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Gas Chromatographic Identification of 2,6-Disubstituted-*trans*-decalins using Retention Indices

KEN'ICHI TAKEDA and SADAO YAMAMOTO

Shionogi Research Laboratory, Shionogi & Co., Ltd.[1]

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Thirty one 2,6-disubstituted-*trans*-decalins were chromatographed on carbowax 20M and OV-1 columns, and the retention volumes were converted into the Kováts' retention indices. The increments of some substituents (OH, OCH_3, Cl, OAc, CN, $COOCH_3$, C_6H_5) were determined on the basis of the additivity rule. Elution characteristics of the investigated stereoisomers were reported and discussed.

Gas chromatography (GC) has been developed as an important technique of identification and separation of volatile organic compounds. The identification is facilitated by combining GC with mass or infrared (IR) spectroscopy. In GC, the nature of a compound is determined by its specific retention volume or relative retention ratio. These data alone are, however, not sufficient to demonstrate a relationship between the structure of a compound and its chromatographic behavior.

For such demonstration, the method of Kováts[2-4] has been considered to be the most advantageous technique. This method is based on the retention index (I) using two *n*-alkanes as reference substances and the difference (ΔI) of retention indices on a polar stationary phase and a nonpolar stationaty phase, while for steroids the "steroid number" concept originally proposed by VanderHeuvel and Horning[5-7] has been applied. The method of Kováts has been applied successfully for the identification of organic compounds in other systems.[8-10] Kováts and Strickler,[11] in the stereoisomers of plinols, reported a relationship between relative configuration of substitutents and ΔI values. However, studies on retention indices of stereoisomers of alicyclic compounds are few.

In a related work we prepared some 2,6-disubstituted-*trans*-decalin derivatives,[12] and those identification and purity check were carried out by GC using polar (carbowax 20M) and nonpolar (OV-1) stationary phase. In this paper we report a relationship between stereochemical structures of the derivatives and their gas chromatographic behaviours observed with an aid of Kováts' retention indices on two columns.

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[1] Location: *Fukushima-ku, Osaka.*
[2] E. Kováts, *Helv. Chim. Acta*, **41**, 1915 (1958).
[3] A. Wehrli and E. Kováts, *Helv. Chim. Acta*, **42**, 2709 (1959).
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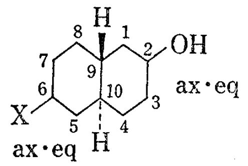
[7] H.C. Horning, C.J.W. Brooks and W.J.A. VanderHeuvel, "Advances in Lipid Research," Vol. 6, ed. by R. Paoletti and D. Kritchevsky, Academic Press, Inc., New York, N.Y., 1968, p. 273.
[8] J. Janák, J. Jones and M. Kratochuíl, *Collection Czech. Chem. Commun.*, **30**, 265 (1965).
[9] H. Widmer, *J. Gas Chromatog.*, **5**, 506 (1967).
[10] Y. Mori and M. Nakamura, *Chem. Pharm. Bull.* (Tokyo), **19**, 499 (1971).
[11] E. Kováts and H. Strickler, *J. Gas Chromatog.*, **3**, 244 (1965).
[12] K. Takeda and S. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **20**, 314 (1972).
The decalin conventions and numbering are used in this paper designating C_9-hydrogen in β-orientation. To simplify the nomenclature of the compounds, the following symbols are used; for 6α- and 6β-substituted-2α-hydroxy-*trans*-decalin derivatives, 6-ax·X-2-eq·OH and 6-eq·X-2-eq·OH, respectively. All the compounds are *dl*-mixtures.

Experimental



1—31

X: H, =O, OCH_3, Cl, OH, CN, OAc, $COOCH_3$, C_6H_5

Chart 1

The chromatographic measurements were carried out on a gas chromatograph Hitachi K53 equipped with a flame ionization detector. The steel columns were 1.0m × 0.3mm packed with 5% stationary phase on Gas chrom Q (100/120 mesh). Stationary phases were carbowax 20M and OV-1 (Applied Science Lab.). The column temperature was 180° for carbowax 20M and 130° for OV-1; the carrier gas was nitrogen. *n*-Tetradecane, *n*-pentadecane, *n*-hexadecane, *n*-heptadecane, *n*-octadecane (Polyscience Corporation), *n*-eicosane, *n*-docosane, *n*-tetracosane, *n*-hexacosane, and *n*-octacosane (Applied Science Lab.) were used as internal standards. Retention indices determined by the method[13] of Kovats are given as mean values.

Materials——The syntheses of all the compounds investigated are reported in the preceding paper,[12] where their structures were determined by IR and/or PMR spectroscopy. A mixture (*ca.* 20/80) of **20** and **21**, and a mixture (*ca.* 20/80) of **22** and **23**, which were obtained from $NaBH_4$ reduction of **15** and **16**, respectively, were chromatographed on two columns.

Table I. Retention Indices of 2,6-Disubstituted-*trans*-decalin Derivatives

No.	Compounds[a]	I_{130}^{OV-1}	I_{180}^{CW20M}	$\Delta I^{CW20M/OV-1}$
0	*trans*-decalin	1082	1283	201
1	2-ax·OH	1280	1906	626
2	2-eq·OH	1284	1943	659
3	2-ax·OAc	1405	1864	459
4	2-eq·OAc	1429	1903	474
5	2-ax·OH-6-ax·OCH_3	1448	2188	740
6	2-ax·OH-6-eq·OCH_3	1482	2282	800
7	2-eq·OH-6-ax·OCH_3	1457	2230	773
8	2-eq·OH-6-eq·OCH_3	1484	2310	826
9	2-ax·OH-6-ax·Cl	1499	2359	860
10	2-ax·OH-6-eq·Cl	1506	2398	892
11	2-eq·OH-6-ax·Cl	1505	2404	899
12	2-eq·OH-6-eq·Cl	1509	2434	925
13	2-ax·OH-6-CO	1503	2578	1075
14	2-eq·OH-6-CO	1506	2620	1114
15	2-ax·OAc-6-CO	1617	2501	884
16	2-eq·OAc-6-CO	1636	2539	903
17	2-ax·OH-6-ax·OH	1485	2572	1087
18	2-ax·OH-6-eq·OH	1495	2616	1121
19	2-eq·OH-6-eq·OH	1499	2659	1160
20	2-ax·OAc-6-ax·OH [b]	1613	2531	918
21	2-ax·OAc-6-eq·OH	1613	2565	952
22	2-eq·OAc-6-ax·OH [b]	1639	2573	934
23	2-eq·OAc-6-eq·OH	1639	2612	973
24	2-eq·OH-6-eq·CO_2CH_3	1665	2656	991
25	2-eq·OAc-6-ax·CO_2CH_3	1771	2512	741
26	2-eq·OAc-6-eq·CO_2CH_3	1804	2597	793
27	2-ax·OH-6-ax·CN	1588	2702	1114
28	2-eq·OH-6-ax·CN	1604	2762	1158
29	2-eq·OH-6-eq·CN	1620	2815	1195
30	2-eq·OH-6-ax·C_6H_5	1919	2923	1004
31	2-eq·OH-6-eq·C_6H_5	1921	2944	1023

a) The symbols are defined in footnote 12).

b) These compounds are chromatographed as a mixture.

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13) L.S. Ettre, *Anal. Chem.*, 36, No. 8, 31A (1964).

Resut and Discussion

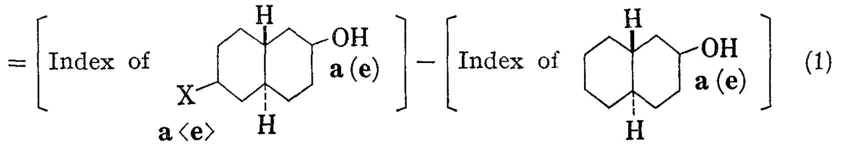
Retention indices obtained from the 2,6-disubstituted-*trans*-decalin derivatives (Chart 1, **1**—**31**) are given in Table I. The third and the forth columns in the table designated by $I_{130}^{\mathrm{OV\text{-}1}}$ and I_{180}^{CW20M} show the retention indices on OV-1 at 130° and on carbowax 20M at 180°, respectively. The last column designated by $\Delta I^{\mathrm{CW20M/OV\text{-}1}}$ indicates the difference of retention indices, $I_{180}^{\mathrm{CW20M}}-I_{130}^{\mathrm{OV\text{-}1}}$.

On an OV-1 column there was no wide deviation among the retention indices of the stereoisomers, though the differences in the retention indices of axial and equatorial substituents were appreciable in the methoxy- and acetoxy-substituted compounds (*e.g.*, **5** and **6**, **7** and**8**, **20** and **22**, and **21** and **23**). The I values on an OV-1 column increased in the following order: $\mathrm{OCH_3}<\mathrm{OH}<\mathrm{Cl}<\mathrm{CN}<\mathrm{OAc}<\mathrm{COOCH_3}\ll\mathrm{C_6H_5}$. This order corresponds approximately to the relative size of substituents. On the other hand, the I values on a carbowax 20M column were very characteristic of additional individual substituents in the basic *trans* decalin skelton and their configuration (axial or equatorial).

On a carbowax 20M column, the four possible stereoisomers were generally eluted in the order; axial-axial<axial-equatorial (or equatrorial-axial)<equatorial-equatorial regarding the configuration of C_2- and C_6-substituents. The order between axial-equatorial and equatorial-axial configurations depended on the magnitude of two increments ($\delta I^{\mathrm{CW20M}}$, which will be discussed later) obtained from the C_2- and C_6-stereoisomers; for example, C_2·axial-C_6·equatirial<C_2·equatorial-C_6·axial for 2-OH-6-Cl, C_2·axial-C_6·equatorial<C_2·equatirial-C_6·axial for 2-OH-6-OCH_3. In addition, the I values on a carbowax 20M column increased in the order, $\mathrm{OCH_3}<\mathrm{Cl}<\mathrm{OAc}<\mathrm{COOCH_3}\simeq\mathrm{OH}\ll\mathrm{CN}\ll\mathrm{C_6H_5}$. The results from a carbowax 20M column were more useful for identification of all possible stereoisomers in the given series than those on an OV-1 column.

The increments (δI, $\delta\Delta I$) determined for additional axial and equatorial C_6-substituents of 2-hydroxy-*trans*-decalins are given in Table II. The increments were obtained from the following equation (1).

[increment for axial- or ⟨equatorial⟩-X]



X: OH, OCH_3, OAc, Cl, CN, $COOCH_3$, C_6H_5

To investigate the validity of the additivity of ΔI, the constancy of δI or $\delta\Delta I$ for additional groups were checked for two pairs of epimers. It can be seen from Table II taht there is a satisfactory constancy for axial and equatorial substituents. The increments for axial substituents were usually smaller than those for equatorial substituents on both the columns. The smaller increments for the axial substituents may be caused by steric hindrance of adhesion between the "adhering zone" of the substrate and the stationary phase. Gas chromatographic behavior of axial substituents of this kind has been observed in steroids.[5,6]

The differences ($\delta\delta I$ and $\delta\delta\Delta I$) in the increments of an equatorial and an axial substituent are also summarized in Table II. The differences ($\delta\delta I$) on a carbowax 20M column increased in the order, $\mathrm{C_6H_5}<\mathrm{Cl}<\mathrm{OH}\simeq\mathrm{OAc}<\mathrm{CN}$ and the values were large enough to identify all possible stereoisomers in the series investigated. On the other hand, the values of the hydroxy and chloro groups obtained from an OV-1 column were too small to identify a pair of epimers.

Wehrli and Kováts[3] suggested several methods for expressing the correlation between retention index and molecular structur more precisely. They gave a detailed discussion about relationship between ΔI values and dipole moments in a series of compounds R–X,

TABLE II. The Increments for Axial- and Equatorial Substituents of 2-Hydroxy-6-substituted-*trans*-decalin Derivatives

Functional groups	Configuration[a] on C_2- or C_6-atom	Corresponding comp. No.	$\delta I^{\text{OV-1}}$[b]	Mean value	$\delta\delta I^{\text{OV-1}}$[c]	δI^{CW20M}[b]	Mean value	$\delta\delta I^{\text{CW20M}}$[c]	$\delta\Delta I^{\text{CW20M/OV-1}}$[b]	Mean value	$\delta\delta\Delta I^{\text{CW20M/OV-1}}$[c]
OH	ax	17–1	205	208	7	666	670	43	461	462	36
		18–2	211			673			462		
	eq	18–1	215	215		710	713		495	498	
		19–2	215			716			501		
OCH_3	ax	5–1	168	171	30	282	285	87	114	114	57
		7–2	173			287			114		
	eq	6–1	202	201		376	372		174	171	
		8–2	200			367			167		
OAc	ax	20–1	333	331	26	625	624	44	292	293	18
		21–2	329			622			293		
	eq	22–1	359	357		667	668		308	311	
		23–2	355			669			314		
Cl	ax	9–1	219	220	6	453	457	35	234	237	29
		11–2	221			461			240		
	eq	10–1	226	226		492	492		266	266	
		12–2	225			491			266		
CN	ax	27–1	308	314	22	796	808	64	488	494	42
		28–2	320			819			499		
	eq	29–2	336	336		872	872		536	536	
C_6H_5	ax	30–2	635	635	2	980	980	21	345	345	19
	eq	31–2	637	637		1001	1001		364	364	
$COOCH_3$	eq	24–2	381	381	—	713	713	—	332	332	

[a] ax: axial configuration, eq: equatorial configuration

[b] The δI values were obtained from equation (1).

[c] The δδI and δδΔI values designate the difference of the increments of equatorial- and axial-substituents, *i. e.* δI (equatorial-X)-δI (axial-X) and δΔI (equatorial-X)-δΔI (axial-X), respectively.

where R is an *n*-pentyl and X is various substituents. Thus, the increments ($\delta\Delta I$) obtained from the present C_6-substituted 2-hydroxy-*trans*-decalins was similarly treated with the group moments of the ethyl derivatives.[14] The results are shown in Fig. 1. In the case of methoxy, chloro, and carbonitrile groups, an approximately linear relationship were obtained between the increments and the group moments. It is suggested that the retention indices of the decalin derivatives reflects strongly a change in the dipole-dipole interaction between the individual functional group and the polar adsorbent. The series containing hydroxy, acetoxy or methyl ester group show large deviation upwards of a straight line. This deviation is explained to be arised from a kind of hydrogen bonding interaction between the functional group and polyethy leneglycols tationary phase. The fact that the deviation of hydroxy group is extraor dinarily large is compatible with this explanation.

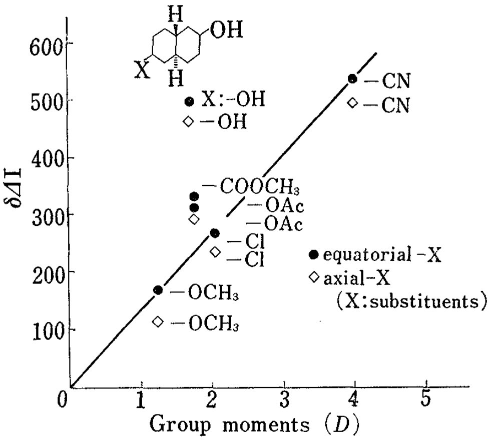


Fig. 1. Relationship between $\delta\Delta I$ and Group Moments[a]

[a] Group moments used here are those of ethyl derivatives.[14]

Further, we examined the compounds containing hydroxy and acetoxy groups on C_2 and C_6 using the additivity rule. The $\delta\Delta I$ values of hydroxy and acetoxy groups obtained from equation (1) were larger by approximately 35—40 units than those obtained by substracting the ΔI values of *trans*-decalin (**0**) from those of monosubstituted-*trans*-decalins (**1**, **2**, **3**, and **4**). The $\delta\Delta I$ values (for ax-OH, 425; for eq-OH, 458; for ax-OAc, 258; for eq-OAc, 273) which are the smallest as the structural effects on the present compounds are used for the following discussion. After repeating of trial-and-error calculations, it was empirically found that the following equation (2) is the best to derive the retention data for these compounds.

$$[\text{calculated retention value}] = [\Delta I\ (\textit{trans}\text{-decalin})] + [\delta\Delta I(C_2\text{-and } C_6\text{-X})] + 37 \qquad (2)$$

X: OH, OAc

The third term in the right hand of the equation (2) is a correction factor, which may be indicative of the structural factor due to the *trans*-decalin ring. The calculated values are shown in Table III. These values are in good agreement with the observed values. It would be possible to dtermine the elution characteristics for a compound containing hydroxy and acetoxy groups in the related series by using the thus-obtained increment values for the substituents.

TABLE III. Comparison of the Observed Retention Values (ΔI) with Calculated Retention Values of Four Stereoisomers attached Hydroxy and Acetoxy Groups to C_2- and C_6-Atoms

Compound No.	Observed ΔI	Calculated ΔI[a]	Compound No.	Observed ΔI	Caclulated ΔI[a]
17	1087	1088	**21**	952	954
18	1121	1121	**22**	934	936
19	1160	1154	**23**	973	969
20	918	921			

[a] These ΔI values are calculated by equation (2).

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[14] C.P. Smyth, "Dielectric Behavior and Structure," McGraw-Hill Book Co., New York, N.Y., 1955, p. 252.

The observations reported in this paper thus indicate that the elution characteristics of all possible stereoisomers of 2,6-disubstituted-*trans*-decalins are obtained from δI and $\delta\Delta$I values.

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