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Gas Chromatography of Sesquiterpenoids. Correlation of Retention Value with Chemical Structure

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Kováts indices of 18 furanosesquiterpenoids on 8 columns were calculated. The additivity rule for substituent index was found to be valid. Using this rule, a tentative identification of substituents was discussed.

Though gas chromatography (GC) is a powerful tool for separation of natural products, identification of chromatogram peaks is not readily achieved. At present, such identification is facilitated by combining GC with mass or infrared spectroscopy. If a retention value could be correlated with chemical structure, GC would afford a powerful means of peak characterization.

For homologous fatty acids, Woodford and Van Gent[2] have introduced the "carbon number" concept using methyl esters of unbranched aliphatic saturated fatty acids as reference compounds. For general compounds, Kováts[3] proposed the "retention index" (RI) concept which is calculated using two *n*-alkanes as reference substances. Further, the difference between RI on a polar stationary phase (emulphor) and that on a nonpolar stationary phase (Apiezon), ΔI, was shown to be measure of the partition energy of a functional group. The additivity rule for ΔI was proved to be valid for monoterpenes, and there are some applications of the rule to other simple compounds.[4] In the steroid field, the "steroid number" concept was suggested by VandenHeuvel and Horning,[5,6] and application of the additivity rule has been demonstrated. It would be very useful if the RI concept could be applied to more complicated compounds. This paper reports application of the additivity rule to the RI of furanosesquiterpenes, isolated from *Lindera strychinifolia vill*, on many columns.

Furanosesquiterpenoids studied are listed in Chart 1. These are two elemane type furanosesquiterpenes and sixteen eudesmane type furanosesquiterpenes, substituted acetoxy, hydroxy, or carbonyl at C_4; or substituted methoxy at C_3; and their polyhydro-compounds.

1) Unsubstituted Compounds (I—IX)

Retention indices obtained for double bond isomers at 170° are shown in Fig. 1. Elemane type compounds (2 rings) were eluted faster than eudesmane type compounds (3 rings). This phenomenon is also seen with steroids,[7] for which it was explained that the electron current in the ring is responsible for the strong affinity with stationary phases. An unsaturated compound has a smaller index value than a saturated one on nonpolar or moderate stationary phases (Apiezon L, OV-1, OV-17, QF-1, and XE-60), the reverse effect is seen on more polar columns (Carbowax, DEGS). The number of double bonds is reflected roughly by retention index on both a nonpolar and a polar stationary phase, but these values are far

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[1] Location: *Fukushima-ku, Osaka.*
[2] F.P. Woodford and C.M. Van Gent, *J. Lipid Res.*, **1**, 188 (1960).
[3] E. Kovats, *Helv. Chim. Acta*, **41**, 1915 (1958).
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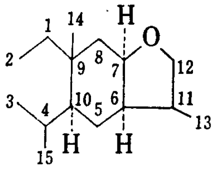
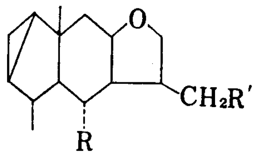


I : 8HG

II : G $\Delta^{1,3,6,11}$



$R=R'=H$

VI : 2HαLN $\Delta^{6,11}-4\alpha-Me$

VII : 2HβLN $\Delta^{6,11}-4\beta-Me$

VIII : LN $\Delta^{4(15),6,11}$

$R=OAc, R'=H$

XIII : 6HLA

XIV : 2HLA $\Delta^{6,11}$

XV : LA $\Delta^{4(15),6,11}$

$R=OH, R'=H$

XVI : 2HLOL $\Delta^{6,11}$

XVII : LOL $\Delta^{4(15),6,11}$

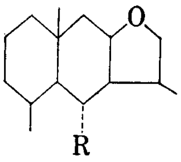
$R=O, R'=H$

XVIII : LON $\Delta^{4,6,11}$

$R=H, R'=OCH_3$

X : LO $\Delta^{4(15),6,11}$

XI : ILO $\Delta^{4,6,11}$



$R=H$

III : 8HLN

IV : 2HAT $\Delta^{6,11}$

V : AT $\Delta^{4(15),6,11}$

IX : LS $\Delta^{1,4(15),6,11}$

$R=OAc$

XII : 8HLA

Chart 1

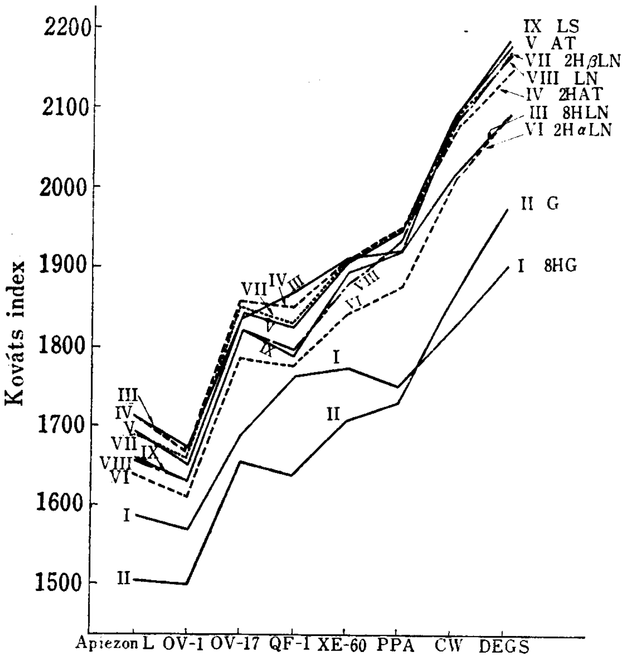


Fig. 1

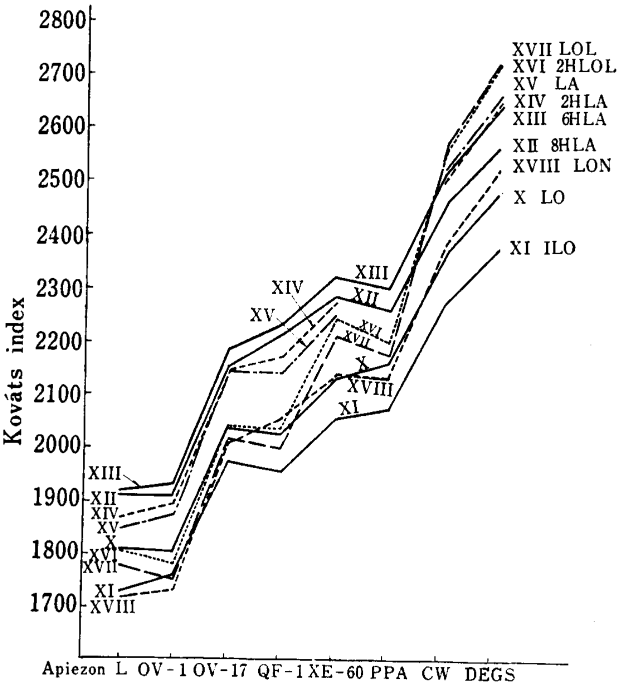


Fig. 2

from quantitative. On a QF-1 column, despite its moderate polarity, the double bond contribution to retention index was similar to that on nonpolar stationary phases such as Apiezon L or OV-1. On a moderately polar column (XE-60, PPA), separation was poor, since the dispersion effect of the double bond cancelled the dipolar effect.

To clarify the relation of unsaturation to the ΔI index, y/Apiezon L terms, differences between RI values on a polar stationary phase and those on a nonpolar stationary phase (Apiezon L), obtained from Fig. 1, are shown in Fig. 3.

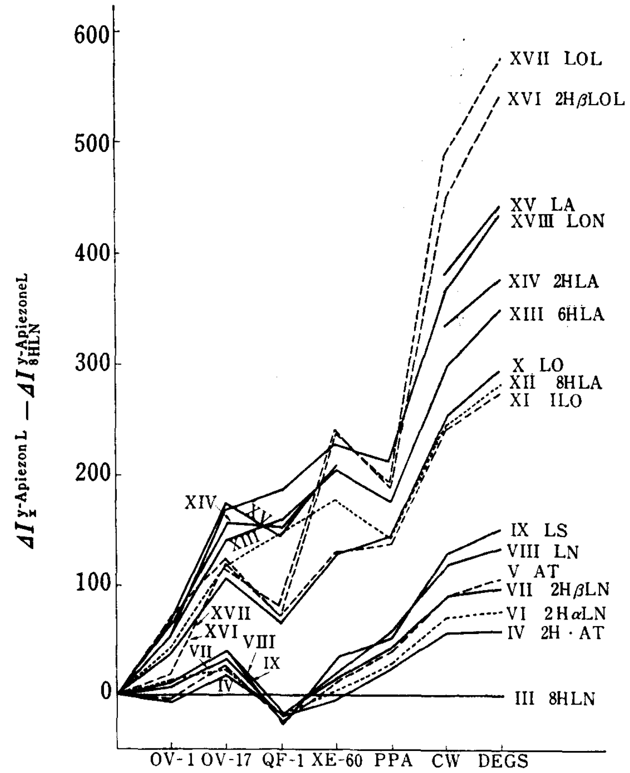


Fig. 4

It is seen in the elemane and eudesmane type series that the contribution of double bonds, and of a cyclopropane ring, to the retention on a polar phase increases with their number. Comparing II (G, fused ring 2) with V (AT, fused ring 3), which have the same degree of unsaturation but large differences in skeleton structure, differences in retention indices were very large on all columns. On the contrary, approximately same values were exhibited in terms of ΔI on polar stationary phases. It is very characteristic that QF-1 could not differentiate double bond. A XE-60 column only poorly resolved saturated and unsaturated compounds.

2) Substituted Compounds

Kovats indices of the substituted furanosesquiterpenes at 170° are shown in Fig. 2. Double bond contribution to RI (XI—XV) on various columns is similar to that in unsubstituted compounds (Fig. 1). The RI values on a nonpolar column were, in increasing order, carbonyl=methoxy<hydroxy≪acetoxy. An OV-17 stationary phase gave differentiation similar to nonpolar Apiezon L or to OV-1. Although a hydroxy group had almost the same contribution to retention on XE-60 as an acetoxy group, it caused a longer retention time than an acetoxy group did on the more polar carbowax and DEGS. Compound (XI) which has a double bond in the ring was eluted faster than compound (X) with an *exo* double bond.

The plot of ΔI against column type is shown in Fig. 3. QF-1 column could not differentiate the compounds having different number of double bond (XI—XV), as was seen with non-substituted furanosesquiterpenes (IV—IX). A hydroxy group had the same contribution to retention index as a methoxy group, and carbonyl group showed a larger retention than acetoxy group. On an XE-60 column, a hydroxy, an acetoxy, and a carbonyl group showed almost the same retention value. The values of ΔI on a polar column (carbowax, DEGS) were in the increasing order: methoxy<carbonyl≈acetoxy<hydroxy.

To investigate the validity of the additivity of ΔI, the constancy of the increment RI for a substituent was checked for many compounds. These results are summarized in Table I.

The RI values of a cyclopropane ring on various stationary phases have approximately the same value as those of a double bond at C_1 or C_4. It is seen that the contribution to retention index by the two double bonds in the furan ring is 1.4 times that of a double bond on a polar phase. Increments in RI not only of double bonds, but also of acetoxy and hydroxy groups have a value, characteristic of each group, which is approximately constant for all stationary phases. A test of the additivity of the RI of a substituent is shown in Table II. For entry 7, RI of cyclopropane plus two double bonds in a furan ring was derived from two different compound series. The mean values of the two series on each stationary phase agree well with the sum of entries 1 and 4. Entry 8, a more complicated system, includes one

Table I. RI Increment for Substituent

	OV-1	OV-17	QF-1	XE-60	PPE	CW	DEGS
1) RI (cyclopropane)							
I_{VII}—I_{IV}	13	15	2	18	18	32	39
I_{VIII}—I_{V}	17	14	7	11	19	28	28
I_{XIII}—I_{XII}	11	22	10	27	33	52	67
mean	14	17	6	19	23	37	45
2) RI (Δ^{1})-RI (4β-Me)							
I_{IX}—I_{V}	16	13	−3	24	11	37	45
3) RI ($\Delta^{4(15)}$)							
I_{VIII}—I_{VII}	7	8	1	7	18	29	36
I_{V}—I_{IV}	3	9	−4	14	17	33	47
I_{XV}—I_{XIV}	3	20	−7	0	—	47	40
I_{XVII}—I_{XVI}	1	5	−9	−4	8	39	35
mean	4	11	−5	4	14	37	40
4) RI ($\Delta^{6,11}$)							
I_{IV}—I_{III}	—5	19	−19	−3	24	57	58
I_{XIV}—I_{XIII}	12	15	−17	3	—	37	53
mean	4	17	−13	0	24	47	56
5) RI (OAc)							
I_{XII}—I_{III}	44	119	148	178	143	242	281
I_{XIV}—I_{VII}	59	122	168	207	—	244	304
I_{XV}—I_{VIII}	55	134	160	186	—	262	308
mean	53	125	159	190	143	249	298
6) RI (OH)							
I_{XVI}—I_{VII}	12	82	98	227	147	358	444
I_{XVII}—I_{VIII}	6	79	88	216	137	368	443
mean	9	81	93	222	142	363	444
7) RI (cyclopropane)+RI($\Delta^{6,11}$)							
I_{VII}—I_{III}	8	34	−17	15	42	89	97
I_{XIV}—I_{XII}	23	37	3	30	—	89	120
mean	16	36	−7	23	42	89	109
1)+4)	18	34	−7	19	47	84	101
8) RI (cyclopropane)+RI ($\Delta^{4(15)}$)+RI ($\Delta^{6,11}$)							
I_{VIII}—I_{III}	15	42	−16	22	40	118	133
I_{XV}—I_{XII}	26	57	−4	30	—	136	160
mean	21	50	−10	26	40	127	147
1)+3)+4)	22	45	−12	23	61	121	141
3)+7)	20	47	−12	27	56	126	149

cycloproapne ring, one vinyl group, and two double bonds in a furan ring; the mean value obtained from the difference of the RI of two compounds series is in accord with the sums of RI of the substituent (entry 1+3+4 and entry 3+7). Therefore, it is proved that a double bond, a hydroxy, and an acetoxy group, each has a characteristic value on each stationary phase, and additivity of the group increment is valid for the furanosesquiterpenes studied.

It would be very valuable for a tentative identification of the peak if it were possible to determine what substituents are contained in the sample by using the additivity rule.

$$\Delta I^{y}_{x} = I^{y}_{x} - I^{\text{Apiezon L}}_{x}$$

where x is compound x, and y is stationary phase tested. Applying the additivity rule:

$$\Delta I_{\mathrm{x}}^{\mathrm{y}}=[I_{\mathrm{x}_0}^{\mathrm{y}}-I_{\mathrm{x}_0}^{\mathrm{Apiezon\ L}}]+\sum_{i=1}^{n}[I_{\mathrm{x}_i}^{\mathrm{y}}-I_{\mathrm{x}_i}^{\mathrm{Apiezon\ L}}] \tag{1}$$

where x_0 is skeleton structure, and x_i is *i*-substituent; for the difference of ΔI on two columns we find

$$\begin{aligned}\Delta\Delta I_{\mathrm{x}}^{\mathrm{y/y'}}&=\Delta I_{\mathrm{x}}^{\mathrm{y}}-\Delta I_{\mathrm{x}}^{\mathrm{y'}}\\&=[I_{\mathrm{x}}^{\mathrm{y}}-I_{\mathrm{x}}^{\mathrm{Apiezon\ L}}]-[I_{\mathrm{x}}^{\mathrm{y'}}-I_{\mathrm{x}}^{\mathrm{Apiezon\ L}}]\\&=I_{\mathrm{x}}^{\mathrm{y}}-I_{\mathrm{x}}^{\mathrm{y'}}\\&=[I_{\mathrm{x}_0}^{\mathrm{y}}-I_{\mathrm{x}_0}^{\mathrm{y'}}]+\sum_{i=1}^{n}[I_{\mathrm{x}_i}^{\mathrm{y}}-I_{\mathrm{x}_i}^{\mathrm{y'}}].\end{aligned}$$

If

$$I_{\mathrm{x}_0}^{\mathrm{y}}-I_{\mathrm{x}_0}^{\mathrm{y'}}\ll[I_{\mathrm{x}_1}^{\mathrm{y}}-I_{\mathrm{x}_1}^{\mathrm{y'}}]\gg\sum_{i=2}^{n}[I_{\mathrm{x}_i}^{\mathrm{y}}-I_{\mathrm{x}_i}^{\mathrm{y'}}],$$

we obtain

$$\Delta\Delta I_{\mathrm{x}}^{\mathrm{y/y'}}\doteqdot[I_{\mathrm{x}_1}^{\mathrm{y}}-I_{\mathrm{x}_1}^{\mathrm{y'}}] \tag{2}$$

As shown in Table III, the two $\Delta\Delta I$ values for a hydroxy group on XE-60/QF-1 and DEGS/XE-60 are very high compared to those of other substituents under study (see Fig. 3). Therefore, equation (2) may be applied to identify a hydroxy group.

TABLE II. $\Delta\Delta I\ {}^{\mathrm{y/y'}}_{\mathrm{x/x_0}}$ Value for Substituent

Substituent	$\Delta\Delta I^{\mathrm{QF\text{-}1/OV\text{-}17}}$	$\Delta\Delta I^{\mathrm{XE\text{-}60/QF\text{-}1}}$	$\Delta\Delta I^{\mathrm{DEGS/XE\text{-}60}}$
OH	12	129	222
OAc	34	31	108
CO	83	11	119
OMe	15	25	56
C=C	−16		21
	−30		56

In the furanosesquiterpene system under study, as the skeleton structure 8HLN (III) shows high ΔI value on polar phases, differentiation of various substituents is very difficult. So another approach to a tentative identification of substituents was tried by application of the alternative equation. Eliminating the effect of the skeleton structure 8HLN (III), we find.

$$\begin{aligned}\Delta\Delta I_{\mathrm{x/x_0}}^{\mathrm{y}}&=\Delta I_{\mathrm{x}}^{\mathrm{y}}-[I_{\mathrm{x}_0}^{\mathrm{y}}-I_{\mathrm{x}_0}^{\mathrm{Apiezon\ L}}]\\&=\sum_{i=1}^{n}[I_{\mathrm{x}_i}^{\mathrm{y}}-I_{\mathrm{x}_i}^{\mathrm{Apiezon\ L}}]\end{aligned} \tag{3}$$

In the same way, difference of increment of the substituent between two columns is

$$\Delta\Delta I_{\mathrm{x/x_0}}^{\mathrm{y/y'}}=\sum_{i=1}^{n}[I_{\mathrm{x}_i}^{\mathrm{y}}-I_{\mathrm{x}_i}^{\mathrm{y'}}] \tag{4}$$

If a characteristic value of any substituent group is known in the right hand terms of Eq. (4), we may tentatively assign the group. Plots of Eq. (3) are shown in Fig. 4.

Applying Eq. (4), where y=QF-1, and y′=OV-17 (see Table II), we find for $\Delta\Delta I^{\mathrm{y/y'}}$ that double bonds have minus values (furan: −30, Δ^1: −16), and the value for the acetoxy group is comparable with that of a furan ring, but opposite in sign. Thus, $\Delta\Delta I^{\mathrm{QF-11/OV-17}}$ values for an acetoxy group change from positive to negative as the number of double bonds in a compound increases (for XII: + +, XIV: +, XV: −; this is shown in the plot of Eq. (2) in Fig. 3). A carbonyl group has a very high positive value, but values for methoxy and hydroxy groups are negligibly small (see Fig. 3). It is characteristic that the effect of a double bond on $\Delta\Delta I^{y/y'}$ is minus in sign on QF-1/OV-17, but positive on DEGS/XE-60. Using the values of RI$_{X}^{QF\text{-}1}$; carbonyl: 194, acetoxy: 159, hydroxy: 93, methoxy: 81, the above observations will enable a tentative identification of substituents. Even when the skeleton structure is unknown, by using Fig. 3 it will be possible to differentiate substituent groups in an unidentified sample. In natural product chemistry, it is common that most compounds produced by a species have the same skeleton structure, in such fields, therefore, application of the above should prove very useful.

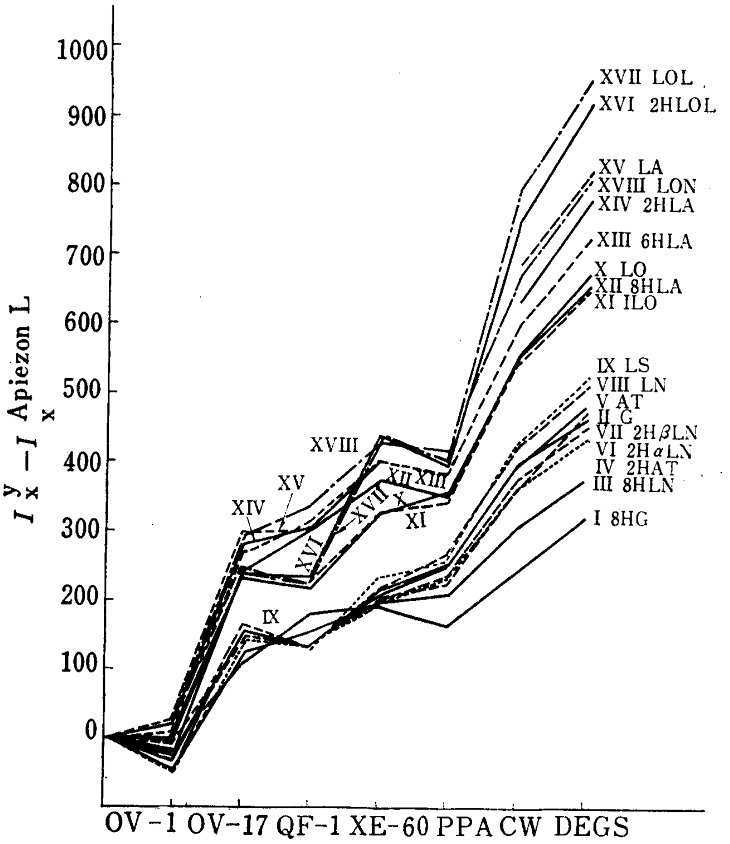


Fig. 3

Experimental

Chromatography was on a Shimadzu GC-4APFT equipped with a flame ionization detector. Glass columns were 1.5m × 0.4 mm packed with 1% stationary phase on Gas Chrom Q (100/120 mesh). Stationary phases were Apiezon L (Associated Electrical Industries Ltd.), OV-1, OV-17, QF-1, XE-60, carbowax (CW), diethylene glycol succinate polyseter (DEGS) (Applied Science Lab.), and polyphenyl ether (6 rings) (PPA) (Nishio Kogyo Ltd.); they were coated by the filtration method. Kováts indices were obtained by using *n*-alkane (Applied Science Lab.) as internal standard at two column temperatures, and interpolated at, or extrapolated to, 170°.

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