase thin-layer chromatography was calculated for each drug in each eluent:

$$R_M = \log(1/R_f - 1) \quad (1)$$

When the coefficient of variation of the parallel determinations was higher than 6% the R_M value was omitted from the following calculations.

To separate the effects of methanol and gamma-CD on the hydrophobicity of steroidal drugs the following equation was fitted to the experimental data:

$$R_M = R_{M0} + b_1 \times C_1 + b_2 \times C_2 \quad (2)$$

where R_M is the R_M-value for a drug determined at given methanol and gamma-CD concentrations, R_{M0} is the R_M-value extrapolated to zero methanol and gamma-CD concentrations, b_1 is the decrease in the R_M value caused by 1% increase in methanol concentration in the eluent (related to the specific hydrophobic surface area of drugs [27], b_2 is the decrease in the R_M value caused by 1 mg/ml concentration-change of gamma-CD in the eluent (related to the relative strength of interaction), C_1 and C_2 are the concentrations of methanol and gamma-CD, respectively. Eq. (2) was applied separately for each steroidal drug.

To test the validity of the hypothesis that in the case of homologous series of solutes the slope and intercept values (b_1 and R_{M0} in Eq. (2)) are strongly intercorrelated [28,29] linear correlation was calculated between the two physicochemical parameters:

$$R_{M0} = A + B \times b_1 \quad (3)$$

where A and B are the intercept and slope values of the linear relationship between R_{M0} and b_1.

In order to determine whether the measured hydrophobicity parameters of steroidal drugs significantly influence their complex-forming capacity, stepwise regression analysis was applied [30]. The relative strength of interaction (b_2) was the dependent variable, whereas the hydrophobicity (R_{M0}), specific hydrophobic surface area (b_1) of Eq. (2) and the complex hydrophobicity parameter R_{M0}/b_1 were the independent variables, respectively. The number of accepted independent variables was not limited and the acceptance limit was set to the 95% significance level. Software of stepwise regression analysis was prepared by CompuDrug, Budapest, Hungary. The relationship between the relative strength of steroidal drug-gamma-CD interaction and the calculated physicochemical parameters was elucidated by principal component analysis (PCA) [31]. The physicochemical parameters included in the calculation as dependent variables were: π, Hansch-Fujita's substituent constant characterizing hydrophobicity; H-Ac and H-Do, indicator variables for proton-acceptor and proton-donor properties, respectively; M-RE, molar refractivity; F and R, electronic parameters characterizing the inductive and resonance effect, respectively; σ, Hammett's constant, characterizing the electron-withdrawing power of the substituent in para and ortho + meta position ($\sigma_{ortho+meta}$, σ_{para}); Es, Taft's constant, characterizing steric effects of the substituent; B_1 and B_4, Sterimol width parameters determined by the distance of substituents at their maximum point perpendicular to attachment. The calculation of the physicochemical parameters of drugs was carried out by using the additivity rule. As the visual evaluation of the multidimensional matrices of PC loadings and variables is complicated, the dimensionality of the matrices was reduced to two by the non-linear mapping technique [32]. The iteration was carried out to the point where the difference between the last two iterations was less than 10^{-8}.

3. Results and discussion

The simultaneous effects of methanol and gamma-CD concentrations on the R_M values of compounds 9 and 12 are shown in Figs. 2 and 3. The R_M values decrease with an increase in the methanol concentration, i.e. these compounds do not show any anomalous retention behaviour in this concentration range which would invalidate the evaluation using Eq. (2). An increase in gamma-CD concentration also caused a decrease in R_M values, indicating complex (probably inclusion-complex) formation. Interaction of the more hydrophilic gamma-CD with the drugs reduces the apparent lipophilicity of the latters, but it does not reduce the inherent molecular lipophilicity of the drug included. Complex formation may result in a more hydrophilic trans-

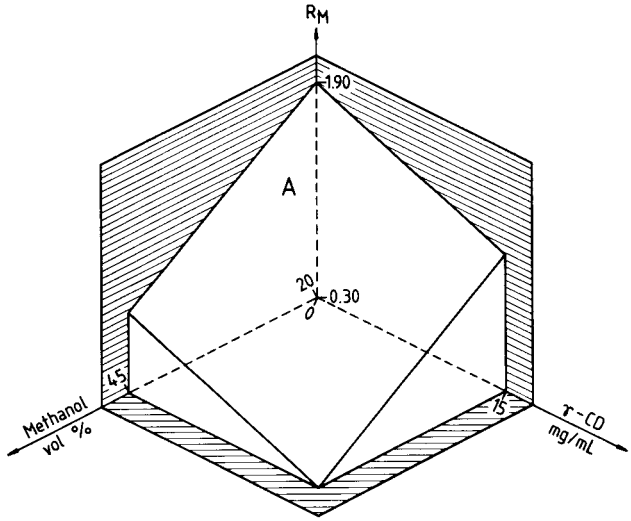

Fig. 2. The effect of methanol and gamma-cyclodextrin concentration on the R_M value of compound 9.

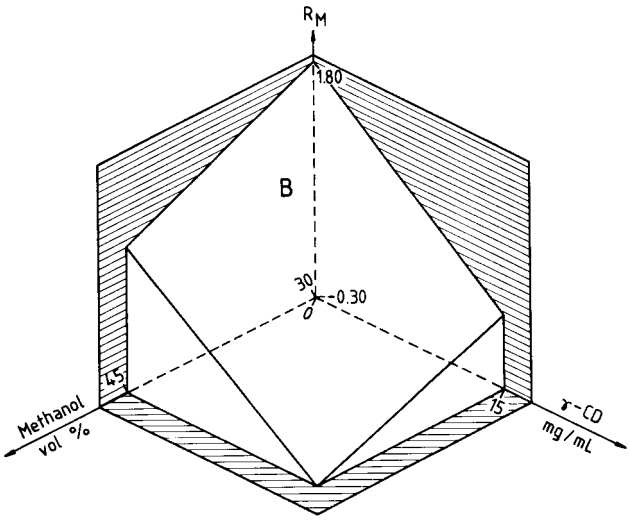

Fig. 3. The effect of methanol and gamma-cyclodextrin concentration on the R_M value of compound 12.

port form for the drugs, i.e. in a faster transport of the steroids to the absorption membrane. The further distribution of the steroids in the body probably happens after the dissociation of the complex, the distribution depending on the physicochemical properties of the non-complexed drugs. This finding suggests that the biochemical and biophysical properties (penetration capacity, uptake, decomposition rate, etc.) of drug-gamma-CD complexes may be different from those of uncomplexed drugs, resulting in modified efficiency. The parameters of Eq. (2) are compiled in Table 1. Eq. (2) fits the experimental data well, the significance levels in each instance being over 99% (see calculated F values). The ratios of variance explained varied between 60–96% (see r^2 values). These statistical data are similar to those obtained with similar methods determining the interaction of hydroxypropyl-beta-CD with antisense nucleotides [33] and with steroidal drugs [34,35] and the interaction between carboxymethyl-gamma-CD and steroidal drugs. Each drug interacts with gamma-CD (b_2 values differ significantly from zero) indicating that in formulations containing both steroidal drugs and gamma-CD their possible interaction has to be taken into consideration. The parameters of Eq. (2) show high variations between the drugs, proving that the lipophilicity (R_{M0}), specific hydrophobic surface area (b_1) and the capacity to form inclusion complexes with gamma-CD (b_2) differ considerably. This result suggests also that the inclusion-complex formation may influence differently the efficiency of the individual drugs. The path coefficients (b'_i % values) indicate that

Table 1

Parameters of linear correlations between the hydrophobicity (R_M) of steroidal drugs and the methanol (C_1 vol. –%) and gamma-cyclodextrin (C_2, mg/ml) in the eluent

Parameter	Steroidal drug number								
	1	2	3	4	5	6	7	8	9
n[a]	18	15	18	22	16	18	17	15	5
R_{M0}	2.01	2.83	2.12	2.64	2.50	1.99	2.40	2.64	2.78
$b_1 \times -10^2$	4.09	4.81	4.32	4.58	5.16	4.96	4.82	3.58	4.18
$s^b_{b1} \times 10^3$	2.61	7.54	2.82	1.93	4.99	2.53	3.40	3.50	3.98
$b_2 \times -10^2$	4.52	6.66	4.80	6.19	5.23	4.33	4.95	4.67	3.92
$s^b_{b2} \times 10^3$	4.33	9.39	4.68	6.63	6.59	4.19	4.97	4.35	4.95
b'_1 (%)[c]	60.04	47.32	59.87	71.75	56.60	65.51	58.72	48.80	56.99
b'_2 (%)[c]	39.96	52.68	40.13	28.25	43.40	34.49	41.28	51.20	43.01
r^{2d}	0.9594	0.8421	0.9576	0.9684	0.9121	0.9705	0.9504	0.9159	0.9123
$F_{calc.}$[e]	177.35	32.00	169.54	293.44	67.47	246.64	134.19	77.19	62.40

Parameter	Steroidal drug number							
	10	11	12	13	14	15	16	17
n[a]	20	16	20	17	15	15	23	13
R_{M0}	3.29	2.20	3.26	2.07	2.48	2.34	8.04	4.46
$b_1 \times -10^2$	5.10	3.80	4.85	4.17	4.33	3.79	8.70	3.16
$s^b_{b1} \times 10^3$	2.87	6.79	4.03	4.87	5.89	7.86	12.64	13.12
$b_2 \times -10^2$	7.39	8.27	9.18	7.99	6.41	7.98	3.75	5.73
$s^b_{b2} \times 10^3$	9.70	8.96	13.61	7.12	7.33	9.79	15.42	15.91
b'_1 (%)[c]	69.98	37.78	64.10	43.28	45.69	37.14	73.88	40.11
b'_2 (%)[c]	30.02	62.22	35.90	56.72	54.31	62.86	26.12	59.89
r^{2d}	0.9540	0.8800	0.8976	0.9270	0.8852	0.8512	0.7095	0.6031
$F_{calc.}$[e]	176.17	47.67	74.52	88.84	46.26	34.33	24.43	7.60

Numbers refer to steroidal drugs in Fig. 1. b_1, decrease in the R_M value caused by a 1% increase in methanol concentration in the eluent (related to the specific hydrophobic surface area of drugs); b_2, decrease in the R_M value caused by a 1 mg/ml concentration change of gamma-CD in the eluent (related to the relative strength of interaction); $F_{calc.}$, calculated F value.[a]Number of data points. [b]Standard deviations of b_1 and b_2 [c]Standard partial regression coefficients of b_1 and b_2, normalized to unity. [d]Coefficient of determination. [e]Calculated F value indicating the fit of Eq. (2) to the experimental data. $R_M = R_{M0} + b_1 \times C_1 + b_2 \times C_2$.

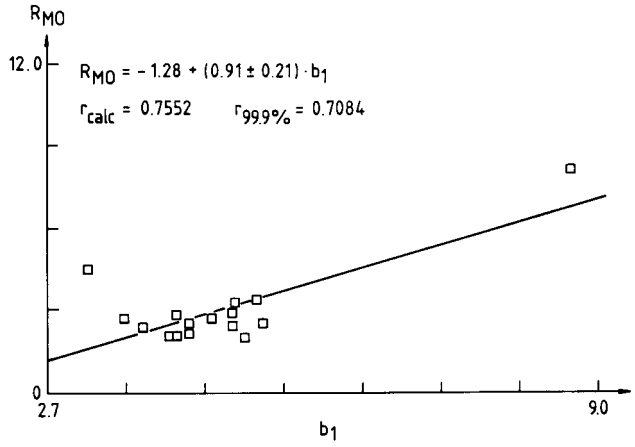

Fig. 4. Relationship between the hydrophobicity (R_{M0}) and specific hydrophobic surface area (b_1) of steroidal drugs.

the change of methanol and gamma-CD concentrations has a similar effect on the retention of steroidal drugs.

Significant linear correlation was found between the intercept (hydrophobicity) and slope (specific hydrophobic surface area) values of steroidal drugs (Fig. 4). This finding indicates that from a chromatographic point of view these drugs behave as a homologous series of compounds, although their chemical structures are different.

Stepwise regression analysis did not find a significant linear relationship between the complex-forming capacity of drugs and their hydrophobicity parameters. This finding indicates that not only hydrophobic forces play a considerable role in the formation of drug-gamma-CD complexes.

Table 2

Similarities and dissimilarities between the physico-chemical parameters of steroidal drugs and their capacity to interact with gamma-cyclodextrin – results of principal component analysis

Number of component	Eigenvalue variance explained (%)		Sum of variance explained (%)
1	7.30	60.86	60.86
2	2.57	21.42	82.28
3	0.93	7.76	90.04
	Principal component loadings		
Parameters	Number of principal components		
	I	II	III
b_2	–0.33	0.37	–0.77
π	0.98	0.07	–0.04
H-Ac	–0.88	0.30	0.25
H-Do	–0.77	0.46	–0.18
M-RE	0.89	0.39	0.13
F	–0.76	0.53	0.28
R	–0.41	–0.83	0.08
$\sigma_{(ortho+meta)}$	–0.85	0.28	0.32
$\sigma_{(para)}$	0.86	–0.29	0.11
Es	–0.38	–0.81	–0.08
B_1	0.93	0.29	0.05
B_4	0.92	0.32	0.10

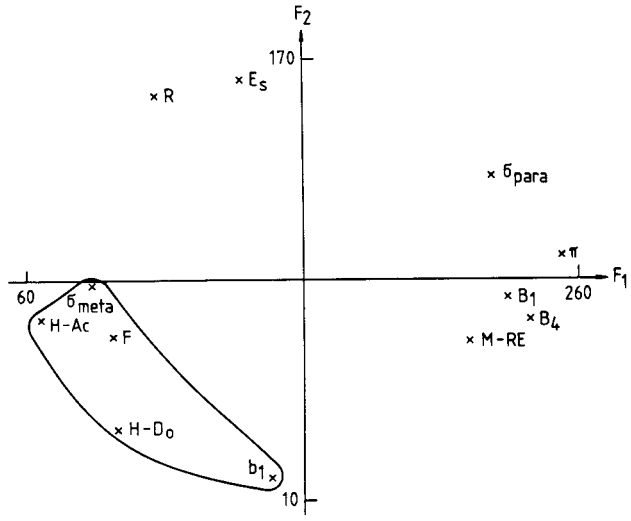

Fig. 5. Relationship between the various physicochemical parameters of steroidal drugs and their capacity to interact with gamma-cyclodextrin. Two-dimensional non-linear map of principal component loadings (number of iterations: 72; maximum error: 1.50×10^{-2}). For symbols see Section 2.

The results of principal component analysis are compiled in Table 2. Three principal components explain the overwhelming majority of the variance, indicating that the 12 original variables can be substituted by three background (abstract) variables with only 9.96% loss of information. Unfortunately, PCA does not prove the existence of such

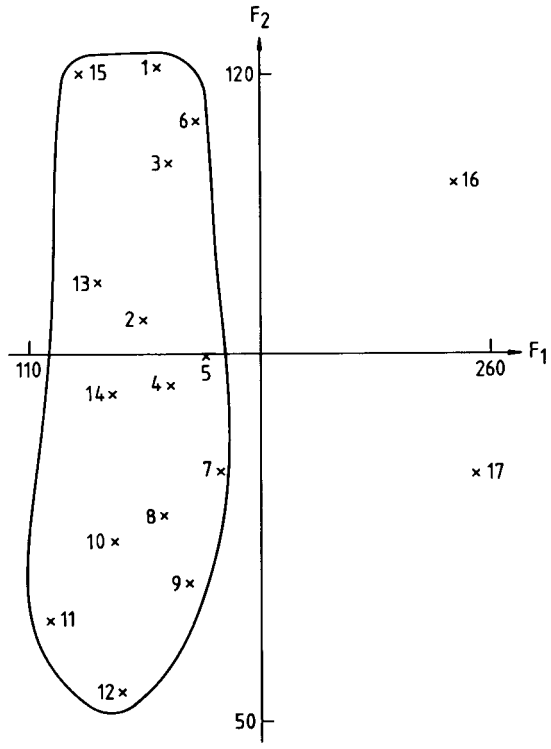

Fig. 6. Similarity and dissimilarity of steroidal drugs. Two-dimensional non-linear map of principal component variables (number of iterations: 171; maximum error: 2.35×10^{-2}). Numbers refer to steroidal drugs in Fig. 1.

background variables as concrete physicochemical entities, but only indicates their mathematical possibility.

The distribution of variables on the two-dimensional non-linear map of PC loadings is shown in Fig. 5. The relative strengths of interaction form a well-defined cluster with the electronic parameters (H-Ac, H-Do, F and σ_{meta}) indicating that more than one molecular characteristic influence the interaction. This result further suggests that more than one type of interactive force are involved in the formation of inclusion complexes. The considerable impact of electronic parameters can be explained by the assumption that the polar substructures of drugs pointing out of the gamma-CD cavity can bind to the hydrophilic substructures on the surface of the gamma-CD molecule, resulting in enhanced stability of the host-guest complex. The distribution of the drugs on the two-dimensional non-linear map of PC variables supports entirely our previous conclusions (Fig. 6). Drugs with highly hydrophobic substructures are not included in the cluster, emphasizing the importance of the polar substituents in the interaction.

It can be concluded from the data that steroidal drugs readily form complexes with gamma-CD. Principal component analysis indicates that electronic forces may contribute to the formation of drug-gamma-CD complexes.

Acknowledgements

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