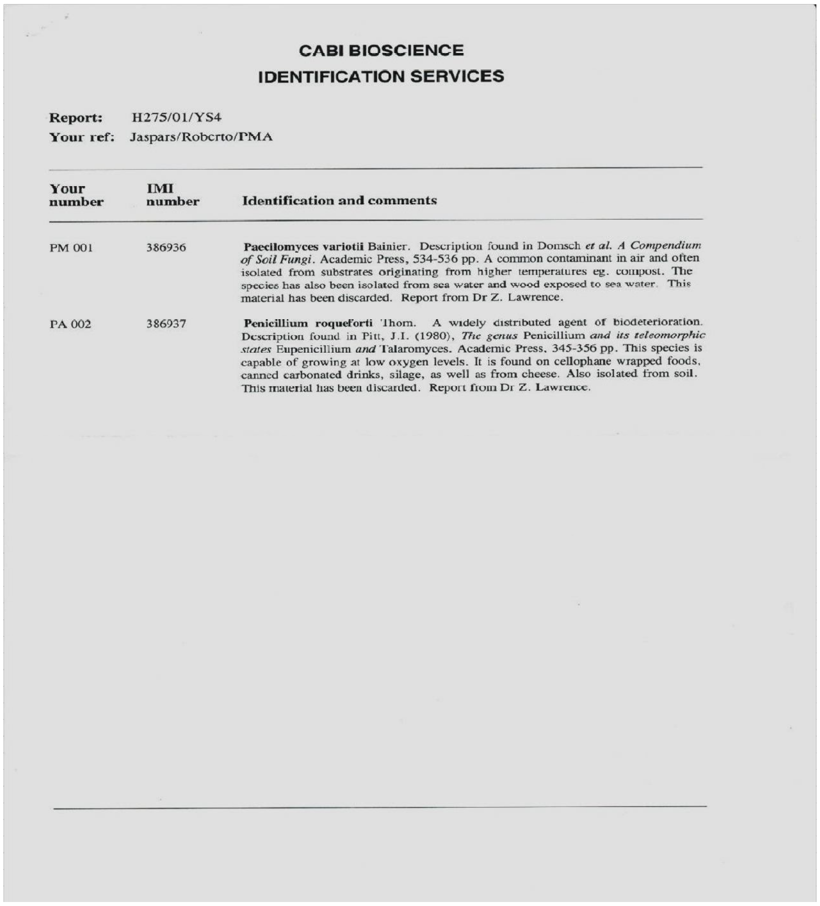
Additional experimental data

Figure 1S - (a) Photograph of the area where the strain of *Penicillium roqueforti* was collected (b) Rocky outcrop on the beach of La Laja, Gran Canaria, Spain.



CABI BIOSCIENCE
IDENTIFICATION SERVICES

Report: H275/01/YS4
Your ref: Jaspars/Roberto/PMA

Your number	IMI number	Identification and comments
PM 001	386936	**Paecilomyces variotii** Bainier. Description found in Domsch *et al. A Compendium of Soil Fungi*. Academic Press, 534-536 pp. A common contaminant in air and often isolated from substrates originating from higher temperatures eg. compost. The species has also been isolated from sea water and wood exposed to sea water. This material has been discarded. Report from Dr Z. Lawrence.
PA 002	386937	**Penicillium roqueforti** Thom. A widely distributed agent of biodeterioration. Description found in Pitt, J.I. (1980), *The genus* Penicillium *and its teleomorphic states* Eupenicillium *and* Talaromyces. Academic Press, 345-356 pp. This species is capable of growing at low oxygen levels. It is found on cellophane wrapped foods, canned carbonated drinks, silage, as well as from cheese. Also isolated from soil. This material has been discarded. Report from Dr Z. Lawrence.

Figure 2S – CABI Bioscience (Surrey, UK) identification report of the fungal strain.

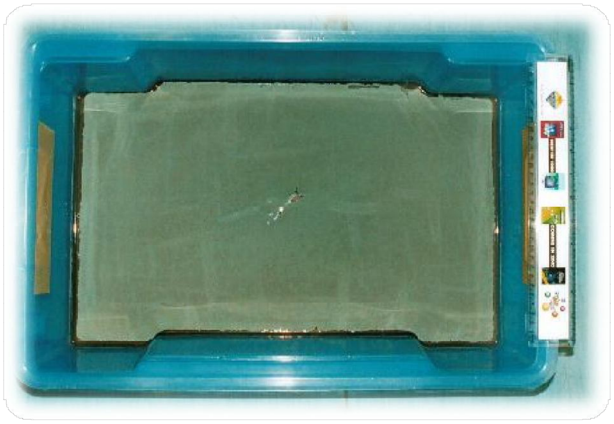


Figure 3S - View from above of the mass cultivation of *Penicillium roqueforti*.

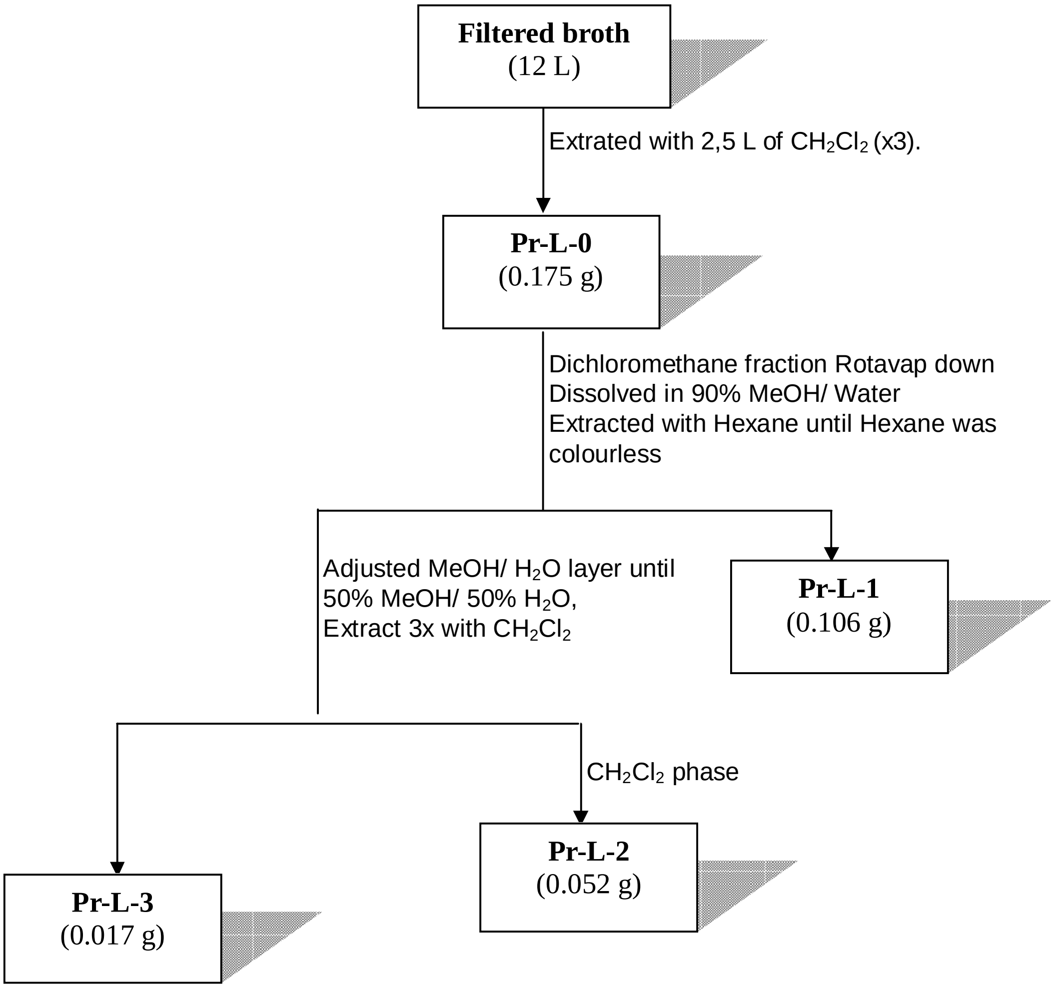


Figure 4S – Solvent-solvent processing scheme applied to partition the culture broth of *Penicillium roqueforti*, adapted from Kupchan et al. (1973).

Description of the chemical analysis of the culture broth from the derived-marine *Penicillium roqueforti* fungus.

Twelve liters of culture broth were extracted in a separatory funnel with 2.5 L of CH_2Cl_2 (x3) to obtain 175 mg of crude extract (**Pr-L-0**), which was subjected to the partitioning scheme described above (Figure 4S).

The ^{1}H-NMR spectrum resulted in seven protons of carboxylic acids at δ 12.5-11.2, aromatics at δ 8.5-7.0; olefinics at δ 5.9-5.0; geminals to heteroatoms at δ 4.3-3.5 and aliphatics at δ 3.0-0.6.

This crude extract was dissolved in 20 ml of water and 180 ml of MeOH and was extracted in a decantation funnel with hexane until it was colorless (10 extractions). The evaporation of the solvent gave 106 mg of the liposoluble fraction (**Pr-L-1**).

The hydrosoluble fraction was diluted with additional water (160 ml) to obtain an aqueous phase of composition 50% H_2O- 50% MeOH, which was reextracted with CH_2Cl_2 (x3) to give the fractions **Pr-L-2** (soluble in CH_2Cl_2, 52 mg) and **Pr-L-3** (17 mg).

Study of the liposoluble fraction-1 (Pr-L-1)

By TLC (normal-phase, Hexane: EtOAc, 80: 20) six major substances were detected. The same was observed by HPLC (normal-phase, Hexane: EtOAc, 80: 20) (Figure 5S):

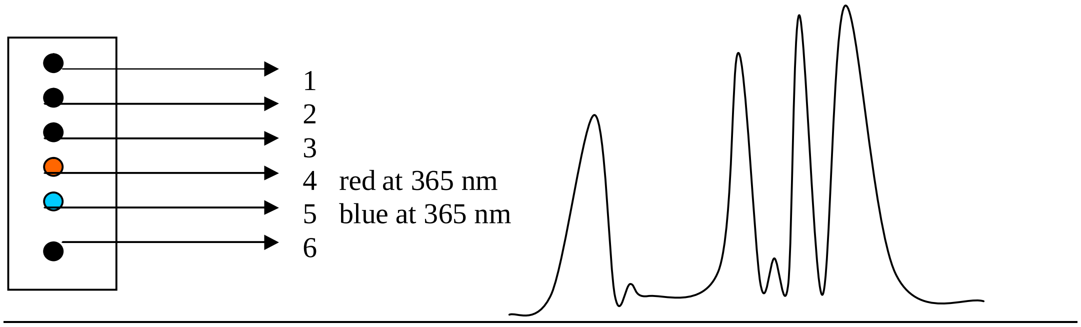


Figure 5S - Analytical chromatography by TLC and HPLC of the **Pr-L-1** fraction**.**

In the ^{1}H-NMR spectrum of this mixture, we detected: aromatic protons (δ 7.70 and 7.53), olefinic (δ 5.38), germinal to heteroatom (δ 4.31) and aliphatic hydrocarbon chains (δ 2.9-0.7).

In the ^{13}C-NMR spectrum, the following were to be observed: carbonilic carbons (δ 178.187), aromatics (δ 167.753), olefinics (δ 130.939 and 128.859), geminals to heteroatoms (δ 77.543-65.914) and from aliphatic chains (δ 37.469-14.132).

This mixture was submitted to a normal-phase semipreparative HPLC (Hexane: EtOAc, 80: 20, UV detector adjusted to 254 nm) giving two fractions: **Pr-L-1-1** and **Pr-L-1-2**.

Pr-L-1-1 fraction

A colourless oil was obtained (50 mg), and the signals were only observed at δ 0.85-1.86 using ^{1}H-NMR spectroscopy, leading to the suspicion that it is a mixture of alkanes. A study by GC-MS allowed for the identification and quantification of the following volatile compounds:

Pentadecane, C_{15} (**1**; n= 12; R_t= 13.990; 0.037 mg)
Heptadecane, C_{17} (**1**; n= 14; R_t= 15.642; 0.030 mg)
Eicosane, C_{20} (**1**; n= 17; R_t= 17.393; 0.063 mg)
Cyclo-ProLeu (**14**; Tr= 17.56; 0.211 mg)
Heneicosane, C_{21} (**1**; n= 18; R_t= 18.327; 0.141 mg)
Docosane, C_{22} (**1**; n= 19; R_t= 18.978; 0.142 mg)
Tricosane, C_{23} (**1**; n= 20; R_t= 19.744; 0.250 mg)
Hexacosane, C_{26} (**1**; n= 23; R_t= 23.242; 1.766 mg)
Heptacosane, C_{27} (**1**; n= 24; R_t= 25.07; 0.588 mg)

This gave a total of 46.772 mg of non-volatile material, that was assigned to the alkanes of higher molecular weight, that did not volatilize at the injector temperature used or were outside the predetermined scanning time in the method.

Pr-L-1-2 fraction

This colourless oil (50 mg), when observed by ^{1}H-NMR, revealed the following: aromatic protons (δ 7.70 and 7.53), olefins (δ 5.48-5.26), geminal to heteroatoms (δ 4.42), in alpha to carbonyls (δ 2.80), of methylenes/ aliphatic methines (δ 1.83-1.20), and methyls (δ 0.96-0.81). Thus, it appears to be a mixture of alkyl phthalates and unsaturated fatty acids. Indeed, a study by GC-MS showed the presence of the following volatile compounds:

Dibutyl Phthalate (**15**; n= 3; R_t= 17.649; 5.126 mg)
7,10-Octadecadienoic acid, methyl ester (**7**; n= 6, m= 5; R_t= 18.378; 0.178 mg)
Hexadecanoic acid, octadecyl ester (**9**; R_t= 18.646; 0.251 mg)
Hexadecanoic acid, 2-(octadecyloxi)-ethyl ester (**12**; R_t= 19.106; 0.119 mg)
9-Octadecenoic acid, (E)- (**5**; R_t= 19.807; 0.039 mg)
Bis(4-methylpentyl) phthalate (**16**; n= 3; R_t= 20.407; 0.968 mg)
2-(Octadecyloxi) ethanol (**11**; R_t= 21.805; 0.555 mg)
Bis(2-ethylhexyl) phthalate (**17**; R_t= 22.645; 0.214 mg)
Dihexyl phthalate (**15**; n= 5; R_t= 22.735; 2.919 mg)
Dioctyl phthalate (**15**; n= 7; R_t= 26.367; 2.349 mg)

There was a total weight of 37.282 mg of non-volatile material that was assigned, using ^{1}H-RMN spectroscopy, to a mixture of alkyl phthalates of high molecular weight, and to free unsaturated fat acids that did not volatilize at the injector temperature used or were outside the predetermined scanning time in the method.

Indeed, rechromatography of this fraction through silica eluting with hexane-ethyl acetate yielded a pure sample (homogeneous by TLC) of one of these phthalates. From its ^{1}H-RMN spectrum, it was assigned the structure of the Dinonyl phthalate (**15**; n= 8): δ 7.70 (2H, dd); 7.53 (2H, dd); 4.32 (4H, t); 1.75 (4H, m); 1.48-1.20 (24H, m) and 0.89 (6H, t).

Study of the liposoluble fraction-2 (Pr-L-2)

52 mg of a mixture was obtained that, by analytical TLC, gave eight substances, with the R_t coinciding with five components of the previous fraction **Pr-L-1**.

The ^{1}H-RMN spectrum showed aromatic protons (δ 7.78-7.47), geminal to heteroatom (δ 4.28 on one side and 3.90-3.15 on the other) and typical saturated hydrocarbonated chains (δ 2.50-0.75), leading to the suspicion, therefore, of the presence of phthalates and saturated lipid components. A GC-MS analysis of this fraction identified/ quantified as follows:

Hexadecanoic acid (2-pentadecyl-1,3-dioxolan-4-yl) methyl ester (**8**; R_t= 12.154; 0.0088 mg)
2-Butyl-1-octanol (**3**; n= 3, m= 3; R_t= 12.519; 0.0025 mg)
Tetradecanoic acid (**4**, n= 12; R_t= 14.776; 0.0063 mg)
1-Tridecanol (**2**; n= 11; R_t= 14.867; 0.0023 mg)
Dietyl phthalate (**15**; n= 1; R_t= 15.062; 0.0083 mg)
1-Hexadecanol (**2**; n= 14; R_t= 16.348; 0.003 mg)
Bis(2-methylpropyl) phthalate (**16**; n= 1; R_t= 17.009; 0.0167 mg)
Dibutyl phthalate (**15**; n= 3; R_t= 17.653; 1.3935 mg)
Stearic acid, 3-(octadecyloxy)-propyl ester (**10**; R_t= 17.948; 0.0024 mg)
9-Octadecenamide (**6**; R_t= 18.358; 0.0181 mg)
9-Octadecenoic acid, (E)- (**5**; R_t= 19.814; 0.0087 mg)

The total weight of 50.529 mg of non-volatile material was assigned by ^{1}H-NMR spectroscopy to a mixture of phthalates and saturated lipid components of the above type but of larger molecular size that, as occurred previously, either did not volatilize at the injector temperature used or went outside the default analysis time for the method. Thus, by rechromatography column, some different enriched fractions were separated:

a) alkanes (**1**; δ 1.62-0.77), b) Dinonyl phthalate {(**15**; n= 8): δ 7.70 (2 H, dd); 7.53 (2 H, dd); 4.32 (4 H, t); 1.75 (4 H, m); 1.48-1.20 (24 H, m) and 0.89 (6 H, t)}, c) Dibutyl phthalate {(**15**; n= 3): δ 7.70 (2 H, dd); 7.53 (2 H, dd); 4.31 (4 H, t); 1.72 (4 H, m); 1.44 (4 H, m); 0.96 (6 H, t)}, d) saturated free fatty acids (**4**; δ 1.85-0.80) e) saturated 1-alkanols (**2**; δ 3.80-3.50; 1.85-0.80).

Study of the liposoluble fraction-3 (Pr-L-3)

The crude fraction (17 mg) has an ^{1}H-NMR spectrum which shifts germinal protons to heteroatoms (δ 4.10-3.15), -NH_2 groups (δ 3.03) and aliphatic chains (δ 2.50-0.75).

The GC-MS analysis of this fraction allowed the identification/quantification of the following volatile organic compounds:

2,6-Octadienal, 3,7-dimethyl-, (E)- (**13**; R_t= 12.179; 0.079 mg)
Pentadecane, C_{15} (**1**; n= 12; R_t= 13.992; 0.005 mg)
9-Octadecenamide (**6**; R_t= 18.366; 0.0489 mg)
9-Octadecenoic acid, (E)- (**5**; R_t= 19.809; 0.03 mg)
2-Ethylhexyl adipate (**18**; R_t= 20.714; 0.143 mg)

That gives a total of 16.694 mg that was assigned by ^{1}H-NMR to a mixture of unsaturated glycerides (δ 5.46-5.14; 4.37-4.06; 2.53-2.23; 1.84-0.80) and polyhydroxy compounds (δ 4.10-3.15) that, as before, either did not volatilize at the injector temperature used or went outside the default analysis time for the method.

Final note: The GC-MS digital fingerprints of the compounds **13**, **15** (n= 1, 3 and 7); and the ^{1}H-NMR spectra of the compound **15 (**n= 3) can be seen below. The GC-MS spectra of the other compounds, and other spectroscopic data, can be obtained by contacting the authors.

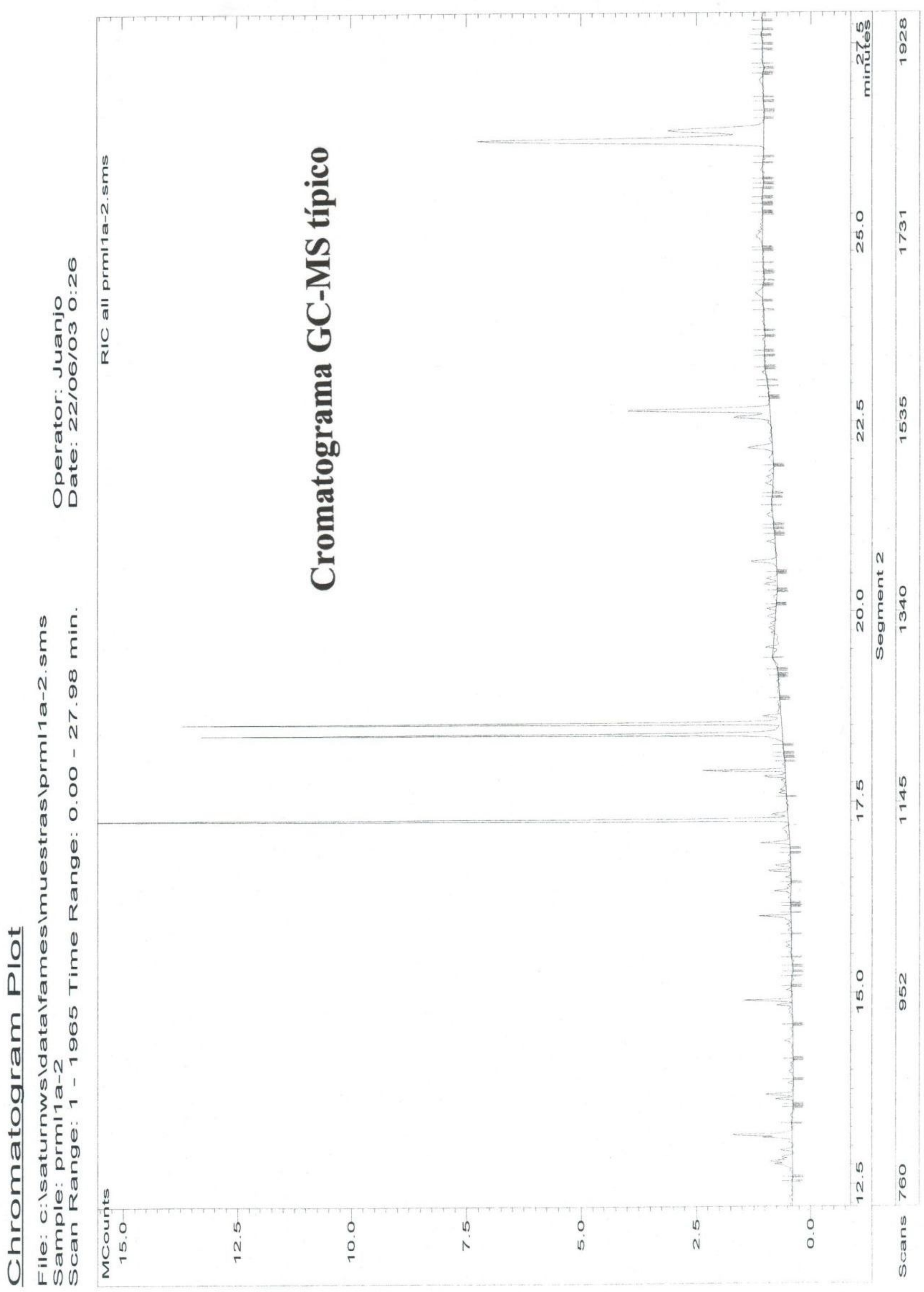


Figure 6S – Typical Gas chromatography–mass spectrometry (GC-MS) chromatrogram.

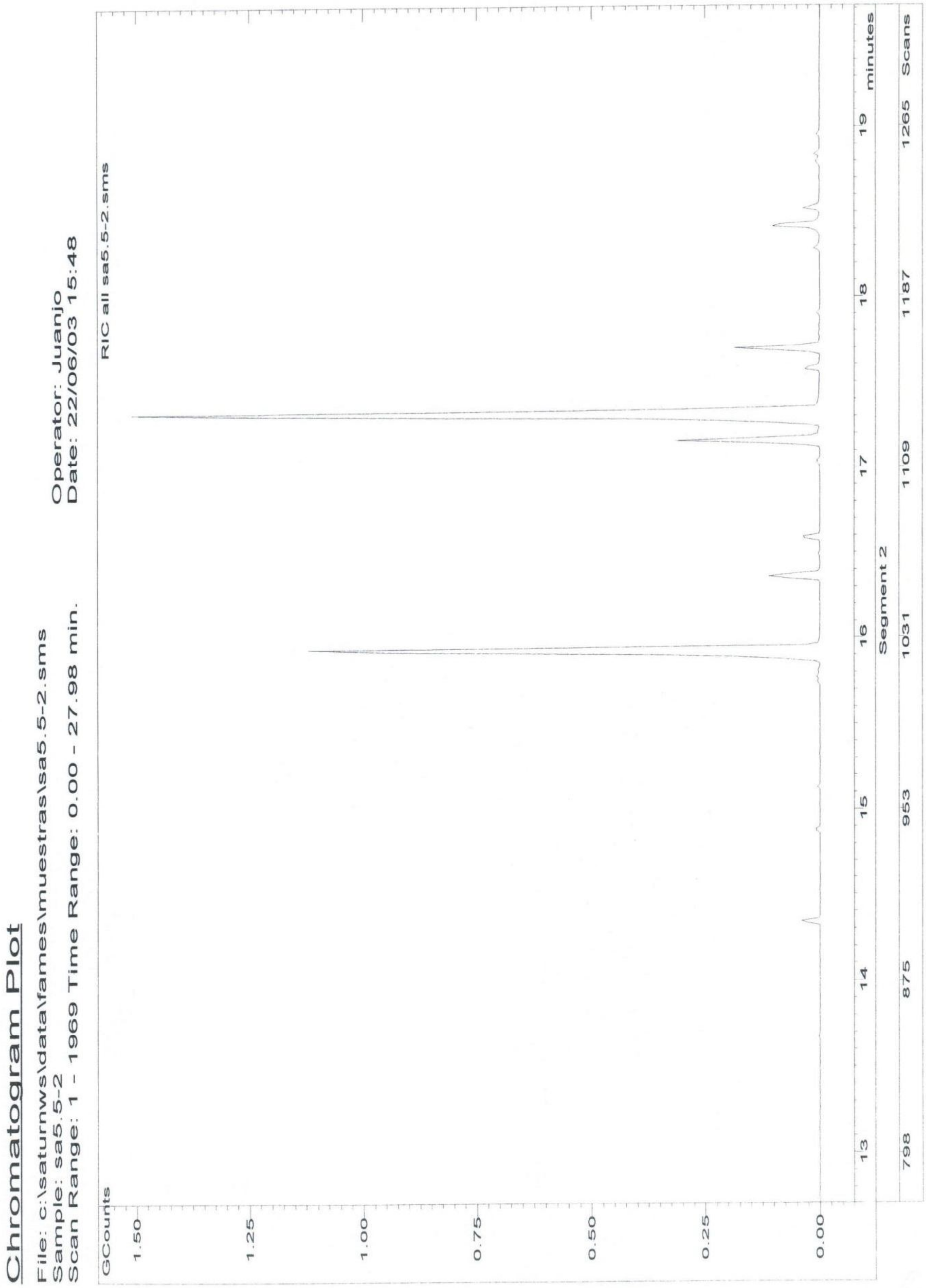
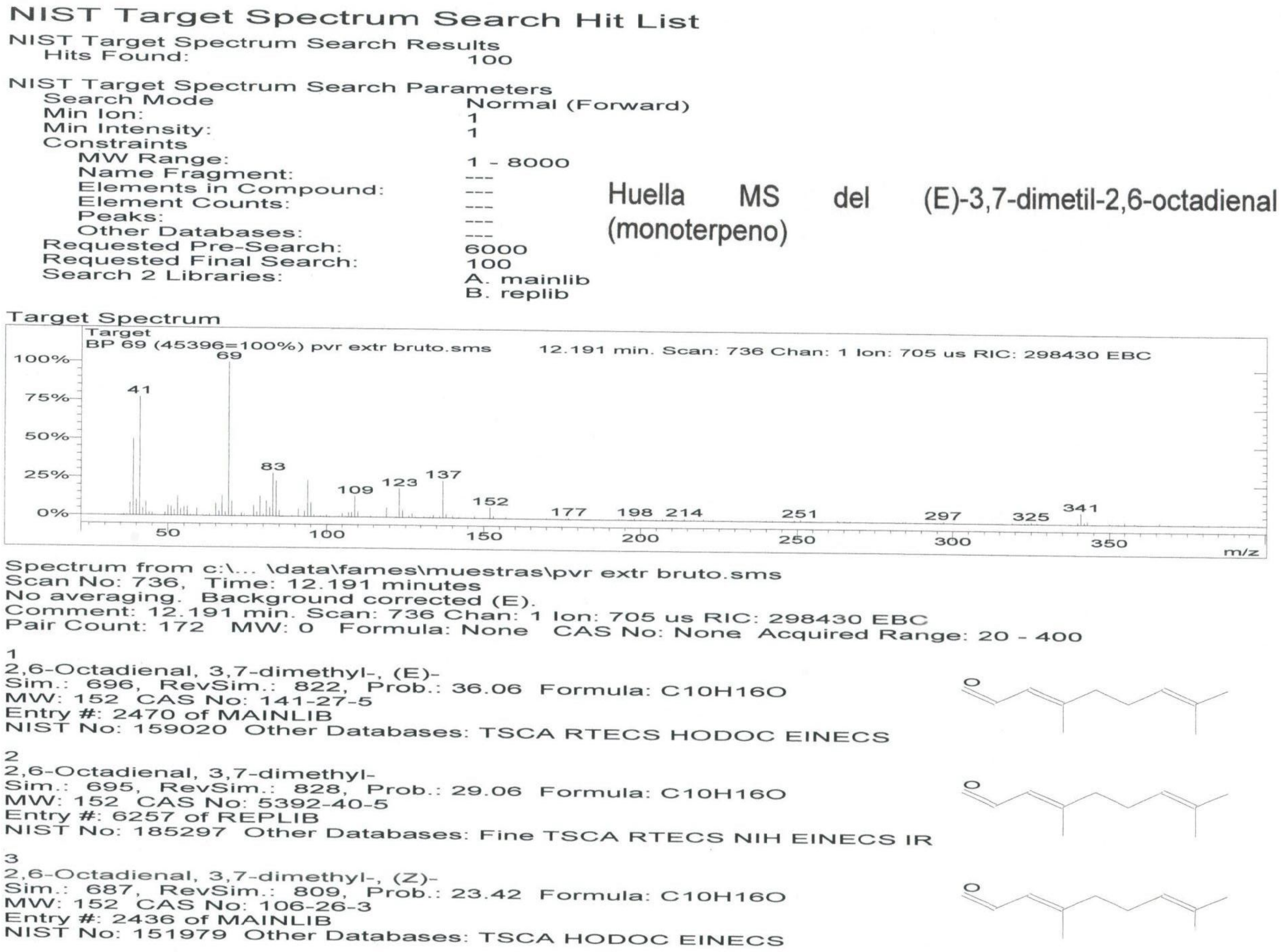


Figure 7S – Typical GC-MS enlarged chromatrogram.



Huella MS del (E)-3,7-dimetil-2,6-octadienal (monoterpeno)

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Target Spectrum
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Pair Count: 172   MW: 0   Formula: None   CAS No: None  Acquired Range: 20 - 400

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2,6-Octadienal, 3,7-dimethyl-, (E)-
Sim.:  696,  RevSim.:  822,  Prob.: 36.06  Formula: C10H16O
MW: 152  CAS No: 141-27-5
Entry #: 2470 of MAINLIB
NIST No: 159020  Other Databases: TSCA RTECS HODOC EINECS

2
2,6-Octadienal, 3,7-dimethyl-
Sim.:  695,  RevSim.:  828,  Prob.: 29.06  Formula: C10H16O
MW: 152  CAS No: 5392-40-5
Entry #: 6257 of REPLIB
NIST No: 185297  Other Databases: Fine TSCA RTECS NIH EINECS IR

3
2,6-Octadienal, 3,7-dimethyl-, (Z)-
Sim.:  687,  RevSim.:  809,  Prob.: 23.42  Formula: C10H16O
MW: 152  CAS No: 106-26-3
Entry #: 2436 of MAINLIB
NIST No: 151979  Other Databases: TSCA HODOC EINECS
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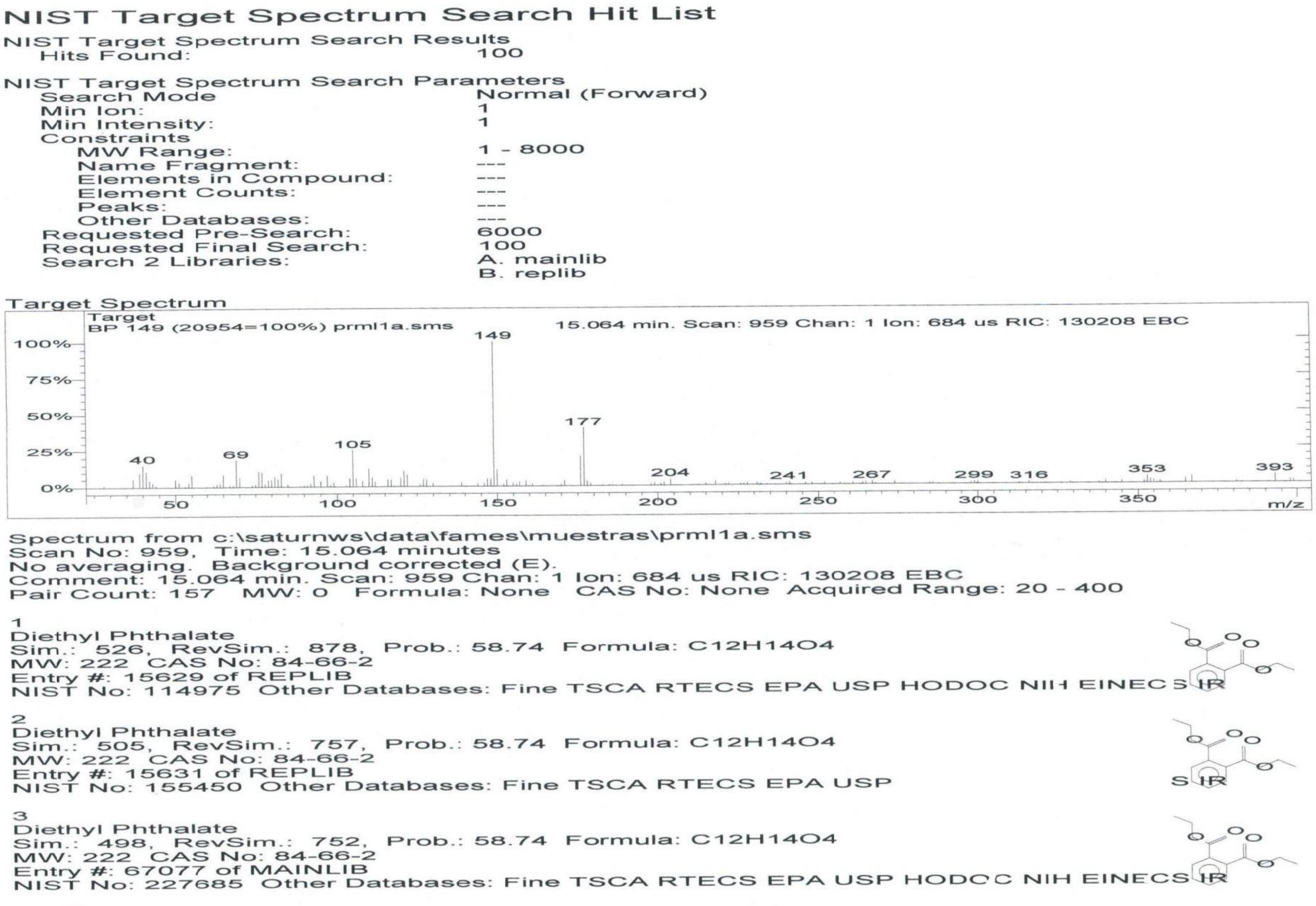
Figure 8S – The GC-MS fingerprint of the 2,6-Octadienal, 3,7-dimethyl-, (E)- (**13**).



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Diethyl Phthalate
Sim.:  526,  RevSim.:  878,  Prob.: 58.74  Formula: C12H14O4
MW: 222  CAS No: 84-66-2
Entry #: 15629 of REPLIB
NIST No: 114975  Other Databases: Fine TSCA RTECS EPA USP HODOC NIH EINECS IR

2
Diethyl Phthalate
Sim.:  505,  RevSim.:  757,  Prob.: 58.74  Formula: C12H14O4
MW: 222  CAS No: 84-66-2
Entry #: 15631 of REPLIB
NIST No: 155450  Other Databases: Fine TSCA RTECS EPA USP                    S IR

3
Diethyl Phthalate
Sim.:  498,  RevSim.:  752,  Prob.: 58.74  Formula: C12H14O4
MW: 222  CAS No: 84-66-2
Entry #: 67077 of MAINLIB
NIST No: 227685  Other Databases: Fine TSCA RTECS EPA USP HODOC NIH EINECS IR
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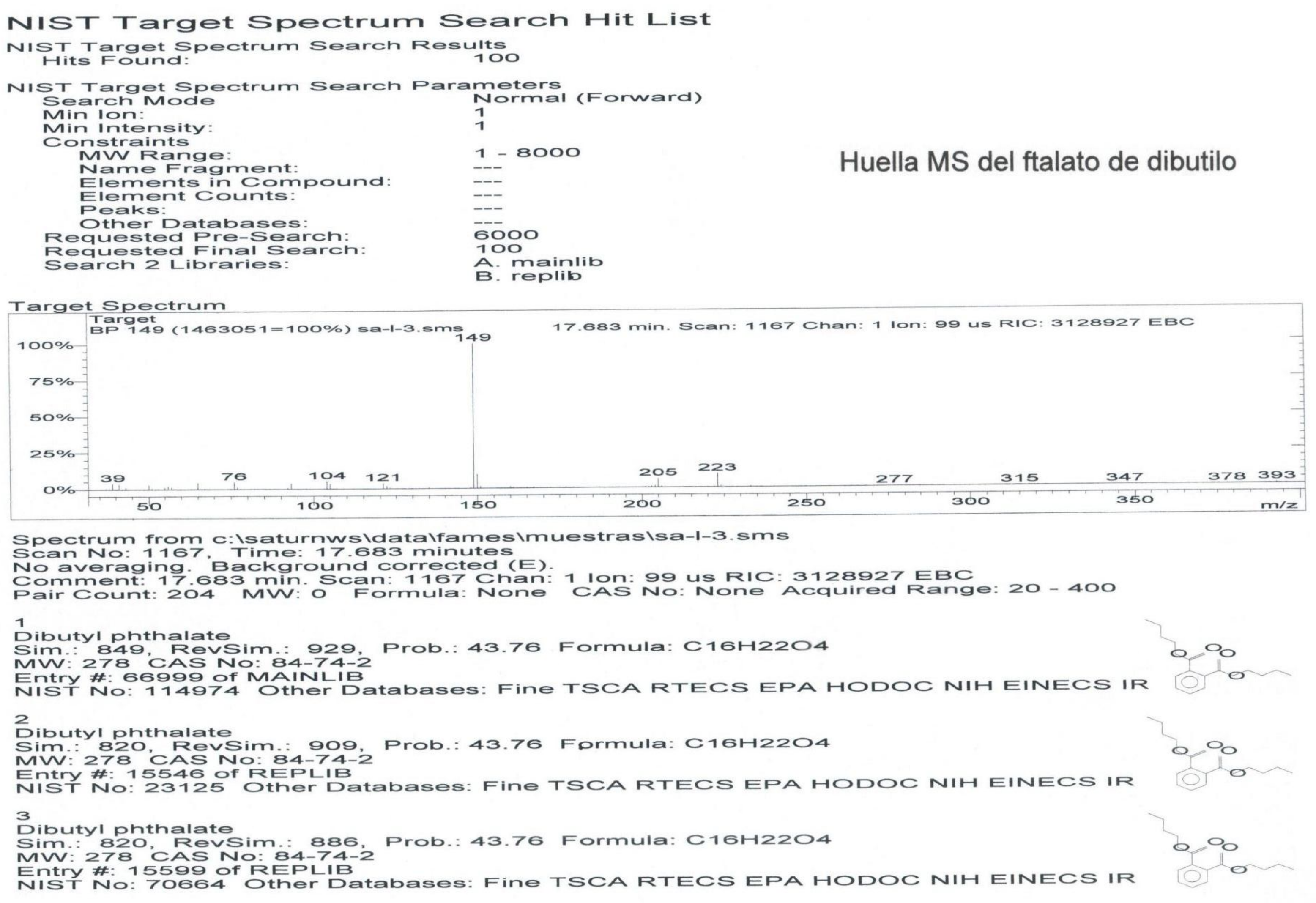
Figure 9S - GC-MS fingerprint of the Diethyl phtalate (**15**; n= 1).



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Huella MS del ftalato de dibutilo

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Target Spectrum
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Dibutyl phthalate
Sim.:  849,  RevSim.:  929,  Prob.: 43.76  Formula: C16H22O4
MW: 278  CAS No: 84-74-2
Entry #: 66999 of MAINLIB
NIST No: 114974  Other Databases: Fine TSCA RTECS EPA HODOC NIH EINECS IR

2
Dibutyl phthalate
Sim.:  820,  RevSim.:  909,  Prob.: 43.76  Formula: C16H22O4
MW: 278  CAS No: 84-74-2
Entry #: 15546 of REPLIB
NIST No: 23125  Other Databases: Fine TSCA RTECS EPA HODOC NIH EINECS IR

3
Dibutyl phthalate
Sim.:  820,  RevSim.:  886,  Prob.: 43.76  Formula: C16H22O4
MW: 278  CAS No: 84-74-2
Entry #: 15599 of REPLIB
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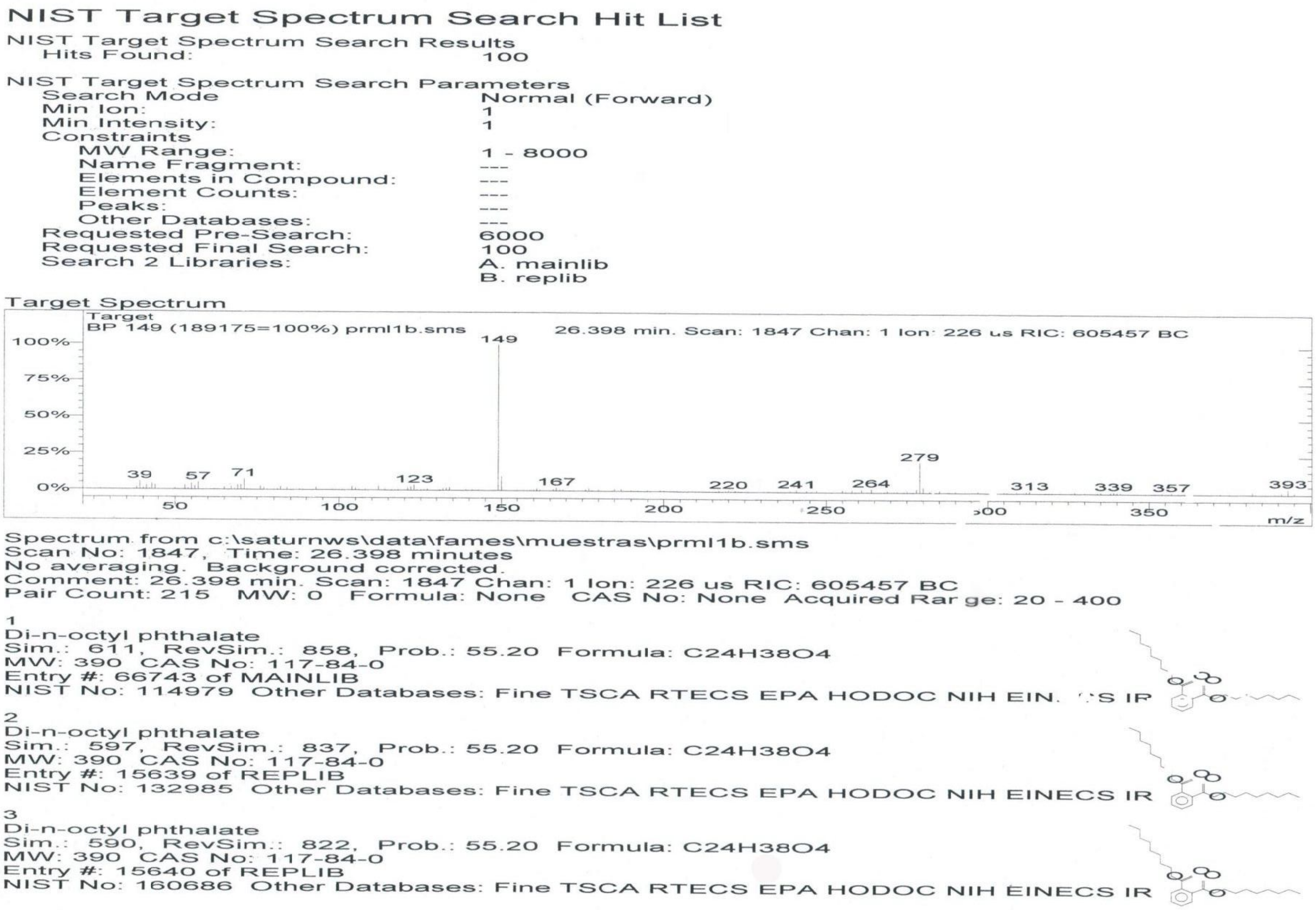
Figure 10S – The GC-MS fingerprint of the Dibutyl phthalate (**15**; n= 3)



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Target Spectrum
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Di-n-octyl phthalate
Sim.:  611,  RevSim.:  858,  Prob.: 55.20  Formula: C24H38O4
MW: 390  CAS No: 117-84-0
Entry #: 66743 of MAINLIB
NIST No: 114979  Other Databases: Fine TSCA RTECS EPA HODOC NIH EIN. [illegible]S IR

2
Di-n-octyl phthalate
Sim.:  597,  RevSim.:  837,  Prob.: 55.20  Formula: C24H38O4
MW: 390  CAS No: 117-84-0
Entry #: 15639 of REPLIB
NIST No: 132985  Other Databases: Fine TSCA RTECS EPA HODOC NIH EINECS IR

3
Di-n-octyl phthalate
Sim.:  590,  RevSim.:  822,  Prob.: 55.20  Formula: C24H38O4
MW: 390  CAS No: 117-84-0
Entry #: 15640 of REPLIB
NIST No: 160686  Other Databases: Fine TSCA RTECS EPA HODOC NIH EINECS IR
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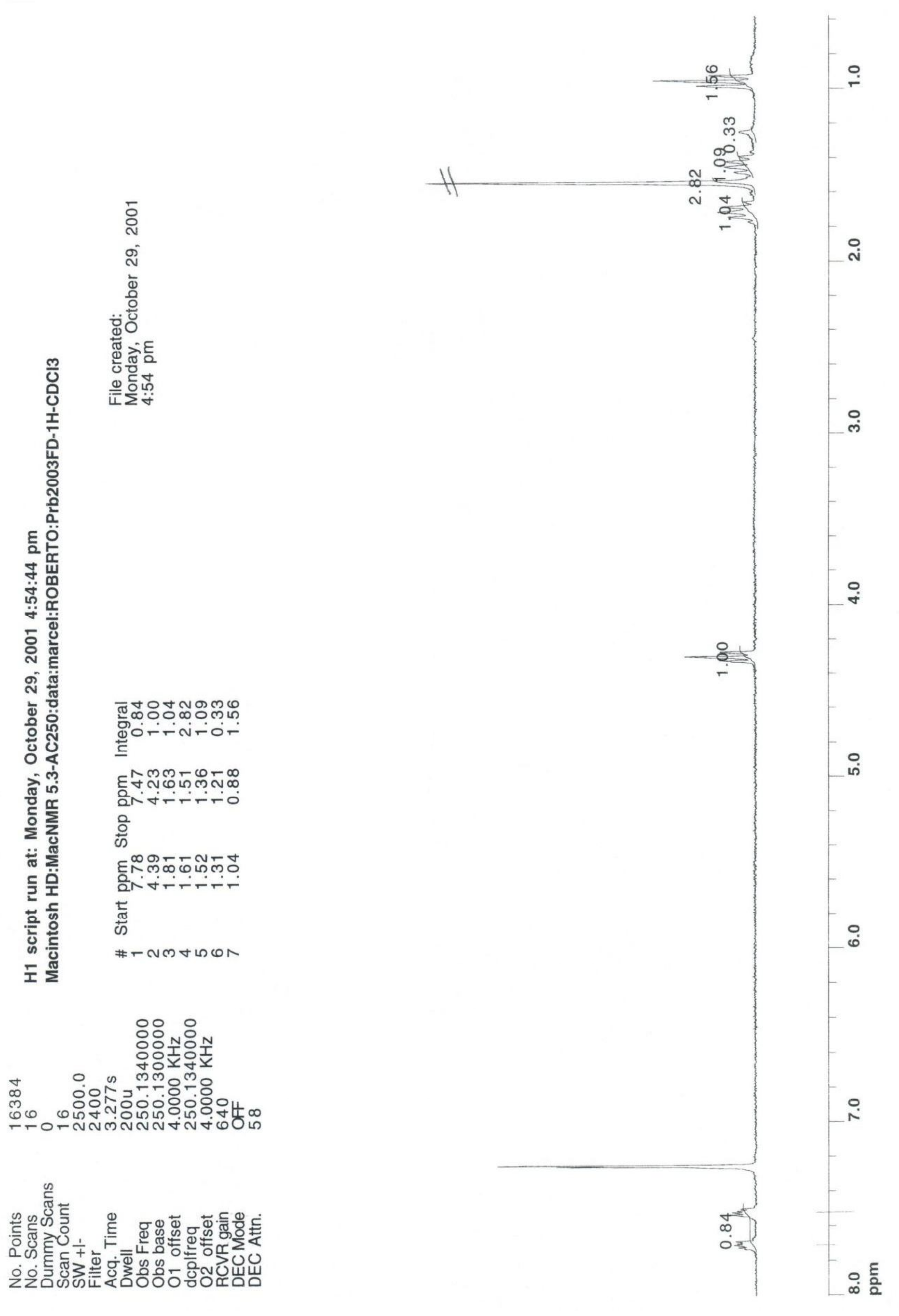
Figure 11S – The GC-MS fingerprint of the Dioctyl phthalate (**15**, n= 7).



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4	1.61	1.51	2.82
5	1.52	1.36	1.09
6	1.31	1.21	0.33
7	1.04	0.88	1.56

File created:
Monday, October 29, 2001
4:54 pm

Figure 12S - The^{1}H-NMR spectrum of the Dibutyl phthalate (**15**; n= 3).