

CHROM. 4225

## THE IDENTIFICATION OF SESQUITERPENE HYDROCARBONS FROM GAS-LIQUID CHROMATOGRAPHY RETENTION DATA

NIELS H. ANDERSEN AND MARK S. FALCONE\*

*Department of Chemistry, University of Washington, Seattle, Wash. 98105 (U.S.A.)*

(Received June 9th, 1969)

## SUMMARY

The identification of sesquiterpenes by gas-liquid chromatography is evaluated. Retention indices (obtained using sesquiterpene standards rather than *n*-alkanes) were found to be the most reproducible form of GLC data. Retention data for fifty-five sesquiterpenes and a number of saturated hydrocarbons obtained by hydrogenation of sesquiterpenes are reported.

## INTRODUCTION

Until recently the study of the sesquiterpenes from natural sources has been plagued by separation problems since most plants produce complicated mixtures of very similar compounds. With the increased use of GLC<sup>1-3</sup> and adsorption chromatography on selective phases (such as AgNO<sub>3</sub>-silica)<sup>3-6</sup> many sesquiterpenes of unquestioned purity have been isolated. However, these unsaturated compounds generally are quite unstable and thus it is virtually impossible to keep large numbers of authentic samples for GLC comparison.

Among the goals of our research are biogenetic studies using radiolabeled sesquiterpene precursors in whole plants and plant extracts. In connection with these studies we required analytical techniques which would allow us to identify the components of gross mixtures available in only small amounts (10  $\mu$ g-10 mg). We felt that GLC on a number of different columns (with appropriate standardization procedures) should serve to identify the major sesquiterpene hydrocarbons in such mixtures.

At present, the less numerous, and more thoroughly studied, monoterpene hydrocarbons can be identified unambiguously by GLC on one or two columns (*e.g.* ref. 7). The retention data from such studies have generally been given in KOVATS' indices or as retention times relative to a standard terpene. We felt that the more numerous sesquiterpenes\*\* could be identified in a similar manner if retention data were obtained on a sufficient number of distinct selective phases.

\* Undergraduate research participant, University of Washington, 1968-1969.

\*\* At present at least a hundred distinct sesquiterpene hydrocarbons have been characterized. However, this probably accounts for only one third of the possible structures. Thus the retention data for a component of a mixture must also be precise (and dependable) enough to indicate that a new substance has been isolated.

## METHODS AND MATERIALS

*Materials*

The sesquiterpenes were either obtained through the generosity of other workers in the field or by isolation from the appropriate essential oils. The identity and purity of these samples were verified through IR and NMR spectroscopy.

H. U. DAENIKER (Givaudan Corp.) supplied  $\alpha$ -cedrene,  $\beta$ -cedrene, valencene, and thujopsene. G. OURISSON (Institut de Chimie, Strasbourg, France) supplied longifolene, cyperene, caryophyllene,  $\alpha$ -gurjunene, and calarene. V. HEROUT (Institute of Organic Chemistry and Biochemistry, Czech. Acad. of Science) supplied  $\beta(\gamma)$ -bisabolene, cyperene,  $\gamma$ -muurolene,  $\epsilon$ -muurolene,  $\gamma$ -cadinene,  $\beta$ -bourbonene, humulene, the santalenes, and  $\gamma$ -amorphene. S. DEV (National Chemical Laboratory, Poona-8, India) supplied  $\alpha$ -himachalene,  $\beta$ -himachalene,  $\alpha$ -copaene, and  $\beta$ -selinene. L. WESTFELT (Swedish Forest Products Research Laboratory, 114 86 Stockholm, Sweden) supplied longicyclene,  $\alpha$ -muurolene, and  $\gamma$ -muurolene. J. E. McMURRY (Univ. of California, Santa Cruz) supplied cyclosativene, sativene, and isosativene. Y. HIROSE AND Y. OHTA (The Institute of Food Chemistry, Osaka, Japan) supplied  $\alpha$ -ylangene,  $\delta$ -cadinene,  $\alpha$ -amorphene, and  $\alpha$ -muurolene.

$\delta$ -Selinene was obtained from  $\beta$ -selinene by treatment with formic acid.  $\alpha$ -Curcumene was isolated from the oil of *Curcuma aromatica*.  $\alpha$ -Copaene,  $\alpha$ -curcumene, longifolene,  $\beta$ -farnesene,  $\beta$ -curcumene,  $\gamma$ -curcumene,  $\gamma$ -cadinene, and  $\delta$ -cadinene were isolated from the needle oil of *Chamaecyparis nootkatensis*<sup>8</sup>.  $\beta$ -Selinene and  $\alpha$ -selinene were isolated from oil of celery.  $\alpha$ -Zingaberene was isolated from oil of ginger.  $\alpha$ -Muurolene and  $\delta$ -cadinene were isolated from rectified cade oil. Humulene and caryophyllene were isolated from wild ginger oil. Zizaene, cyclocopacamphene,  $\beta$ -vetivenene,  $4\beta$ H-nootkata-1(10),7(11),8-triene, and the antipodes of  $5\alpha$ H-selina-4(14),7-diene, and  $5\alpha$ H-selina-4(14),7(11)-diene were isolated from oil of vetiver and this work will be the subject of another communication.

*Methods*

The analyses were performed on a F & M Scientific Series 700 laboratory chromatograph (Hewlett-Packard) equipped with WX filaments. The columns used were 16-50 ft.  $\times$  0.125 in. The stationary phase loading was 0.5-3% on silanized Chromosorb G. The columns had between 300-800 theoretical plates per foot under normal operating conditions (80-200°, 15-40 cc/min flow of helium).

The sesquiterpenes and *n*-alkane standards were introduced as 2-10% solutions in *n*-decane. The injection volumes were 0.1-0.6  $\mu$ l, delivered with a 1  $\mu$ l Hamilton syringe. KOVATS' indices were calculated using *n* (even) *n*-alkanes or by the method given below. The odd *n*-alkanes generally lie on a different line than the even ones on a plot of *n* vs.  $\log t_R'$ . KOVATS' indices<sup>9</sup> were calculated only when the peaks for the unknown and co-injected standards corresponded to those obtained from 0.02-0.2  $\mu$ l of a 5% solution of the pure substances. Careful studies indicated that overloading effects (skewing to longer retention time, peak broadening, etc.) could not be detected until the injection volume was 0.9  $\mu$ l of a 5% solution. Thus all measurements were obtained from traces on which all of the important peaks were Gaussian and far below maximum loading.

The method of calculating KOVATS' indices (using two sesquiterpenes as

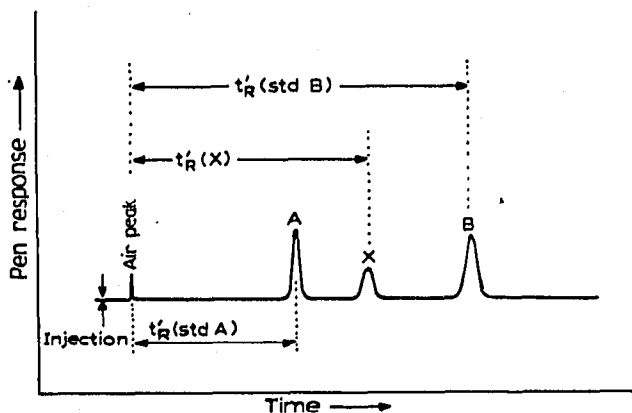


Fig. 1. Schematic GLC trace.

standards, A and B) and relative retentions is shown below, together with an illustrative trace (Fig. 1):

$$RR_X (\text{rel. to A}) = \frac{t'_R (X)}{t'_R (\text{std A})}$$

$$I_X = I_{\text{std A}} + \left( \frac{\log t'_R (X) - \log t'_R (\text{std A})}{\log t'_R (\text{std B}) - \log t'_R (\text{std A})} \right) (I_{\text{std B}} - I_{\text{std A}})$$

## RESULTS AND DISCUSSION

The first problem was to determine the most reproducible form of retention data. Preliminary studies indicated that relative retention times (from  $RR = 0.4-2.0$ ) could be reproduced ( $\pm 0.8\%$ ) on a single column as long as the temperature was kept with a  $5^\circ$  range and the flow rates were controlled to  $\pm 10\%$ . However, relative retentions could not be reproduced as well on other columns containing the same stationary phase. KOVATS' indices were more reproducible from column to column but varied significantly with temperature (see Table I). We found that  $\Delta I/\Delta T \sim + 1.1$  for sesquiterpenes on Carbowax, and that  $\Delta I/\Delta T$  increased on polar columns and was somewhat less on less selective non-polar phases. In all cases, the KOVATS'

TABLE I

KOVATS' INDICES OF SELECTED SESQUITERPENES ON CARBOWAX 20 M

Compound	I		$\Delta I$
	$132^\circ$	$205^\circ$	
Longicyclene	1518.8	1598.5	80
$\alpha$ -Copaene	1521.5	1593	71.5
$\alpha$ -Cedrene	1597.5	1689	91.5
Longifolene	1600.0	1697	97
Caryophyllene	1618.5	1695.5	77
Humulene	1681.5	1765	83.5
Valencene	1725.5	1801	75.5
$\alpha$ -Muurolene	1726	1792	66
$\gamma$ -Cadinene	1762	1835.5	73.5

indices increased with increasing temperature. Under normal laboratory conditions the oven temperature can be reproduced to  $\pm 3^\circ$  and thus KOVATS' indices could be reproduced to only  $\pm 3.5$  units (out of 1400-1900 units) with all other sources of error discounted.

The temperature dependence variation in KOVATS' indices<sup>1</sup> within the sesquiterpenes is much less ( $\Delta I/\Delta T = + 1.1 \pm 0.25$  for the entire group) and thus KOVATS' indices can be reproduced to  $\pm 1$  unit by using sesquiterpenes as standards rather than using even *n*-alkanes as is usually done. Our usual procedure consisted of determining (in the usual manner<sup>9</sup>) the KOVATS' indices of a number of sesquiterpenes on a single phase on one day so as to minimize temperature variation. From then on, the indices of additional sesquiterpenes were determined using sesquiterpenes as standards (see *Methods*). We generally used  $\alpha$ -copaene- $\alpha$ -cedrene-humulene- $\gamma$ -cadinene mixtures for standardization. The indices for sesquiterpenes on seven different stationary phases are collected in Table II. In addition, approximate indices can be calculated for other sesquiterpenes from GLC traces in the literature if the traces also show: (1) the air peak and peaks due to two sesquiterpenes for which standardized indices are available; or (2) peaks due to three sesquiterpenes for which standardized indices are known. Approximate KOVATS' indices obtained in this way are included in Table II, and are indicated by an asterisk. Chromatographic data from refs. 1-3 were used for these calculations. A trace of the hydrocarbon portion of the oil of *Mentha piperita* on Apiezon L<sup>3</sup> was particularly informative. The trace included peaks for ylangene, humulene, and  $\gamma$ -cadinene which were used for standardization. The indices calculated for  $\beta$ -bourbonene, caryophyllene,  $\gamma$ -muurolene,  $\alpha$ -muurolene, and  $\delta$ -cadinene (also appearing in this oil) were within  $\pm 3$  units (average) of the values obtained by us.

Examination of the data presented in Table II quickly shows that all of these sesquiterpenes can be distinguished by the use of only two to three different phases. Thus other workers should be able to confirm the identity of these substances by co-injection with two identified sesquiterpenes appearing in Table II. The types of selectivity displayed by various stationary phases will be discussed in detail in a later communication. However, some correlations deserve comment at this point. In every case except the himachalenes the *exo*-methylene isomers are eluted after the corresponding trisubstituted olefins. Comparison of the indices for  $\alpha$ -curcumene and  $\beta$ -curcumene (or  $\delta$ -cadinene) reveals an increasing affinity (and therefore retention) for aromatics in going from Apiezon L to Carbowax and particularly DEGS. There are many other cases of position reversals not only on changing stationary phase but even with temperature changes on a single phase (see longicyclene- $\alpha$ -copaene, longifolene-caryophyllene-calarene, thujopsene-santalene, valencene- $\beta$ -bisabolene- $\alpha$ -muurolene all on Carbowax 20M).

In the structure elucidation of sesquiterpenes it is a common practice to identify the carbon skeleton of a new sesquiterpene by hydrogenation to the fully saturated ring system. The saturated hydrocarbons are usually identified from their physical properties and by comparison of thick-film IR spectra with the published standards<sup>11</sup>. However, this method is not foolproof. The IR spectra of saturated hydrocarbons are particularly devoid of useful detail for such comparisons and these substances have very low rotatory powers. In addition, the hydrogenations involved usually introduce new asymmetric centers and the resulting products are mixtures of dia-

TABLE II

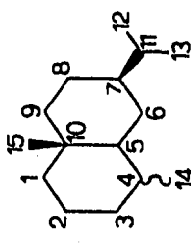
## MODIFIED KOVATS' INDICES OF SESQUITERPENES

The values for the sesquiterpenes indicated by an asterisk are approximate only, since they are obtained from traces in the literature<sup>1-3</sup>. Values in parentheses have been obtained from a single trace only.

	Apiezon L 155°	SF-96 170°	SE-30 (UC-W98) 130°	DC-710 <sup>d</sup> 165°	QF-1 132°	Carbowax 20 M			DEGS 160°
						132°	165°	205°	
1	Cubebene*	1368							
2	$\alpha$ -Longipinene*		1359.5	1454.5		1538.5		1653	
3	$\alpha$ -Ylangene	1401.5	1396.1						
4	$\beta$ -Elemene*	1410							
5	$\alpha$ -Bourbonene*	1410							
6	$\alpha$ -Copaene	1410.2	1400.5	1459	1447	1551.3	1593	1665	(1684) <sup>c</sup>
7	Cyclosativene	1411.9	1399.7			1549		1684	
8	Longicyclene	1417.1	1409.1	1454	1465	1554	1598.5	1685.6	
9	Cyclocopacamphene	1417.8			1467.2	1555.4		1714	
10	$\beta$ -Bourbonene	1418.3	1411.7	1477.3	1477.5	1586.5	1618	1818.5	(1738) <sup>c</sup>
11	$\beta$ -Farnesene	1429.2			1509	1668			
12	Sativene	1434.7	1420.7			1594.5			
13	Cyperene	1446.6	1432.5	1501	1493	1606	1650	1736.5	
14	$\alpha$ -Gurjunene		1435.2	1500.5	1471	1558	1633	1712.5	
15	Caryophyllene	1451.7	1445.3	1523	(1587)	1618.5	1695.5	1835.5	
16	Longifolene	1464.0	1440.2	1517.5	1520	1600	1697	1802.5	
17	Isosativene	1464.4	1440.9			1639		(1797) <sup>c</sup>	
18	Calarene	1466.0	1459.7	1535.5	1513	1618	1700	1806	
19	$\beta$ -Ylangene*								
20	$\beta$ -Copaene*		~1417.5						
21	$\alpha$ -Cedrene	1473.4	1445.0	1516	1518	1597.5	1689	1788.5	
22	Thujopsene	1476.1	1458.3	1542.3	1540	1643	1732	1858.5	
23	Aromadendrene*	~1477							
24	$\alpha$ -Maaliene*	~1477							
25	$\gamma$ -Curcumene	1481.9							
26	$\beta$ -Cedrene	1482.4	1454.8	1533.5	1539.5	1624.5	1714	1834.5	
27	$\alpha$ -Curcumene	1483	1480.4	1589	1557.5	1787.5	(1814)	1992.5	
28	$\epsilon$ -Muurolole	1484.8	(1474.0)	(1501.5)	1552.5	1713.8	(1739.5)	1893.5	
29	Humulene	1487.2	1476.6	1561.5	1583.5	1719	1765	1929.5	
30	Santalene (minor)		1459.5	1535	1522	1644.5	1702	1830	
31	Santalene (major)		1470.5	1548	1533.5	1683	1714.5	1843.5	
32	Selina-4(14), 7-diene <sup>a</sup>	1491.9	1475.7		1542	1694		1852.5	
33	$\delta$ -Selinene <sup>a</sup>	1504.5				1728.5		1889	
34	$\gamma$ -Muurolole	1505.7			1545	1725		1896.5	
35	$\gamma$ -Amorphene	1506.4			1544.5	1724			

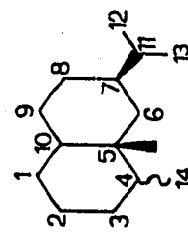
36	$\alpha$ -Himachalene	1508.0	1444	1561.5	1533.5	1002.5	1704.5	1755	1870
37	$\alpha$ -Amorphene	1509.5	1491.7	1582.5	1535	1724.5	1724.5	1755	1897
38	Zizaene	1511.6	1481.8		1562	1706.3	1706.3		1879.5
39	$\beta$ -Bisabolene	1512.9	1510.3	1592.5	1548	1745.5	1745.5	1772	1909.5
40	$\beta$ -Curcumene	1513.6	1510.4	1547.5	1547.5	1756	1756		1922.5
41	$\alpha$ -Zingaberene		(1479.6)	1583.5		1738		(1762)	
42	Valencene <sup>b</sup>	1525.6	1508.8	~1600	1581	1725.5	1760	1801	1948
43	$\beta$ -Himachalene	1529.7		1607.5	1578	1717	1752.5	1799	
44	$\beta$ -Selinene <sup>a</sup>	1530.2	1506.3	1595	1597.5		1766.5	1815.5	1958
45	$\gamma$ -Bisabolene	1531.3	(1505)	1601			1765.5	1815	
46	$\alpha$ -Muurolene	1531.3	1507.7	~1600	1558.5	1726	1752.5	1792	1927.5
47	$\alpha$ -Pyrovetivene <sup>b</sup>	1533.9	1521.5				1817		2026.0
48	$\alpha$ -Selinene <sup>a</sup>	1534.5							
49	$\epsilon$ -Bulgarene <sup>a</sup>	1538							
50	$\delta$ -Cadinene	1546.4	(1526.4)	~1504 <sup>d</sup>	1628.5	1784		1818	1959
51	Calamenene <sup>a</sup>	~1550							
52	$\gamma$ -Cadinene	1554.9	1523.5	1623.5	1587	1762	1792.3	1835.5	1978.5
53	Selina-4(14), 7(11)-diene <sup>a</sup>	1572.0			1611.5		1816.3		(2018) <sup>c</sup>
54	Selina-3,7(11)-diene <sup>a</sup>	1580							
55	$\beta$ -Vetivenene <sup>b</sup>	1583.0	1563.3				1885		2111

<sup>a</sup> Selinane (eudesmane) hydrocarbons are designated using THEOBALD'S system of nomenclature based on the numbering shown<sup>10</sup>:

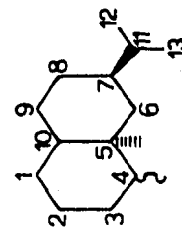


$\alpha$ -selinene [= 3,11-diene],  $\beta$ -selinene [= 4(14), 11-diene] and  $\delta$ -selinene [= 4,6-diene].

<sup>b</sup> Eremophilane and nootkatane sesquiterpenes are named based on the following structure projections and numbering systems.



Eremophilane



Nootkatane

Valencene = 4 $\beta$ H-nootkata-1(10),11-diene;  $\beta$ -vetivenene = 4 $\beta$ H-nootkata-1,7(11),9-triene;  $\alpha$ -pyrovetivene = 4 $\beta$ H-nootkata-1(10),7(11),8-triene.

<sup>c</sup> Extrapolated from measurement at 175° on the DEGS column:  $\alpha$ -copaene (1692.1), cyclosativene (1711.8), sativene (1766.7), isosativene (1827.9), selina-4(14),7-diene (1888.3), zizaene (1923.4),  $\beta$ -bisabolene (1935.0),  $\delta$ -cadinene (1990.4),  $\gamma$ -bisabolene (1997.6),  $\gamma$ -cadinene (2015.0),  $\alpha$ -curcumene (2018.6), selina-4(14),7(11)-diene (2049), and  $\alpha$ -pyrovetivene (2074.0).

<sup>d</sup> A 15° increase leads to Kovars' indices from 7.5-15 units higher than those listed.  $(AI/AT)_{ave} = 0.75$ .

TABLE III

## KOVATS' INDICES OF HYDROGENATION PRODUCTS OF SESQUITERPENES

Hydrogenations were performed using Adam's catalyst in all cases. The solvent was acetic acid unless otherwise specified.

Compound <sup>a</sup>	Hydrogenation product of	Retention index			
		Apiezon L 155°	SF-96 170°	Carbowax 20 M 165°	DEGS 175°
Farnesane	$\beta$ -farnesene	1367.6		1354	1350
Bisabolane I	$\beta$ -bisabolene	1458.4		1501	1557
Bisabolane II <sup>b</sup>	$\beta$ -curcumene <sup>b</sup>	1463.6		1520.7	1586.5
Copaane I	$\alpha$ -copaene	1477.2	1455.4	1598.8	1740
Copaane II <sup>c</sup>		1437.3	1420.6	1550	1676
Muurolane I	$\alpha$ -muurolene	1479.2	1454.7	1582	1713
Muurolane II		1535.6	1505.6	1659	1808.5
Cadinane I	$\gamma$ -cadinene <sup>d</sup>	1503.2	1483.4	1618	1760
Cadinane II		1493.5	1478.4	1597	1728
Amorphane I	$\alpha$ -amorphene	1489.8	1463.4	1593	1718.2
Amorphane II		1524.4	1498.4	1648.5	1794.2
Calarane	calarene	1508.1	1489.3	1670.5	1845.5
4,5 $\alpha$ H-Eudesmane = selinane	$\beta$ -selinene	1539.6	1509.5	1664.5	1819.4
Nootkatane	valencene	1530.6	1500.9	1648	1796.2
Vetivane I = nootkatane	<sup>e</sup>	1529.8	1500.4	1649	1797
Vetivane II = 7-epinootkatane		1553	1518.7	1683	1843.5
Cedrane I	$\beta$ -cedrene	1521.3	1479.4	1662	1841.5
Cedrane II		1509.1	1470.3	~1651	~1826
Zizaane	zizaene	1513.1	1479.4	1660	1842.5

<sup>a</sup> When several diastereomeric products were obtained they are listed in order of decreasing proportion in the mixture obtained.

<sup>b</sup> The two bisabolanes are obtained in a 1:1 ratio from  $\beta$ -bisabolene. Hydrogenation of  $\beta$ -curcumene gives a 1:1.7 mixture of bisabolanes I and II respectively.

<sup>c</sup> Produced in higher yield when hydrogenation is performed with benzene as the solvent.

<sup>d</sup>  $\delta$ -Cadinene yields cadinane II, cadinane I, and muurolane II as the major hydrogenation products.

<sup>e</sup> From either  $\beta$ -vetivenene or  $\alpha$ -pyrovetivene.

stereomers—the exact composition varying with the placement of the double bonds in the original sesquiterpene. Here again, precise and reproducible GLC retention data could offer a more dependable basis for identification. To this end, we have hydrogenated some common sesquiterpenes and determined the Kovats' indices of the resulting hydrocarbons. The products obtained from the hydrogenation reactions were GLC resolvable mixtures of diastereomers in all cases for which inspection of Dreiding models suggested nonstereospecific hydrogenation. The compositions of these mixtures and the structure assignments for the components will be the subject of another communication. Table III gives the indices for the major saturated products obtained. Kovats' indices for the hydrogenation products showed temperature

dependence similar to that of the sesquiterpenes themselves; for this reason the indices were calculated using sesquiterpene standards.

Preliminary examinations of monoterpenes and oxygenated sesquiterpenes indicate that highly reproducible retention indices can be obtained by the internal standardization method used above for the sesquiterpene hydrocarbons. Extensions of Tables II and III and tables of retention indices for other natural products will be published periodically in the Chromatographic Data section of this journal.

#### ACKNOWLEDGEMENTS

The authors wish to thank professors G. OURISSON, V. HEROUT, S. DEV, L. WESTFELT, and Y. HIROSE for their generosity in supplying authentic sesquiterpenes and Givaudan Corporation and Fritzsche Brothers, Inc. for supplying oil samples.

Partial support of this research by a Frederick Gardner Cottrell Grant from the Research Corporation is acknowledged.

#### REFERENCES

- 1 R. D. HARTLEY AND C. H. FAWCETT, *Phytochemistry*, 8 (1969) 637.
- 2 L. WESTFELT, *Acta Chem. Scand.*, 20 (1966) 2829.
- 3 R. VLAHOV, M. HOLUB, I. OGNJANOV AND V. HEROUT, *Collection Czech. Chem. Commun.*, 32 (1967) 808.
- 4 S. WITEK AND J. KREPINSKY, *Collection Czech. Chem. Commun.*, 31 (1966) 113.
- 5 A. S. GUPTA AND S. DEV, *J. Chromatog.*, 12 (1963) 189.
- 6 N. P. DAMODARAN AND S. DEV, *Tetrahedron*, 24 (1968) 4113.
- 7 T. SAKAI, H. MAARSE, R. E. KEPNER, W. G. JENNINGS AND W. M. LONGHURST, *J. Agr. Food Chem.*, 15 (1967) 1070.
- 8 N. H. ANDERSEN AND D. SYRDAL, unpublished work.
- 9 E. KOVATS, *Helv. Chim. Acta*, 41 (1958) 1915.
- 10 D. W. THEOBALD, *Tetrahedron*, 19 (1963) 2261.
- 11 J. PLIVA, M. HORAK, V. HEROUT AND F. SORM, *Die Terpene, Sammlung der Spektren und physikalischen Konstanten, Vol. I, Sesquiterpene*, Akademie Verlag, Berlin, 1960.

*J. Chromatog.*, 44 (1969) 52-59