The mutagenicity of benzimidazole and benzimidazole derivatives
III. The influence of the 2-substituent in benzimidazole on the mutagenic activity*

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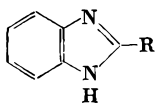
Summary

A number of 2-substituted benzimidazoles has been prepared and the compounds were characterized by their physico-chemical and spectral properties. – Mutagenicity tests with two strains of *Salmonella typhimurium* revealed that on one hand the mutagenic activity of these compounds tends to parallel their respective pK_a's, but that on the other hand the benzimidazoles with larger sized 2-substituents are comparatively less active mutagenic compounds.

As benzimidazole proved to be mutagenic in the his G 46 and TA 1530 strains of *Salmonella typhimurium*[1] and as the mutagenic activity seemed to be enhanced by a substituent in 2-position, we undertook a comparative study of the influence the size of the group has on the mutagenicity of benzimidazole. Since benzimidazole acts through base substitutions by direct incorporation of the molecule into nucleic acids[2] it was supposed that a certain limit in the size of the substituent should exist, beyond which the substance would be no more mutagenic or be mutagenic by acting through another mechanism. We wish to report the results of this study.

We synthesized the 2-substituted benzimidazoles



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[1] J. P. SEILER, *Mutation Res. 15* (1972) 273.
[2] J. P. SEILER, *Mutation Res. 17* (1973) 21.

Table 1

No.	Compound	Molecular Weight	Melting Point	pK_a	E_{280} [a]	GC retention time
1	Benzimidazole	118.15	171–172	5.5	3200	32′40″ [b]
2	2-Methylbenzimidazole	132.17	176	6.2	6240	28′10″ [b]
3	2-Ethylbenzimidazole	146.19	174–175	6.3	7110	27′40″ [b]
4	2-Isopropylbenzimidazole	160.21	236–237	6.2	7370	21′20″ [b]
5	2-tert. Butylbenzimidazole	174.23	320 subl.	6.1	7350	14′20″ [b]
6	2-Cyclohexylbenzimidazole	200.23	287–288	5.9	7860	–
7	2-Benzylbenzimidazole	208.25	191–192	5.6	7330	25′ [c]
8	2-Diphenylmethylbenzimidazole	284.34	230–232	< 3	5450	111′ [c]
9	2-(α,α) Diphenylethylbenzimidazole	298.36	233–234	< 3	5340	41′ [c]
10	2-(4′-Biphenylyl) benzimidazole	270.33	303–305	< 3	11670	230′ [c]

[a] in methanolic solution
[b] column temperature 200°
[c] column temperature 260°

listed in table 1 by the following general method[3]: *o*-Phenylenediamine was heated in a water or oil bath with a ca. 2-fold excess of the corresponding acid for 2 to 8 hours. The solution was neutralized with 10% NaOH after cooling and the precipitate filtered off. This precipitate was then dissolved in either boiling water or hot aqueous ethanol and treated with charcoal. After hot filtration the clear solution was allowed to cool slowly to room temperature for crystallization of the benzimidazole. The crystals were then collected, recrystallized once (without charcoal) and finally dried at 80° over P_2O_5.

Melting point determination and GC analysis was used to check the purity of these compounds. Also UV-, IR- and NMR-spectra were recorded to investigate comparatively their spectral properties.

GC-Analyses were performed on a Varian 2100 gas chromatograph equipped with a flame ionization detector; the benzimidazoles were separated on a 2.0 m glass column (i.D. 3 mm) packed with 3% Carbowax 20 M on Chromosorb W 100/120 mesh. Column temperature was 200 °C or 260 °C resp., as indicated in table 1, detector temperature 235 °C, injector temperature 270 °C and He flow 26 ml/min. The retention times are shown in table 1.

The influence of the various substituents on the electron density around N-1 of the benzimidazole nucleus was determined by measuring the pK_a of the different compounds. Owing to the very slight solubility of these benzimidazoles in water the determination by titration of a benzimidazole solution proved to be very difficult if not impossible. We therefore determined these values by using equation (1), which is valid for a salt of a strong acid with a weak base, and combining it with the general equation (2).

$$pH = -\log K_w - \frac{1}{2}(pK_b + \log c_{Salz}). \qquad (1)$$

$$pK_a = pK_w - pK_b. \qquad (2) \text{ (from [4])}$$

In order to determine the pK_a we prepared first the hydrochlorides of the various benzimidazoles by passing dry hydrogen chloride through a dry ethereal solution of the benzimidazole. The dried hydrochloride was then dissolved in water and the *p*H of this solution was measured. The results obtained with the first four benzimidazoles agreed fairly well with the values already published[5,6]. Difficulties were only encountered with the last three benzimidazoles, the hydrochlorides of which were not completely soluble.

The main issue was but the influence of the size of the 2-substituent on the mutagenic activity of the benzimidazole. For these tests we used the strains his G46 and his D3052 for the identification of base substitution and frameshift mutagenicity, resp. The tests were done as described elsewhere[1,7]; the benzimidazoles were dissolved in dimethylsulfoxide to a concentration of about 15 mg/ml or 0.25 mg per plate. After 3 days of incubation at 37 °C revertant colonies were scored and the mutagenicity of the tested compounds relative to the background of spontaneous reversions calculated. It can be seen from the results presented in table 2 that aliphatic substituents in 2-position in the first instance tend to enhance the relative mugenicity, while aromatic ones depress the mutagenic activity. This behavior can be accounted for by the different electronic properties of alkyl- and aryl-groups, resp., which finds its expression in the change of pK_a's. In addition to the decreased mutagenic activity of aryl substituted benzimidazoles with respect to base substitutions there is no corresponding increase of frameshift mutagenicity. Furthermore by comparing pK_a's and relative mutagenic activities, it can be seen that the mutagenicity is de-

[3] E.C. WAGNER and W.H. MILLETT, Benzimidazole, in *Organic Syntheses*, Coll. Vol. II (A.H. BLATT ed.), p. 65, J. Wiley & Sons, New York/London 1943.
[4] *Biochemisches Taschenbuch* (H.M. RAUEN ed.), Springer, Berlin 1964, Vol. II, p. 46.
[5] C.P. WHITTLE and R.K. ROBINS, *J. Amer. Chem. Soc. 87* (1965) 4940.
[6] *Handbook of tables for organic compound identification* (Z. RAPPOPORT ed.), The Chemical Rubber Comp., Cleveland 1967, p. 436.
[7] B.N. AMES, The detection of chemical mutagens with enteric bacteria, in *Chemical Mutagens* (A. HOLLAENDER ed.), Plenum Press, New York/London 1971, Vol. I, p. 267.

Table 2

Compound	Relative mutagenic activity for strain	
	his G46	his D3052
Benzimidazole	4.0 (± 0.7)	1.0
2-Methyl-	5.5 (± 0.5)	1.0
2-Ethyl-	6.0 (± 0.5)	1.0
2-Isopropyl-	5.5 (± 0.7)	1.0
2-tert. Butyl-	4.5 (± 0.5)	1.0
2-Cyclohexyl-	3.5 (± 0.7)	1.0
2-Benzyl-	2.5 (± 0.8)	1.0
2-Diphenylmethyl-	3.0 (± 1.0)	1.0
2-(α,α-Diphenyl)ethyl-	1.5	1.0
2-(4'-Biphenylyl)-	2.0 (± 0.8)	1.0

pressed by the increasing sizes of the 2-substituents, obviously because of an increasingly difficult incorporation.

It has been proposed[8] to use benzimidazole-based fungicides as additives in cheese, flour and other food to enhance their resistivity against moulds. From the present study it can be concluded, that, if a certain danger of genetic influence by the way of such food should exist, such a hazard might certainly be smaller by using large aryl substituents in the 2-position of the benzimidazole nucleus.

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[8] Swiss Patents No. 467020 and 478526 (Merck & Co., Inc., Rahway, N.J., USA).