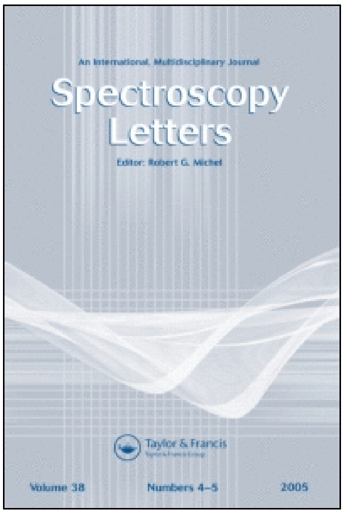

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GC(Retention Indices), GC-MS, and ^{13}C NMR of Two Citral-Rich *Cymbopogon* Leaf Oils: *C. Flexuosus* and *C. tortilis*

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GC(Retention Indices), GC-MS, and ^{13}C NMR of Two Citral-Rich *Cymbopogon* Leaf Oils: *C. Flexuosus* and *C. tortilis*

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ABSTRACT Leaf oils of *Cymbopogon flexuosus* and *Cymbopogon tortilis* were submitted to combined analysis by CG(retention indices), Gas Chromatography Mass Spectrometry (GC-MS), and ^{13}C Nuclear Magnetic Resonance (NMR). The composition was dominated by geranial (39.2% and 32.0%) and neral (24.1% and 19.1%). *Cis*- and *trans*-7-hydroxy-3,7-dimethyl-3,6-oxyoctanal (synonym: *cis*- and *trans*-tetrahydro-5-(1-hydroxy-1-methylethyl)-2-methyl-2-furanacetaldehyde) were identified by ^{13}C NMR by comparison with literature data.

KEYWORDS *cis*- and *trans*-7-hydroxy-3,7-dimethyl-3,6-oxyoctanal, combined analysis, *cymbopogon*, ^{13}C NMR, essential oil composition, GC-MS

INTRODUCTION

Essential oils are complex mixtures of several tens of components, mainly mono- and sesquiterpenes, more scarcely diterpenes, phenylpropanoids, or acyclic nonterpenic compounds. Their analysis is carried out in a conventional way by matching against computerized mass spectral libraries and comparison of the retention indices (RI) on two columns of different polarities with those of authentic samples [GC(RI) and GC-MS].[1] However, due to the fact that sesquiterpenes and diterpenes sometimes exhibit insufficiently differentiated mass spectra, more sophisticated techniques are needed for the analysis of complex essential oils, such as GC-MS-MS,[2] Gas Chromatography Mass Spectrometry Fourier Transform Infra Red (GC-MS-FTIR),[3] or High Performance Liquid Chromatography Gas Chromatography Mass Spectrometry (HPLC-GC-MS).[4]

Despite the improvement of "on-line" analytical techniques, the misidentification of some compounds, especially sesquiterpenes, still occurs.[5] Consequently, in order to carry out a detailed analysis, a two-step procedure has been developed: a small quantity of a substance is separated by a chromatographic technique and then identified by comparison of its spectral data, including ^{1}H Nuclear Magnetic Resonance (NMR) and sometimes ^{13}C NMR, with those of reference compounds.[6] This "off-line" sequence is obviously more accurate but is very time consuming.

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A third way to identify the individual components of an essential oil is using ^{13}C NMR spectroscopy without previous separation. In their pioneering work, Formácek and Kubeczka[7] used ^{13}C NMR to confirm the identification of various oil components previously suggested by GC. An analytical method was developed, based on the computerized analysis of the ^{13}C NMR spectrum, which allows the direct identification of the (major) components.[8–10]

Among aromatic and medicinal plants, several *Cymbopogon* species (Poaceae), mostly distributed in Asia and in Africa, produce essential oils that are of commercial interest.[11] Citronella oil is produced from *Cymbopogon nardus* (L.) Rendle (Ceylon citronella oil), or by *C. winterianus* (Java citronella oil), and it exhibits diverse chemical compositions dominated by geraniol, citronellal, and citronellol.[11] Palmarosa oil, obtained from *C. martinii* (Roxb.), is essentially constituted by geraniol. Lemongrass oil is obtained from *C. citratus* (DC. ex Nees) (West Indian or Guatemala lemongrass) or from *C. flexuosus* (Nees ex Steud.) (Indian lemongrass oil), and it is a citral-rich oil.[11] Other *Cymbopogon* oils have been punctually investigated. For instance, only one study reported the composition of *C. tortilis* leaf oil from China, methyl eugenol being by far the major component.[12]

In continuation of the on-going studies relative to the characterization of aromatic plants through the composition of their essential oil investigated, this article reports here on the chemical composition of two leaf oils from *Cymbopogon flexuosus* and *Cymbopogon tortilis* of Vietnamese origin. Analysis was achieved by combination of chromatographic and spectroscopic techniques—column chromatography (CC), GC(RI), GC-MS, and ^{13}C NMR.

MATERIALS AND METHODS

Plant Material, Essential Oil Isolation, and Fractionation

Aerial parts (leaves) of *Cymbopogon flexuosus* and *C. tortilis* were collected from plants cultivated near Hanoi (Vietnam). A voucher specimen has been deposited at the Herbarium of the Institute of Medicinal Materials (Hanoi). The essential oils were obtained by hydrodistillation using a Clevenger type apparatus.

Cymbopogon flexuosus essential oil (1.0 g) was separated by flash chromatography into six fractions (silica gel, 200–500 µg, elution with a mixture of solvents of increasing polarity: pentane/diethyl oxide: 100/0 to 0/100).

GC Analysis

GC analyses were carried out using a Perkin-Elmer Autosystem GC apparatus (Courtaboeuf, France) equipped with two flame ionization detectors and fused-silica capillary columns (50 m × 0.22 mm, film thickness 0.25 µm), BP-1 (polydimethylsiloxane), and BP-20 (polyethylene glycol). Oven temperature was programmed from 60°C to 220°C at 2°C/min and then held isothermal (20 min); detector temperature, 250°C; injector temperature, 250°C (injection mode, split, 1/60); carrier gas, helium (0.8 mL/min). Injected volume was 0.5 µL of a solution of 50 µL of the mixture (oil or fraction of chromatography) diluted in 350 µL of CCl_4. The relative proportions of the oil constituents were expressed as percentages obtained by peak area normalization, without using correcting factors.

GC-MS Analysis

The samples were analyzed with a Perkin-Elmer TurboMass detector (quadrupole), directly coupled to a Perkin-Elmer Autosystem XL, equipped with a fused-silica capillary column (60 m × 0.22 mm, film thickness 0.25 µm), Rtx-1 (polydimethylsiloxane). Carrier gas, helium at 1 mL/min; split, 1/80; injection volume, 0.2 µL; injector temperature, 250°C; oven temperature programmed from 60°C to 230°C at 2°C/min and then held isothermal (45 min); ion source temperature, 150°C; energy ionization, 70 eV; electron ionization mass spectra were acquired over the mass range 35–350 Da.

^{13}C NMR Analysis

^{13}C NMR spectra of the two oils and the fractions of chromatography were recorded on a Bruker 400 Avance Fourier Transform spectrometer (Wissenburg, France) operating at 100.13 MHz for ^{13}C, equipped with a 5-mm probe, in deuteriochloroform, with all shifts referred to internal tetramethylsilane (TMS). ^{13}C NMR spectra were recorded with the following parameters: pulse width = 4 µs (flip angle 45°); acquisition time = 2.7 s for 128-K data table with a spectral width of 25,000 Hz (250 ppm); Composite Phase Decoupling (CPD) mode decoupling; digital resolution = 0.183 Hz/pt. The number of accumulated scans was 5,000 for each sample (around 60 mg of the oil or fraction of chromatography in 0.5 mL $CDCl_3$).

Identification of Individual Components

Identification of the components was based (1) on comparison of their GC(RI) on polar and apolar columns, determined relative to the retention times of a series of *n*-alkanes with linear interpolation with those of authentic compounds; (2) on computer matching against commercial mass spectral libraries[13–15] and by comparison of spectra with literature data[16–18]; and (3) by ^{13}C NMR spectroscopy, following the methodology developed and computerized in our laboratories, using a home-made software and spectral data library.[8–10] In both oil samples, two compounds present at appreciable contents (A: RI = 1252 and 1940 and B: RI = 1257

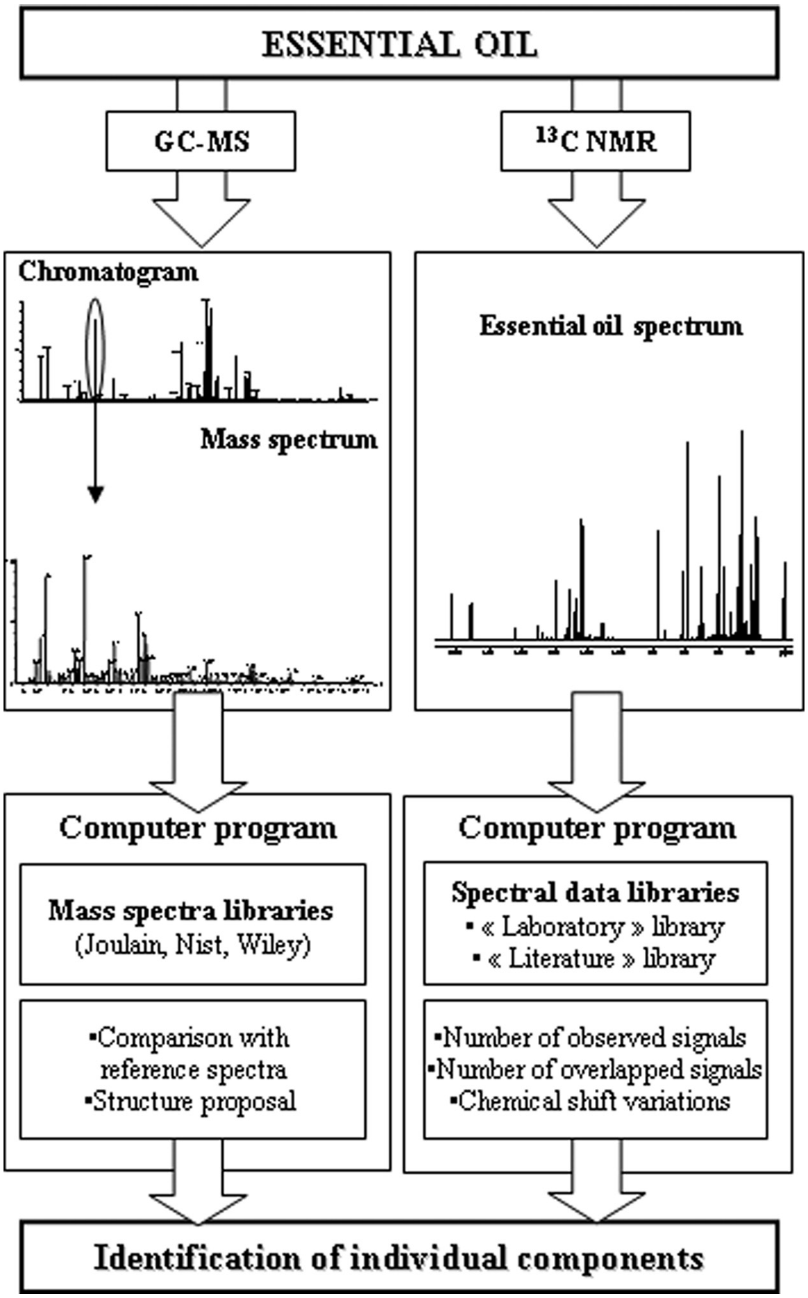

FIGURE 1 Identification of essential oil components by combined methods: GC-MS and ^{13}C NMR.

TABLE 1 **Chemical Composition of Essential Oils of *Cymbopogon flexuosus* and *Cymbopogon tortilis***

Compound	RI$_a$	RI$_p$	*C. flex.*	*C. tort.*	Identification mode
Heptanal	883	1168	—	0.1	RI, MS
Camphene	944	1066	0.5	—	RI, MS
6-Methylhept-5-en-2-one	961	1343	0.9	1.0	RI, MS, ^{13}C NMR
Octanal	980	1294	0.1	0.1	RI, MS
p-Cymene	1013	1276	0.1	0.7	RI, MS, ^{13}C NMR
Limonene	1020	1199	—	0.2	RI, MS
cis-Linalool oxide THF	1058	1446	—	0.5	RI, MS, ^{13}C NMR
trans-Linalool oxide THF	1073	1475	—	0.5	RI, MS, ^{13}C NMR
Linalol	1082	1544	0.1	2.6	RI, MS, ^{13}C NMR
Citronellal	1134	1486	—	0.4	RI, MS
Borneol	1142	1703	0.2	—	RI, MS,*^{13}C NMR*
Pinocampheol	1166	1712	—	0.3	RI, MS
α-Terpineol	1169	1712	0.5	—	RI, MS, ^{13}C NMR
Decanal	1186	1503	0.2	—	RI, MS, *^{13}C NMR*
2,3-Epoxyneral	1198	#	—	0.3	RI, MS
2,3-Epoxygeranial	1208	#	—	0.4	RI, MS
Nerol	1213	1804	0.1	—	RI, MS, *^{13}C NMR*
Neral	1220	1691	24.1	19.1	RI, MS, ^{13}C NMR
Piperitone	1229	1722	0.2	0.2	RI, MS
Geraniol	1240	1850	0.3	2.1	RI, MS, ^{13}C NMR
Geranial	1249	1743	39.2	32.0	RI, MS, ^{13}C NMR
trans-7-hydroxy-3,7-dimethyl-3,6-oxyoctanal	1252	1940	4.5	2.5	^{13}C NMR
cis-7-hydroxy-3,7-dimethyl-3,6-oxyoctanal	1257	1957	4.4	1.4	^{13}C NMR
Neryl formate	1263	1666	—	0.5	RI, MS, ^{13}C NMR
Lavandulyl acetate	1275	1586	—	0.3	RI, MS
Isoascaridole	1283	1853	—	0.7	RI, MS, ^{13}C NMR
6,7-Epoxyneral	1302	1956	—	1.9	RI, MS, ^{13}C NMR
Methyl geraniate	1305	1678	0.7	—	RI, MS, ^{13}C NMR
6,7-Epoxygeranial	1337	2041	—	5.0	RI, MS, ^{13}C NMR
Neryl acetate	1341	1734	—	1.6	RI, MS, ^{13}C NMR
Eugenol	1341	2179	0.5	—	RI, MS, ^{13}C NMR
Geranic acid	1343	2356	1.3	1.6	RI, MS, ^{13}C NMR
Geranyl acetate	1363	1761	0.3	0.2	RI, MS, *^{13}C NMR*
β-Elemene	1391	1587	0.3	0.5	RI, MS, ^{13}C NMR
trans-α-Bergamotene	1430	1587	0.1	0.2	RI, MS, *^{13}C NMR*
1,5-*diepi*-Aristolochene	1470	1672	tr	—	RI, MS, *^{13}C NMR*
Tridecan-2-one	1478	1592	—	0.5	RI, MS
(Z,E)-α-Farnesene	1483	1723	—	0.9	RI, MS, ^{13}C NMR
γ-Cadinene	1496	1766	1.2	—	RI, MS, ^{13}C NMR
δ-Cadinene	1507*	1756	0.2	—	RI, MS, *^{13}C NMR*
Cuparene	1507*	1830	0.1	—	RI, MS, *^{13}C NMR*
β-Elemol	1538	2077	0.1	0.5	RI, MS, *^{13}C NMR*
Caryophyllene oxide	1576	1980	1.6	3.2	RI, MS, ^{13}C NMR
Humulene epoxide	1600	2049	—	0.4	RI, MS
α-Cadinol	1644	2229	—	0.3	RI, MS
(2E,6E)-Farnesol	1704	2361	0.1	—	RI, MS, *^{13}C NMR*

Order of elution and percentages are given in the apolar (BP-1) column, except for compounds with an asterisk (*); percentages on the polar column (BP-20); RI$_a$ and RI$_p$: RI measured on apolar (BP-1) and polar (BP-20) columns, respectively.

C. flex.: *Cymbopogon flexuosus, C. tort.*: *Cymbopogon tortilis*.

#: RI not determined.

^{13}C NMR (italic letters): components identified by NMR in various fractions of chromatography.

and 1957) were identified by ^{13}C NMR after partitioning on CC (see "Results" section).

RESULTS

Hydrodistillation of leaves of *Cymbopogon flexuosus* and *C. tortilis* yielded a yellowish essential oil. The two oil samples were analyzed by GC(RI), GC-MS, and ^{13}C NMR. ^{13}C NMR analysis was carried out following a computerized method developed in our laboratories (Fig. 1).[8–10] In that procedure, there is neither separation nor individualization of the components before their identification. The computer program, made up from the Access® software of Microsoft (ISSY–les–Moulineaux, France), compared the chemical shift of each carbon in the experimental spectrum with the spectra of pure compounds compiled in two spectral data libraries. The first contains spectra of mono-, sesqui-, and diterpenes and phenylpropanoids recorded in our laboratories with the same experimental conditions (solvent, concentration, pulse sequence). The reference compounds were commercially available or isolated from essential oils and extracts. The second library was constructed with literature data.

Each compound is then identified, taking into account three parameters directly available from computer program: (1) the number of observed carbons with respect to the number of expected signals, (2) the number of overlapped signals of carbons that possess the same chemical shift, (3) the difference of the chemical shift of each signal in the mixture spectrum and in the reference spectra.

Using this method, up to 39 of compounds in an essential oil[19] were identified, without isolation of the components, with respect to their percentage, which should reach at least 0.3–0.4%. This method appeared extremely useful for the identification of isomers, stereoisomers, or thermolabile compounds.[20,21]

Analysis of the two oil samples by GC(RI), GC-MS, and ^{13}C NMR led to the identification of 46 components (Table 1). They were classical components found in essential oils, and they were identified by GC-MS by computer matching against commercial mass spectral data libraries and by comparison of their RI with those of reference compounds on two columns of different polarity. Twenty-three components present, in one or the other oil samples and at appreciable content, were also identified by ^{13}C NMR following the computerized method developed in our laboratories and summarized above.

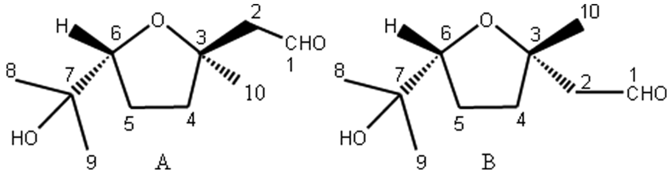

FIGURE 2 Structure of *trans*- and *cis*-7-hydroxy-3,7-dimethyl-3,6-oxyoctanal ((A) and (B)).

However, two compounds, A (RI = 1252 and 1940) and B (RI = 1257 and 1957), accounting for 4.5% and 4.4% in *C. flexuosus* oil and for 2.5% and 1.4% in *C. tortilis* oil, remained unassigned by GC(RI) and GC-MS. Moreover, the two compounds were not present in the ^{13}C NMR data library constructed with spectra recorded in our laboratory. Computer matching of the ^{13}C NMR data against a library constructed with literature data, suggested the occurrence of *cis*- and *trans*-7-hydroxy-3,7-dimethyl-3,6-oxyoctanal (synonym: *cis*- and *trans*-tetrahydro-5-(1-hydroxy-1-methylethyl)-2-methyl-2-furanacetaldehyde) (Fig. 2 and Table 2). The RI belonging to A (RI = 1252 and 1940) and B (RI = 1257 and 1957) were assigned to *trans* and *cis* isomers, respectively, on the basis of the mean intensity of the two sets of signals in the ^{13}C NMR spectrum of *C. tortilis* oil and their percentage on GC (2.5% and 1.4%, respectively).

To ensure the occurrence of both compounds, the oil from *C. flexuosus* was partitioned by flash chromatography on silica gel. Indeed, in the most polar

TABLE 2 ^{13}C NMR and MS Data of *trans*- and *cis*-7-hydroxy-3,7-dimethyl-3,6-oxyoctanal

trans-7-hydroxy-3,7-dimethyl-3,6-oxyoctanal	*cis*-7-hydroxy-3,7-dimethyl-3,6-oxyoctanal
^{13}C NMR (ppm): 202.29 (C1), 86.15(C6), 81.17 (C3), 70.90 (C7), 54.08 (C2), 38.05 (C4), 27.57 (C10), 27.27 (C9), 26.08 (C5), 24.15 (C8)	^{13}C NMR (ppm): 202.49 (C1), 85.71 (C6), 81.28 (C3), 71.15 (C7), 54.18 (C2), 38.16 (C4), 27.38 (C9), 26.84 (C10), 26.22 (C5), 24.45 (C8)
EI MS 70 eV, *m/z* (rel. int.): 127 (23), 85 (21), 84 (48), 83 (19), 81 (50), 71 (19), 59 (100), 55 (18), 43 (71), 41 (27)	EI MS 70 eV, *m/z* (rel. int.): 127 (19), 85 (21), 84 (46), 83 (21), 81 (39), 71 (20), 59 (100), 55 (18), 43 (66), 41 (26)

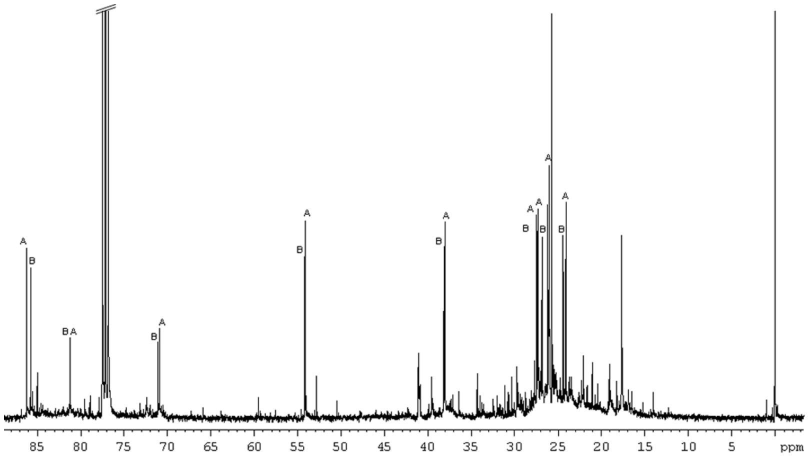

FIGURE 3 **Part of the ^{13}C NMR spectrum of the diethyl oxide fraction from *Cymbopogon flexuosus* essential oil. (A) *trans*-7-hydroxy-3,7-dimethyl-3,6-oxyoctanal and (B) *cis*-7-hydroxy-3,7-dimethyl-3,6-oxyoctanal.**

fraction, eluted with diethyl oxide, both compounds accounted for 21.5% and 21.3%, respectively. Their NMR data were in agreement with those reported by Yarovaya et al.,[22] who obtained the two oxides by transformation of 6,7-epoxycitral in acidic media. A partial spectrum of the diethyl oxide eluted fraction is reproduced in Fig. 3. The signals of carbons are assigned to A and B. The mass spectra of the two isomers are quite similar (Table 2). They exhibited two abundant fragments at m/z 43 and m/z 59 belonging to the oxygenated substituents of the THF moiety ($^{+\bullet}CH_2CHO$ and $^{+\bullet}C(CH_3)_2OH$), as well as various fragments belonging to a substituted THF skeleton (m/z 127, for instance), which confirmed the identification of these two compounds.

In parallel, analysis of the other fractions of chromatography by ^{13}C NMR confirmed the presence of minor monoterpenes, borneol, nerol, and geranyl acetate, as well as sesquiterpenes, *trans*-α-bergamotene, 1,5-*diepi*-aristolochene, δ-cadinene, cuparene, β-elemol, and (2E, 6E)-farnesol.

Concerning ^{13}C NMR analyses, at least 80% of expected signals of each compound identified by this technique were observed in the spectrum of either the crude oils or of the fractions of chromatography. The number of overlapped signals ranged from 0 to 4 for monoterpenes and from 0 to 6 for sesquiterpenes. This observation means that every compound was identified by at least 50% of its signals, which belonged solely to that molecule.

The composition of the two oil samples from *C. flexuosus* and *C. tortilis* was dominated by geranial (39.2% and 32.0%, respectively) and neral (24.1% and 19.1%, respectively). The leaf oil from *C. flexuosus* afforded certain likeness with that of various samples reported in the literature.[11] The content of citral (63.3%) makes it interesting for commercial use. Otherwise, the citral-rich oil from *C. tortilis* investigated in the present study differed drastically from the methyl eugenol-rich oil from China.[12]

In conclusion, 46 components were identified in these two oil samples from *C. flexuosus* and *C. tortilis*. The identification of most components was ensured by three techniques—GC(RI), GC-MS, and ^{13}C NMR. A combination of CC and ^{13}C NMR analysis of the fractions of chromatography allowed the identification of two monoterpenic oxides, reported for the first time as components of an essential oil or solvent extract.

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