Analyses by GC–MS and GC–MS–MS of the hantzsch synthesis products using hydroxy- and methoxy-aromatic aldehydes

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Abstract

EI mass spectra of products of the dihydropyridine Hantzsch synthesis using hydroxy and methoxy aldehydes as starting materials are reported. The reaction products (C-4 hydroxy- and methoxyphenyl-1,4-dihydropyridines and chromeno[3,4,c]-pyridines) were derivatized with *N*-methyl-*N*-(trimethylsilyl)-trifluoracetamide to be analyzed by gas chromatographic techniques. Fragmentation pathways for 1,4-dihydropyridines and chromeno-pyridines are proposed. The study provides (mainly through MS–MS technique) useful data for the confirmation of the structure of the compounds and also is a valuable tool for further analytical purposes to follow both photostability and reactivity studies with free radicals for these types of compounds.

Keywords: GC–MS; GC–MS–MS; Trimethylsilyl C-4 hydroxy- and methoxyphenyl-substituted 1,4-dihydropyridines; Chromeno[3,4-c]pyridine

1. Introduction

1,4-Dihydropyridines (1,4-DHPs)-type calcium channel antagonists are important drugs in the treatment of hypertension and coronary heart disease [1,2].

GC–MS technique has been widely used to quantify 1,4-dihydropyridines with different analytical purposes [3–5]. On the other hand, studies on the GC–MS characterization of several series of 1,4-DHPs exhibiting variations on the substituents involving the 3-, 4- and 5-positions have also been reported [6–9]. In a previous paper [9] we proposed two common characteristics for the EI fragmentation pattern of some C-4 substituted 1,4-DHPs, i.e., loss of the C-4 substituent on the dihydropyridine ring giving rise to the common base peak, *m/z* 224 and loss of the C-3 substituent ($COOCH_3$) giving $[M - 59]^+$ fragment ions.

Suárez et al. [7] presented a study of the ESI and sequential product ion fragmentation of 1,4-DHPs derivatives endowed with long alkyl chains in 5-position, concluding that these chains were eliminated as ions in the MS^2 fragmentation or as relatively stable olefins in the MS^3 fragmentation. Furthermore, Salehi et al. [6,8] reported the EI-TOFMS characterization of 1,4-dihydro-4-substituted-2,6-dimethyl-3,5-bis(alkoxycarbonyl)pyridines.

In previous work [10–14], we have found significant *in vitro* reactivity of commercial and new synthesized 1,4-dihydropyridines with ABAP-derived alkyl and alkylperoxyl radicals and superoxide anion. In consequence, the compounds here studied have been synthesized and characterized in the search of compounds with enhanced pharmacological properties.

This paper reports an electron ionization mass spectrometric study of products obtained in the dihydropyridine Hantzsch synthesis using hydroxy- and methoxy-aromatic aldehydes as starting materials. Additionally, we have also analyzed some selected fragment ions using GC–MS–MS.

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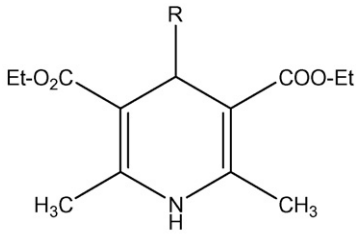
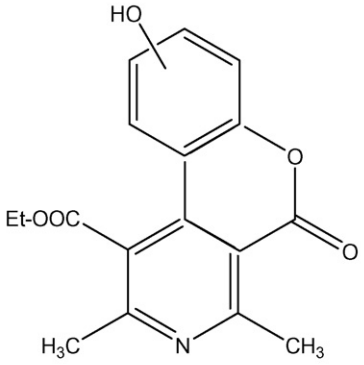


Compound I: R= 3-hydroxyphenyl
Compound II: R= 4-hydroxyphenyl
Compound III: R= 3,4-dihydroxyphenyl
Compound IV: R= 3,5-dihydroxyphenyl
Compound V: R= 4-(4-methoxy-3-hydroxyphenyl)
Compound VI: R= 4-(4-hydroxy-3-methoxyphenyl)



Compound VII: 7-hydroxy-1-ethoxycarbonyl-2,4-dimethyl-chromeno[3,4-c]-pyridine-5-one
Compound VIII: 8-hydroxy-1-ethoxycarbonyl-2,4-dimethyl-chromeno[3,4-c]-pyridine-5-one
Compound IX: 9-hydroxy-1-ethoxycarbonyl-2,4-dimethyl-chromeno[3,4-c]-pyridine-5-one

Fig. 1. Chemical structures of C4-substituted 1,4-dihydropyridines and hydroxychromeno[3,4-c]pyridines.

2. Experimental

2.1. Compounds

2.1.1. Chemistry

All the studied compounds were synthesized in our laboratory according to previously published methods [15].

As can be seen in Fig. 1, the compounds obtained are 1,4-dihydropyridines in the case of 3-, 4-, 3,4-, 3,5-, and methoxyaldehydes (compounds I–VI). However, in the case of 2-hydroxyaldehydes, surprisingly under the same experimental conditions the products corresponded to chromeno[3,4-c]pyridines (compounds VII–IX). The cyclization leading to the chromeno-pyridine moiety is due to the interaction between the OH group at 2-position of the aromatic ring and the ethoxycarbonyl group at 3-position of the dihydropyridine ring followed by oxidation of the dihydropyridine ring to pyridine leading to the corresponding chromeno-pyridine derivatives.

2.1.2. Equipments

A Gas Chromatograph/Mass Selective 6890/5973 Detector (Hewlett-Packard, Palo Alto, California, USA) and Hewlett Packard 7673 Autosampler were used for the analyses. Hewlett Packard Data System was used to control the instrumentation and data processing. The *m/z* monitored range was 43-600, 1 scan/s scan rate; normal energy electron, 70 eV.

2.1.3. Chromatography column

Hewlett-Packard Ultra-1 column, 25 m × 0.2 mm i.d. × 0.11 μm film thickness (Little Falls, Wilmington, Delaware, USA).

2.1.4. Chromatographic conditions

Detector temperature, 300 °C; injector temperature, 250 °C; split ratio, 1:10; pressure, 25 psi; purge flow, 50 ml min^{-1}; purge time, 0.5 min.

2.1.5. Temperature program

Oven temperature was programmed from 120 to 310 °C (hold for 3 min) at 17 °C min^{-1}; run time, 14 min. Helium was used as carrier gas, inlet pressure, 35 kPa.

2.1.6. Derivatization

Ethanolic solution of compounds was evaporated to dryness under a gentle helium flux and derivatized with

100 μl MSTFA (*N*-methyl-*N*-(trimethylsilyl)-trifluoracetamide, Sigma–Aldrich) by heating at 75 °C for 30 min. The derivatized extracts were worked up as usual.

2.1.7. MS–MS studies

A gas chromatographic/ion trap detector Varian 3900/Saturno 2100T GC–MS–MS and a CP-8400 Autosampler were used for the analyses. *m/z* was monitored for all the compounds in the range 100–550. Ionization mode was selected to auto EI.

Chromatography column: Varian CPsil 5CB-MScolumn 15 m × 0.25 mm i.d. film thickness.

Chromatographic condition: Injector temperature, 250 °C, pression pulse 20 psi, time pulse, 0.1 min, column flow, 0.8 ml/min, split ratio 1:10.

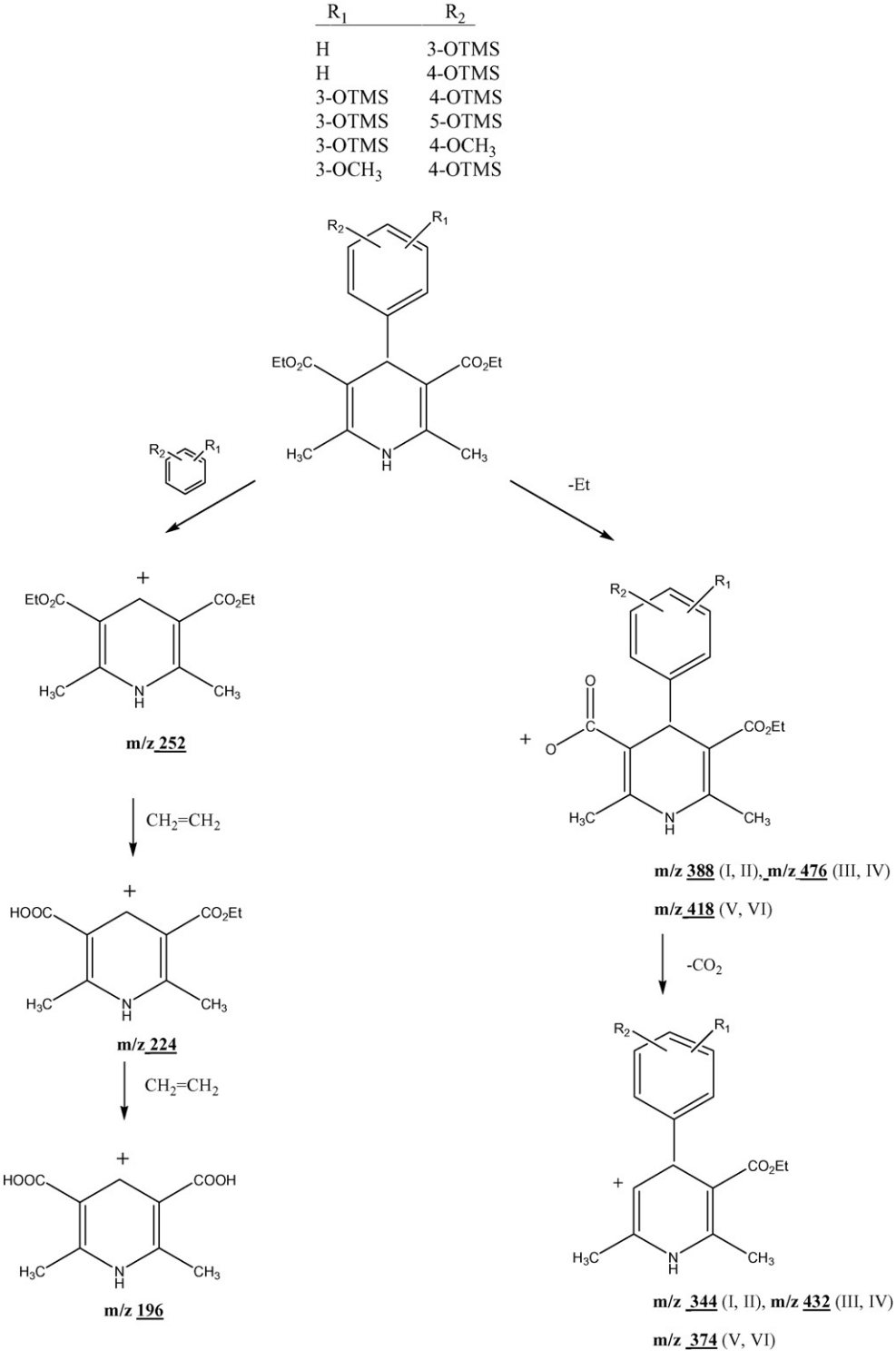


Fig. 2. Proposed fragmentation pathways of silylated compounds I–VI.

Temperature program: Oven temperature programmed from 180 to 310 °C (hold for 5 min) at 15 °C/min, run time, 12 min. Helium (99.99%, flux 0.9 ml/min) was used as carrier gas.

Equipment: temperature trap, 210 °C, temperature manifold, 50 °C, scan time, 1 s/scan, emission, 90 μA.

- Excitation storage level (ESL), *m/z*: 100–130.
- Excitation amplitude (EA, V): 0.10–0.74.

3. Results and discussion

Dihydropyridines have been classically synthesized using the "Hantzsch dihydropyridine synthesis" described in 1882. Variations using acidic medium or catalytic reagents are widely described in the literature [15]. The reports of the use of this method always described that the products of this reaction are dihydropyridine or pyridine derivatives, nevertheless, we found in our research that 2-hydroxy aromatic aldehydes give rise to chromeno[3,4-c]pyridine derivatives instead of the corresponding 4-(2-hydroxyphenyl)-1,4-dihydropyridines.

In the present work, we report a systematic study on the chromatographic behaviour of the above-mentioned products (Fig. 1). These studies were conducted by using EI GC–MS and GC–MS–MS with the silylated derivatives.

3.1. Fragmentation pathways forming fragments with a 1,4-DHP nucleus

The most abundant fragment correspond to *m/z* 252 formed directly from the molecular ion by loss of the substituent at 4-position. So, the presence of hydroxy, methoxy or any other group on the phenyl ring at 4-position does not affect the main fragmentation pathway.

The fragmentation pathways proposed for the formation of fragments containing the trimethylsilylated 1,4-dihydropyridine ring are shown in Fig. 2 (I–VI). In this Figure it can be seen that a first general pathway is the cleavage and loss of the C-4 substituents, to give rise a common mass fragment with *m/z* 252 which corresponds to the peak base with the highest abundances (100%). Retention time values (R_t) of mono-and di-sylilated compounds did not show significant differences, varying between 8.7 and 9.4 min under the experimental conditions previously described. On the other hand, the consecutive loss of ethylene produced common fragments at *m/z* 224 (6–9%) and *m/z* 196 (20–30%).

Cleavage of ethyl from the ethoxycarbonyl group at 3-position of the DHP with a total mass loss of 29 units produced fragments *m/z* 388 (I, II, 10%), *m/z* 476 (III, IV, 10%), *m/z* 418 (V, VI, 30%). For compound II we are in good agreement with

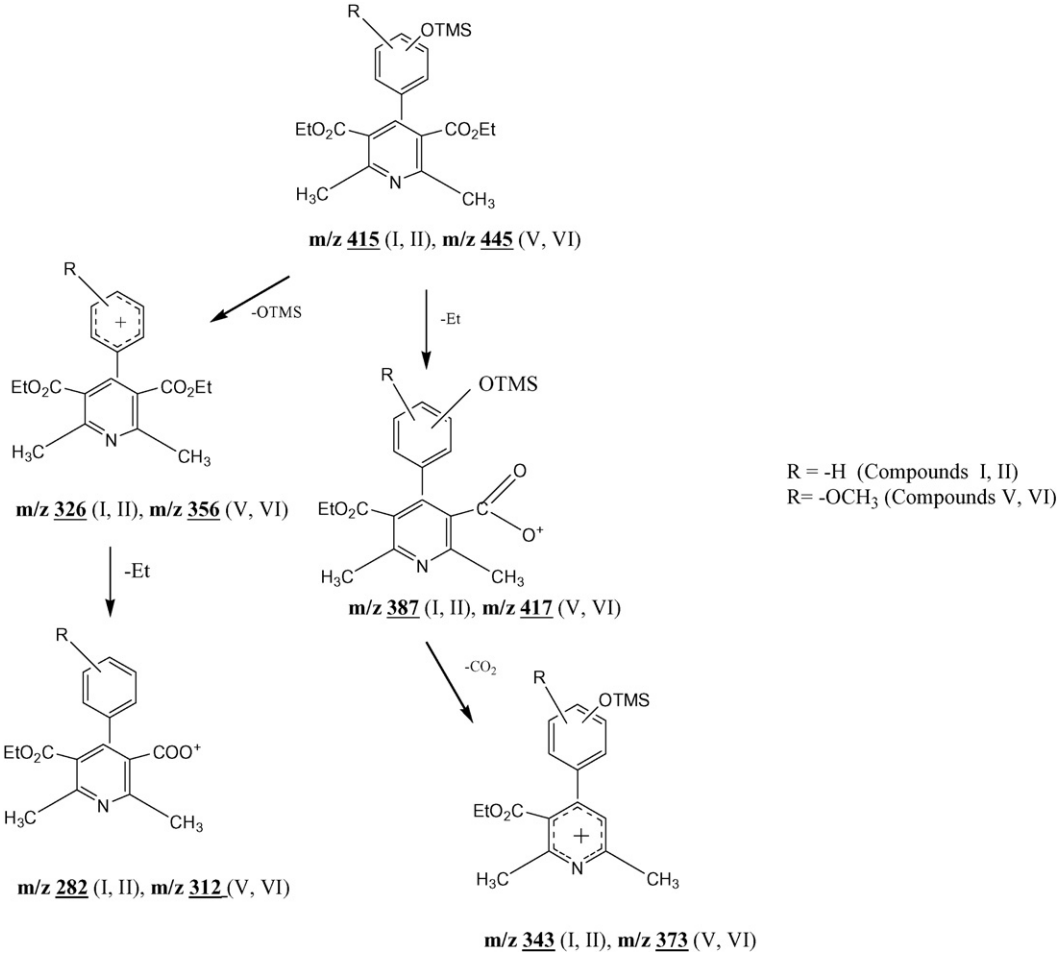


Fig. 3. Proposed fragmentation pathways for monohydroxy silylated moieties containing pyridine nucleus.

Table 1
EI-MS–MS fragmentation data for silylated compound I–IX

Derivative	M^+	Precursor ion	Relative abundance (%)	MS^{2a}	Main loss fragment
3-*O*-TMS phenyl-DHP	417	$[M-15]^+$ (403)	31	374	Ethyl
4-*O*-TMS phenyl-DHP	417	$[M-15]^+$ (403)	15	374	Ethyl
3,4-di-*O*-TMS-phenyl DHP	505	$[M-15]^+$ (491)	33	462	Ethyl
3,5-di-*O*-TMS-phenyl DHP	505	$[M-15]^+$ (491)	34	444	Ethoxy
4-(3-*O*-TMS-4-methoxy)-phenyl DHP	447	$[M-29]^+$ (418)	22	391	Ethyl
4-(4-*O*-TMS-3-methoxy)-phenyl DHP	447	$[M-29]^+$ (418)	16	390	Ethyl
7-*O*-TMS-chromeno[3,4-c]pyridine	385	M^+ (385)	5	370	Methyl
8-*O*-TMS-chromeno[3,4-c] pyridine	385	M^+ (385)	12	370	Methyl
9-*O*-TMS-chromeno[3,4-c] pyridine	385	M^+ (385)	39	370	Methyl

[a] Relative abundances of all MS^2 fragments were 100%.

the results previously obtained by Salehi et al. [6] with the only difference that we used the silylated derivative. Molecular ions (M^+) of compounds I–IV showed relative intensities varying between 4 and 7%. However, the average relative intensities for M^+ corresponding to compounds V–VI with a percentage of 30%.

3.2. Fragmentation pathways forming fragments with a pyridine ring

Retention times for these compounds varied between 7.7 and 8.6 min. A relevant characteristic of these fragmentation pathways is the preservation of the substituent at C-4 position. The fragmentation involves mainly partial or total cleavage of lateral branches. Probably, pyridine formation prevents the elimination reaction of the substituent at 4-position by stabilizing the internal charge delocalized on the whole system. In all the cases, the most abundant fragment corresponded to the molecular ion, M^+.

On the other hand, pyridines corresponding to silylated monohydroxy derivatives (I–VI) having *m/z* 415 (I and II, 100%), *m/z* 445 (V and VI, 100%) exhibit two different fragmentation pathways. The first one forms fragments with *m/z* of 326 (I and II, 30%), *m/z* 356 (V and VI, 10%), corresponding to the loss of OTMS fragment followed by loss of an ethyl fragment giving rise to *m/z* 282 (I and II, 22%), *m/z* 312 (V and VI, <5%). The second pathway with loss of ethyl for these compounds leads to fragments *m/z* 387 (I and II, <5%), *m/z* 417 (V and VI, 70%). Further loss of CO_2 produced *m/z* 343 (I and II, <5%), *m/z* 373 (V and VI, <5%). The general fragmentation pathway accounting for the ion fragments containing pyridine ring for I–VI is shown in Fig. 3.

Silylated dihydroxy derivatives (III and IV) with *m/z* 503 (100%) undergo consecutive loss of OTMS giving rise to *m/z* 414 (20%) and *m/z* 324 (80%). Loss of ethoxy group from *m/z* 414 gives *m/z* 369 (<5%). Fig. 4 shows a general fragmentation pathway for these compounds.

3.3. Fragmentation pathways for the chromeno[3,4-c]pyridines

Retention times for these compounds varied between 9.3 and 9.9 min. In chromeno derivatives exhibiting a cyclic stabilized structure, the main fragmentation pathway involved the branch at 3-position. In these compounds, M^+ was the most abundant fragment. Chromeno[3,4-c]pyridines (M^+ 385, 100%) exhibit three different fragmentation pathways leading to fragments with *m/z* 312 (5%), which is due to the cleavage of ethoxycarbonyl group, *m/z* 370 (50%) corresponding to the loss of a methyl group and *m/z* 340 (18%) corresponding to the cleavage of ethoxy group. Further loss of CO from *m/z* 340 gives rise to fragment *m/z* 312 as an alternative pathway as can be seen from Fig. 5.

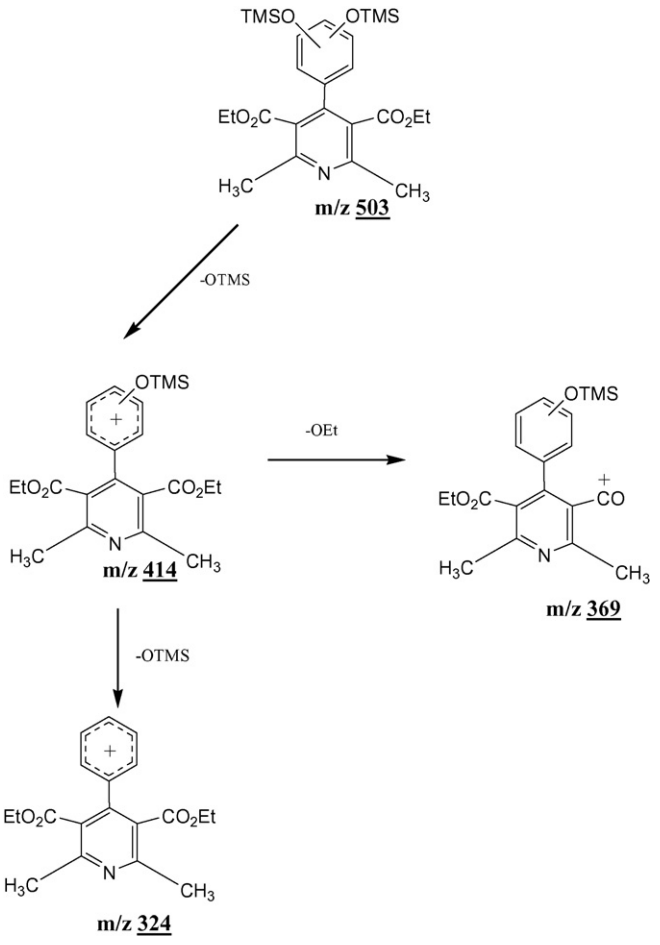


Fig. 4. Proposed fragmentation pathways for dihydroxy silylated moieties containing pyridine nucleus.

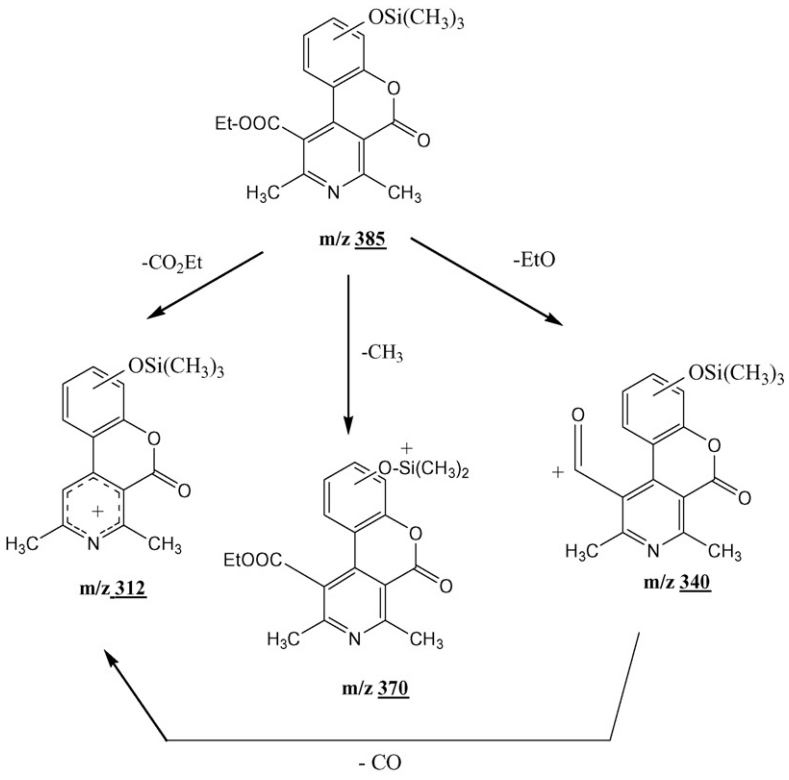


Fig. 5. Proposed fragmentation pathways of silylated compounds VII–IX.

3.4. MS–MS fragmentation analysis

We repeated the experiments using EI-MS–MS ion trap spectrometer. To accomplish the experimental work, we selected certain fragments corresponding one to one to each of the compounds I–IX. The most representative fragments were selected. Therefore, in some cases we selected the M^+ fragment (VII–IX), the $[M-15]^+$ fragment for compounds I–IV and the $[M-29]^+$ fragment for compounds V and VI. The results are shown in Table 1. Compounds having the 1,4-dihydropyridine ring experiment the cleavage of an ethyl group (I–VI) giving rise to the MS^2 ions from the precursors as shown in Table 1. In the case of the chromeno[3,4-c]-pyridine derivatives (compounds VII–IX) the MS^2 corresponded to the loss of methyl from the silyl group. Compound IV is the only one that experiments an ethoxy loss to produce the MS^2 from the precursor.

4. Conclusions

A few general conclusions on the study can be summarized as follows. The mass fragmentation pattern corresponding to compounds mono- or dihydroxy-substituted at 4-position with the dihydropyridine nucleus exhibits the same main fragment (*m/z* 252, Fig. 2). The chromeno[3,4-c]pyridine derivatives (VII–IX) show three common different fragmentation patterns. Such patterns involve loss of methyl, ethoxy and ethoxycarbonyl fragments (Fig. 4). These patterns are independent of the position of the hydroxyl group on the phenyl ring.

Finally, the present study provides (mainly through MS–MS technique) useful data for the confirmation of the structure of the compounds and also is a valuable tool for further analytical purposes to follow both photostability and reactivity studies with free radicals of this type of compounds.

Acknowledgements

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