Use of ^{13}C -NMR as complementary identification tool in essential oil analysis

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Abstract. A program using ^{13}C -NMR data was developed to improve GC/MS identifications of chemical constituents in essential oils. Compiled data uses 2290 and 2370 chemical shifts of 229 monoterpenes and 158 sesquiterpenes, respectively, stored into two reference libraries. Each carbon atom was codified using rules that correlates its chemical shift with structural features of the carbon hybridization and vicinity.

Keywords: *Alpinia speciosa*, Zingiberaceae, ^{13}C -NMR spectroscopy, monoterpenes, sesquiterpenes, essential oils

1. Introduction

Isolation of individual components from essential oils for NMR analysis, is somewhat difficult, time consuming and is not practical for complex oils. On the other hand, NMR essential oil analysis as a mixture is problematic because the chance of superimposing chemical shifts increases with the mixture complexity. Furthermore, many of the absorptions belonging to compounds present in low yields are not detectable due to background superposition. This explains why ^{13}C -NMR spectroscopy is largely used in identification and structural determination of pure compounds, but rarely applied when the analyte is a complex mixture like an essential oil [1–3]. It is also known that volatile oils constituents analysis only by mass spectroscopy, may present some problems [4], making desirable the use of other spectroscopic data to complement it. In this study is used GC/MS analysis of *Alpinia speciosa* essential oil presented in Table 3 with the help of an experimental procedure based on data of the ^{13}C -NMR overall spectrum of the oil presented in Fig. 2. The ^{13}C -NMR procedure compares each carbon chemical shift hybridization with the same parameters stored into libraries of mono and sesquiterpenes.

2. Experimental

2.1. Plant material

Alpinia speciosa leaves were collected in the Medicinal Plants Garden, Laboratory of Natural Products Universidade Federal do Ceará in Fortaleza-Ceará, Brazil. Voucher is deposited in “Herbário

Prisco Bezerra, Universidade Federal do Ceará" under number 17624. Essential oil was first analysed using a HP-5971 GC/MS instrument equipped with a 25 m fused silica capillary SE-54 column (0.25 mm id), using helium as carrier gas (1 ml/min) and temperature programmed initially from 50 to 180°C at 4°C/min and then from 180 to 250°C at 20°C/min. ^{13}C -NMR spectrum of the oil was obtained on a Bruker A200 instrument (200 MHz, 12 hours of acquisition).

2.2. Reference libraries

Two references libraries were compiled using 2290 encoded carbon atoms from 229 monoterpenes and 2370 encoded carbon atoms from 158 sesquiterpenes.

2.3. ^{13}C -NMR experimental data

In the Proton-Noise-Decoupled (PND) ^{13}C -NMR spectrum, recorded for *Alpinia speciosa* essential oil were detected 118 chemical shifts shown in Table 5. Chemical shifts hybridization patterns were deduced from Distortionless Enhancement by Proton Transfer (DEPT), at angles $\phi = 135^\circ$ and $\phi = 90^\circ$.

2.4. Software

Computer programs to match experimental ^{13}C -NMR oil chemical shifts and carbon hybridization against those existing in the reference libraries, were written using FoxPro for Windows language.

All carbon atoms in the reference libraries were stored using a general code represented by **C.αααα.rrr.ββ.γγ** using letters separated by dots into five sub-codes as described below: The sub-code **C** identifies the substitution carbon atom pattern according to the rules shown in Table 1.

The sub-code **αααα** represents the four substituents directly bonded to the carbon atom **C**, fulfilled using the **C** and heteroatoms sub-codes defined in Table 1 completing the four digits with zero if necessary.

The sub-code **rrr** was created to designate rings that the carbon atom participates. The first two digits indicate the ring size in the increasing order of the carbon number. The last digit in this sub-code defines the stereochemistry of ring junction using capital letters **Z** for cis and **E** for trans, respectively.

The sub-code **ββ** represents the two most important β effects induced by substituents linked to the carbon being analysed. When the hybridization of the selected **C** carbon is sp^2 , the two beta

Table 1

C sub-code	Carbon type		Heteroatom sub-code	
1	sp^3 primary carbon	RCH_3	A	OH
2	sp^3 secondary carbon	R_2CH_2	B	OR
3	sp^3 tertiary carbon	R_3CH	C	$\text{C}=\text{O}$
4	sp^3 quaternary carbon	R_4C		
5	sp^2 carbon methylenic	$=\text{CH}_2$		
6	sp^2 carbon methynic	$=\text{CHR}$		
7	sp^2 quaternary carbon	$=\text{CR}_2$		
8	sp carbon methynic	$\equiv\text{CH}$		
9	sp carbon without protons	$\equiv\text{CR}$		

Table 2

Carbon number	Carbon atoms codes
1	3.4320.49E.72.40
2	2.3200.900.44.B0
3	2.4200.900.B1.40
4	4.B321.900.22.30
5	3.B420.900.21.70
6	2.3200.900.B7.1b
7	2.7200.900.35.B0
8	7.5320.900.32.40
9	3.7320.49E.54.1a
10	2.4300.400.73.50
11	4.3211.400.32.70
12	1.4000.000.33.30
13	1.4000.000.33.30
14	5.7000.000.32.30
15	1.4000.000.B3.20

Fig. 1. Caryophyllene oxide.

substituents effects to be considered are two atoms apart from this carbon as shown in the example below [9]:

The first digit in sub-code $\gamma\gamma$ represents the most important gamma substituent effect of carbon **C** and the second digit is reserved for the alpha (= *a*) or beta (= *b*) steric effect on this carbon.

Another example for the sesquiterpene caryophyllene oxide illustrates how the codes were constructed and stored in the library (see Table 2).

3. Results and discussion

3.1. Compound identification

The chemical composition of the *A. speciosa* essential was obtained by GC/MS library search best retrievals compatible with retention indices expressed as Kovats indices. Confirmations were made by visual comparison of each mass spectrum present in the oil with the corresponding mass spectrum in Refs [5–8] and by the ^{13}C -NMR procedure described here.

Table 3
 ^{13}C -NMR absorptions detected for p-cymene in the essential oil of *Alpinia speciosa*

C-atom	Code in the monoterpene library	Substitution pattern	Library stored chemical shifts	Experimental observed chemical shifts	Δ^*
1	7.6610.600.66.70	s	135.0	135.1	0.1
2	6.7600.600.71.60	d	129.1	128.2	0.9
3	6.7600.600.77.10	d	126.3	125.9	0.4
4	7.6630.600.11.70	s	145.3	144.8	0.5
5	6.7600.600.73.10	d	126.3	125.9	0.4
6	6.7600.600.71.60	d	129.1	128.2	0.9
7	3.7110.060.66.60	d	33.9	33.5	0.4
8	1.3000.000.70.60	q	24.2	24.7	0.5
9	1.3000.000.70.00	q	24.2	24.7	0.4
10	1.7000.000.66.60	q	21.0	21.0	0.0

* Absolute differences between observed and stored chemical shifts.

Fig. 2. p-cymene.

3.2. ^{13}C -NMR matching program

^{13}C -NMR matching program applied to *A. speciosa* essential oil of were done when GC/MS identifications were already known. To illustrate how the program retrieves the library standards during comparisons, the chief constituent p-cymene and one of the minor constituents, caryophyllene oxide present in the oil were selected as example. As final result the program was able to detect all ^{13}C -NMR absorptions of p-cymene and 14 from the 15 chemical shifts of caryophyllene oxide as illustrated in Tables 3 and 4.

3.3. Application of ^{13}C -NMR matches to essential oil of *A. speciosa*

As stated before, compound identifications were first made by mass spectra library search by retrieving the best unknown structurally similar to the reference in the library, using GC/MS experimental data expressed in percent of agreement (0.0 = null match, 1.0 = full match), as shown in Table 5, column MIG.

3.4. ^{13}C -NMR positive agreement

A positive ^{13}C -NMR agreement results when the chemical shifts compared for a pair of carbon atoms in the library and in the oil have exactly the same hybridisation pattern and their absolute chemical shift difference is no more than 1.5 ppm. A compound full agreement occurs when all

Table 4
 ^{13}C -NMR absorptions detected for caryophyllene oxide in the essential oil of *Alpinia speciosa*

C-atom	Code in the sesquiterpene library	Substitution pattern	Library chemical shifts	Experimental chemical shifts	Δ^*
1	3.4320.49Z.72.40	d	50.8	50.0	0.8
2	2.3200.900.44.B0	t	30.2	30.3	0.1
3	2.4200.900.B1.30	t	39.1	38.4	0.7
4	4.B321.39Z.22.30	s	59.9	58.9	1.0
5	3.B420.39Z.22.10	d	63.7	62.7	1.0
6	2.3200.900.B7.50	t	30.2	30.3	0.1
7	2.7200.900.53.B0	t	29.9	29.4	0.5
8	7.5320.900.32.40	s	151.8	150.7	0.9
9	3.7320.49Z.54.10	d	48.7	48.0	0.7
10	2.4300.400.73.50	t	39.8	39.0	0.8
11	4.3211.400.23.70	s	39.1	—	—
12	1.4000.000.33.2b	q	29.8	29.8	0.0
13	1.4000.000.33.2a	q	21.7	20.7	1.0
14	5.7000.000.32.40	t	112.8	112.0	0.8
15	1.4000.000.33.20	q	17.0	16.8	0.2

* Absolute differences between observed and stored chemical shifts.

Table 5
 Chemical composition of the essential oil of *Alpinia speciosa* with the aid of ^{13}C -NMR matches

Compound	KI	Yield %	MIG	Match 1	Match 2
p-cymene	1011	33.1	0.9	1.0	1.0
1,8-cineole	1017	18.9	0.9	1.0	1.0
(E)-terpinen-4-ol	1173	18.8	1.0	1.0	1.0
Sabinene	963	8.2	1.0	1.0	1.0
(Z)-caryophyllene oxide	1556	2.4	0.9	0.9	0.8
α -thujene	921	2.1	1.0	0.9	0.6
Linalool	1084	1.8	0.9	0.8	0.7
β -pinene	966	1.6	1.0	0.8	0.5
α -terpineol	1176	1.1	0.9	0.9	0.4
α -pinene	927	0.9	1.0	0.8	0.4
(Z)-sabinene hydrate	1101	0.9	0.8	0.7	0.4
(E)-sabinene hydrate	1128	0.8	0.8	0.6	0.3
p-cymene-9-ol	1181	0.7	0.8	0.9	0.7
β -terpineol	1051	0.7	0.8	0.9	0.3
Piperitol	1196	0.3	0.8	0.7	0.2
(Z)-nerolidol	1527	0.3	1.0	0.9	0.3
β -caryophyllene	1410	0.2	0.9	0.7	0.2
Bornyl acetate	1269	0.2	0.9	0.7	0.1
Myrcene	978	0.2	1.0	0.6	0.0

data being compared are retrieved from the library. In this case the comparison is expressed by the unity (1.00) otherwise it is expressed by the number of positive agreements divided by the number of chemical shifts existing in compound in the ^{13}C -NMR library.

3.5. ^{13}C -NMR matches

The program make two runs using the positive agreement defined above. In the first run (match 1) ^{13}C -NMR library reference compounds corresponding to those identified by GC/MS, independently

Illustration how the ^{13}C -NMR program subtracts chemical shifts in the spectrum of the oil and identifies the ten major constituents

[illegible]

Table 6
(Continued)

PND	HYB	p-cym	1,8-cin	ter-4-ol	sab	car-ox	α -thuj	lin	β -pin	α -ter-ol	α -pin	
32.2	d	32.5	*	—	—	—	—	—	—	—	—	1,8-cin
31.9	d	31.9	31.9	31.9	31.9	31.9	*	—	—	—	—	α -thu
31.5	d	31.5	31.5	31.5	*	—	—	—	—	—	—	sab
30.6	d	30.6	30.6	30.6	30.6	30.6	30.6	30.6	30.6	30.6	30.6	sht
30.3	d	30.3	30.3	30.3	30.3	30.3	30.3	30.3	30.3	30.3	30.3	shc
30.1	d	30.1	30.1	30.1	30.1	30.1	*	—	—	—	—	α -thu
29.2	d	29.2	29.2	29.2	*	—	—	—	—	—	—	sab
28.1	d	28.1	28.1	28.1	28.1	28.1	28.1	28.1	28.1	28.1	28.1	shc
26.8	d	26.8	26.8	26.8	26.8	26.8	26.8	26.8	26.8	26.8	26.8	pip
26.2	d	26.2	26.2	26.2	26.2	26.2	26.2	26.2	26.2	26.2	26.2	unk
18.7	d	18.7	18.7	18.7	18.7	18.7	18.7	18.7	18.7	18.7	18.7	unk
112.0	t	112.0	112.0	112.0	112.0	112.0	112.0	112.0	112.0	112.0	112.0	myr
110.6	t	110.6	110.6	110.6	110.6	110.6	110.6	*	—	—	—	lin
107.8	t	107.8	107.8	107.8	107.8	107.8	107.8	107.8	107.8	107.8	107.8	β -ter-ol
105.5	t	105.5	105.5	105.5	105.5	105.5	105.5	105.5	*	—	—	β -pin
101.2	t	101.2	101.2	101.2	*	—	—	—	—	—	—	sab
44.7	t	44.7	44.7	44.7	44.7	44.7	44.7	44.7	44.7	44.7	44.7	unk
41.6	t	41.6	41.6	41.6	41.6	41.6	41.6	*	—	—	—	lin
39.0	t	39.0	39.0	39.0	39.0	39.0	39.0	39.0	39.0	39.0	39.0	β -ter-ol
38.4	t	38.4	38.4	38.4	38.4	38.4	38.4	38.4	38.4	38.4	38.4	β -ter-ol
36.6	t	36.6	36.6	36.6	36.6	*	—	—	—	—	—	car-ox
35.8	t	35.8	35.8	35.8	35.8	35.8	*	—	—	—	—	α -thu
35.1	t	35.1	35.1	35.1	35.1	35.1	35.1	35.1	35.1	35.1	35.1	ac-b
33.6	t	33.6	33.6	*	—	—	—	—	—	—	—	ter-4-ol
32.4	t	32.4	*	—	—	—	—	—	—	—	—	1,8-cin
30.8	t	30.8	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4	myr
30.5	t	30.5	30.5	30.5	30.5	30.5	30.5	30.5	30.5	30.5	*	α -pin
30.1	t	30.1	30.1	30.1	30.1	30.1	30.1	30.1	30.1	30.1	*	α -pin
29.8	t	29.8	29.8	*	—	—	—	—	—	—	—	ter-4-ol
29.5	t	29.5	*	—	—	—	—	—	—	—	—	1,8-cin
28.8	t	28.8	28.8	28.8	28.8	28.8	28.8	28.8	28.8	28.8	28.8	β -car
28.6	t	28.6	28.6	28.6	28.6	28.6	28.6	28.6	28.6	28.6	28.6	β -car
28.4	t	28.4	28.4	28.4	28.4	28.4	28.4	28.4	28.4	28.4	28.4	pip
28.1	t	28.1	28.1	28.1	*	—	—	—	—	—	—	sab
27.2	t	27.2	27.2	27.2	27.2	27.2	27.2	27.2	27.2	*	—	α -ter-ol
26.6	t	26.6	26.6	*	—	—	—	—	—	—	—	ter-4-ol
26.5	t	26.5	26.5	26.5	26.5	26.5	26.5	26.5	26.5	26.5	26.5	β -ter-ol
26.3	t	26.3	26.3	26.3	26.3	26.3	26.3	26.3	26.3	26.3	26.3	β -ter-ol
26.2	t	26.2	26.2	26.2	26.2	26.2	26.2	26.2	26.2	26.2	26.2	myr
26.0	t	26.0	26.0	26.0	*	—	—	—	—	—	—	sab
25.6	t	25.6	25.6	25.6	25.6	25.6	25.6	25.6	*	—	—	β -pin
24.6	t	24.6	*	—	—	—	—	—	—*	—	—	1,8-cin
23.3	t	23.3	*	—	—	—	—	—	—	—	—	1,8-cin
22.8	t	22.8	22.8	22.8	22.8	22.8	22.8	22.8	*	—	—	β -pin
22.1	t	22.1	22.1	22.1	22.1	22.1	22.1	22.1	*	—	—	β -pin
20.7	t	20.7	20.7	20.7	20.7	20.7	*	—	—	—	—	α -thu
15.2	t	15.2	15.2	15.2	*	—	—	—	—	—	—	sab
29.2	q	29.2	*	—	—	—	—	—	—	—	—	1,8-cin
29.0	q	29.0	*	—	—	—	—	—	—	—	—	1,8-cin
27.5	q	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	myr
25.3	q	*	—	—	—	—	—	—	—	—	—	p-cym
24.9	q	24.9	24.9	24.9	24.9	24.9	24.9	24.9	*	—	—	β -pin
24.7	q	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	*	α -pin
22.5	q	22.5	22.5	22.5	22.5	22.5	22.5	22.5	*	—	—	β -pin
21.0	q	21.0	21.0	21.0	*	—	—	—	—	—	—	sab

Table 6
(Continued)

PND	HYB	p-cym	1,8-cin	ter-4-ol	sab	car- ox	α -thuj	lin	β -pin	α -ter-ol	α -pin	
20.5	q	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	*	α -pin
20.1	q	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	*	α -pin
19.3	q	19.3	19.3	19.3	19.3	19.3	*	–	–	–	–	α -thu
18.7	q	18.7	18.7	18.7	18.7	18.7	*	–	–	–	–	α -thu
17.9	q	17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9	9-ol
17.5	q	17.5	17.5	*	–	*	–	–	–	–	–	ter-4-ol
17.2	q	17.2	17.2	17.2	17.2	17.2	17.2	*	–	–	–	lin
16.8	q	16.8	16.8	16.8	16.8	16.8	16.8	16.8	16.8	16.8	16.8	myr
15.5	q	15.5	15.5	*	–	–	*	–	–	–	–	ter-4-ol
15.2	q	15.2	15.2	15.2	15.2	15.2	*	–	–	–	–	α -thu
10.8	q	10.8	10.8	10.8	10.8	11.8	10.8	10.8	10.8	10.8	10.8	ac-b

* Positive agreement; – chemical shift subtracted from the oil's PND.

Abbreviations: PND = proton noise decoupled; HYB = carbon atom hybridization; p-cym = para-cymene;

1,8-cin = 1,8-cineole; ter-4-ol = terpinen-4-ol; sab = sabinene; car-ox = trans-caryophyllene oxide; α -thu = α -thujene;

lin = linalool; β -pin = β -pinene; α -ter-ol = α -terpineol; α -pin = α -pinene; ner = nerolidol; β -ter-ol = β -terpineol;

unk = unknown; 9-ol = p-cymen-9-ol; myr = myrcene; shc = sabinene hydrate cis; sht = sabinene hydrate trans;

β -car = beta caryophyllene; pip = piperitol; ac-b = bornyl acetate.

of their yields in the oil, are matched against the overall spectrum of the oil. This inform how many absorptions of the compound being compared are present in the mixture. In the second run (match 2) comparisons follow decreasing order of the yields obtained by the GC/MS experiments shown in Table 5. When a positive agreement is found, the chemical shift just being compared is subtracted from the PND of the oil to avoid its use in a further comparison. Due to this approach, values in match 2 are represented by lower figures than those in match 1, but have the advantage of informing how many chemical shifts in the spectrum of the oil belong exclusively to the compound being matched (see Table 5, match 1 and match 2 columns).

Table 6 shows in the PND column, the 118 chemical shifts found in the ^{13}C -NMR spectrum of the oil. When a chemical shift is present in all columns mean that this absorption does not belong to any of the ten major compounds listed on the head of Table 6. An asterisk in a given column means that the corresponding chemical shift belongs to the compound in the head of the column. An asterisk is always followed by minus (–) signal to indicate that chemical shift was subtracted from the PND, to avoid their use in another comparison. The last column in Table 6 shows abbreviations of all compounds identified in the oil as well as those not identified represented by the abbreviation unk.

4. Conclusions

From Table 5, considering this particular essential oil analysis, some conclusions may be anticipated:

1. Compounds with high GC/MS MIG values are the best candidates for confirmation using match 1 and match 2.
2. Compounds with high values for both MIG and match 1 may be considered unequivocally identified, i.e., sabinene, p-cymene, 1,8-cineole, terpinen-4-ol.
3. Figures with high MIG value, present in the oil in low yield – α -thujene, α -pinene, β -pinene, myrcene, linalool, α -terpineol – have their identifications confirmed by high value of match 1 (>0.70).

4. Even low values in match 2, but with significative figures in match 1 for compounds in low yield, are of great importance for identification purposes because identify in the mixture data belonging exclusively to the compound being compared.

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