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Effect of carboxymethyl-β-cyclodextrin on the hydrophobicity parameters of steroidal drugs

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Abstract

The interaction between 17 steroidal drugs and carboxymethyl-β-cyclodextrin (CMβCD) was determined by charge–transfer chromatography and the relative strength of interaction was calculated. CMβCD interacted with the majority of steroidal drugs decreasing the hydrophobicity of the guest molecules. The relative strength of interaction considerably depended on the structure of the drug molecule. The hydrophobicity parameters of the drugs significantly influenced the strength of interaction indicating the involvement of hydrophobic forces in the binding of drugs to CMβCD. The marked influence of CMβCD on the hydrophobicity of drugs suggests that this interaction may have a considerable impact on the biological properties (adsorption, uptake, half-life etc,) of drug — CMβCD complexes resulting in modified efficacy.

Keywords: Carboxymethyl-β-cyclodextrin; Hydrophobicity; Steroidal drugs

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, and even inorganic compounds (Szejtli, 1982; Szejtli, 1989). The formation of various drug–CD inclusion complexes has been extensively studied. Thus, the formation of inclusion complexes with antimycotic agents (Pedersen et al., 1993), insulin (Watanabe et al., 1992a; Watanabe et al., 1992b), anticancer drugs (Distelmans et al, 1991) etc. has been reported. The physicochemical and pharmacological characteristics of drug–CD inclusion complexes deviate considerably from those of uncomplexed drug molecules. The formation of inclusion complexes improves the performance of intravenous formulation (Estes et al., 1990), prolongs the pulmonary absorption (Marques et al., 1991), sustains the release rate (Uekama et al, 1989), increases the stability of the guest molecule (Djedainipilard et al., 1993), enhances the peak concentration of drugs in blood (Hostetler et al., 1992), improves bioavailability (Müller and Albers, 1991; Hatanaka et al., 1993), and enhances the extent and rate of absorption in organs (Betlach et al., 1993).

Much effort has been devoted to the elucidation of the binding forces in the drug–CD interaction. It was assumed that dipole–dipole, van der Waals and hydrophobic interactions (Müller and Albers, 1992; Suzuki et al., 1991), and hydrogen bond formation (Park et al., 1992; Inoue et al., 1993) influence the strength of the drug–CD interaction.

Various chromatographic techniques can be successfully used for the study of molecular interactions (Cserháti and Valkó, 1994). A number of advantages are associated with the use of chromatographic techniques, such as the small quantity of material required. The interacting molecules used do not need to be of high purity, as any impurities are separated during the chromatographic process. Due to their considerable pharmaceutical importance, the interaction of steroidal drugs with CDs has been extensively studied (Loftsson et al., 1993; Chun and Yun, 1993). Chromatographic methods such as thin-layer (Cserháti and Forgács, 1996; Cserháti and Forgács, 1997) and high performance liquid chromatography have also been used for the study of such interactions (Sadley-Sosnowska, 1995; Sadley-Sosnowska, 1996).

Principal component analysis (PCA) (Mardia et al., 1979) has frequently been employed in biochemistry and biophysics. Thus, it has been applied in the multivariate characterisation of amino acids (Cocchi and Johansson, 1993), for the evaluation of aquatic toxicity data (Eriksson et al., 1995), and for the study of ligand selectivity (Pastor and Cruciani, 1995). Although PCA reduces the dimensionality of the original data matrix, the resulting matrices of the principle component (PC) and PC variables are sometimes even multidimensional. As the capacity of the human brain to evaluate data distributed in multidimensional space is

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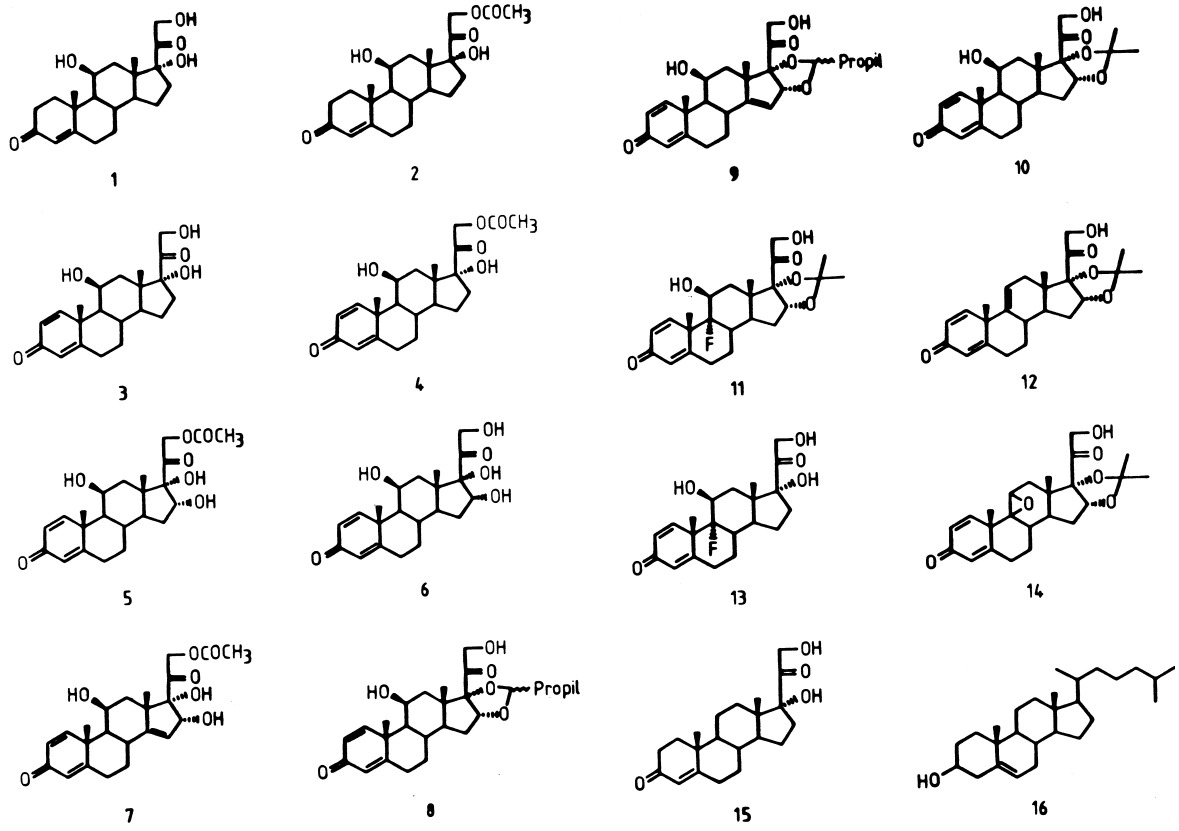

Fig. 1. Chemical structures of steroidal drugs.

limited, the dimensions of the matrices of PC loadings and variables can be reduced to two by nonlinear mapping technique (Sammon, 1969). This method takes into consideration the positive or negative signs of the correlations by constructing the corresponding maps. Necessarily, the variables with strong negative correlation are far from each other on the map. The situation is the same when two variables are not intercorrelated: they are also a large distance from each other on the map. This means that without the previous knowledge of the individual coefficients of regression, the evaluation of the similarities or dissimilarities between the variables is subjected to error when both negative and positive correlations occur between the members of the original data matrix. Theoretically, this discrepancy can be avoided by using only the absolute values for the construction of the map and dendogram.

The objectives of this work were to study the interaction of steroidal drugs with carboxymethyl-β-cyclodextrin (CMβCD) 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 by using PCA followed by nonlinear mapping.

2. Experimental

Polygram UV_{254} (Macherey–Nagel, Dürren, Germany) plates were impregnated by overnight predevelopment in *n*-hexane–paraffin oil 95:5 (v/v). The chemical structures of steroidal drugs are shown in Fig 1. Drugs were the gift of Professor Sándor Görög, Gedeon Richter, Ltd. Budapest, Hungary. The drugs were separately dissolved in methanol 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 20 to 30 vol.%. As the object was to study the complex formation between the solutes and CMβCD and not the study of the effect of CMβCD 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 CMβCD was excluded, Methanol was chosen as the organic solvent miscible with water because it forms only weak inclusion complexes with β-cyclodextrins (Buvári et al., 1983/1984; Harada and Takahashi, 1984), Carboxymethyl-β-cyclodextrin (CYCLOLAB Research and Development Laboratory, Budapest, Hungary) was added to the eluents in the concentration range of 0–15 mg/ml. Developments were carried out in sandwich chambers (22 × 22 × 3 cm) at room 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.

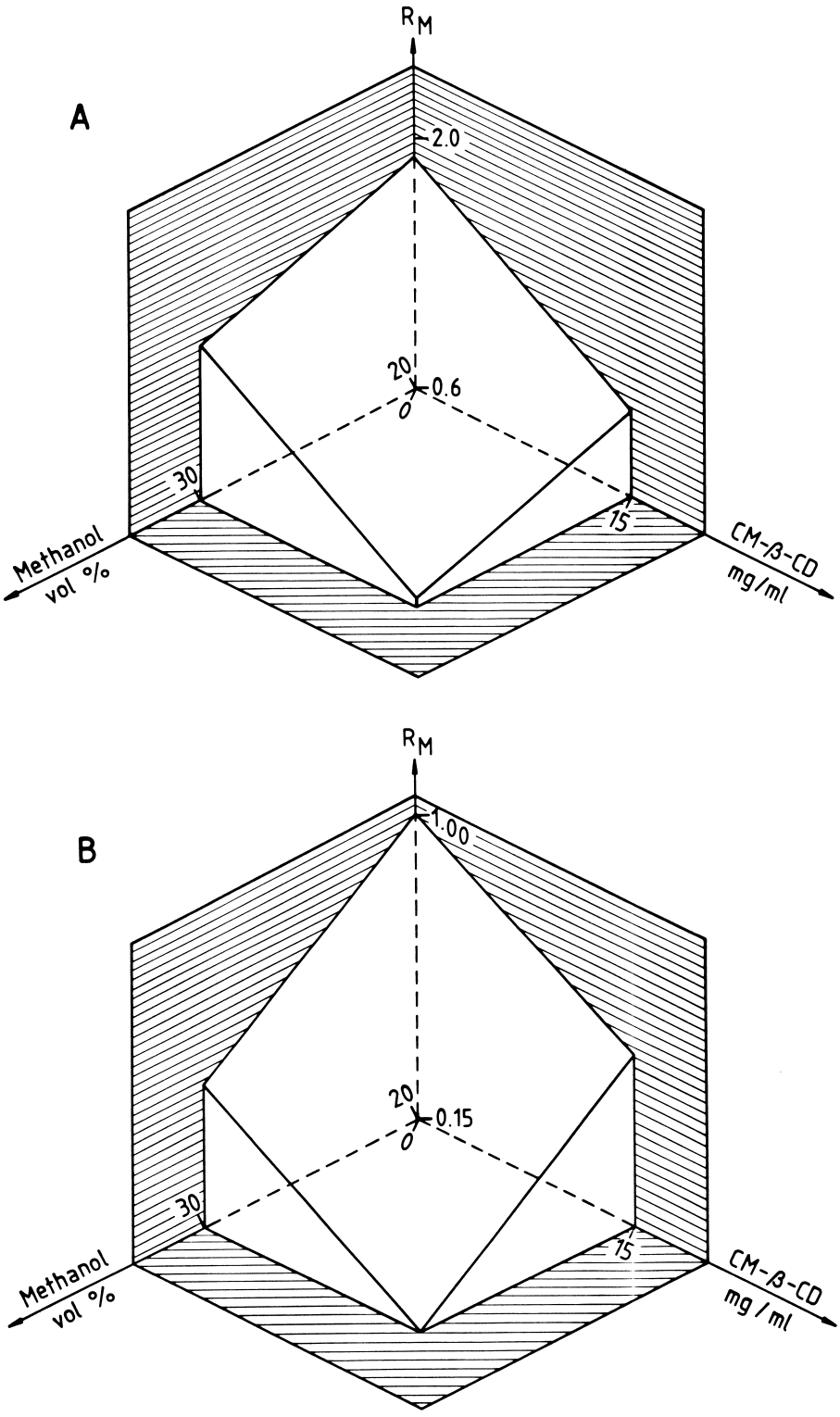

Fig. 2. Effects of methanol and carboxymethyl-β-cyclodextrin (CMβCD) concentrations on the RM value of steroidal drugs 2 (A) and 6 (B) in Fig. 1.

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

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

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 CMβCD on the hydrophobicity of steroidal drugs, the following equation

Table 1
Relationship between the R_M values of steroidal drugs and the concentrations of methanol (C_1) and carboxymethyl-β-cyclodextrin (C_2) in the eluent. Numbers refer to steroidal drugs in Fig. 1.$R_M = R_{MO} + b_1 \cdot C_1 + b_2 \cdot C_2$

Parameter	No of steroidal drugs														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
R_{M0}	1.98	2.81	2.10	2.72	2.48	1.94	2.37	2.48	2.55	3.40	2.25	3.56	2.06	2.29	2.37
$-b_1 \cdot 10^2$	3.88	4.52	4.14	4.69	4.92	4.64	4.55	2.95	3.42	5.24	3.60	5.29	3.88	3.63	3.64
$S_{b1} \cdot 10^3$	3.63	8.02	3.44	2.16	6.30	3.89	4.24	6.47	7.97	3.21	5.26	3.50	4.40	8.14	6.68
$-b_2 \cdot 10^2$	3.54	5.39	3.44	4.61	3.36	2.64	3.92	3.39	2.81	5.25	3,25	5.14	4.78	– –	5.86
$S_{b2} \cdot 10^3$	6.02	9.98	5.70	7.41	8.31	5.45	6.20	8.05	9.91	10.84	6.96	11.81	6.43	—	8.31
b_1%	64.53	51.10	66.59	77.73	65.88	74.45	62.91	52.00	60.21	77.13	59.42	77.68	54.28	—	43.58
b_2%	35.47	48.90	33.41	22.27	34.12	25.55	37.09	48.00	39.78	22.87	40.58	22.32	45.72	—	56.42
R^2	0.9085	0.7810	0.9235	0.9652	0.8332	0.9140	0.9090	0.6933	0.6214	0.9525	0.8096	0.9456	0.8944	0.6042	0.8262
$F_{calc.}$	74.47	21.40	90.49	262.89	32.47	79.74	69.93	13.56	9.85	170.34	27.63	147.87	59.29	19.84	28.51

was fitted to the experimental data:

$$R_M = R_{M0} + b_1.c_1 + b_2.c_2 \quad (2)$$

where R_M = R_M value for a drug determined at given methanol and CMβCD concentrations; R_{M0} = R_M value extrapolated to zero methanol and CMβCD concentrations; b_1 = 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 (Horvath et al., 1976; b_2 = decrease in the R_M value caused by 1 mg/ml concentration change of CMβCD in the eluent (related to the relative strength of interaction); and C_1 and C_2 = concentrations of methanol and CMβCD**,** respectively. Eq. 2 was applied separately for each steroidal drug.

Charge-transfer chromatography carried out on reversed-phase thin-layer plates shows some advantages: the method is rapid, and it is suitable for the simultaneous determination of more than one interaction. However, the drawback of the method is that the exact stoichiometry of the complexes cannot be determined and only the relative strength of the interaction can be calculated.

To find the physicochemical parameters of steroidal drugs significantly influencing their complex forming capacity, principal component analysis was applied. The relative strength of interaction (b_2), the measured hydrophobicity (R_{M0}), the specific hydraphobic surface area (b_1) of Eq. 2, and the following calculated physicochemical parameters were the variables: π = Hansch–Fujitas substituent constant characterising 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 characterising the inductive and resonance effect, respectively; σ = Hammett's constant, characterising the electron-withdrawing power of the substituent in para and ortho + meta position ($\sigma_{ortho+meta}$, σ_{para}); Es = Taft's constant, characterising steric effects of the substituent; B_1 and B_4 = Sterimol width parameters determined by distance of substituents at their maximum point perpendicular to attachment. The calculation of the physicochemical parameters of solutes was carried out using the additivity rule. The physiochemical parameters were the variable and the steroid drugs were the observations in the principle component analysis. The dimensionality of the matrices was reduced to two by the nonlinear mapping technique. The iteration was carried out to the point where the difference between the last two iterations was less than 10^{-8}. Map of PC loadings was prepared from the original loadings and from the absolute value of the loadings.

The software for PCA and nonlinear mapping was written by Dr Barna Bordás (Plant Protection Institute of Hungarian Academy of Sciences, Budapest, Hungary).

3. Results and discussion

Compounds 16 and 17 remained at the origin in each eluent system, indicating that they are more hydrophobic than the other steroidal drugs investigated, and therefore their interaction with CMβCD cannot be studied under these experimental conditions.

The simultaneous effect of methanol and CMβCD concentrations on the R_M values of drugs 2 and 6 are shown in Fig. 2.. The R_M value decreases in each instance with increase in methanol concentration, i.e. these compounds do not show any anomalous retention behaviour in this concentration range that would invalidate the use of Eq. 2. An increase in the CMβCD concentration also caused a decrease in R_M values, indicating complex (probably inclusion complex) formation. Interaction of the more hydrophilic CMβCD with the steroidal drugs decreases the hydrophobicity of the latter. As the hydrophobicity of a bioactive compound influences the capacity to penetrate the hydrophobic cell membrane (Vaara et al., 1990) and to bind to the target organs (Camilleri et al., 1990) or organisms (Fini et al., 1990) the hydrophobicity values have been frequently used in various quantitative structure–activity relationship studies (Franke, 1985). Due to the modification of the hydrophobicity of steroidal drugs by CMβCD, it can be expected that the biological properties (adsorption, uptake, half-life etc,) of drug–CMβCD complexes may be different from that of uncomplexed drug.

The parameters of Eq. 2 are compiled in Table 1. The

Table 2
Similarities and dissimilarities between the physico–chemical parameters of steroidal drugs and their capacity to interact with carboxymethyl-β-cyclodextrin. Results of principal component analysis.

No. of component	Eigenvalue	Variance explained (%)		Sum of variance explained (%)	
1	6.12	43.72		43.72	
2	2.58	18.45		62.17	
3	2.26	16.16		78.33	
4	1.27	9.05		87.39	
5	0.83	5.95		93.34	
	Principal component loadings				
Parameters	No of principal components				
	I	II	III	IV	V
R_{MO}	0.50	− 0.51	0.52	0.25	− 0.22
b_1	0.17	0.01	0.86	0.01	− 0.39
b_2	− 0.14	− 0.20	0.54	0.56	0.52
π	0.03	0.14	0.45	− 0.75	0.45
H-Ac	0.82	− 0.09	− 0.15	0.37	0.21
H-Do	0.51	− 0.53	− 0.56	0.07	− 0.01
M-RE	0.90	0.24	0.23	0.06	0.12
F	0191	0.13	− 0.32	− 0.05	− 0.02
R	− 0.78	0.58	0.11	0.19	− 0.02
$\sigma_{(ortho+meta)}$	0.78	0.47	− 0.34	0.00	− 0.02
$\sigma_{(para)}$	− 0.37	0.87	− 0.02	0.24	− 0.18
Es	− 0.79	0.24	− 0.29	0.25	0.22
B_1	0.74	0.58	0.18	− 0.05	0.02
B_4	0.84	0.44	0.16	0.12	0.09

equation fits the experimental data well, the significance level in each instance being over 95.0% (see calculated F values). Except for compound 14, each steroidal drug interacts with CMβCD (b_2 values differ significantly from zero) suggesting that in pharmaceutical formulations containing both steroidal drugs and CMβCD, their possible interaction has to be taken into consideration. The parameters of Eq. 2 show high variations between the drugs proving that the hydrophobicity (R_{M0}), specific hydrophobic-surface area (b_l) and their capacity to form inclusion complexes with CMβCD (b_2) differ considerably. This finding suggests also that the inclusion complex formation may influence differently the biological effect of individual steroidal drugs, The path coefficients (b values) indicates that the impact of the change of methanol and CMβCD concentrations on the reversed-phase mobility of steroidal drugs is commensurable, i.e. the retention of steroidal drugs can be equally modified by changing either the methanol or the CMβCD concentration in the eluent. The results of principal component analysis are compiled in Table 2. Five principal components explain the overwhelming majority of variance indicating that the 14 original variables can be substituted by 5 background (abstract) variables with only 6.66% loss of information. Unfortunately, PCA does not prove the existence of such background variables as concrete physicochemical entities, but only indicates their mathematical possibility.

The complex forming capacities of steroidal drugs — together with many physicochemical parameters — have high loadings in the first three PCs, indicating the marked influence of these parameters on the complex forming capacity of drugs. The two-dimensional nonlinear maps calculated from the original PC loadings and from the absolute values of PC loadings are shown in Fig. 3. Maps show marked differences in the distribution of variables indicating the considerable impact of the modification of the mode of calculation. The measured (R_{MO} = I, b_1 = II) and calculated hydrophobicity parameters (π = IV) form a distinct cluster with the capacity of steroidal drugs to interact with CMβCD (b_2 = III) on both PCL maps indicating the strong relationship between these variables. The fact that the hydrophobicity parameters exert a considerable influence on the strength of interaction suggests that hydrophobic forces are involved in the interaction. This result supports the assumption that steroidal drugs enter into the lipophilic cyclodextrin cavity and they are retained by hydrophobic forces. Steroidal drugs did not form distinct clusters on the two-dimensional map PC variables (Fig.4). This finding indicates that more than one molecular substructure of drugs influence their capacity to form inclusion complexes with CM(CD and the strength of interaction is the results of the interplay of the contribution of the various substructures to the interaction.

It can be concluded from the data that steroidal drugs form complexes (probably inclusion complexes) with CM(CD. The strength of complex formation markedly depends on the structure of the drug molecule and is significantly influenced by the hydrophobicity parameters of the drugs. It is probable that the complex formation of drugs with CM(CD modifies the various biological parameters uptake, transfer, decomposition rate, etc.) and consequently

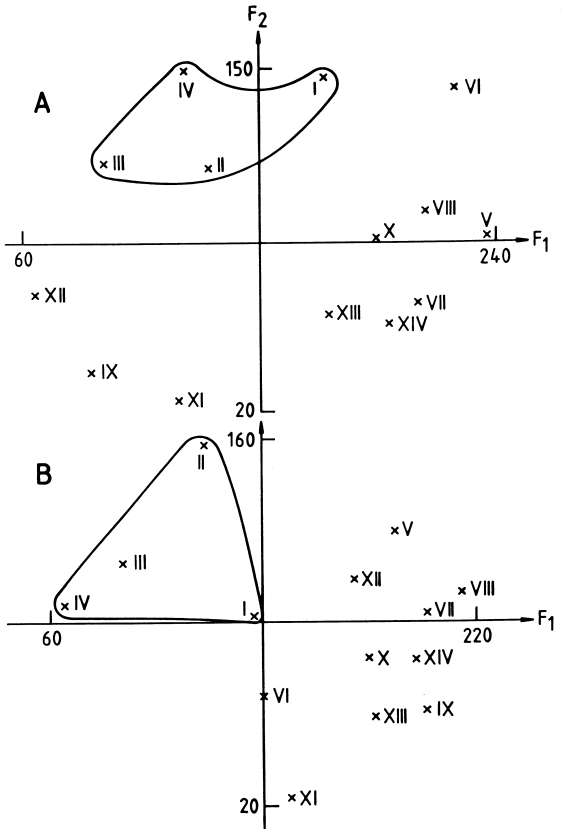

Figure 3. Two-dimensional nonlinear map calculated from the original PC loadings (A) and from the absolute values of PC loadings (B). A = number of iterations: 228; maximum1 error: 5.35×10^{-2}. B = number of iterations: 86; maximum error: 2.32×10^{-2}. I = R_{M0}; II = b_1; III = b_2; IV = π; V = H-Ac; VI = H-Do; VII = M-RE; VIII = F; IX = R; X = $\sigma_{ortho+meta}$; XI = σ_{para}; XII = Es; XIII = B_1; XIV = B_4.

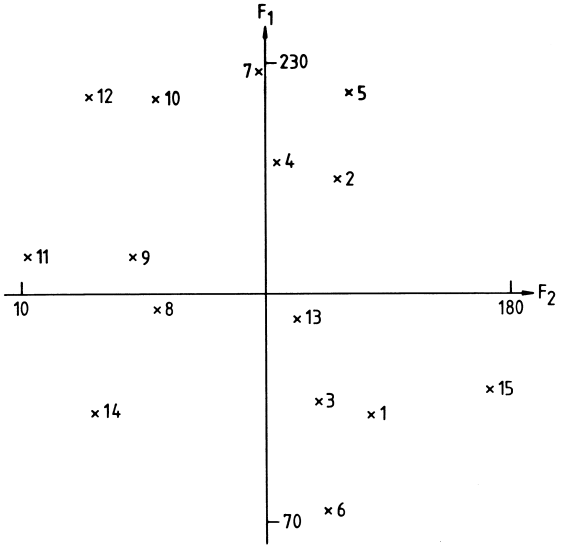

Figure 4. Two-dimensional nonlinear map of PC variables. Number of iterations: 184; maximum error: 5.32×10^{-2}.

the biological efficacy of the steriodal drugs in living organisms.

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