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## Note

### Kováts indices of diterpene hydrocarbons on fused-silica capillary columns

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More than 25 cyclic diterpene hydrocarbons have been isolated from a range of natural sources<sup>1,2</sup>, and several are intermediates in the biosyntheses of oxygenated diterpenes such as the gibberellins<sup>3</sup>. Considerable confusion has arisen in the past (see for example ref. 4) because the diterpene hydrocarbons often co-occur and the mixtures can be difficult to separate. The usefulness of gas chromatography (GC) in this context was demonstrated early in the history of the technique<sup>5</sup>.

The Kováts retention index is the preferred method of reporting GC retention data of a compound, as this quantity is dependent only on the stationary phase and the column temperature<sup>6,7</sup>. Tables of retention indices have been compiled for monoterpenes<sup>8</sup>, sesquiterpenes<sup>9</sup>, and some oxygenated diterpenes<sup>10</sup>. However, values for only a few diterpene hydrocarbons have been reported<sup>4</sup>. While involved in a study of the variation of diterpene levels in the foliage of some New Zealand conifers, we have built up a collection of retention index values which were very useful in making identifications<sup>11</sup>. These are reported here. The level of confidence in identifications by retention indices is improved by using high resolution GC columns, stationary phases of different polarities and measurements at different temperatures<sup>12</sup>. Accordingly, measurements were made on two different fused-silica capillary columns. The two stationary phases used were those which had been employed in previous work on terpenes<sup>4,8,9</sup>, and in a recent large compilation of retention index data<sup>13</sup>.

#### EXPERIMENTAL

Samples were generously supplied by Professors R. C. Cambie (hibaene, kaurene and trachylobane), R. M. Carman (isohibaene), W. G. Dauben (cembrene), M. Kitadani (abieta-8,11,13-triene, isopimara-8,15-diene, rimua-5(10),15-diene and sandaracopimaradiene), M. D. Sutherland (abieta-7,13-diene, isopimara-8,15-diene and sandaracopimaradiene), and A. Yoshikoshi (abieta-8,11,13-triene, dolabradiene and hibaene). Lauren-1-ene, rimuene, isophyllocladene and phyllocladene were isolated from the foliage of *Dacrydium cupressinum* Lamb. (Podocarpaceae)<sup>14,15</sup>. Laurenane and 1 $\beta$ H-laurenane were synthesised from lauren-1-ene<sup>16</sup>, and dihydrorimuene and rimuane from rimuene<sup>14</sup>. Rosa-5,15-diene was isolated from the foliage of *D. intermedium* Kirk, and was converted into rosa-5(10),15-diene by acid-catalysed rearrangement<sup>11</sup>. Sclarene and *cis*- and *trans*-biformene were prepared by dehydrating

manool<sup>17</sup>. Isopimaradiene was isolated from the heartwood of *D. biforme* Pilger<sup>18</sup>. Isokaurene was prepared by the acid-catalysed rearrangement of kaurene.

Fused-silica capillary columns were supplied by Scientific Glass Engineering, and were fitted in a Varian 3700 chromatograph. The oven temperature was checked, and found to be reproducible to  $\pm 0.2^\circ\text{C}$ . Dilute solutions of each diterpene were coinjected with solutions of *n*-alkanes with carbon numbers 16, 18, 19, 20 and 22 (and 24 when necessary). The use of an injection splitter and a flame ionisation detector allowed low sample loadings, so that asymmetric peaks were avoided. Retention times were measured with an electronic integrator. An interactive computer program was used to calculate retention indices\*. The procedure for each run was:

(1) The correction for column hold-up was calculated from the retention times of three *n*-alkanes with evenly spaced carbon numbers<sup>19</sup>.

(2) Regression analysis of the logarithms of the corrected *n*-alkane retention times and their retention indices gave the regression constant and intercept (correlation coefficients  $> 0.999990$ ).

(3) The retention index of the sample peak was calculated.

## RESULTS AND DISCUSSION

The retention indices of 26 diterpene hydrocarbons on Carbowax 20M and SE-30 at  $170^\circ\text{C}$  and  $190^\circ\text{C}$  are given in Table I. Semi-systematic names are given in brackets, when these differ from the common name<sup>20</sup>. The values given are the means of at least two measurements. The 95% confidence limits (for those values based on five or more measurements) had a mean of  $\pm 0.6$  index units, which is similar to the uncertainty in retention indices due to retention time measurements accurate to  $\pm 0.6$  sec as in this work<sup>21</sup>. The values in Table I differ from those reported previously by between one and eight index units<sup>4</sup>.

The temperature gradients of the retention indices are also given in Table I, as these can be used to extrapolate to other temperatures (for discussion, see Ettre<sup>22</sup>). The plots of retention index and temperature were close to linear for rimuene, sclarene and phyllocladene from  $150^\circ\text{C}$  to  $210^\circ\text{C}$  on Carbowax 20M, and from  $150^\circ\text{C}$  to  $230^\circ\text{C}$  on SE-30. A straight line drawn through the points given in Table I deviated by at most three index units from the experimental values over these temperature ranges. The temperature gradients of the diterpenes show a strong positive correlation with the number of rings present in their structures, as shown in Table II. Ettre noted that the retention indices of cycloalkanes and aromatic compounds had greater temperature gradients than those of acyclic hydrocarbons, but offered no explanation<sup>22</sup>.

Most of the known diterpene hydrocarbons can be identified using the data presented here. However, isolation and further examination may be generally advisable, since enantiomeric diterpenes have been found in plants of the same genus<sup>23</sup>, and even of the same species<sup>3,11</sup>.

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\* A listing of the Fortran program ISORI will be supplied on request.

TABLE I

## KOVÁTS INDICES AND TEMPERATURE GRADIENTS FOR DITERPENE HYDROCARBONS

	<i>Carbowax 20M</i>			<i>SE-30</i>		
	<i>170°C</i>	<i>190°C</i>	$\delta I/\delta T$	<i>170°C</i>	<i>190°C</i>	$\delta I/\delta T$
Rimua-5(10),15-diene (8,10-Friedoisopimara-5(10),15-diene)	2114	2143	1.4	1864	1885	1.0
Lauren-1-ene	2123	2161	1.9	1869	1897	1.4
Dihydrorimuene (8,5-Friedoisopimar-5-ene)	2154	2187	1.6	1917	1940	1.1
Rimuene (8,5-Friedoisopimara-5,15-diene)	2156	2187	1.5	1884	1906	1.1
Rosa-5(10),15-diene (8,10-Friedopimara-5(10),15-diene)	2156	2187	1.5	1896	1917	1.0
Rimuane (8,5-Friedoisopimarane)	2164	2198	1.7	1942	1966	1.2
Laurenane	2165	2204	1.9	1912	1940	1.4
Isopimara-8,15-diene	2175	2210	1.7	1898	1921	1.1
Cembrene (Cembra-2,4,7,11-tetraene)	2180	2207	1.3	1916	1935	0.9
1 $\beta$ H-Laurenane	2191	2229	1.9	1925	1953	1.4
Rosa-5,15-diene (8,5-Friedopimara-5,15-diene)	2200	2233	1.6	1914	1936	1.1
Hibaene (Beyer-15-ene)	2200	2241	2.0	1913	1941	1.4
Isohibaene (13 $\alpha$ -Beyer-15-ene)	2204	2245	2.0	1914	1942	1.4
Sclarene (Labda-8(17),13(16),14-triene)	2233	2259	1.3	1914	1933	0.9
Isophyllocladene (13 $\beta$ -Kaur-15-ene)	2238	2277	1.9	1946	1974	1.4
Sandaracopimaradiene (Isopimara-8(14),15-diene)	2243	2279	1.8	1943	1969	1.3
Trachylobane (13,16-Cycloatisane)	2279	2318	1.9	1965	1993	1.4
Isokaurene (Kaur-15-ene)	2280	2321	2.0	1967	1995	1.4
Dolabradiene (8,4-Friedoisopimara-4(18),15-diene)	2283	2318	1.7	1945	1970	1.2
Isopimaradiene (Isopimara-7,15-diene)	2295	2332	1.8	1968	1994	1.3
cis-Biformene (Labda-8(17),12Z,14-triene)	2306	2330	1.2	1962	1979	0.8
Phyllocladene (13 $\beta$ -Kaur-16-ene)	2319	2362	2.1	1985	2015	1.5
trans-Biformene (Labda-8(17),12E,14-triene)	2344	2370	1.3	1985	2004	0.9
Kaurene (Kaur-16-ene)	2350	2393	2.1	2003	2033	1.5
Abieta-7,13-diene	2410	2447	1.8	2044	2068	1.2
Abieta-8,11,13-triene	2443	2476	1.6	2013	2034	1.0

TABLE II

TEMPERATURE GRADIENTS OF RETENTION INDICES OF MONO-, BI-, TRI-, TETRA- AND PENTACYCLIC DITERPENES

Rings	Cases	Carbowax 20M		SE-30	
		Mean	Range	Mean	Range
1	1	(1.3)	—	(0.9)	—
2	3	1.27	1.2-1.3	0.87	0.8-0.9
3	12	1.64	1.4-1.8	1.13	1.0-1.3
4	9	1.98	1.9-2.1	1.42	1.4-1.5
5	1	(1.9)	—	(1.4)	—

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