Characteristics Of The GC-MS Mass Spectra Of Terpenoids ($C_{10}H_{16}$)

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Abstract

The terpenoids, sometimes referred to as isoprenoids, are a large and diverse class of naturally occurring organic chemicals similar to terpenes, derived from five-carbon isoprene units assembled and modified in thousands of ways. Most are multicyclic structures that differ from one another not only in functional groups, but also in their basic carbon skeletons. These lipids can be found in all classes of living things, and are the largest group of natural products. The features of mass spectra of mono-, bi- and tri-cyclic terpenoids: 1-isopropyl-4-methyl-1, 3-cyclohexadiene (I) terpilene; 1-methyl-4-(1-methylethylidene)-1-cyclohexene (II) terpinolene; 4-methyl-3-(1-methylethylidene)-1-cyclohexene (III); 1,5,5-trimethyl-3-methylene-1-cyclohexene (IV); 2,6,6-trimethylbicyclo [3.1.1]-2-heptene (V) α -pinene; 2,2,6-trimethylbicyclo [3.1.1]-2-heptene (VI) β -pinene; 2,2-dimethyl-3-methylenebicyclo [2.2.1]-heptane (VII) camphene; 1-isopropyl-4-methylbicyclo [3.1.0]-2-hexene (VIII) β -thugene; 5-isopropyl-2-methylbicyclo [3.1.0]-2-hexene (IX) α -thugene; 1-methyl-6-(1-methylidene) bicycle [3.1.0] hexane (X); 3,7,7-trimethylbicyclo [4.1.0]-3-heptene (XI) δ -3-carene; 4,7,7-trimethylbicyclo [4.1.0]-2-heptene (XII) (+)-4-carene; 3,7,7-trimethylbicyclo [4.1.0]-2-heptene (XIII) (+)-2-carene; 1,7,7-trimethyltricyclo [2.2.1.0(2.6)]-heptane (XVI) tricyclene; 1,3,3-trimethyltricyclo [2.2.1.0(2.6)]-heptane (XVII) cyclofenchene extracted from natural raw materials and comprising a number of drugs were investigated. The mass spectra for: cyclohexene-3-(tretbutyl) peroxide (XIV) and 3-isopropyl-1-cyclohexene (XV) were investigated as well. It was established that the mass spectra of these compounds are absolutely identical in mass values of peaks of fragment ions, where their relative intensities have minor differences. In the spectra of all compounds the observed characteristic ions were [M-CH $(CH_3)_2$] and [M-CH $(CH_3)_2$ –H_2]. The latter has a structure with m/z 91 that is attributed to pathing. All fragment ions correspond to even-electron cations. The identity of mass spectra of the studied tepenoids is explained by isomerization of molecular ions, similar to isomerization of terpenes in chemical transformations under the action of acids, also under the influence of other catalysts, temperature and light.

Keywords: Mass; GC-MS; Spectra; Ions; Spectrometry; Terpenoids; Oil.

1. Introduction

Currently, there is an increased interest in natural substances with valuable medicinal properties, such as terpenoids (hydrocarbon composition) and multiple C_5H_8. The terpenoids, sometimes called isoprenoids, are a large and diverse class of naturally-occurring organic chemicals similar to terpenes, derived from five-carbon isoprene units assembled and modified in thousands of ways. Most are multicyclic structures that differ from one another not only in functional groups but also in their basic carbon skeletons. These lipids can be found in all classes of living things, and are the largest group of natural products [0]. Terpenes are hydrocarbons resulting from the combination of several isoprene units.

Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions. Terpenoids contribute to the scent of eucalyptus, the flavors of cinnamon, cloves, and ginger, and the color of yellow flowers. Well-known terpenoids include citral, menthol, camphor, salvinorin A in the plant salvia divinorum, and the cannabinoids found in cannabis. The steroids and sterols in animals are biologically produced from terpenoid precursors. Sometimes terpenoids are added to proteins, e.g., to enhance their attachment to the cell membrane; this is known as isoprenylation [1]. These compounds and their derivatives also belong to other drugs as validol, bromkamfora, menovasin, turpentine etc. Turpentine is widely used for external use drugs; it is the main raw material for other products on the basis of terpenoids. The basis of turpentine are α-and β-pinene [2,3,5]. Basic research about terpenoids by various methods, including chromatography, was carried out in the early 60's late 70-ies of the last century [2-6]. A comprehensive review about terpenoids, their sources, structures, uses can be found in [7].

During the study of the terpenoid chemistry it was noted that this class of compounds is fascinating for ease of mutual conversion of compounds belonging to different subgroups. For example, the transitions of cyclic compounds are cyclic, monocyclic to bicyclic and vice versa, etc. Terpenoids have a very special chemical characteristic embedded in their highly developed ability to isomerization, which is caused by the acidic environment, high temperature, exposure to light and different catalysts. Systematic chromatographic-mass spectrometric analytical studies for the presence of drugs outside of impurity substances were conducted extensively [8-10] . Studying the regularities of mass-spectrometric behavior of the investigated class of substances is extremely difficult [11]. For this purpose, and was carried out this work.

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2. Methods

In this study we found that the mass-spectrometric decomposition, under electron impact, exhibit some features similar to the chemical transformations of terpenoids. This primarily refers to monocyclic (I-IV), bicyclic (V-XIII) and tricyclic (XVI-XVII) terpenoids. In literature and in the database of mass spectra of National Institute of Standards and Technology (NIST) [12] one can find that the mass spectral libraries show the mass spectra of large number of mono-, bi- and tricyclic terpenoids, but the mass spectra of terpenoids were not studied in details. In this study, the mass spectra of compounds were obtained by the instrument Varian 3900/2100T – quadrupole GC-MS (gas chromatography – mass spectrometry) with a source of ions as "ion trap".

3. Results and Discussion

The results shown in Tables 1 and 2 show the first feature in the mass spectra of monocyclic terpenoids I-IV, bicyclic V-VII (α-and β-pinene), bicyclic VIII-XIII, tricyclic XVI-XVII composition of $C_{10}H_{16}$ with a molecular weight of 136 kg/kg mol, as well as all other known terpenoids with the observed elemental composition that is absolutely identical to the mass values of peaks of fragment ions with minor differences in their relative intensities. Monocyclic terpenoids I-IV that have double bonds in the cycle, compared with other terpenoids, are characterized by higher relative intensities of molecular ion peaks. For I-II (50%) and III-IV (30%), accordingly, the stability of the molecular ion W_M, $W_M = I_M / \sum I$, by electron impact consists of 11% (I), 10% (II), 7% (III) and 6.2% (IV). These differences may in some cases be used to identify the presence of double bonds in the chemical structures of these compounds.

The second feature that can be seen from Tables 1 and 2 is that the main peak in the mass spectra of the terpenoid compounds is responsible for the collapse of such unusual compounds ion $[M - CH(CH_3)_2]^{+\cdot}$. This usually does not occur for compounds with fully or partially aromatic cycle of elimination from the molecular ion of alkyl radicals (CH_3, C_2H_5, $CH(CH_3)_2$, etc.) [13,14]. From our point of view in the case of mass-spectrometric decomposition identity of the mass spectra of monocyclic terpenoids (I-IV) with separation and fragmentation of molecular ion isopropyl radical, can be explained by the fact that the ionization of molecules is a complete or partial isomerization of the molecular ion. It is occurring, apparently, before the collapse of the fragments. Rudakov [15] proposed a scheme of isomerization of α-and β-pinenes in the presence of acidic catalysts, involving carbocation intermediates (Figure 1), in this figure the numbering of compounds of the sources cited is given. Figure 2 shows an example of mass-spectrometric decomposition of compound I, caused by possible isomerization under electron impact in the molecular ions of substances I-IV.

While ionization of the molecules of compound I is equally by the two double bonds, the structure of molecular ion with a positive charge on the carbon atom C-4 is preferred. In this case the methyl group at the expense of a higher + I-inductive effect effectively stabilizes the positive charge on the atom C-4, than if it was located on the atom C-1. Figure 2 shows that molecular ion $M^{+\cdot}$ is in transitional state which resulted in the formation of ions of $M_1^{+\cdot}$ and $M_2^{+\cdot}$, these are molecular ions of β-and α-pinenes, respectively. In this way, the isomerization is carried out with a breakage in the bonds C3-C4 of six-membered ring with migration of reaction-mobile hydrogen atoms of the isopropyl group at C-6 from methyl group at C-1. A similar analogue of such rearrangement ($IV \leftrightarrow V$) is shown in Figure 1. The necessary condition for isomerization transitions is that all carbon atoms must be located on the cycle-sp^3. Only in this case, the possible existence of cycles in the form of mobile shapes "armchair" or "bath" which are necessary for the formation of bicyclic structure with bridged atoms of carbon. Such conditions are satisfied by the structure of the transitional molecular ion $M^{+\cdot}$ shown in square brackets in Figure 2. From the $M^{+\cdot}$ structure as well as isomerized molecular ions of $M_1^{+\cdot}$ and $M_2^{+\cdot}$ one can easily imagine elimination of the isopropyl group, this will be shown later in other figures of compounds' decomposition. Decay of compound I from $M^{+\cdot}$ is shown in Figure 3.

Table 1: Schemes of Terpenoids.

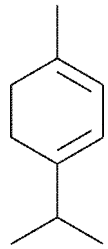
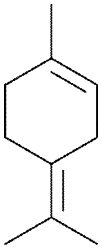
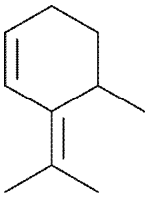
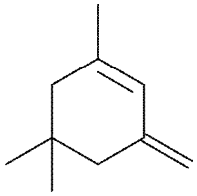
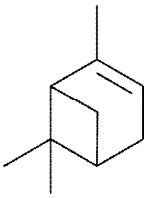
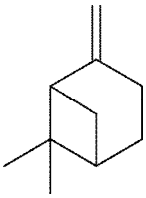
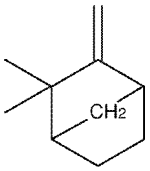
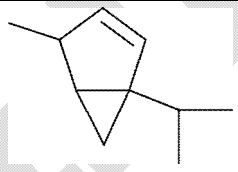
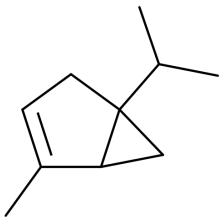
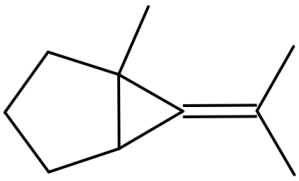
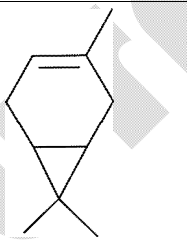
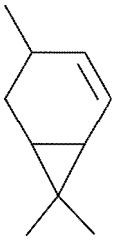
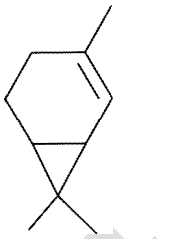
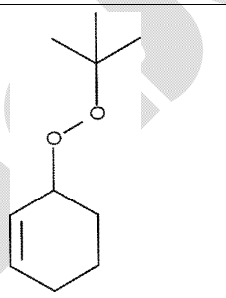
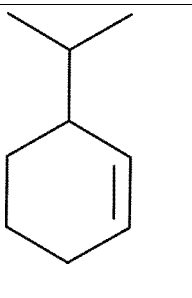
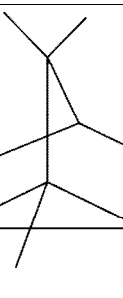
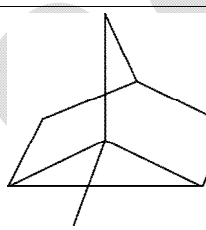
			
I	**II**	**III**	**IV**
			
V	**VI**	**VII**	**VIII**
			
IX	**X**	**XI**	**XII**
			
XIII	**XIV**	**XV**	**XVI**
			
XVII			

Table 2: Relative Intensity and m/z Values for the Terpenoid Schemes Shown in Table 1.

Compound	Values of m/z (relative intensity of ion peaks in % to maximum)
I	137 (5); 136 (43); 122 (9); 121 (100); 120 (8); 108 (4); 107 (8); 106 (4); 105 (20); 94 (7); 93 (85); 92 (12); 91 (40); 80 (5); 79 (28); 78 (6); 77 (34); 67 (5); 66 (2); 65 (10); 64 (2); 63 (5); 55 (6); 53 (8); 52 (4); 51 (12); 50 (6); 43 (20); 41 (25)
II	137 (6); 136 (61); 122 (6); 121 (78); 120 (18); 108 (5); 107 (12); 106 (5); 105 (26); 94 (8); 93 (100); 92 (18); 91 (68); 80 (6); 79 (46); 78 (8); 77 (44); 67 (12); 66 (4); 65 (12); 64 (4); 63 (12); 57 (12); 55 (14); 53 (6); 52 (6); 51 (12)
III	137 (4); 136 (38); 122 (7); 121 (78); 120 (4); 119 (7); 108 (6); 107 (8); 106 (6); 105 (28); 94 (7); 93 (100); 92 (12); 91 (45); 80 (6); 79 (49); 78 (7); 77 (40); 67 (14); 66 (4); 65 (12); 55 (12); 53 (14); 52 (6); 51 (8)
IV	137 (8); 136 (35); 122 (12); 121 (100); 119 (7); 108 (7); 107 (20); 106 (10); 105 (30); 103 (8); 94 (18); 93 (70); 92 (10); 91 (38); 81 (8); 80 (10); 79 (67); 78 (10); 77 (35); 67 (5); 55 (12); 53 (6); 55 (8); 53 (12); 52 (6); 51 (12)
V	136 (7); 121 (12); 107 (5); 106 (4); 105 (8); 94 (10); 93 (100); 92 (35); 91 (32); 80 (8); 79 (25); 78 (6); 77 (30); 68 (4); 67 (8); 65 (7); 55 (8); 53 (10); 51 (6)
VI	136 (8); 121 (10); 107 (5); 94 (12); 93 (100); 92 (14); 91 (31); 80 (12); 79 (28); 78 (12); 77 (28); 69 (35); 68 (5); 67 (12); 65 (10); 55 (6); 53 (12); 52 (6); 51 (6); 41 (61)
VII	136 (12); 122 (5); 121 (58); 103 (6); 108 (6); 107 (22); 105 (6); 95 (18); 94 (20); 93 (100); 92 (20); 91 (37); 80 (6); 79 (40); 78 (6); 77 (28); 69 (6); 68 (10); 67 (29); 66 (7); 65 (12); 55 (7); 53 (20); 52 (5); 51 (10); 41 (30)
VIII	136 (18); 121 (6); 94 (18); 93 (100); 92 (10); 91 (22); 80 (10); 79 (18); 77 (26)
IX	136 (8); 121 (6); 105 (6); 94 (10); 93 (100); 92 (40); 91 (43); 79 (12); 78 (8); 77 (46)
X	137 (10); 136 (56); 122 (10); 121 (100); 108 (8); 107 (35); 106 (10); 105 (25); 95 (10); 94 (12); 93 (81); 92 (8); 91 (35); 81 (20); 80 (18); 79 (53); 78 (10); 77 (31); 67 (25); 66 (5); 65 (10); 55 (27); 53 (20); 41 (37)
XI	136 (18); 121 (22); 119 (6); 107 (10); 105 (12); 94 (12); 93 (100); 92 (30); 91 (50); 80 (20); 79 (32); 78 (6); 77 (32)
XII	137 (6); 136 (62); 122 (10); 121 (88); 119 (6); 108 (6); 107 (12); 106 (6); 105 (20); 94 (10); 93 (100); 92 (12); 91 (37); 80 (8); 79 (34); 77 (32)
XIII	137 (10); 136 (55); 122 (10); 121 (90); 119 (8); 107 (12); 105 (18); 94 (12); 93 (100); 92 (14); 91 (40); 80 (10); 79 (39); 78 (6); 77 (32); 67 (10); 65 (8); 55 (10); 52 (10)
XIV	$M^{+\cdot}$ отсутствует. 82 (7); 81 (100); 80 (28); 79 (18); 68 (5); 57 (16); 53 (7)
XV	124 (15); 109 (12); 97 (7); 95 (6); 82 (21); 81 (100); 80 (18); 79 (26); 77 (9); 68 (6); 67 (25); 55 (14); 53 (14)
XVI	136 (18); 121 (20); 107 (6); 105 (12); 94 (10); 93 (100); 92 (28); 91 (32); 80 (10); 79 (22); 78 (12); 77 (20); 67 (8); 66 (6); 65 (7); 55 (10); 53 (12); 51 (10)
XVII	136 (18); 121 (22); 107 (8); 105 (10); 94 (12); 93 (100); 92 (25); 91 (22); 80 (10); 79 (20); 78 (4); 77 (18); 55 (10); 53 (12); 51 (6)

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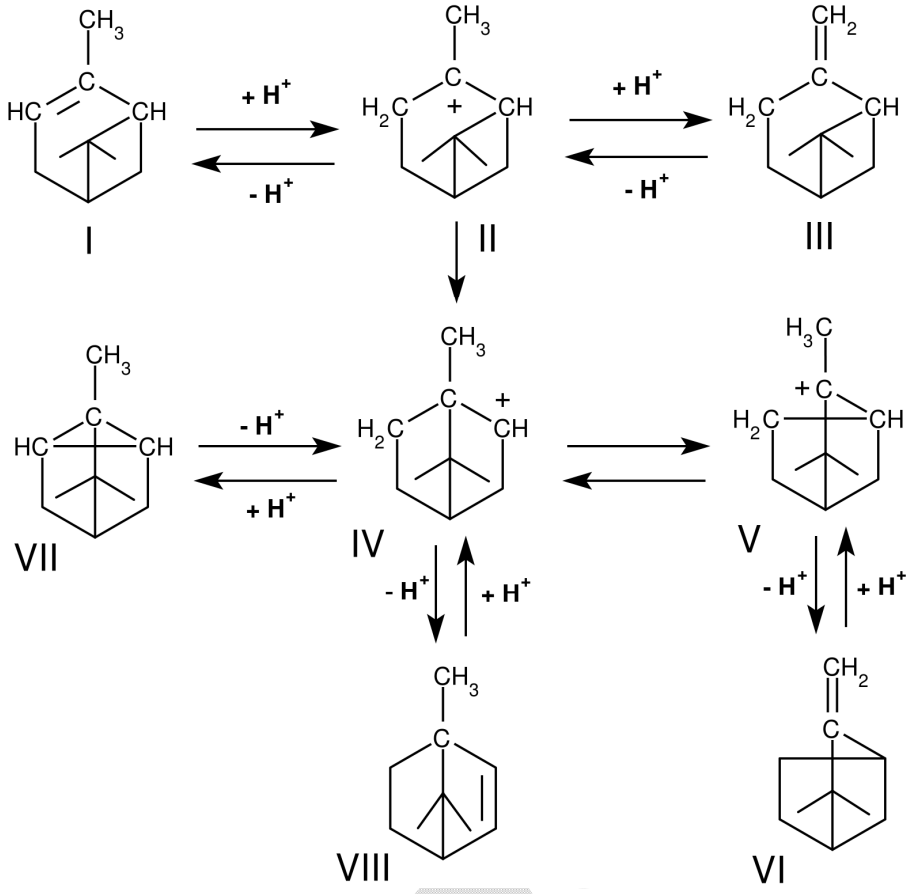

Figure 1: Isomerization of α-and β-pinenes.

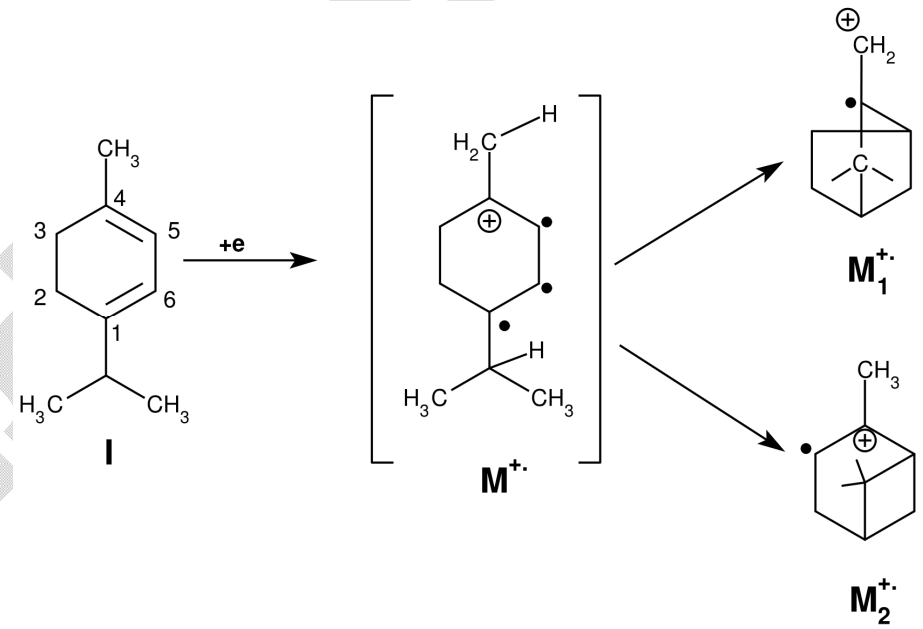

Figure 2: Transitional State of $M^{+\cdot}$.

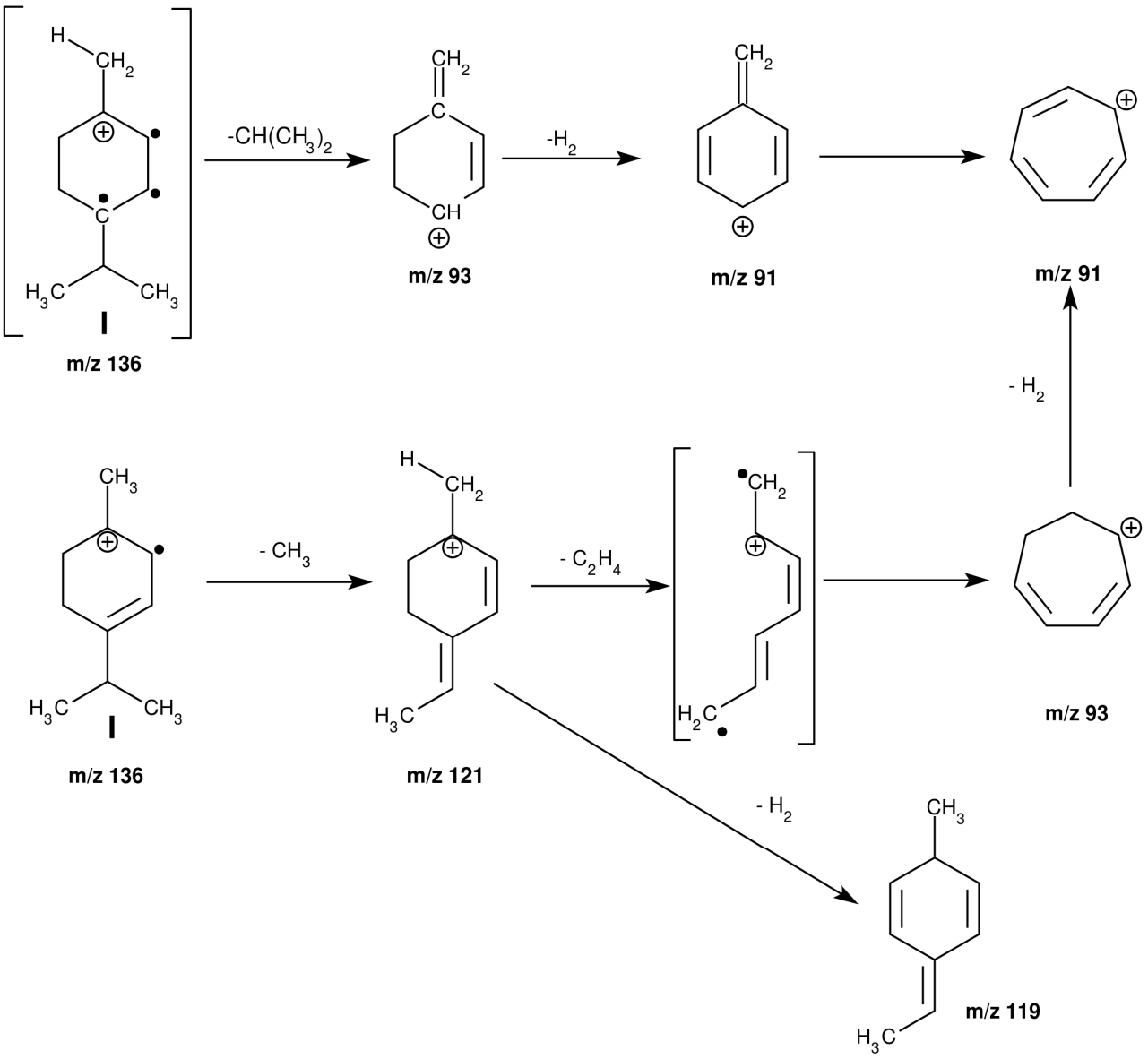

Figure 3: Decay of Compound I.

From this figure and based on the analysis of the mass spectra of all studied compounds the other reason for the formation of an ion $M^{+\cdot}[M-CH(CH_3)_2]$ with m/z 93 is the sequential separation of CH_3 and C_2H_4. For the formation of ions with m/z 93 from $M^{+\cdot}$, hydrogen ion (H^+) must migrate from the methyl group to C1. The cleavage of H_2 from this fragment results in the formation of an ion with m/z 91. It is logical to ascribe the last fragment of the structure to benzyl cation C_7H_7, the collapse of benzyl cation will be denoted, in the schemes, as an ion with m/z 91. Spectra of a- and b- pinenes (V, VI) did not differ significantly from each other but the intensities of peaks with m/z is less than 93. This is also evident in the case of compounds V-VII (Figure 4). The collapse of compounds VIII-X is shown in Figure 5.

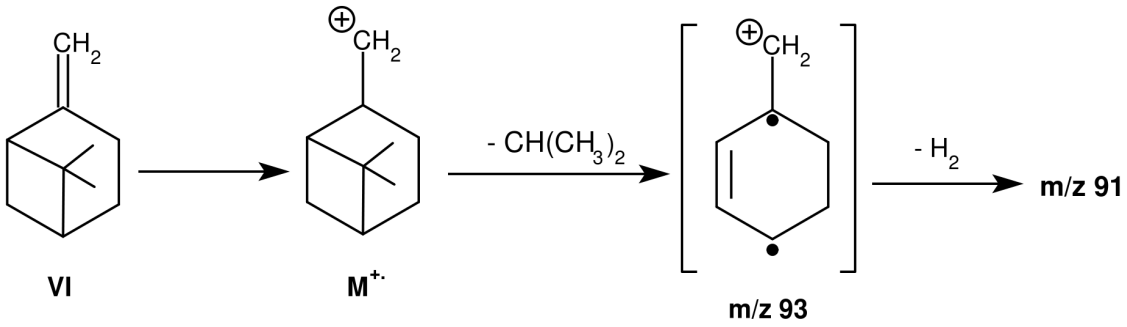

Figure 4: Collapse of Compounds V-VII.

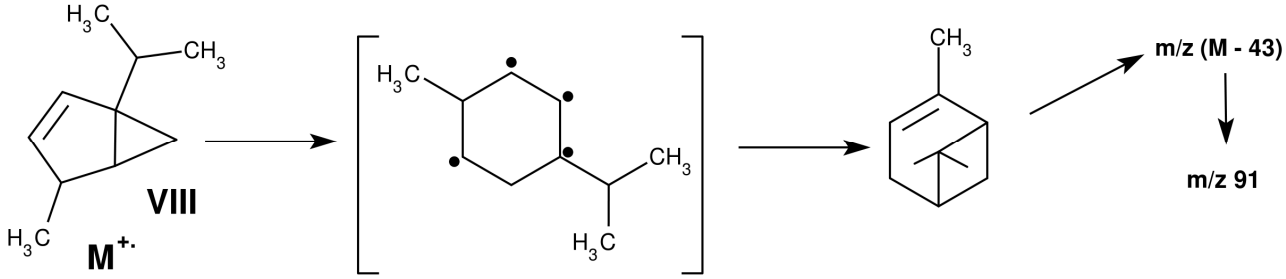

Figure 5: Collapse of Compounds VIII-X.

From the mass spectra of XI-XIII compounds, it follows that isopropyl radical is eliminated from the molecular ion. Having a gap in the form of carbene ($C(CH_3)_2$) is more beneficial as this leads to partial aromatization of the initial six valence electron structure. Hence the third feature of the mass spectra of the above compounds is that all the major intense peaks of fragment ions have odd mass with even value of the mass of the molecular ion. Thus, the fragment ions are even electron cations. In the case of elimination of carbene formed cation-radical, the appearance of even electron, in the early stages of the collapse of the molecular ion fragments, characterizes the fact that the structure of many terpenoids saturated in the spectra at low mass numbers is observed with intense peaks of fragments formed with a gap of aliphatic bonds - this is the fourth feature of mass spectra of terpenoids. In the decay of alicyclic compounds one can observe a large number of fragments with low mass values and both even and odd number of electrons [8].

Figure 6 shows the structure of the ions A and B, formed in the separation of the radical and carbene. Obviously, the cation A has lower activation energy of formation than the cation-radical of B. This hypothesis is confirmed by the mass spectra of substances XIV and XV which can be used as model compounds (Figure 7). In the spectra of these substances only a fragment of type $A^{'}$ with m/z 81 is formed where no formation of ions with smaller mass can take place, in the meantime the spectrum has intense (28%) peak ion $(A^{'} - H_2)^{+}$ with m/z 79.

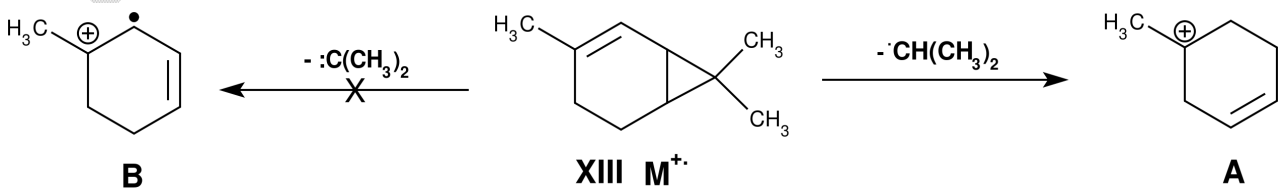

Figure 6: Structure of A and B ions.

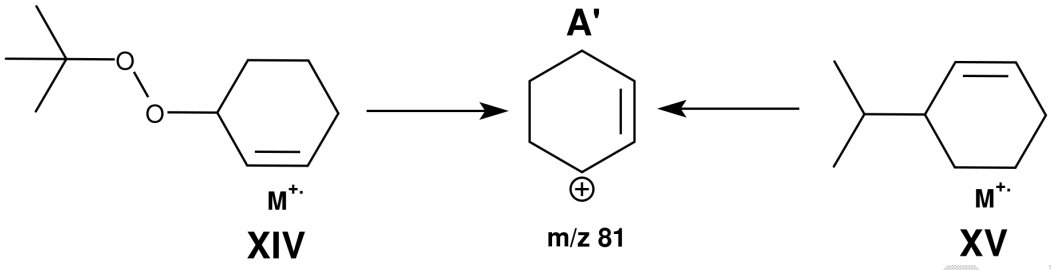

Figure 7: Model Compound XIV and XV.

Mass spectra of structural isomers-tricyclene XVI-XVII are also characterized by the same values of fragment masses, which are practically identical. Their identity is due to rupture of the most intense connection C1-C7 or C4-C7 (or C2-C6). Chemical isomerization of tricyclene XVI and XVII, according to Rudakov [6], proceeds following the mechanism shown in Figure 8.

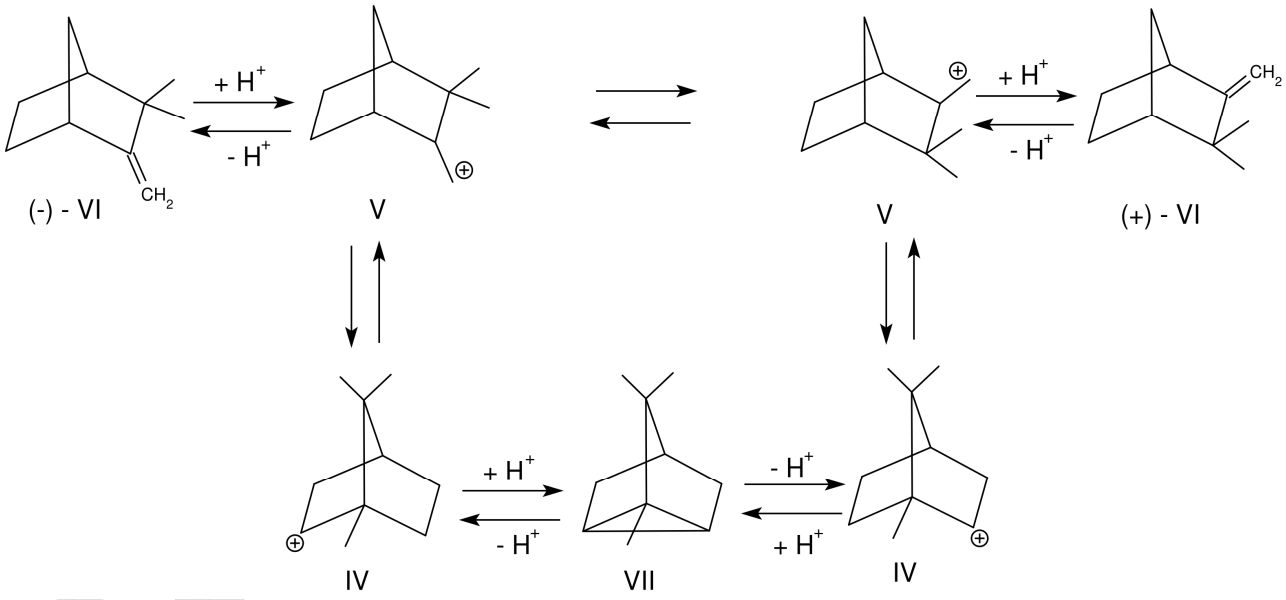

Figure 8: Chemical Isomerization of Tricyclene XVI and XVII.

Figure 9 shows the parallel and series reaction of the chemical transformations of the mass spectra of two structural isomers of tricyclene isomerization. The characteristics behavior, under electron impact of compounds XVI-XVII, can be explained by fragmentation of the open molecular ion formed by the breakage the most mobile bonds between atoms C1-C7 or C4-C7 followed by migration of one hydrogen atom and the structural rearrangement.

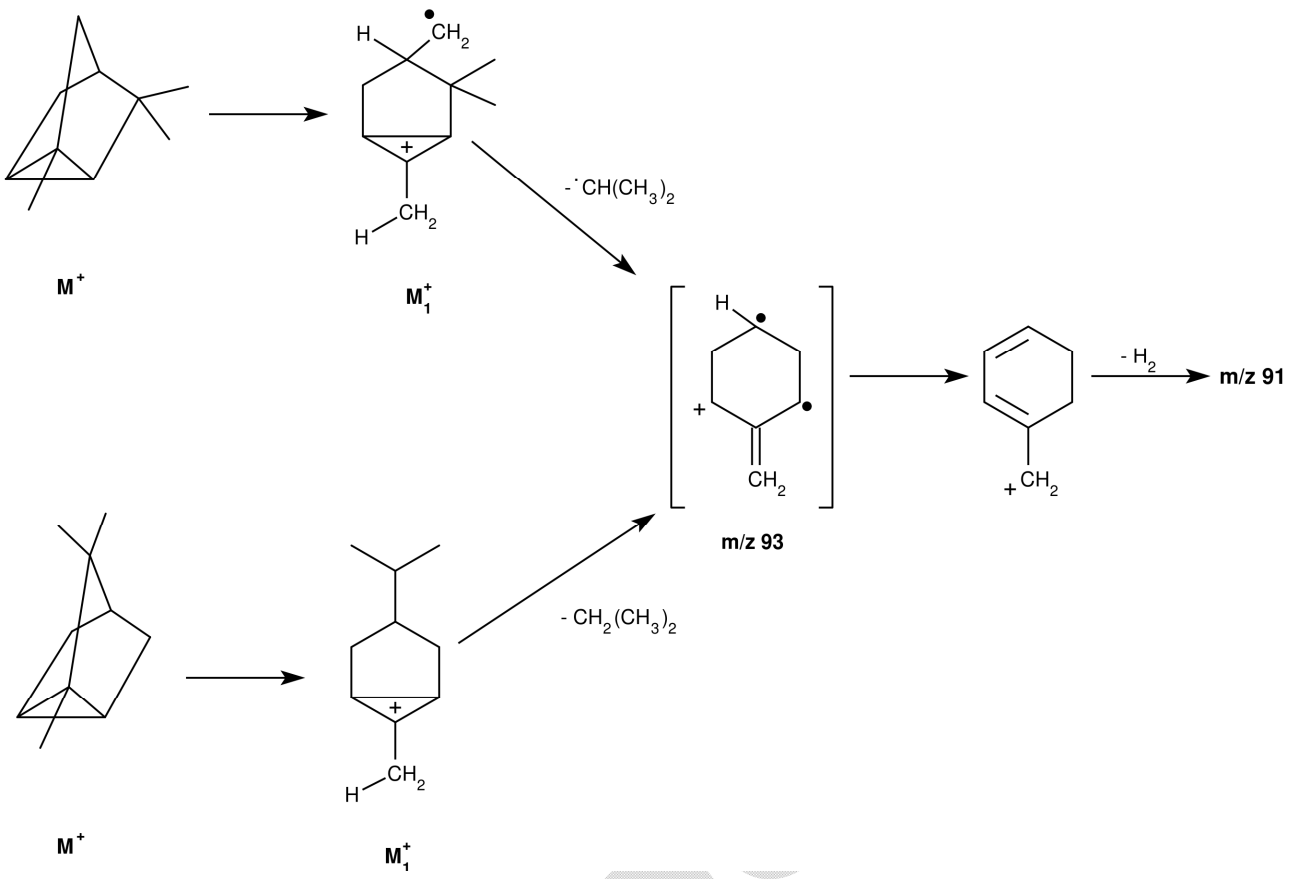

Figure 9: Chemical Transformations of the Mass Spectra of Two Structural Isomers of Tricyclene Isomerization.

4. Conclusion

This study presented the first efforts in evaluating a vast number of terpenoid mass spectra obtained by GC-MS in details. The following conclusions can be drawn from the conducted work:

1. During the gas-phase mass spectrometric reactions, occurring under electron impact, isomerization processes occur on the positively charged molecular ions terpenoids. The occurred isomerization processes are more or less analogous to the chemical processes in condensed matter.
2. Isomerization processes are most evident in the decay of monocyclic terpenoids such as 1,3-cyclohexadene and cyclohexene (compounds I-IV), bicyclic compounds such as bicyclo [3.1.0]-2-hexenenes (compounds VIII-X) and bicyclo [4.1.0]-2-heptenes (compounds XI-XIII). Features of their decay were explained based on the isomerization of the molecular ion.
3. Fragmentation of tricyclo [2.2.1.0 (2,6)] heptanes (compounds XVI-XVII) was explained based on an open form of the molecular ion formed by breaking the cycle and having the most intense C-C bonds. Apparently, isomerization processes in molecular ions occur for these compounds. Regrouping processes was so helpful in explaining the fragmentation of these compounds.

5. Competing Interests

The authors declare that they have no competing interests.

6. Authors' Contributions

AY developed and supervised the research, analyzed the data; AK prepared the manuscript and performed data analysis; HQ contributed towards manuscript preparation; RA and YK conducted the experimental work, studied different samples, operated the GC-MS and helped with spectral analysis.

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