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Determination of sulphurated compounds in *Tagetes patula* cv. *nana* essential oil by gas chromatography with mass spectrometric, Fourier transform infrared and atomic emission spectrometric detection

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ABSTRACT

The determination of the sulphurated compounds in the essential oil of *Tagetes patula* cv. *nana* by gas chromatography (GC)–mass spectrometry with different ionization techniques (electron impact and positive- and negative-ion chemical ionization), GC–Fourier transform infrared spectrometry and GC–atomic emission spectrometry is reported. *Tagetes* essential oils contain a series of acetylenic thiophenes in various amounts; the most abundant have already been described in the literature, whereas the structures of some others, especially trace components, are still unknown. These compounds are known to be repellent to insects. The contribution that these combined techniques can make to the identification and structure elucidation of acetylenic thiophene compounds in the essential oil of *T. patula* cv. *nana* is discussed.

INTRODUCTION

The identification of the components of a complex mixture, such as an essential oil, is generally carried out by combining a separation technique [mainly gas chromatography (GC)] with a structure identification technique, of which the best established are mass spectrometry (MS) and Fourier transform infrared spectrometry (FT-IR).

The coupling between GC and atomic emission spectrometry (AES) has recently been made available commercially for the routine analysis of complex mixtures. The use of microwave-induced helium plasma (MIP)–AES at low pressure for GC detection has been known since 1965 [1,2]. In 1976, Beenakker [3,4] described an atmospheric pressure helium plasma with a new cavity concept which was immediately successful. More recently, new commercial instruments have become available which combine an atmospheric Beenakker-like plasma with a photodiode-array detector as a multi-channel detector [5]. The main feature of MIP–AES is the multi-element selective detection, which permits the calculation of the elemental formulae of the components separated by GC [6–8].

During a study on the composition of the essential oils of some ornamental plants [9], we focused our attention on the flowers of a cultivar of *Tagetes patula*, in particular *Tagetes patula* cv. *nana* Furia. A class of compounds characteristic of these species is the acetylenic thiophenes, a group of minor com-

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ponents which are relatively abundant in the early vegetation stages of the plant. Acetylenic thiophenes constitute an important group of secondary metabolites, found in many species of Asteraceae [10–12]. These compounds are widely studied for their biological activity, in particular as photoinduced insecticides, nematocides, fungicides and bactericides. Extracts of *Tagetes* are used in folk medicine to treat ailments which include conjunctivitis, mumps, sore eyes, coughs and dysentery [13].

This paper describes the contribution that combined techniques (GC–MS with different ionization modes, GC–FT-IR, GC–AES) can make to the identification and structure elucidation of acetylenic thiophenes in complex mixtures such as *T. patula* cv. *nana* essential oils without the need for preliminary isolation.

EXPERIMENTAL

Essential oil samples

T. patula cv. *nana* was identified and supplied by Professor E. Cappelletti (Dipartimento di Biologia, Università di Padova, Padova, Italy). The essential oils were obtained by submitting dried flowers to steam distillation, either in a modified Marcusson apparatus (100 g) [14] or in a modified Marcusson microapparatus, developed in the authors' laboratory (10 g) [15].

Capillary gas chromatographic (cGC) analysis

cGC analyses were carried out on a fused-silica open tubular (FSOT) capillary column (23 m × 0.25 mm I.D., film thickness d_f = 0.3 μm) coated with OV-1, installed in a Carlo Erba Mega 5360 gas chromatograph provided, with both flame ionization (FID) and sulphur-selective detection (SSD) [16]. The operating conditions were as follows: column temperature, increased from 50°C (held for 1 min) to 220°C at 3°C/min; carrier gas, hydrogen; flow-rate, 1.6 ml/min; injection system, split; splitting ratio, 1:30; and detector temperature, 250°C. cGC data were processed by a Hewlett-Packard 3393A integrator.

cGC–MS analysis

cGC–MS analyses were carried out on a Hewlett-Packard Model 5988A GC–MS system. The same column as for cGC analysis was used, with cGC conditions as above except that the carrier gas was helium. Positive-ion (PICI) and negative-ion chemical ionization (NICI) were carried out with methane at an ion source temperature of 100°C and a pressure of 0.7 Torr.

cGC–FT-IR analysis

A Hewlett-Packard Model 5965 xGC–IR system was used. A 1-μl volume of the essential oil diluted 1:100 in hexane was injected. cGC analysis was carried out on the FSOT column and under the chromatographic conditions described above.

The FT-IR conditions were as follows: cGC–FT-IR interface, 100-μl light-pipe (10 cm length × 1.2 mm I.D.); temperature, 240°C; make-up gas, nitrogen; flow-rate, 0.2 ml/min; time resolution (repetition rate), 3 scans/s at 8 cm^{-1} resolution. In most cases five interferograms were added in real time, resulting in an effective time slice of about 2 s. The FT-IR detector was an HgCdTe type of narrow band width (4000–750 cm^{-1}).

cGC–AES analysis

The MIP system was an HP 5921 AES instrument coupled with an HP 5890 gas chromatograph provided with an autosampler (HP 7673A). The same column as for cGC analysis was used, with the cGC conditions as described above except that the carrier gas was helium. AES was used to measure the C:S ratio at the wavelengths C = 193 nm and S = 181 nm. The AES conditions were as follows: cavity temperature, 270°C; spectrometer purge flow, nitrogen at 2 l/min; scavenger gases, oxygen and hydrogen; make-up flow, helium at 60 ml/min; window purge helium at 30 ml/min.

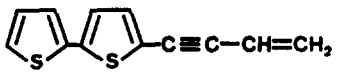
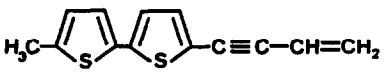
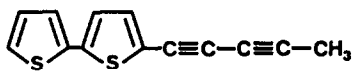
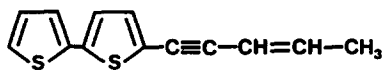
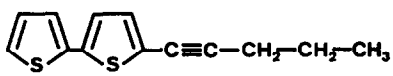
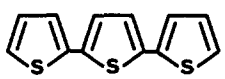
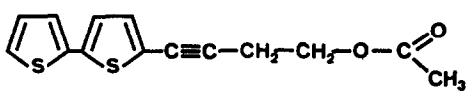
RESULTS AND DISCUSSION

Acetylenic thiophenes constitute a classical example of constituents of a complex mixture that are quantitatively minor but of high biological interest. Recently, Hathcock *et al.* [17] reported the presence of two of them (compounds **6** and **7** in Table I) in the essential oils of *Tagetes minuta*, together with a brief discussion of the techniques for their identification. To the authors' knowledge, compounds belonging to this class have never been reported before in *Tagetes* essential oils.

Table I gives the elemental formulae, structural

TABLE I

ELEMENTAL FORMULAE, STRUCTURAL FORMULAE AND SYSTEMATIC NAMES OF THE ACETYLENIC THIOPHENES IDENTIFIED IN *T. PATULA* CV. *NANA* FLOWERS ESSENTIAL OIL

Compound No.	MW	Elemental formula	Structural formula	Systematic
1	216	$C_{12}H_8S_2$		5-(3-Buten-1-ynyl)-2,2′-bithienyl
2	230	$C_{13}H_{10}S_2$		5′-Methyl-5-(3-buten-1-ynyl)-2,2′-bithienyl
3	228	$C_{13}H_{10}S_2$		5-(1,3-Pentadiynyl)-2,2′-bithienyl
4	230	$C_{13}H_{10}S_1$		5-(3-Penten-1-ynyl)-2,2′-bithienyl
5	232	$C_{13}H_{14}S_2$		5-(1-Pentynyl)-2,2′-bithienyl
6	248	$C_{12}H_8S_2$		α-Terthienyl
7	276	$C_{14}H_{12}S_2O_2$		5-(4-Acetoxy-1-butynyl)-2,2′-bithienyl

formulae and names of the sulphurated compounds identified in the essential oil of *T. patula* cv. *nana* flowers. These compounds must be identified through coupled techniques when their isolation is impossible, as here, where the plant material was scarce and the essential oil content low. The classical methods of detection (FID) and identification [electron impact (EI) MS] can often be insufficient for identification for several reasons, *e.g.*, the complexity of the essential oils in question, the small amounts of the compounds under investigation, the compounds under investigation elute in very complex parts of the gas chromatogram and the compounds under investigation elute very close to quantitatively major components.

Fig. 1 reports the cGC–EI-MS pattern of the *T. patula* essential oil.

The first problem to deal with is the qualitative location of acetylenic thiophenes within the cGC pattern and their quantitative evaluation, if necessary. This was achieved by simultaneous FID–SSD [16]. Fig. 2 shows the cGC–SSD pattern of the sample previously reported, where seven sulphurated compounds are detected.

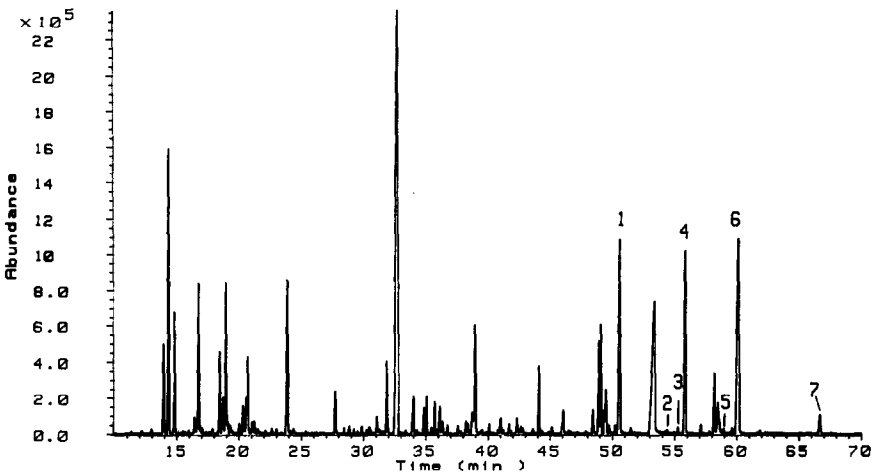


Fig. 1. cGC–EI-MS pattern of the *T.* patula cv. *nana* flower essential oil.

SSD also affords quantitative results, although these are affected by the quadratic detector response related to the excited species $^*S_2$ produced in SSD. Retention indices were used to identify the acetylenic thiophenes in the various combined GC–identification techniques patterns.

Identification of sulphur-containing compounds can also be achieved by GC–MIP-AES. This technique provides not only information that is multi-element sensitive, selective, qualitative and linearly quantitative, but also information about the elemental formulae of the eluting components.

Elemental formulae can be calculated from GC–AES results through the following element-to-carbon (E/C) ratio expression [18]:

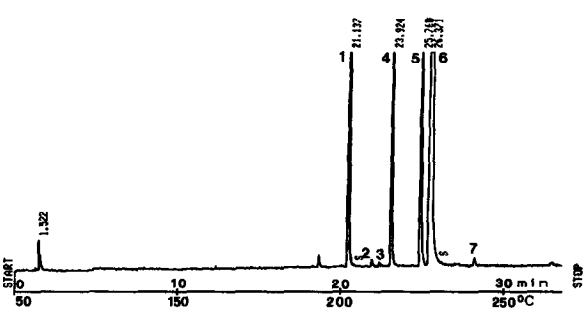


Fig. 2. cGC–SSD pattern of the *T. patula* cv. *nana* flower essential oil.

$$\frac{E}{C} = \frac{E \text{ at. in ref.}}{C \text{ at. in ref.}} \frac{E \text{ (area of uk.)}}{E \text{ (area of ref.)}} \frac{C \text{ (area of ref.)}}{C \text{ (area of uk.)}}$$

where at. = atoms, ref. = reference and uk. = unknown.

As the original molecular structure of the analytes could influence the element responses [7,8,18], one of the acetylenic thiophene components unambiguously identified in the sample (compound **7**) through its IR and EI mass spectra was used as a reference for calculation. One of the most critical points for the determination of the empirical formula is a reliable and unambiguous evaluation of the peak area of each element in the formula: this requires a baseline cGC separation of the peaks under investigation and a careful selection of the integration codes. For complex mixtures such as those under investigation, a cGC column coated with a different stationary phase can often be necessary to obtain a baseline separation of the components in question.

Table II reports the C:S ratios calculated for acetylenic thiophenes detected in the essential oil under investigation. Fig. 3 shows those parts of the S and C AES patterns where the acetylenic thiophenes elute. This example clearly shows the reason why the C:S ratio for compound **1** is in very good agreement with the supposed elemental formula whereas the C:S ratios for compounds **2**, **4** and **6** are not particularly close to the theoretical values. In these

TABLE II

C:S RATIOS CALCULATED FOR ACETYLENIC THIOPHENES DETECTED IN THE ESSENTIAL OIL OF *TAGETES PATULA* CV. *NANA* FLOWERS

Compound No.	MW	Elemental formula	C:S ratio	
			Theoretical	Experimental
1	216	$C_{12}H_8S_2$	0.166	0.164
2	230	$C_{13}H_{10}S_2$	0.154	0.175
4	230	$C_{13}H_{10}S_2$	0.154	0.160
6	248	$C_{12}H_8S_3$	0.25	0.261
7	276	$C_{14}H_{12}S_2O_2$	0.142	Reference

instances, an exact C peak-area value is difficult to determine because of overlapping with major or equally abundant peaks. Moreover, the C pattern at 193 nm in the part of the chromatogram where the acetylenic thiophenes elute is very complex, and compounds **3** and **5** are present only in trace amounts; as a consequence, their C peak areas do not provide a correct calculation of the C:S ratio.

AES results are very useful for identification, and often also to calculate the elemental formula, but they do not provide any structural information. Structure elucidation without isolation can only be carried out using GC–MS with different ionization mode and GC–FT-IR.

The EI mass spectra of acetylenic thiophenes are characterized by a very intense molecular ion M^+, which is often the base peak of the spectrum; other less intense but diagnostic ions corresponding to the acetylenic thiophene structure are, for instance, the fragments at *m/z* $[M-HS]^+$, *m/z* 215 ($[C_{12}H_7S_2]^+$) and *m/z* 171 ($[C_{11}H_7S]^+$). The fragmentation patterns of the acetylenic thiophenes identified here are in agreement with that reported by Hartough [19] and Horn and Lamberton [20].

GC–MS with both PICI and NICI using methane as reactant gas produces high-intensity diagnostic ions affording the confirmation of the molecular weight of acetylenic thiophenes. Methane PICI-MS gives a high-intensity quasi-molecular ion $[M+1]^+$ due to a proton addition, while methane NICI-MS produces high-intensity molecular ions (M^-) as a consequence of an electron-capture phenomenon. In the essential oil under investigation, the electron-capture phenomenon associated with methane

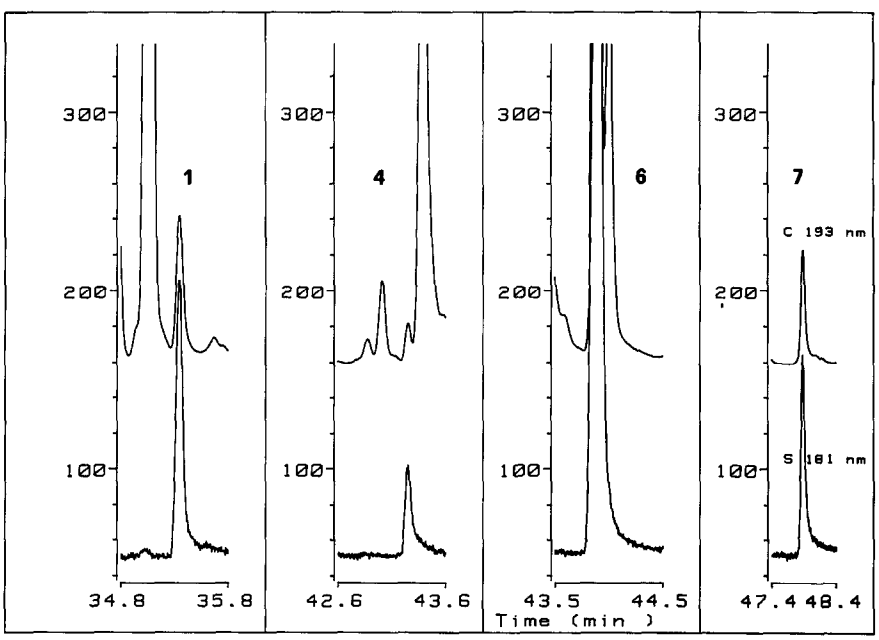


Fig. 3. S and C AES patterns of compounds **1**, **4**, **6** and **7**.

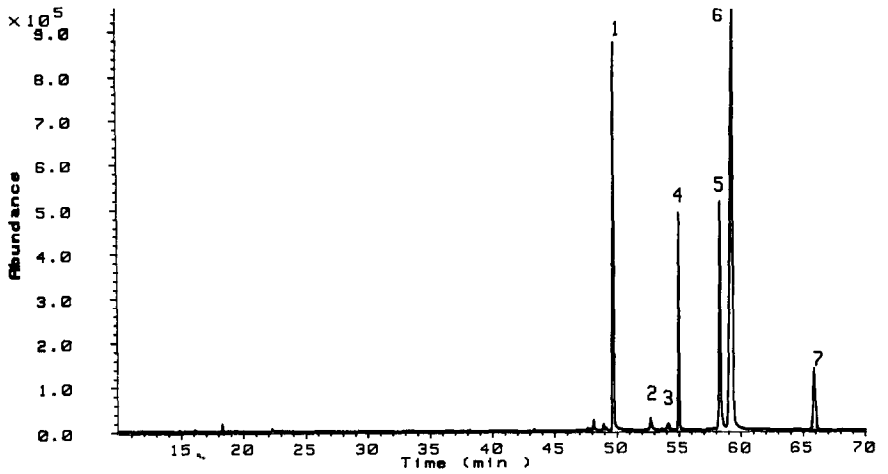


Fig. 4. cGC–methane NICI-MS pattern of *T. patula* cv. *nana* flower essential oil.

NICI-MS is specific for the acetylenic thiophenes, and is complementary to GC–SSD and to GC–AES for their correct identification in the total chromatogram. Fig. 4 reports the cGC–methane NICI-MS pattern of *T. patula* cv. *nana* essential oil. In both the CI and EI mass spectra, the peaks at m/z M^+ and $[M+2]^+$ exhibit the characteristic intensity ratio peculiar to ions containing sulphur. Fig. 5 reports the EI, NICI and PICI mass spectra of compound **1**. Table III reports the main CI- and EI-MS ions characteristic of the acetylenic thiophenes present in the essential oil under investigation.

GC–FT-IR also gives significant spectra useful and complementary to MS for an unambiguous identification of acetylenic thiophenes. They can be identified by their characteristic absorptions in the following regions [19–22]: 3080 cm^{-1}, medium-high-intensity band, CH = stretch in the thiophene ring; 2195 cm^{-1}, medium-low-intensity band, $-C\equiv C-$ stretch of the acetylenic triple bond; 1505, 1415, 1380 cm^{-1}, medium-low-intensity bands, substitution in position 2 of the thiophene ring; and 835, 795 cm^{-1}, high-intensity band characteristic of the thiophene ring.

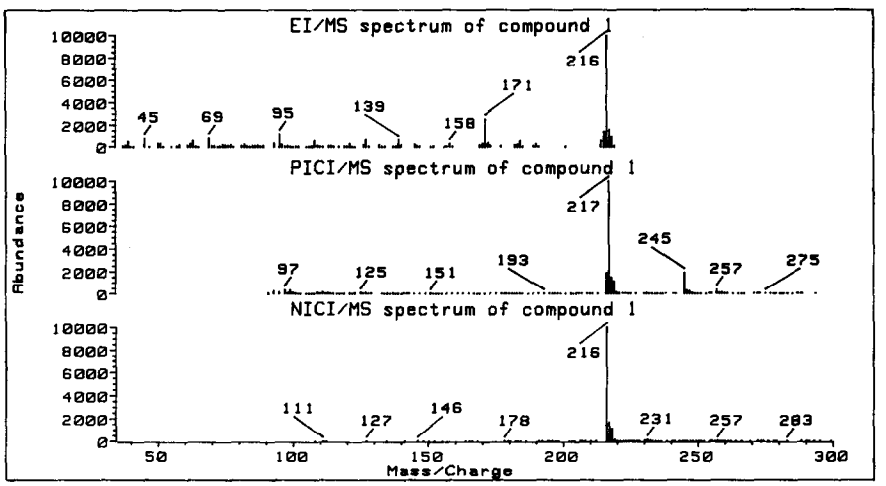


Fig. 5. EI, NICI and PICI mass spectra of compound **1**.

TABLE III

EI- AND CI-MS IONS CHARACTERISTIC OF THE ACETYLENIC THIOPHENES PRESENT IN THE ESSENTIAL OIL OF *TAGETES PATULA* CV. *NANA* FLOWERS

Compound No.	MW	Ionization mode	Main fragments (*m/z*, %)
1	216	EI	45(8.38), 69(10.15), 95(10.31), 127(6.13), 139(6.43), 171(26.32), 172(3.67), 173(1.35), 215(12.57), 216(100), 217(14.83), 218(9.55)
2	230		39(4.71), 45(5.47), 83(15.60), 102(6.33), 121(4.25), 152(6.09), 184(4.78), 197(22.36), 229(36.07), 230(100), 231(18.10), 232(11.38)
3	228		45(1.39), 69(5.32), 121(3.92), 139(5.99), 141(5.01), 169(4.27), 183(4.78), 195(18.71), 227(34.78), 228(100), 229(17.72), 230(11.40)
4	230		39(9.64), 45(10.23), 69(8.32), 115(6.86), 165(5.76), 171(7.20), 184(7.15), 197(10.75), 229(25.94), 230(100), 231(17.48), 232(9.82)
5	232		41(53.38), 43(62.52), 83(31.30), 84(100), 97(28.89), 111(22.15), 149(16.35), 203(19.72), 231(14.69), 232(49.56), 233(9.01), 234(8.46)
6	248		45(13.96), 69(10.92), 121(5.71), 124(6.33), 127(13.49), 171(10.23), 190(4.12), 203(16.35), 247(5.93), 248(100), 249(15.75), 250(13.82)
7	276		43(24.56), 45(4.32), 69(5.52), 171(9.44), 203(12.41), 215(5.06), 216(100), 217(15.77), 218(9.76), 276(8.45), 277(1.42), 278(0.75)
1	216	NICI	216(100), 217(17), 218(11), 231(1.47)
2	230		230(100), 231(189), 232(12)
3	228		228(100), 229(25), 230(17), 243(1.39)
4	230		230(100), 231(18), 232(119; 245(1.56)
5	232		232(100), 233(16), 234(11)
6	248		248(100), 249(18), 250(16), 263(1.90)
7	276		276(100), 277(18), 278(16)
1	216	PICI	216(18.99), 217(M + 1) (100), 218(15.11), 219(11.55), 245(18.96)
2	230		230(24.68), 231(M + 1) (100), 232(20.32), 233(13.59), 259(15.12)
3	228		Non-significant spectrum
4	230		230(25.05), 231(M + 1) (100), 232(17.90), 233(11.57), 259(16.81)
5	232		233(M + 1) (100), 234(14.70), 235(10.03), 261(14.16)
6	248		248(23.74), 249(M + 1) (100), 250(17.34), 251(13.96), 277(16.25)
7	276		217(37.11), 277(M + 1) (100), 278(14.15), 279(10.97), 305(10.06)

Fig. 6 reports the FT-IR spectra of **1** and **6**. The two spectra clearly show that the IR spectra contribute to the characterization of the thiophenic part of the structure, with absorptions near 3083, 1509, 1414, 1385, 841 and 795 cm^{-1} for **1**, and with those near 3080, 1503, 1418, 1379, 832 and 796 cm^{-1} for **6**. In addition, **1** exhibits the absorptions characteristic of the acetylenic group, with the absorption at 2195

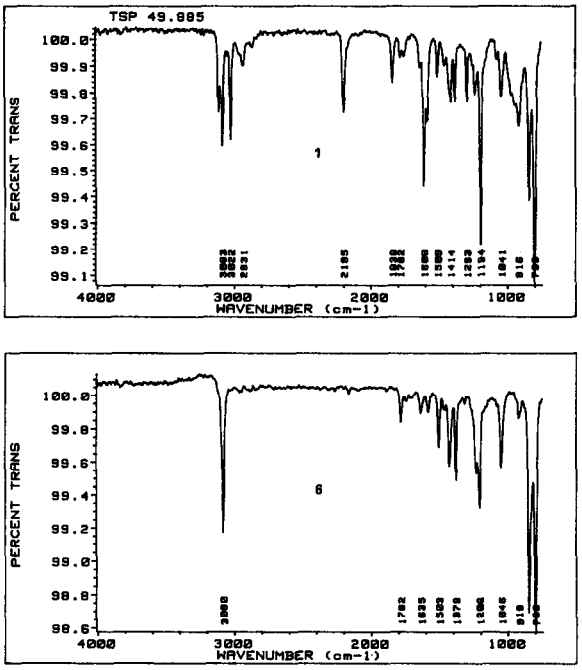


Fig. 6. FT-IR spectra of compounds **1** and **6**.

cm^{-1} and those at 3100, 3022 and 2931 cm^{-1} characteristic of the substituent in position 2 on the thiophene ring.

Table IV reports the characteristic absorptions for the acetylenic thiophenes present in the essential oil under investigation. FT-IR also afforded an unambiguous distinction between the two isomers at MW 230 (**2** and **4**), which cannot be distinguished by MS. Compound **2** actually shows medium-low-intensity bands at 3040, 2870 and 2748 cm^{-1}, corresponding to the stretching of a methyl substituent in position 4 of the thiophene ring [19,22]. The lower sensitivity of GC–FT-IR than GC–MS allowed us to record significant IR spectra only for four of the seven detected acetylenic thiophenes.

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TABLE IV

CHARACTERISTIC ABSORPTIONS FOR THE ACETYLENIC THIOPHENES PRESENT IN THE ESSENTIAL OIL OF *TAGETES PATULA* CV. *NANA* FLOWERS

Compound No.	Molecular mass	IR band position (cm^{-1})[a]
1	216	3083(m), 3022(m), 2931(w), 2195(m), 1838(m), 1782(w), 1509(w), 1414(w), 1380(w), 1194(s), 830(m), 798(s)
2	230	3110(w), 3081(m), 3025(w), 2940(w), 2205(w), 1615(w), 798(s)
4	230	3079(m), 3040(w), 3016(m), 2932(s), 2869(m), 2231(w), 1747(w), 1621(s), 1516(w), 1443(w), 1383(w), 1194(m), 910(m), 798(s)
6	248	3080(s), 1782(w), 1635(w), 1503(w), 1420(m), 1396(m), 1206(s), 1046(w), 918(s), 835(s), 798(s)

[a] m = medium; s = strong; w = weak.

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