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Short communication

Gas chromatography–mass spectrometry coupled with pseudo-Sadtler retention indices, for the identification of components in the essential oil of *Curcuma longa* L.

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Abstract

Gas chromatography–mass spectrometry was applied to the cyclohexane extract of *Curcuma longa* L. The chromatographic conditions generated retention indices very close i.e., greater than 99.9%, to those reported for structures in the Sadtler Standard Gas Chromatography Retention Index Library. In addition to the extensively reported sesquiterpene ketones, this essential oil extract contained a series of saturated and unsaturated fatty acids. Wiley mass spectra library matching for the free fatty acids, their trimethylsilyl esters and methyl esters narrowed their identity down to a few candidates. Combining this information with the retention indices of the fatty acid methyl esters in the Sadtler library allowed the identification of some of the double bond positions.

Keywords: *Curcuma longa*; Sequiterpene ketones; Fatty acid esters; Fatty acids; Essential oils

1. Introduction

Gas chromatography–mass spectrometry (GC–MS) is a powerful analytical tool. It is however insufficient for identifying substances which lack reference spectra. It has been shown that the combination of retention indices (I_{exp}) with GC–MS for identification can have a much higher performance than GC–MS alone [5,13]. Unfortunately a problem lies in the inter-laboratory comparison of retention indices. Capillary column GC can generate retention indices very precisely, assuming standardisation of stationary film polarity, carrier gas flow-rate, stationary phase film thickness and temperature programming rate. However, most published retention indices have unfortunately not been produced under standardised conditions.

Initial development work [2,3] to circumvent this problem, involved the successful discovery of "pseudo-Sadtler" conditions. The strategy used was as follows: one dataset was adopted as a founding core (i.e., the 8°C on OV-1 stationary phase subset of the excellent Sadtler compilation), then experimental conditions were established that mimicked this kernel. The resultant retention indices allowed a direct comparison with those in the Sadtler library. This

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presented work is similar (although more analytically rigorous) to that in [5], where matching of an unknown GC peak's spectrum from a cinnamon essential oil mixture with the Wiley EI library produced three candidates i.e., γ-pinene, α-pinene and δ-3-carene. All three had a Hewlett-Packard (HP) search quality index >90/100: but subsequent comparison with the Sadtler retention index library clearly pointed to γ-pinene.

This methodology was applied to the study of the cyclohexane extracts of the spice turmeric (*Curcuma longa* L.) from two different sources. Sample 1 was of whole cured rhizomes, Allepey fingers, in which the identification and structural elucidation of the sesquiterpene ketones α- and β-turmerone have been previously reported [6]: sample 2 was of a ground spice of Peruvian origin [15]. *Curcuma longa* L. has been the subject of numerous previous studies [6–8,12,14,16–18]. The novel part of the presented work is the (highly probable) location of the double bond positions for some of the fatty acids, whose existence was reported in Ref. [19]. Secondly, the analytical data reported here can be readily reproduced and thus constitutes a reference for further studies on *Curcuma longa* L. constituents.

2. Experimental

2.1. Instrumentation

A Finnigan TSQ-70B quadrupole mass spectrometer was used as a GC detector, running in either 70 eV electron-impact ionisation (EI) or methane gas positive ion chemical ionisation (CI) mode, coupled with a HP 5890 series II gas chromatograph. The GC injector was maintained at 250°C, the GC transfer line at 320°C, the source at 150°C and the vacuum manifold at 70°C. Thermogreen LB-1 GC injector septa from Supelco were used. The mass range was scanned from m/z 10 to 770 per 0.5 s for EI and from m/z 70 to 770 per 0.5 s for CI. The GC program was from 35°C to 320°C at 15°C/min, followed by 10 min isothermally at 320°C. The mass spectrometer electron multiplier was at +1100 V, the conversion dynode at −15 kV and the electron current at 400 μA. The Wiley library was the 118 137 entry edition (Finnigan part No. 70001-30098, version 1.00, 1992).

Table 1
Representative (first and last three eluting) structures of calibration mixture for EPA method 625

I_{exp}	M_r	Identity	I_{Sad}
951.52±0.54	142	Bis(2-chloroethyl)ether	950.80
986.21±0.34	146	1,3-Dichlorobenzene	985.10
991.21±0.41	146	1,4-Dichlorobenzene	990.59
2416.64±0.06	252	3,3′-Dichlorobenzidine	2416.56
2433.59±0.36	228	Benz[*a*]anthracene	2432.77
2442.66±0.35	228	Chrysene	2442.51

All extracts were measured with simultaneous injection of *n*-alkanes standards (C_5 to C_{28}). The average retention indices ±standard deviation expressed in Tables 1–4, under the I_{exp} column heading, were determined from six measurements. A HP Ultra-1 capillary column (25 m×0.32 mm I.D. and a film thickness of 0.52 μm) was used for consistency with the Sadtler compilation.

2.2. Retention index calibration

The Base/Neutrals mixture for EPA method 625

Table 2
GC–MS of underivatised sample No. 1 cyclohexane extract

I_{exp}	M_r	MS library match	I_{Sad}
1304.39±0.68	166	1,1′-Bicyclohexyl	1300.00
1445.28±0.46	202	Unidentified C15.H12	–
1472.96±0.28	202	α-Curcumene	–
1489.28±0.35	204	α-Zingiberene	–
1503.99±0.51	204	β-Bisabolene	–
1518.34±0.65	204	β-Sesquiphellandrene	–
1559.59±0.70	200	–	–
1638.36±0.56	216	–	–
1648.84±0.22	218	–	–
1680.82±0.08	218	–	–
1724.25±0.55	220	1-Bisabolon	–
1737.27±0.46	228	Tetradecanoic acid	–
1744.01±0.46	232	–	–
1753.93±0.00	218	Zerumbone	–
1791.76±0.58	234	–	–
1916.29±0.49	254	9-Hexadecenoic acid	–
1938.97±0.99	256	Hexadecanoic acid	–
2106.20±0.72	280	–	2105.07
2113.48±1.35	282	–	–
2138.54±0.68	284	Octadecanoic acid	2139.86

Table 3
GC–MS of methylated sample No. 1 cyclohexane extract

I_{exp}	M_r	MS library match	I_{Sad}
1707.44±0.58	242	Methyl tetradecanoate	1707.40
1885.86±0.45	268	Methyl 9- or 11-hexadecenoate	–
1908.78±0.42	270	Methyl hexadecanoate	1907.60
2074.30±0.65	294	Methyl octadecadienoate	–
2082.49±0.68	296	Methyl *cis*-9-octadecenoate	2080.42
2087.87±0.80	296	Methyl *trans*-9-octadecenoate	2086.58
2109.49±0.72	298	Methyl octadecanoate	–
2311.59±0.10	326	Methyl eicosanoate	–

(Sigma part No. 38,463-1; 41 structures with retention index 952 to 3199), was manually injected in a purged splitless mode, simultaneously with a C_5 to C_{28} *n*-alkane mixture (Sadtler Marker Kit No. 40, mixture No. 6) and the retention indices measured by simple linear interpolation between bracketing *n*-alkanes, using the Van den Dool and Kratz equation [4]. This was used to find the "pseudo-Sadtler" conditions. Of the 41 structures, 29 are reliably in the Sadtler data set. Various temperature programming rates were evaluated between 15 and 18°C/min together with various helium carrier gas flow-rates (between 45.0 and 70.0 kPa head pressure) to find the "pseudo-Sadtler" conditions. A least squares unweighted fit of these 29 retention indices with those in the 8°C/min on OV-1 subset of the Sadtler compilation allowed adjustment of the temperature programming and helium flow-rate to find the best alignment i.e., the "pseudo-Sadtler" conditions. The best combination found was a linear temperature programming rate of 15°C/min from 35 to 320°C and a HP 5890A GC column head-pressure of 70.0 kPa helium.

Table 4
GC–MS of trimethylsilylated sample No. 1 cyclohexane extract

I_{exp}	M_r	MS library match	I_{Sad}
1842.12±0.53	300	TMS tetradecanoate	–
2016.29±0.51	326	TMS *cis*-9-hexadecenoate	–
2039.10±0.48	328	TMS hexadecanoate	–
2201.12±0.38	352	TMS *cis,cis*-9,12-octadecadienoate	–
2208.86±0.89	354	TMS *cis*-9-octadecenoate	–
2215.05±0.59	354	TMS *trans*-9-octadecenoate	–
2236.43±1.03	356	TMS octadecanoate	–
2416.85±0.96	384	TMS eicosanoate	–

2.3. Preparation of the extracts

Sample 1: cured, dried *Curcuma longa* L. rhizomes (Allepey fingers, 2.00 g), a gift from Quest, Ashford, UK, were ground with a cutter grinder (Condux CS-150) at 1450 rpm to a particle size of 0.01–0.5 mm. The resulting fine yellow powder was suspended in cyclohexane (100 ml) under an argon atmosphere and stirred vigorously for 15 min. The suspension was filtered and the extraction was repeated (cyclohexane, 2×100 ml). The combined filtrates were concentrated in vacuo to give a bright yellow oil (38 mg). Sample 2: a commercially labelled "Palillo", (labelled also Safran des Indes, Turmeric, produce of Peru) packed by Doña Isabel, Passaic, NJ, USA (2.00 g), was subjected to an analogous procedure, to give a reddish-brown oil (86 mg).

2.4. Derivatisation

A 10-mg amount from each of the original 38 mg (sample 1) and 86 mg (sample 2) cyclohexane residues was dissolved in 500 μl of dichloromethane and 0.2 μl injected splitless into the GC–MS to give the results in Table 2. An equivalent volume of the cyclohexane used for extraction was concentrated, dissolved in dichloromethane and injected as a control. For trimethylsilylation, 250 μl of the initial extract No. 1 dichloromethane solution was added to 250 μl of N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA; Pierce part No. 38830) and allowed to react in a sealed Reacti-Vial for 24 h at room temperature. This 500-μl solution was then blown down under nitrogen to 50 μl to remove the majority of the BSTFA, 250 μl of dichloromethane added and 0.2 μl injected. For methylation, 50 μl of the initial extract No. 1 dichloromethane solution was added to 100 μl of methanolic hydrochloric acid reagent (3 *M*, anhydrous; Supelco part No. 3-3051) and allowed to react at room temperature in a sealed Reacti-Vial for 24 h. This 150-μl solution was then blown down to complete dryness to protect the capillary column from residual hydrochloric acid, resuspended in 100 μl dichloromethane and 0.1 μl injected.

Table 1 shows the first and last three eluting structures (within the Sadtler dataset retention index

range of 600 to 2500) from the Environmental Protection Agency method 625 mixture, illustrating the proximity of the I_{exp} values to the I_{Sad} values using a 15°C/min programming rate and 70.0 kPa helium head pressure i.e., the "pseudo-Sadtler" conditions. These conditions were chosen from the linear fit of I_{Sad} versus I_{exp} (r=0.9999).

3. Results and discussion

The GC experimental calibration method discussed, allowed a very close approach to I_{Sad} values and so a comparison of unknown structures with the defined structures in the Sadtler library. This method is simple and robust because of the existence of a plateau of "pseudo-Sadtler" calibration conditions ([2] table 1). It has also been reported [9] that OV-1 columns other that those of HP manufacture can reproduce the I_{Sad} values, indicating that I_{Sad} interlaboratory reproducibility is easier to attain than previously reported [10].

The sample 1 GC–MS chromatogram is shown in Fig. 1. GC–MS identified ar-turmerone, α- and β-turmerone, and components with molecular mass= 202 and 204, in both cyclohexane extracts. Sample 2 contained proportionally more ar-turmerone than sample 1, but lacked the fatty acids.

In Table 2, methane CI GC–MS was used to confirm the EI derived molecular masses of the underivatised structures in the cyclohexane extract of sample No. 1. CI is a softer ionisation technique compared with EI and generates in the molecular ion region principally $[M+H]^+$ and trace diagnostic $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$ ions. CI was especially necessary for the M_r estimation of the unidentified components with I_{exp} values of 1559.58 and 1744.01, which both have no molecular ion but an intense EI ion at m/z 119, indicative of the isopropylbenzene cation. A typical identification was as follows: for the structure with molecular mass (M_r)=280 and I_{exp}=2106.20, library matching with the purity method gave no reasonable formula. However methylation and then library fitting gave

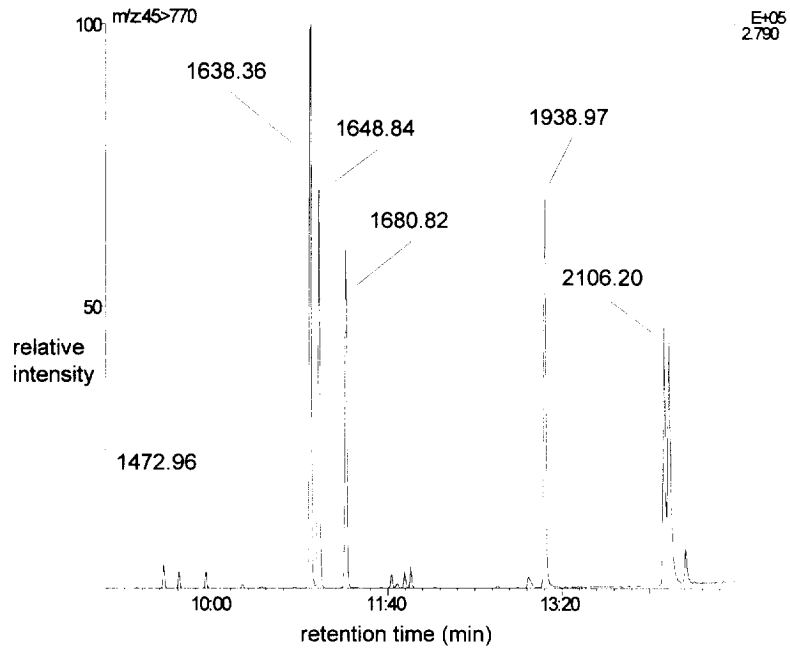

Fig. 1. GC–MS (EI mode) chromatogram of sample No. 1 cyclohexane extract. The GC peak labels refer to I_{exp} values (see Table 2). The reconstructed ion current that is represented, is from 45 to 770 u.

four good matches, all methyl esters of octadecadienoic acid with possible double bond positions at 7:10, 9:12, 11:14 and 8:11. Trimethylsilylation and library matching (purity method) gave only one good fit i.e., the trimethylsilyl (TMS) ester of *cis,cis*-9,12-octadienoic acid (Table 3), consistent with one of the methyl ester fits. Finally *cis,cis*-9,12-octadienoic acid has I_{Sad}=2105.07, very close to the I_{exp} value.

A further interesting possibility exists when I_{Sad} values are unavailable. The calibration methods developed in [1,2] can generate two different types of retention index: firstly experimental "pseudo-Sadtler" indices i.e., I_{exp} values with narrow search windows and secondly corrected "pseudo-Sadtler" indices with generally wider windows derived from the application of experimental offsets to literature values. Methyl octadecanoic acid has no entry in the Sadtler collection [1], but has one in a lower quality compilation [11], in which the indices were calculated using a "simplified extended" Kováts [20] rather than the Van den Dool and Kratz equation [4]. The salvage technique described in [2,3] and as applied here is insensitive to the use of the two different equations. A corrected "pseudo-Sadtler" retention index value of 2094.83 (with a search window of ±24.95) was created from this entry and this is close to the I_{exp} value of 2109.49 reported here.

Structures with I_{exp}=1638.36, 1648.84 and 1680.82 have been previously identified as ar-turmerone, α-turmerone and β-turmerone, respectively [6]. 1,1′-Bicyclohexyl was found by comparison with a control to originate from the cyclohexane extraction solvent. Tables 3 and 4 show only structures that displayed a derivatisation-induced change in molecular mass and retention index compared to those in Table 2.

4. Conclusions

The GC–MS results on the underivatised *Curcuma longa* L. extracts gave an initial identification (especially a stoichiometric formula) for the majority of GC peaks. Methylation and trimethylsilylation offered then a second and third round of library fits. In the specific case of the fatty acids here, a weak point of the library matching was in the distinguishing of double bond positions and geometry, although the TMS esters gave clearly more information on this than the methyl esters and underivatised fatty acids. This weakness was compensated for by the use of GC retention indices which are relatively powerful in labelling separate structures on the basis of double bond positions and geometry. The eight fatty acids found in *Curcuma longa* L. were, in order of elution, tetradecanoic acid, *cis*-9-hexadecenoic acid, hexadecanoic acid, *cis,cis*-9,12-octadecadienoic acid, *cis*- and *trans*-9-octadecenoic acid, octadecanoic and eicosanoic acid.

In conclusion, retention indices when measured under "pseudo-Sadtler" GC conditions offer a powerful and much under-valued supplement to the GC–MS identification of unknown organic structures.

Acknowledgments

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