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RELATION OF GAS CHROMATOGRAPHIC BEHAVIOUR TO THE CHEMICAL STRUCTURE OF PYRIDO[1,2a]PYRIMIDINE DERIVATIVES

O. PAPP, G. SZÁSZ and K. VALKÓ

Institute of Pharmaceutical Chemistry, Semmelweis Medical University, Budapest (Hungary)

SUMMARY

The gas chromatographic behaviour of about 40 derivatives was studied, several of which exert biological activity. Kováts' indices of compounds were determined and δI values were calculated there from. The parts of the molecules which significantly influence the indices are indicated. With the aid of the δI values, further information is obtained on the properties of the derivatives.

INTRODUCTION

The examined compounds represent a group of pharmacologically active substances. One of them is a preparation registered in Hungary under the trade-name Probon®. Numerous physical and chemical properties of this and related compounds are already known[1-5], but they have not yet been examined by gas chromatography (GC).

For the study of the correlation between GC behaviour and chemical structure we used the Kováts' index and the δI values which can be calculated from it. We hope that the data obtained will be of some help in the GC examination of the metabolites of future drugs.

EXPERIMENTAL

The conditions of the GC procedure are summarized in Table I.

The retention times were measured to 0.1 sec accuracy, with the aid of an integrator. For the determination of the Kováts' indices, not less than three measurements were used. The accuracy of the method has been calculated[6] to be ±1 index unit. The total error is probably greater than that due to individual effects, *e.g.*, the ageing of the column.

The formulae of the compounds and measured indices are presented in Tables II–V. All compounds were synthesized by Mészáros *et al.*[7].

TABLE I

GAS CHROMATOGRAPHIC CONDITIONS

Chromatograph: Hewlett-Packard 5710A
Integrator: Digint 21 Chinoin
Column: 6 ft. × ¹/₄ in., glass
Column packings: Chromosorb W CMDS
Stationary phases: 3% OV-17, 3% OV-1
Carrier gas: nitrogen
Carrier gas flow-rate: 30 ml/min
Column temperature: 240°
Detector temperature: 300°
Injector temperature: 300°
Sample size: 1 μl, applied with Hamilton syringes
Solvent: chloroform
Attenuator: 128 × 10

TABLE II

TETRAHYDROPYRIDO[1,2a]PYRIMIDINE COMPOUNDS INVESTIGATED
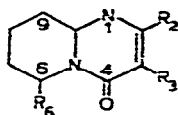
No.	R_2	R_3	R_6	Kováts' index	
				OV-1	OV-17
1	–H	–H	–CH$_3$	1539	1960
2	–H	–H	–H	1576	2000
3	–H	–CH$_3$	–CH$_3$	1641	1996
4	–CH$_3$	–H	–CH$_3$	1695	2032
5	–CH$_3$	–C$_2$H$_5$	–CH$_3$	1764	2057
6	–H	–C$_2$H$_5$	–CH$_3$	1800	2142
7	–H	–CN	–CH$_3$	2000	2433
8	–H	–COOC$_2$H$_5$	–CH$_3$	2088	2533
9	–H	–COOCH(CH$_3$) with CH$_3$	–CH$_3$	2101	2548
10	–H	–COOC$_2$H$_5$	–H	2120	2616
11	–H	–COOC$_3$H$_7$	–CH$_3$	2172	2638
12	–H	–CONH$_2$	–CH$_3$	2183	2638
13	–H	–COOC$_4$H$_9$	–CH$_3$	2269	2735
14	–H	–CONH$_2$	–H	2270	2681
15	–H	–C$_6$H$_5$	–CH$_3$	2305	2755
16	–H	–CH$_2$COOC$_2$H$_5$	–CH$_3$	2080	2519

RESULTS AND DISCUSSION

On the basis of the tabulated data the following conclusions can be made:

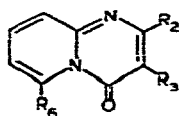(1) The compounds may be divided into three groups according to their

TABLE III

N₁-SUBSTITUTED HEXAHYDROPYRIDO[1,2a]PYRIMIDINE COMPOUNDS INVESTIGATED
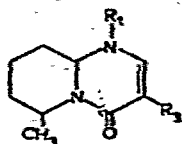
No.	R_1	R_3	Kováts' index	
			OV-1	OV-17
17	–CH₃	–CONH₂	2477	2975
18	–CH₃	–COOC₂H₅	2570	2958
19	–C₂H₅	–CONH₂	2480	2963
20	–C₄H₉	–CONH₂	2631	3071
21	–COCH₃	–COOC₂H₅	2272	2746
22	–H	–COOC₂H₅	1856	—

TABLE IV

PYRIDO[1,2a]PYRIMIDINE COMPOUNDS INVESTIGATED

No.	R_2	R_3	R_6	Kováts' index	
				OV-1	OV-17
23	–H	–CH₃	–H	1668	2051
24	–CH₃	–H	–CH₃	1712	2065
25	–H	–CH₃	–CH₃	1732	2080
26	–CH₃	–C₂H₅	–CH₃	1812	2128
27	–C₂H₅	–H	–CH₃	1830	2162
28	–CH₃	–H	–C₂H₅	1834	2116
29	–C₃H₇	–C₂H₅	–CH₃	1979	2285
30	–H	–COOC₂H₅	–CH₃	2199	2639
31	–H	–C₆H₅	–H	2347	2789
32	–H	–C₆H₅	–CH₃	2396	2812

TABLE V

PYRROLOPYRIMIDINE COMPOUNDS INVESTIGATED
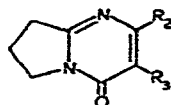
No.	R_2	R_3	Kováts' index	
			OV-1	OV-17
33	–H	–COOC₂H₅	2060	2523
34	–H	–CN	1962	2437
35	–H	–C₆H₅	2252	2783

Kováts' indices. Compounds 1–6 and 23–29 containing cyclic tertiary N_1 with only alkyl substituents at other positions have the lowest indices. Within this group, the unsaturated molecules containing alkyl substituents with high C-atom number have higher index values. Medium index values are shown by molecules with cyclic tertiary N_1 and polar substituents at C_3 (compounds 7–16, 30–35). The highest index values are exhibited by the N_1-alkyl derivatives 17–21.

Thus significant differences in the Kováts' indices are caused by different valence states of the N_1 atom and by polar substitution at C_3.
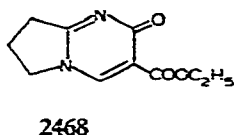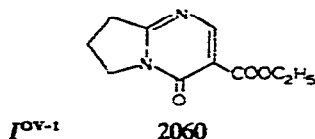
(2) The effect of the number of methylene groups in the ring containing only one nitrogen is demonstrated by the following data:
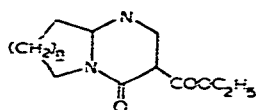
n	Kováts' indices on OV-1 stationary phase
1	2060
2	2120
3	2150

Increasing the ring size decreases the retention index increment.

(3) The isomerism of the oxo group an increase of 408 index units:

| I^{OV-1} | 2060 | 2468 |

The explanation of this is that the 2-oxo group does not form a carboxamide type of configuration with the neighbouring nitrogen which has planar symmetry. However in the 4-oxo case the neighbouring nitrogen has tetragonal symmetry and this isomer is less polar. Irreversible adsorption of the 2-oxo isomer to the column was observed.

(4) In Table VI it can be seen that the index values of the unsaturated derivatives are always higher. These derivatives exhibit stronger inductive interactions due to their planar aromatic character.

TABLE VI

EFFECT OF UNSATURATION ON KOVÁTS' INDICES

R_1	R_2	I_{240}^{OV-1}			I_{240}^{OV-17}		
		saturated	unsaturated	δI	saturated	unsaturated	δI
–CH₃	–H	1695	1712	17	2032	2065	33
–CH₃	–C₂H₅	1764	1825	61	2057	2151	94
–H	–CH₃	1641	1732	91	1996	2080	84
–H	–C₆H₅	2305	2396	91	2755	2813	58
–H	–COOC₂H₅	2088	2199	111	2533	2639	106

The δI values

The δI value is the difference between the Kováts' indices of two compounds which differ from one another in one substituent, measured under identical conditions. Such values reflect the size of the substituent, its polarity and its interaction with the whole molecule.

In Table VII the δI values of the CH_3 group are shown. Substitution of the methyl group at C_2, C_3, C_6, C_7 and C_8 demonstrates that the distribution of the electrons within the molecule is different in each case. Thus on the OV-1 stationary phase, the electron density on N_1 is larger ($\delta I = 156$) with the C_2-methyl substituent than with the C_3-methyl substituent ($\delta I = 102$). Substitution at C_7 and C_8 places the methyl groups at greater distances from both nitrogens, resulting in lower index increases. The direction of the effect in the case of the C_6-methyl substituted derivative is opposite to that expected. It may be interpreted only in terms of shielding of part of the molecule which would result in an increase of retention index. Since the CH_3 group at C_6 is in an axial position, as proved by nuclear magnetic resonance (NMR) spectroscopy[7], it is presumed that this effect is exerted on the N_5 atom. The effect has also been examined in other pairs of molecules (Table VIII). The δI values of the C_6-methyl substituent are positive in the case of unsaturated compounds, but negative in all saturated derivatives. The cause of this difference is that in the case of the

TABLE VII

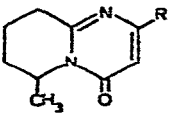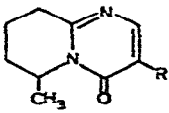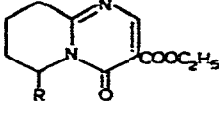δI VALUES OF METHYL SUBSTITUENTS IN DIFFERENT POSITIONS

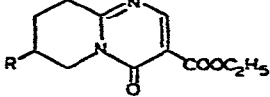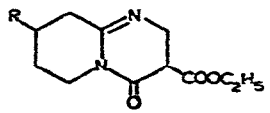| Compound	OV-1			OV-17		
	$R = H$	$R = CH_3$	δI	$R = H$	$R = CH_3$	δI
	1539	1695	156	1960	2032	72
	1539	1641	102	1960	1996	36
	2120	2088	−32	2616	2533	−83
	2120	2164	44	2616	2618	2
	2120	2193	73	2616	2625	9

TABLE VIII

δI VALUES OF C_6 METHYL GROUPS

(a) Unsaturated derivatives:
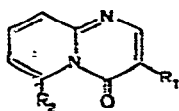
R_1	R_2	I_{240}^{OV-1}	δI_{240}^{OV-1}	I_{240}^{OV-17}	δI_{240}^{OV-17}
–CH₃	–H	1668	64	2051	29
–CH₃	–CH₃	1732		2080	
–C₆H₅	–H	2347	59	2789	24
–C₆H₅	–CH₃	2396		2813	

(b) Saturated derivatives:
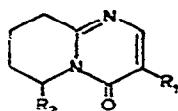
R_1	R_2	I_{240}^{OV-1}	δI_{240}^{OV-1}	I_{240}^{OV-17}	δI_{240}^{OV-17}
–H	–H	1599	−60	2000	−40
–H	–CH₃	1539		1960	
–COOC₂H₅	–H	2120	−32	2616	−83
–COOC₂H₅	–CH₃	2088		2533	
–CONH₂	–H	2270	−87	2681	−43
–CONH₂	–CH₃	2183		2638	

unsaturated derivatives the ring has been aromatized and is planar and the CH_3 group is located in the plane of the ring. The shielding effect exerted at N_5 is thus weakened significantly.

Table IX shows the effect of the nature of the bonding at N_1 on the value of the Kováts' index. N-Methyl substitution resulted in an increase of about 700 index units
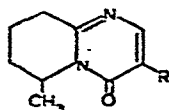
TABLE IX

EFFECT OF BONDING AT N_1 ON KOVÁTS' INDEX OF SOME COMPOUNDS ON OV-1 STATIONARY PHASE AT 240°
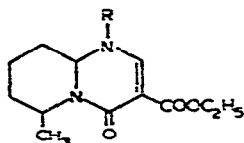
R	I_{240}^{OV-1}
–CH₃	2570
–COCH₃	2272
–Δ1–10*	2088
–H	1856

* Δ1–10 indicates double bond between N-1 and C-10.

TABLE X

δI VALUES OF DIFFERENT SUBSTITUENTS AT C_3

R	I_{240}^{OV-1}	δI_R	I_{240}^{OV-17}	δI_R
–H	1539	—	1960	—
–CN	2000	461	2430	473
–CH$_2$COOC$_2$H$_5$	2082	543	2519	559
–COOC$_2$H$_5$	2088	549	2539	573
$\begin{array}{c}CH_3\\|\\-COOCH\\|\\CH_3\end{array}$	2101	562	244H	583
–COOC$_3$H$_7$	2172	633	2658	678
–CONH$_2$	2183	644	2658	678
–COOC$_4$H$_9$	2269	730	2735	775
–C$_6$H$_5$	2305	766	2755	795

in comparison to the molecule containing N_1–H. The index value of the first molecule is lower than expected.

It may be supposed that value of the Kováts' indices of the four molecules in Table IX is also related to the strength of the basicity of the N_1 nitrogen. Acylation results in the introduction of a polar group, but it causes only a minor increase of the index in comparison to methyl substitution. Methylation results in an increase of basicity, acylation in a decrease.

Table X shows the δI values of the C_3 substituents on both stationary phases. The δI value of the COOC$_3$H$_7$ substituent is significantly higher than that of CH$_2$COOC$_2$H$_5$ on both stationary phases. This may be due to the interaction of the ester group with the skeleton, which increases the retention. The interaction is decreased by the insertion of a methylene group. The δI value of the carboxamide group is only about 100 index units greater than that of the ethyl ester group. Presumably there is intramolecular hydrogen bond formation between the carboxamide and the 4-oxo group.

REFERENCES

1 Gy. Szász, Z. Mészáros, Zs. Ignáth and G. Kovács, J. Chromatogr., 117 (1976) 345–353.
2 Gy. Szász, Á. Dávid, Z. Mészáros and Zs. Ignáth, Acta Pharm. Hung., 47 (1977) 120–124.
3 T. Vajda, T. Szabó and M. Mészáros, Acta Pharm. Hung., 44 (1974) 200.
4 Z. Mészáros, Arzneim.-Forsch., 22 (1972) 815.
5 K. Sasvári, Acta Crystallogr., Sect. B, 28 (1972) 2405.
6 J. Takács and D. Králik, J. Chromatogr., 50 (1970) 379–384.
7 Z. Mészáros, I. Hermecz, L. Vasvári-Debreczeny, Á. Horváth, Á. Dávid, P. Dvortsák, M. Pongor-Csákvári and V. Kovács-Mindler, Magy. Kém. Lap., 31 (1976) 281–292.