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PREDICTIONS OF GAS CHROMATOGRAPHIC RETENTION CHARACTERISTICS OF BARBITURATES FROM MOLECULAR STRUCTURE

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SUMMARY

Gas chromatographic retention indices of 23 5,5-disubstituted barbituric acid derivatives have been related to the numbers of carbon atoms, molecular weights and molecular connectivity values of the substituent groups. Correlations are low when all compounds are considered, but are excellent when the barbiturates are divided into chemically similar sub-groups. Retention data can be predicted with great accuracy. Overall correlations can be improved either by combination of selected connectivity terms or by modification of existing rules for their calculation. General relationships with very high predictive power are described and their applications discussed.

INTRODUCTION

The use of chromatographic methods for the confirmation of identity relies on the availability of reference data or authentic samples of compounds for comparative purposes. If neither data nor sample are at hand, as is often the case in forensic analyses, the problem of identification is particularly acute. Chromatographic discrimination of barbiturates has recently been examined, and an effective approach to the separation of these closely related compounds has been proposed[1]. However, the usefulness of these data is necessarily restricted to those compounds which have been studied. The wide range of barbiturates means that a sample of any particular one may not always be immediately available for an analysis. It is therefore desirable that accurate predictions of the chromatographic behaviour of uncommon barbiturates can be made so that such barbiturates can be excluded as possible identities in qualitative analyses.

Relationships have been demonstrated between retention properties and a variety of physico-chemical parameters (*e.g.* boiling point, heat of solution). All of these parameters must be determined experimentally, although calculation of some is possible using substituent constants. In many cases, procedures for obtaining a particular value are complex and impractical in routine use. In the following work the gas chromatographic (GC) retention data of barbiturates differing only in the nature of their substituent groups have been related to parameters based on molecular structure (*e.g.* carbon number, molecular weight, connectivity (χ) terms[2]), and which do

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not need to be experimentally determined. The study concentrates on predictions based on the fundamental and simple GC system, since correlations between GC and high-performance liquid chromatographic (HPLC) retention data have been demonstrated[1]. The work is further restricted to a series of 23 5,5-disubstituted barbiturates.

METHODS

GC retention indices for 23 5,5-disubstituted barbiturates, abstracted from data presented by Gill *et al.*[1], were obtained using a 2 m × 4 mm I.D. glass column packed with 3% SE-30 on Chromosorb G HP (80–100 mesh) at a column temperature of 200°C and a nitrogen flow-rate of 45–50 ml/min.

The number of carbon atoms and the combined molecular weight of the two substituents at the C-5 position were calculated.

Molecular connectivity indices (χ), characteristic of the combined topological structure of the two substituent groups at the C-5 position, were also calculated. Indices of this type are derived from the size of, and numerical degree of branching in, molecular skeletons. A discussion of molecular connectivity and an introduction to the determination of χ values are given by Kier and Hall[2]. The quidelines they presented have since been modified (*e.g.* ref. 3), and other workers have used or interpreted them in different ways (*e.g.* refs. 4 and 5). There appears to be no single unified set of principles for calculating the connectivity index of a complicated structure. In the present work, first-order substituent connectivity indices ($^1\chi^o$) and valence connectivity indices ($^1\chi^v$) were calculated using the rules of Kier and Hall[2] (see i and ii below). Valence connectivity indices following our own empirical modification of conventional rules to suit the barbiturate group ($^1\chi^v_N$) were also calculated (see (iii) below). Calculations were performed as follows:

(i) $^1\chi^o$ — a skeleton structure of the substituent groups is drawn (including the carbon atom at the 5-position of the barbiturate ring). Each atom is assigned a number (δ), which is the number of atoms other than hydrogen attached to it. Each bond is assigned a value which, for a bond between atoms i and j, is $(\delta_i\delta_j)^{-\frac{1}{2}}$. The sum of these values for all bonds in the substituent groups, *i.e.* $\Sigma(\delta_i\delta_j)^{-\frac{1}{2}}$, is the substituent connectivity index ($^1\chi^o$). In calculating $^1\chi^o$ in the above way, double and triple bonds are considered as single bonds.

(ii) $^1\chi^v$ — as for $^1\chi^o$ above, except that δ^v values are used instead of δ values. Each atom is assigned a valence value, δ^v, being the difference between the number of valence electrons of an atom (Z^v) and the number of hydrogen atoms (h) bonded to it, *i.e.* $\delta^v = Z^v - h$. Double and triple bonds are thus accounted for. The bromine atom (in brallobarbitone, ibomal and sigmodal) takes an empirical δ^v value of 0.254. $^1\chi^v$ is equal to $\Sigma(\delta_i^v\delta_j^v)^{-\frac{1}{2}}$.

(iii) $^1\chi^v_N$ — as for $^1\chi^v$ above, except that δ^v_N values are used instead of δ^v values. δ^v_N values are obtained as follows. Where a carbon atom is involved in a double bond within a chain, 1 is substracted from its δ^v value for each substituent group attached to the atom. Where double bonds are at the end of a chain, 1 is subtracted from the δ^v value of the terminal carbon atom. In addition, 0 is subtracted from the δ^v value of the penultimate carbon if no substituent group other than the rest of the chain is bonded to it; 2 is subtracted if a substituent group, as well as the chain, is attached. Where a ring is involved it is opened at the C-1 position to give the

equivalent chain isomer; the broken bond should be considered to be still attached to the distal carbon atom. The number of valence electrons (Z^v) for the C-1 carbon atom is therefore reduced to three. If a double bond occurs at the C-1 position and it is the only one in the ring, then the ring should be opened at that bond. δ^v_N values are designated as described above and the ring is rejoined before the calculation of $^1\chi^v_N$. If there is a double bond at C-1 then 1 is subtracted from the δ^v value of that carbon atom (*cf.* subtracting 1 for a substituent on a double bond in a chain, see above). $^1\chi^v_N$ is equal to $\Sigma(\delta^v_{i(N)}\ \delta^v_{j(N)})^{-\frac{1}{2}}$.

In all the above calculations no changes were made in the rules for the extra bond of a ring structure relative to the equivalent chain isomer (*cf.* ref. 6), and the connectivity index of the core barbiturate structure was not added.

RESULTS AND DISCUSSION

The relationship between GC properties and the structures of molecules in a congeneric series is well known (*e.g.* the carbon numbers of straight-chain hydrocarbons are used as the basis for retention index calculations). In the present work, correlations have been made between retention indices of a series of 23 5,5-disubstituted barbiturates and the carbon number, molecular weight, and molecular connectivity terms ($^1\chi^o$ and $^1\chi^v$) of the two substituents at the C-5 position. Retention data and structural parameters are given in Table I. Poor overall correlations are observed (Table II) and thus predictions of retention characteristics are poor. However, if the barbiturates are separated on the basis of their substituents into chemically similar sub-groups (*e.g.* dialkyl or alkyl-allyl derivatives, where the barbiturate core-structure remains constant throughout), correlation coefficients increase markedly (Table II), and hence the accuracy of predictions within a sub-group improves considerably. The situation is reflected in the regression lines calculated for the different sub-group relationships; the lines are separate but parallel in all cases (*e.g.* retention indices *vs.* molecular weight, Fig. 1). Since retention characteristics are dependent on non-topological characteristics (not described by carbon number, molecular weight or connectivity) as well as structural differences, similar parallel lines are to be expected for all sub-groups (including those not examined in the present work).

The more rigorous and sophisticated definition of the structure of a molecule provided by connectivity terms has been widely used for correlating chromatographic retention data of various types of compounds[5,7–9]. In the present work, $^1\chi^o$ values of compounds in chemically similar sub-groups are shown to provide more accurate predictions than do either carbon number or molecular weight, although for practical purposes correlations between retention characteristics and the latter two parameters are as good. Relationships using either carbon number or molecular weight are disadvantageous however, since neither can adequately represent complex molecular structures. Further, they cannot be modified in order to combine the different parallel regression lines associated with derivatives with different substituent or functional groups (*e.g.* Fig. 1). Molecular connectivity indices can be modified in this way. More complicated terms (*e.g.* higher order connectivity indices accounting for more than one bond in the substituent group[2], valence δ values (*e.g.* $^1\chi^v$, accounting in part for electrostatic forces in a substituent group[5,8], and combination of the above-mentioned parameters and introduction of interactive terms[5] have all been reported to

TABLE I

SOME STRUCTURAL PARAMETERS, CALCULATED FOR THE SUBSTITUENTS AT THE C-5 POSITION* AND GAS CHROMATOGRAPHIC RETENTION DATA FOR 23 BARBITURATES

Name	*Number of carbon atoms*	*Molecular weight*	$^1\chi^o$	$^1\chi^v$	$^1\chi^v_S$	*Retention Index***
Allobarbitone	6	82.15	3.121	2.340	2.678	1586
Amylobarbitone	7	100.21	3.477	3.477	3.477	1700
Aprobarbitone	6	84.16	3.004	2.613	2.783	1600
Barbitone	4	58.12	2.121	2.121	2.121	1482
Brallobarbitone***	–	161.04	3.477	3.228	4.303	1842
Butalbital	7	98.19	3.477	3.087	3.256	1658
Butobarbitone	6	86.18	3.121	3.121	3.121	1645
Cyclobarbitone	8	110.20	4.166	3.861	4.753	1945
Cyclopentobarbitone	8	108.18	4.166	3.442	4.193	1858
Heptabarbitone	9	124.23	4.666	4.361	5.253	2035
Hexethal	8	114.23	4.121	4.121	4.121	1835
Ibomal***	–	163.06	3.360	3.496	4.407	1866
Idobutal	7	98.19	3.621	3.231	3.400	1698
Nealbarbitone	8	112.22	3.768	3.377	3.546	1720
Pentobarbitone	7	100.21	3.542	3.542	3.542	1733
Phenobarbitone	8	106.17	4.166	3.221	4.975	1934
Phenylmethylbarbituric acid	7	92.14	3.605	2.661	4.414	1875
Probarbitone	5	72.15	2.504	2.504	2.504	1550
Quinalbarbitone	8	112.22	4.042	3.651	3.821	1770
Secbutobarbitone	8	86.18	3.042	3.042	3.042	1650
Sigmodal***	–	191.11	4.398	4.534	5.445	2031
Talbutal	7	98.19	3.542	3.151	3.321	1704
Vinbarbitone	7	98.19	3.542	3.215	3.828	1755

* Connectivity indices also include the carbon atom at the 5 position.
** Obtained using a 3% SE-30 column, see Methods section.
*** Barbiturates which contain a bromine atom in the substituent group.

TABLE II

LINEAR CORRELATION COEFFICIENTS FOR THE COMBINATION OF STRUCTURAL PARAMETERS* AND GAS CHROMATOGRAPHIC RETENTION DATA

Combination	*Overall (n = 23)***	*Alkyl-alkyl derivatives (n = 7)*	*Alkyl-allyl derivatives (n = 6)*
Number of carbon atoms	0.884	0.991	0.913
Molecular weight	0.747	0.991	0.913
$^1\chi^o$	0.887	0.997	0.981
$^1\chi^v$	0.801	0.997	0.980
$A\ ^1\chi^o + B\ ^1\chi^v$	0.891	0.997	0.981
$^1\chi^v_S$	0.996	0.997	0.981

* Calculated for the substituents at the C-5 position.
** Except for combination with carbon number where bromoallyl derivatives are not included; $n = 20$ in this case.

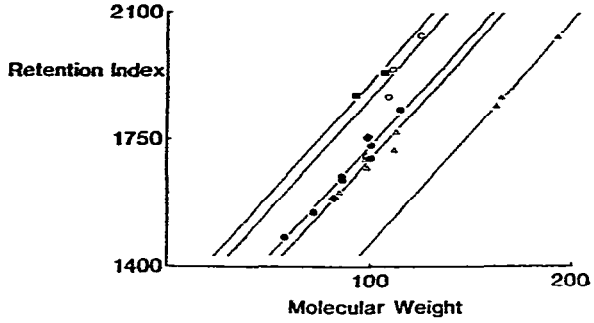

Fig. 1. Correlation of GLC retention data (SE-30 stationary phase) for 23 barbiturates with the molecular weight of the substituents at the C-5 position. ● = 5,5-Dialkylbarbituric acids; △ = 5-alkyl-5-allylbarbituric acids; ▲ = 5-alkyl-5-bromoallylbarbituric acids; ■ = 5-alkyl-5-phenylbarbituric acids; ○ = other cyclic barbiturates; ◆ = others.

improve overall correlations with retention data by drawing the parallel lines together.

The more complicated higher-order connectivity indices are relatively difficult to calculate and are therefore of limited practical value. The separate parallel lines remain when $^{4}\chi^{v}$ values alone are used (Fig. 2), and consequently overall correlations are not improved. In all cases where parallel regression lines exist, the retention properties of a barbiturate can only be predicted if at least one compound within the same chemical sub-group has already been characterised such that a regression line parallel to those relating to other sub-groups can be drawn. Use of substituent interactive terms[5], accounting for the differences between related molecules and designed to combine a family of parallel regression lines, is undesirable for the same reason. Thus, at least one compound in the same sub-group must again be characterised before an interactive term can be applied. Correlations are improved by combination of the simple connectivity terms $^{4}\chi^{o}$ and $^{4}\chi^{v}$ discussed individually above (*e.g.* with $^{4}\chi^{v}$, $r = 0.801$ and $n = 23$, whereas with a combination of $^{4}\chi^{o}$ and $^{4}\chi^{v}$, $r = 0.891$ and $n = 23$). Obviously the form of the best-fit equations provided by such

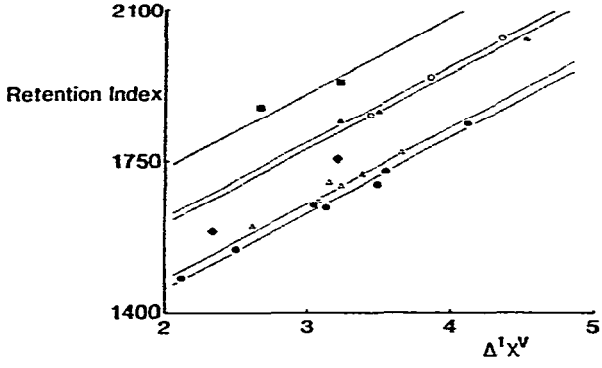

Fig. 2. Correlation of GLC retention data (SE-30 stationary phase) for 23 barbiturates with the molecular valence connectivity index of the substituents at the C-5 position ($^{4}\chi^{v}$). ● = 5,5-Dialkylbarbituric acids; △ = 5-alkyl-5-allylbarbituric acids; ▲ = 5-alkyl-5-bromoallylbarbituric acids; ■ 5-alkyl-5-phenylbarbituric acids; ○ other cyclic barbiturates; ◆ = others.

combinations is regulated by the available data, and the usefulness of the equations is generally limited because extrapolations cannot always be performed with confidence.

In contrast, very highly correlated relationships are obtained by simple modification of the conventional rules for calculating connectivity indices. The modifications performed, described in full in the Methods section, account for all the differences in structure of the 23 barbiturates examined. They consider the whole series of compounds rather than just a sub-group (*cf.* interactive terms). This suggests that use of such modifications has a wider application than does either use of interactive terms or combination of connectivity indices. Recalculation of substituent connectivity indices following empirical modification of conventional rules, to give $\Delta^1\chi_N^V$ values, allows the separate parallel regression lines associated with each chemical sub-group (*e.g.* Fig. 2) to be drawn as a single line (Fig. 3). This line, accounting for all the available data, has a very high correlation coefficient ($r = 0.996$). The retention indices of four barbiturates not included in the original group of derivatives have been predicted from this regression line following calculation of their $\Delta^1\chi_N^V$ values. It can be seen from the data in Table III that reliable extrapolations can be made as predictions are excellent. Further, the fact that such good predictions can be made for vinylbitone and reposal, barbiturates which represent previously unexamined sub-groups (alkyl-vinyl- and alkyl-bicyclo-oct-2-enyl-barbiturates, respectively), indicates the more general applicability of connectivity indices calculated using the modified rules.

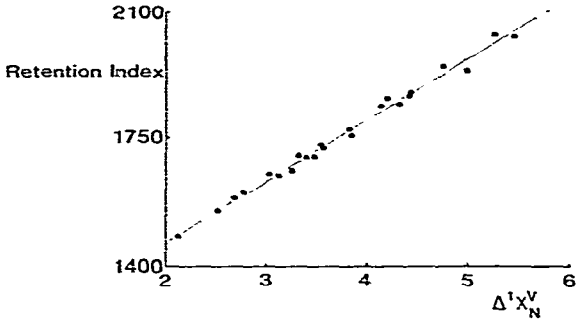

Fig. 3. Correlation of GLC retention data (SE-30 stationary phase) for 23 barbiturates with the molecular connectivity index of the substituents at the C-5 position following modification of rules for their calculation ($\Delta^1\chi_N^V$). Correlation coefficient, $r = 0.996$, $n = 23$. Barbiturates not in the original group (○, see Table III), are also included to compare them with the regression line.

While accurate overall predictions are possible with best-fit combinations of connectivity indices or recalculated values, there are disadvantages associated with their use. Thus, neither approach can be applied to chromatographic systems other than the one for which it was originally designed, as both are derived for a specific set of experimental data. However, use of combinations, and particularly of modified rules, has an immediate appeal because both approaches account for a far wider range of barbiturate types than does sub-group regression analysis. Simple connectivity terms, carbon numbers and molecular weights can only be used for accurate prediction of retention data after division of the compounds into chemically similar sub-groups. Compared with these three parameters (*i.e.* simple connectivity terms,

TABLE III

OBSERVED AND PREDICTED* RETENTION INDICES FOR BARBITURATES IN DIFFERENT CHEMICAL SUB-GROUPS**

	$^{A}\chi_{S}^{1}$	*Retention index*	
		Observed	*Predicted*
Ethyl-allylbarbituric acid (alkyl-allyl)	2.400	1532***	1532
Vinylbitone (alkyl-vinyl)	3.347	1730 §	1691
Butallylonal (alkyl-bromoallyl)	4.945	1969***	1960
Reposal (alkyl-bicyclo-oct-2-enyl)	5.720	2092 §	2090

* Using modified connectivity rules.

** The individual compounds are not included in the original group of 23 barbiturates examined; examples of the sub-groups alkyl-vinyl and alkyl-bicyclo-oct-2-enyl barbituric acids are not included in the original group.

*** Estimated from data in Machata and Battista[10].

§ Estimated from data in Menez *et al.*[11], and Stead *et al.*[12].

carbon number, and molecular weight) there is little difference in sub-group correlations when simple connectivity indices are combined or recalculated following modification of conventional rules (Table II).

The present work demonstrates the versatility of molecular connectivity terms in providing relationships which allow accurate prediction of chromatographic retention indices of disubstituted barbiturates. Predictive relationships of this type can be used to advantage in determining the retention characteristics of new and related compounds (*i.e.* those which may be produced illicitly and for which no authentic specimen may be available). Further, they can be used to determine whether a derivative whose retention characteristics are unknown is likely to interfere in an analysis for a specific barbiturate. This has obvious potential for aiding the forensic toxicologist in analyses of the wide range of barbiturates available.

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