

Principal Component Analysis for a Better Understanding of the Herbicidal Effectivity of Some Benzonitriles

Z. SZIGETI¹ T. CSERHÁTI² and B. BORDÁS²

1 *Department of Plant Physiology, Eötvös University, Budapest, P.O.B. 324, H-1445, Hungary*

2 *Research Institute for Plant Protection, Hungarian Academy of Sciences, Budapest, P.O.B. 102, H-1525, Hungary*

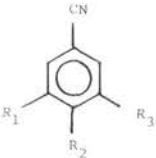
Abstract. To gain more insight in mode of action of ten different 4-hydroxy-benzonitrile derivatives, their biological activities in eight bioassays, and their lipophilicity and adsorptivity determined by thin-layer chromatography in nine different systems were subjected to principal component analysis. Four background components explained about 90 % of total variance. Only three of eight biological activities, the inhibition of the 2,6-dichlorophenol-indophenol reduction by spinach and wheat chloroplasts and the CO₂ fixation of wheat seedlings had not any common background components with the physico-chemical parameters of the compounds. The nonlinear mapping of principal component loadings and variables showed, that the *in vivo* and *in vitro* biological activities differed considerably and depended on the object investigated. The effectivity of compounds is governed mainly by the number of substituents and by the presence of free hydroxy group.

Key words: Principal component analysis — Benzonitriles — Herbicidal effectivity

Introduction

In computerized quantitative structure-activity relationship (QSAR) approach of drug and pesticide design it has become apparent that biological activity can rarely be coupled to a single parameter, but should rather be envisaged as a result of interplay of various parameters. In biological experiments the results are determined by a great number of variables, which can be measured, but there are hidden factors, which can be extracted by principal component analysis (PCA) and by factor analysis. By these methods we can reduce the number of variables (parameters) to be taken into account. These multivariate methods are specially useful when the variables show significant intercorrelations (Mardia et al. 1979). Up till now, these methods have received relatively little attention in QSAR studies.

Table 1. Chemical structure of benzonitrile derivatives investigated

General structure	Compound N°	R ₁	R ₂	R ₃
	I	H	OH	H
	II	H	OH	Br
	III	H	OH	NO ₂
	IV	NO ₂	OH	Br
	V	Br	OH	Br
	VI	Cl	OH	NO ₂
	VII	Br	OH	NO ₂
	VIII	I	OH	NO ₂
	IX	Br	O—CO—OCH ₃	NO ₂
	X	Br	O—CO—OCH(CH ₃) ₂	NO ₂

The herbicidal character of 3,5-dihalogeno-4-hydroxy-benzonitriles has been known since 1963 (Carpenter and Heywood 1963; Wain 1963). They are primarily inhibitors of photosynthetic electron transport chain (Paton and Smith 1965) and uncouplers of oxidative and photophosphorylation (Ferrari and Moreland 1969). The 3,5-disubstituted 4-hydroxy-benzonitriles containing nitro group have a similar mode of action (Szigeti et al. 1982).

Lipophilicity is one of the physico-chemical parameters frequently applied in quantitative structure-activity relationship studies (Fujita et al. 1964). Lipophilicity can be determined by the classical partition method between water and *n*-octanol (Hansch and Anderson 1967; Papp et al. 1982), by high performance liquid chromatography (McCall 1975) and by reversed-phase thin-layer chromatography (RPTLC) (Boyce and Milborrow 1965; Biagi et al. 1969). To increase the reliability of lipophilicity determination different water: organic phase ratios have been frequently applied (Ekiert et al. 1980; Cserháti 1984). The salt concentration (Cserháti et al. 1982) and the pH value (Rittich et al. 1980) of eluent can considerably influence the lipophilicity. Sometimes the values determined on non-impregnated silica correlate better with the biological activity than the values measured on impregnated silica (Guerra et al. 1981).

To gain more insight in mode of action of ten different 4-hydroxy-benzonitrile derivatives, their biological effectivity in eight assays, and their physico-chemical parameters determined in nine different thin-layer chromatographic systems were subjected to principal component analysis.

Materials and Methods

The benzonitrile derivatives were synthesized in the Research Centre of Chinoïn Pharmaceutical and Chemical Works LTD. Budapest. The chemical structures of the compounds are shown in Table 1.

The reversed-phase thin-layer chromatographic (RPTLC) and thin-layer chromatographic (TLC)

Table 2. Chromatographic systems to study the reversed-phase and adsorptive thin-layer chromatographic behaviour of benzonitrile derivatives

System	Layer	Eluent composition
1	reversed-phase	water: methanol 1:1
2	reversed-phase	water: methanol 4:1
3	reversed-phase	2 mol l ⁻¹ NaCl
4	reversed-phase	water
5	reversed-phase	Britton-Robinson buffer pH 7.96
6	reversed-phase	Britton-Robinson buffer pH 5.02
7	reversed-phase	2 mol l ⁻¹ CaOHCl pH = 7.00
8	silica	water
9	silica	2 mol l ⁻¹ NaCl

systems used for determination of lipophilicity and adsorptivity are compiled in Table 2. Layer thickness was always 0.25 mm, 5 µl-s of solution of 2 mg compound per cm³ acetone were spotted to the plates. After development the exact localization of spot maximums was carried out by a video-densitometer (Telechrom OE 976, Chinoi, Hungary). Lipophilicity and adsorptivity of compounds were characterized by R_M and R_f values, respectively. For RPTLC, the plates were impregnated in a 5 % solution of paraffin oil in n-hexane overnight. Because of the acidic character of CaCl₂, 2 mol l⁻¹ CaCl₂ solution was titrated to pH 7.0 with 2 mol l⁻¹ Ca(OH)₂ solution and the resulting neutral solution was used as eluent.

For biological activity measurements, the following assays were used: Photosystem 2 (PS 2) activity was characterized by reduction of 2,6-dichlorophenol-indophenol (DCPIP) by broken chloroplasts isolated from spinach or wheat in presence of 30 µmol l⁻¹ benzonitrile (for other conditions, see Szigeti et al. 1981). CO₂ fixation: after 2 h treatment of the leaves with 30 µmol l⁻¹ benzonitrile, they were placed in an atmosphere containing 1 % CO₂ (0.1 % ¹⁴CO₂), illuminated for one minute at 12 klx. Radioactivity of the samples was determined by a liquid scintillation technique (Sárvári et al. 1976). The activity of the carboxylating enzymes, ribulose-1,5-bisphosphate carboxylase (RuBPC) and phosphoenolpyruvate carboxylase (PEPC) was determined in vitro using crude enzymes, extracted from spinach leaves or 7-day-old wheat seedlings and was characterized by the amount of H¹⁴CO₃ fixed. The enzymes were preincubated with 30 µmol l⁻¹ benzonitriles. The reaction was started by adding the corresponding substrate (Nagy et al. 1973).

In PCA the R_M and R_f values of compounds and their biological activities were the variables 1-9 and 10-17, respectively; the compounds were taken as observations. The two dimensional nonlinear mapping of principal component (PC) loadings and variables were also carried out according to Sammon (1969). To separate the individual loadings varimax rotation was applied to the PC loadings (Weber 1974). PCA was also carried out separately for the chromatographic and biological data. The principal component variables of biological data exceeding the eigenvalue 1 were correlated with the principal component variables of chromatographical data exceeding the eigenvalue 1:

$$y = a + b_1 \cdot x + b_2 \cdot x^2 + b_3 \cdot \frac{1}{x}$$

where y = principal component variables of biological data

x = principal component variables of chromatographic data

To exclude non-relevant information stepwise regression analysis was applied, the partial F value of independent variables was set to $F = 0.8$.

Table 3. $100 \cdot R_M$ (columns 1—7) and $100 \cdot R_f$ (columns 8, 9) values of some benzonitrile derivatives. For symbols, see Tables 1 and 2

Compound	Chromatographic system								
	1	2	3	4	5	6	7	8	9
I	-25	4	99	46	36	12	90	87	77
II	-37	-36	127	36	71	115	152	89	64
III	-76	-79	85	-39	11	-5	57	91	56
IV	-72	-83	82	-48	-7	-16	58	97	82
V	-75	-58	121	-21	26	74	105	90	53
VI	-65	-75	78	-47	9	-12	78	96	71
VII	-65	-66	94	-34	8	-4	87	96	74
VIII	-63	-57	117	-15	22	30	117	95	73
IX	28	117	93	300	300	300	300	42	0
X	66	168	300	300	300	300	300	0	0

Results and Discussion

The R_M and R_f values are shown in Table 3. Each value is the mean of five independent parallel determinations. In most cases the compounds move well in distilled water as eluent. According to our earlier results with other compounds, molecules containing hydrophilic substituents have a lower lipophilicity in ionic environment than in an ion free one (Cserháti and Szőgyi 1981). However, these benzonitrile derivatives containing highly hydrophilic substituents show higher lipophilicity in ionic environment. It means that the ions may promote the penetration of these compounds through the lipophilic membrane structures of plant cells. We assume that this phenomenon can be explained by the strong intramolecular forces between the substituents, which are modified by the ions.

Similar results were obtained concerning the adsorption of benzonitrile derivatives on silica. Due to the competition of ions and hydrophilic substituents of compounds for the polar adsorption sites (silanol groups) on silica surface a decrease of adsorptivity of molecules containing hydrophilic substituents can be expected. In case of benzonitrile derivatives — in spite of their hydrophilic substituents — their adsorptivity is enhanced by ions. On the basis of this finding their lower mobility and smaller leaching rate in soils of high salt content can be expected.

The biological effects are summarized in Table 4. The biological activity data were discussed in details in our earlier publications (Szigeti and Nagy 1980; Szigeti et al. 1981). In the present paper the most important statements are reported only: (a) In the inhibition of PS 2 activity only the 3,5-disubstituted 4-hydroxy-benzonitriles were active. (b) The spinach chloroplasts were more sensitive to these compounds than the chloroplasts isolated from wheat. (c) In the *in vivo* CO_2

Table 4. Biological activity of some benzonitrile derivatives. (Percentage of inhibition)

Compound	Biological parameter							
	10	11	12	13	14	15	16	17
I	14.2	4.4	-7.0	35.1	2.8	9.3	3.9	34.9
II	8.3	31.1	6.3	18.8	16.7	4.6	1.7	33.9
III	2.9	36.0	0.7	43.5	11.1	9.3	9.1	28.4
IV	98.7	-12.3	-1.6	32.5	51.4	-21.3	5.8	32.1
V	91.2	89.3	0.0	27.9	51.4	52.8	10.5	30.4
VI	80.9	23.7	8.0	16.9	52.8	-2.8	0.0	7.3
VII	91.6	84.9	-3.0	55.8	62.5	4.6	1.4	41.3
VIII	91.7	33.6	12.9	27.3	76.4	16.7	7.2	15.6
IX	71.4	89.0	6.1	0.0	45.8	8.3	15.4	-10.1
X	44.2	74.6	19.3	12.3	45.8	-5.6	21.5	6.4

Biological parameters: 10 = DCPIP reduction by spinach chloroplasts; 11 = CO₂ fixation of spinach leaves in vivo; 12 = RUBPC activity of spinach; 13 = PEPC activity of spinach; 14 = DCPIP reduction by wheat chloroplasts; 15 = CO₂ fixation of wheat seedlings in vivo; 16 = RUBPC activity of wheat; 17 = PEPC activity of wheat. For compound numbers, see Table 1.

Table 5. Eigenvalues of principal component analysis

Principal component	Eigenvalues	Sum of total variances explained [%]
1	10.71	63.00
2	2.22	76.07
3	1.54	85.11
4	1.05	91.27
5	0.64	95.04

fixation assay the tolerance of wheat is more pronounced. (d) None of the compounds affected significantly the activity of the RuBPC isolated from spinach and wheat, respectively. However, PEPC was inhibited to various extent — by almost all compounds.

The results of PCA (Tables 5 and 6) showed that there were four PCs which explained 90 % of structure-activity relationships, but up till now we could not identify the PCs with any real physico-chemical or chemical parameters. (The PCs do not prove the existence of real parameters, they only indicate that within the set of measured data their existence is mathematically possible.)

The two dimensional nonlinear mappings of PC loadings and variables are shown in Figures 1 and 2. From Fig. 1 it can be seen that molecular lipophilicities (points 1—7) and adsorptivities (points 8—9) form separate clusters on the opposite poles of the map indicating that hydrophobic substituents decrease the

Table 6. Loadings of the principal components. The number of variables corresponds to physico-chemical parameters (1–9) and biological activities (10–17), respectively. (See Tables 2, 3 and 4)

Variables	Components				
	1	2	3	4	5
1	<u>0.94</u>	0.22	-0.08	0.12	0.11
2	<u>0.96</u>	0.15	0.02	0.09	0.13
3	<u>0.73</u>	-0.04	-0.03	<u>0.59</u>	-0.32
4	<u>0.97</u>	0.14	0.02	-0.03	0.17
5	<u>0.99</u>	0.07	0.01	-0.05	0.14
6	<u>0.97</u>	0.02	0.10	-0.06	0.05
7	<u>0.98</u>	-0.01	0.02	-0.06	0.09
8	<u>-0.96</u>	-0.07	0.03	-0.23	-0.03
9	<u>-0.96</u>	0.11	-0.13	0.09	0.02
10	<u>-0.30</u>	<u>-0.87</u>	-0.20	0.16	0.17
11	<u>0.52</u>	<u>-0.43</u>	<u>0.64</u>	0.02	0.18
12	<u>0.68</u>	-0.34	-0.36	0.06	-0.48
13	<u>-0.74</u>	0.03	0.26	<u>0.51</u>	0.19
14	<u>0.06</u>	<u>-0.95</u>	-0.22	0.05	0.15
15	<u>-0.07</u>	<u>-0.31</u>	<u>0.83</u>	-0.23	-0.32
16	<u>0.85</u>	-0.09	<u>0.17</u>	0.17	-0.06
17	<u>-0.75</u>	0.22	0.28	0.49	0.01

adsorption energy of 4-hydroxy-benzonitrile derivatives on hydrophilic surfaces. The DCPIP reduction (points 10–14) and PEPC activity (points 13–17) of spinach and wheat are close to each other, indicating the similarity of mode of inhibition in these *in vitro* systems used. RuBPC activities (points 12, 16) differ more strongly than the two activities mentioned above. The highest deviation in the *in vivo* CO₂ fixation was observed between wheat and spinach (points 11, 15). This finding emphasizes again that the more complicated the biological systems investigated, the higher the differences between their responses to herbicides. This result also reflects the different *in vivo* sensitivity of wheat and spinach to benzonitriles.

The compounds differ considerably from each other in their biological activities and physico-chemical parameters (Fig. 2). The ester derivatives (points IX, X) separate clearly from the other compounds on the map, indicating that their herbicidal effect deviates highly from those of free phenole derivatives. These latter compounds form clusters according to the number of substituents. The first substitution — in position 3 — may hardly modify the behaviour of 4-hydroxy-benzonitrile molecule (point I is not so far from points II and III), however, further substitutions (mainly with halogens in position 5) change considerably the characteristics of the parent molecule (points IV–VIII).

The results of varimax rotation support our previous conclusions (Table 7). The physico-chemical parameters show marked similarities, however, only the CO₂

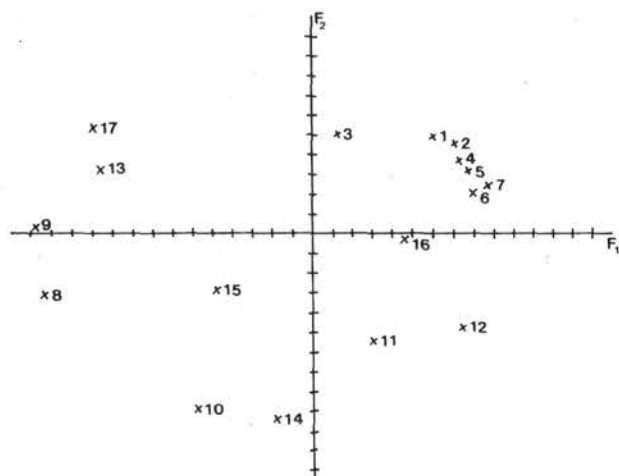


Fig. 1. Two-dimensional nonlinear mapping of principal component loadings. Numbers indicate physico-chemical parameters and biological activities of Tables 3 and 4. Number of iterations: 20, error of mapping: 0.030.

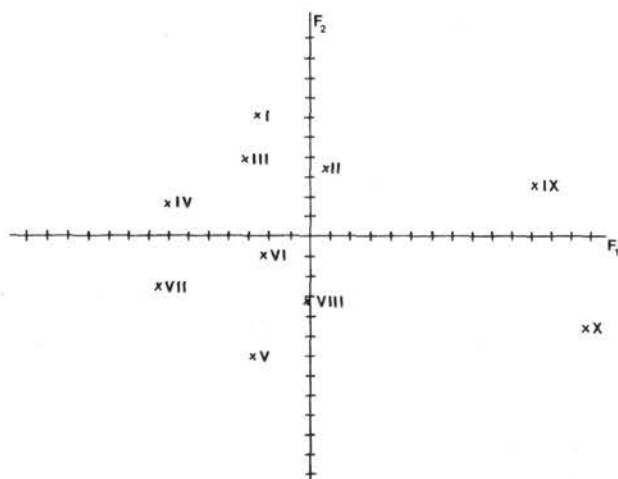


Fig. 2. Two-dimensional nonlinear mapping of principal component variables. Roman numbers indicate compounds listed in Table 1. Number of iterations: 20, error of mapping: 0.026.

fixation of spinach *in vivo* (11) and the RuBPC activity of wheat (16) may primarily correlate to the physico-chemical parameters. The DCPIP reduction by spinach (10) and wheat chloroplasts (14), the *in vivo* CO₂ fixation (11, 15) and PEPC activities (13, 17) have high loadings in different components, demonstrating that

Table 7. Varimax rotation of principal component loadings. Number of iterations: 12

Variables	Rotated matrix				
	1	2	3	4	5
1	<u>0.89</u>	0.17	-0.15	-0.23	-0.21
2	<u>0.92</u>	0.13	-0.05	-0.23	-0.17
3	<u>0.63</u>	-0.02	-0.02	0.06	<u>-0.75</u>
4	<u>0.93</u>	0.13	-0.03	-0.31	-0.08
5	<u>0.92</u>	0.07	0.00	-0.36	-0.11
6	<u>0.87</u>	0.07	0.12	-0.36	-0.15
7	<u>0.88</u>	0.01	0.05	-0.38	-0.15
8	<u>-0.90</u>	-0.05	0.06	0.18	0.34
9	<u>-0.82</u>	0.02	-0.21	0.40	0.20
10	<u>-0.26</u>	<u>-0.90</u>	0.01	0.09	-0.01
11	<u>0.58</u>	-0.28	<u>0.63</u>	0.04	0.05
12	<u>0.34</u>	-0.23	-0.05	<u>-0.53</u>	<u>-0.66</u>
13	-0.42	-0.07	0.02	<u>0.80</u>	0.11
14	0.01	-0.97	0.07	-0.16	-0.08
15	-0.12	0.02	<u>0.95</u>	0.01	0.00
16	<u>0.70</u>	-0.02	0.18	-0.15	-0.30
17	<u>-0.49</u>	0.16	0.03	0.78	0.01

Table 8. Results of principal component analysis carried out separately on the chromatographic (A) and biological (B) data

Principal component	Eigenvalues		Sum of total variance explained (%)	
	A	B	A	B
1	8.16	3.24	90.63	40.53
2	0.55	1.89	96.80	64.20
3		1.46		82.39
4		0.57		89.54

Principal component loadings						
components Variables	A		Variables	B		
	1	2		1	2	3
1	<u>0.97</u>	0.04	10	0.34	<u>-0.85</u>	0.33
2	<u>0.98</u>	0.00	11	<u>0.56</u>	-0.32	<u>-0.64</u>
3	<u>0.75</u>	<u>0.65</u>	12	<u>0.81</u>	0.21	0.19
4	<u>0.98</u>	-0.14	13	<u>-0.77</u>	-0.42	-0.10
5	<u>0.99</u>	-0.15	14	<u>0.52</u>	<u>-0.77</u>	0.33
6	<u>0.97</u>	-0.13	15	0.07	-0.35	<u>-0.81</u>
7	<u>0.98</u>	-0.13	16	<u>0.73</u>	0.26	<u>-0.32</u>
8	<u>-0.98</u>	-0.16	17	<u>-0.88</u>	-0.25	-0.15
9	<u>-0.94</u>	0.16				

there are some similarities between the responses of wheat and spinach to the 4-hydroxy-benzonitrile derivatives. The importance of this similarity is, however, small because the second PC explains 13 % of total variance, while the third PC only 9 % of that.

The data of PCA carried out on the chromatographic data shows clearly that a "general lipohydrophilic property" of compounds can be defined by this procedure (Table 8). However, three hidden components governs the eight biological activities. The distribution of principal component loadings is similar to that of the common PCA.

Stepwise regression analysis found linear correlation between the "general hydrophobic property" of compounds and the first components of biological activity:

$$y = (0.48 \pm 0.15) \cdot x \quad r_{\text{calc.}} = 0.7564 \quad r_{98\%} = 0.7155$$

This finding proves again that the molecular characteristics of compounds determined by chromatographic procedures explain a considerable part of their overall biological activity. No correlation was observed between the chromatographic and the other biological parameters.

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Received February 15, 1984/Accepted May 29, 1984