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Features of the Chromatography-Mass Spectrometric Identification of Condensation Products of the Carbonyl Compounds

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Abstract—By the example of the condensation products of acetone with the simplest aromatic carbonyl compounds it was shown that the joint interpretation of mass spectrometric and chromatographic data in conjunction with a priori assumptions about the nature of the chemical structure allows the detection of the components of reaction mixtures that were not previously characterized by standard mass spectra or by retention indices on standard stationary phases. A necessary condition for solving such problems is creating a hypothesis about the sample composition that is possible for the expected products of the known organic reactions.

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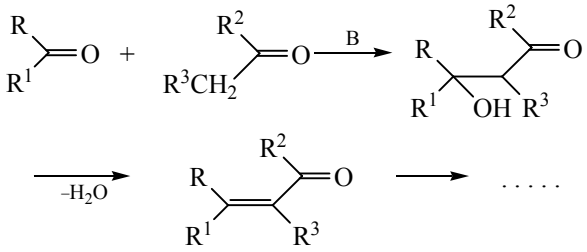
The carbonyl fragment is among the most common functional groups in the composition of organic compounds. The C=O group reactivity is sufficiently high, especially with respect to nucleophilic reagents. [1]. For this reason, for example, the derivatization of carbonyl compounds for gas chromatographic analysis [1] consists not in the transformation of analytes in more volatile derivatives [2]. but in their conversion into less reactive products that are more stable in mixtures with other substances. Both in alkaline and in acidic media carbonyl compounds readily enter in the intermolecular condensation reactions [2]. For example, the base-catalyzed aldol condensation followed by dehydration of the intermediate products leads to α,β-unsaturated carbonyl compounds:



Despite the fact that such products may be present and found in many reaction mixtures, the possibility of their gas chromatography-mass spectrometric identification still is very limited. This is due to the formation (especially in the case of aliphatic aldehydes and ketones) of a large number of the products of more "profound" (oligomeric) condensation, whose molecules, moreover, may be capable of cyclization, migration of C=C double bonds, and (*Z*,*E*)-isomerism. For compounds with different R and $R^1 \neq R'$ the number of potential products increases even more due to the lack of regioselectivity of the reaction. The main difficulty consists in the lack of the reference data for the most of the condensation products necessary for the interpretation of the results of gas chromatography-mass spectrometry analysis, namely, the usual electron impact mass spectra and gas chromatography retention indices (*RI*) on the standard (especially non-polar) stationary phases.

To illustrate the current state of information concerning the condensation products of even the simplest carbonyl compounds, it is interesting to compare the data for the acetone [reaction (1)] and 2-butanone [reaction (2)] oligomers, corresponding to the following sequence of molecular formula and mass numbers (in parentheses):

$$C_3H_6O\ (58) \rightarrow C_6H_{10}O\ (98) \rightarrow C_9H_{14}O\ (138) \rightarrow C_{12}H_{18}O\ (178) \rightarrow \ldots, \quad (1)$$

$$C_4H_8O\ (72) \rightarrow C_8H_{14}O\ (126) \rightarrow C_{12}H_{20}O\ (180) \rightarrow \ldots\ . \quad (2)$$

One of the most detailed database of mass spectrometric and chromatographic data NIST/EPA/NIH[3] [3] includes both the mass spectrum and no less than 12 references to original sources of the *RI* values for the 4-methyl-3-penten-2-one (mesityl oxide), the primary condensation product of acetone. Inasmuch as the database [3] is in the process of forming, the number of sources of retention indices for each compound is continuously growing. As the main products formed in the next stage of condensation can be assumed to be 2,6-dimethyl-2,5-heptadien-4-one (phorone, **I**), 4,6-dimethyl-3,5-heptadien-2-one (**II**), and 3,5,5-trimethyl-2-cyclohexen-1-one (α-isophorone, **III**). Of these isomers, the mass spectra are known only for compounds **I** and **III**, and the *RI*, according to the publications [4–16], only for α-isophorone **III** (the values vary in the range 1074–1099 with an average value of 1089±7). The reason for such strong discrepancy in the number of data for different isomers is that α-isophorone is one of the components forming the odor of many foods and, therefore, was described in detail. For the possible compounds of the same composition, $C_{12}H_{18}O$ (M = 178) (and also for more complex ones) the currently available information is limited to the mass spectrum of a single isomer, 1-(3,5,5-trimethyl-2-cyclohexenylidene)acetone (**IV**) [3]. It is noteworthy that for all compounds in the database [3] the *RI* estimates using the classical incremental additive scheme are available [17] whose accuracy is unfortunately low.

Even more spectacular is the poor state of information for the condensation products of 2-butanone [reaction (2)]. The expected primary products of its condensation ($C_8H_{14}O$, M = 126) are 5-methyl-4-heptyl-3-one (**V**) and 3,4-dimethyl-3-hexen-2-one (**VI**); each one exists in the form of (*Z*)- and (*E*)-isomers that has been characterized by the mass spectra, which, naturally, for each pair of isomers should be virtually identical. The database [3] includes an alternative mass spectrum of compound **V** which does not coincide with the first one (in one of them the maximum peak is at *m*/*z* 55, while in another its intensity is negligible). For the same compound **V** the value of *RI* (1007) is know [10] attributed to the isomer with an unknown geometry, but even in this case this value appears to be erroneous (too high). Thus, when solving a real problem of identification it is necessary to consider also the problem of the possible unreliability of reference data and the necessity of their checking and refinement. For this purpose the information from independent sources fits best, but when such information is inavailable (as commonly occurs), it is necessary to use estimates based on the data for the simpler and therefore more reliably characterized structural analogs.

As another illustrative example of the mentioned complexity of the representation of chromatographic data in modern databases (by the example of [3]), we may consider a relatively simple compound such as 3-penten-2-one. Only one value of *RI* = 712 is known, which is uniquely attributed to (*E*)-isomer of the ketone [18]. For the corresponding (*Z*)-isomer are known two *RI* values not coinciding with each other (711 [19] and 652 [20]). In addition, the *RI* values for this ketone falling to the range 697–755 [without reffering to (*E*)-and (*Z*)-isomers] are given in other 15 publications. Such data are clearly insufficient, not only for an unambiguous conclusion about the value of *RI* of (*Z*)-3-penten-2-one, but even about the order of the isomers elution.

Thus, the essential features of chromatographic-mass spectrometric identification of the condensation products of carbonyl compounds are as follows:

(1) Informativity of the mass spectra of this class of compounds is limited, and for many isomers the spectra are virtually identical, but for most products they are unknown;

(2) For the identification at the limited informativity of mass spectrometry data it is necessary to use chromatographic retention indices, but for most compounds of this group they are also unknown;

(3) In the absence of reference values of the indices their estimates by various methods should be used, including simple additive schemes;

(4) The necessity to obtain such estimates involves the formation of *a priori* assumptions about the structure of the condensation products.

The present communication discusses the features of chromatography-mass spectrometric identification of previously characterized condensation products of acetone (as one of the simplest representatives of aliphatic carbonyl compounds and one of the most common organic solvents) and the simplest aromatic carbonyl compounds, based on joint interpretation of their mass spectra and chromatographic retention parameters on standard nonpolar phases.

Among the methods of identification used in modern practice of gas chromatography-mass spectro-

metry the most popular is the comparison of mass spectra of the analytes with reference data. The basis for the effectiveness of this approach is the availability of sufficiently detailed database of mass spectra. So, the latest version of one of the most well-known database of the National Institute of Standards and Technology (NIST, USA, 2008) contains 220460 standard mass spectra of 192108 compounds [3]. Such a library search does not exclude the methods of interpretation based on known patterns of fragmentation of organic compounds, but they are significantly less used. Since 2005, the base [3] was supplemented by chromatographic retention indices (*RI*) on standard nonpolar and polar phases. The version of 2008 included 293247 *RI* values for 44008 compounds, whose use in conjunction with the mass spectra should enhance the reliability of the identification results.

However, despite the existence of such information, the solution for a sufficiently large number of real analytical problems concerns the compounds that have not been characterized by mass spectra or chromatographic retention parameters. When a direct comparison of experimental and reference data is impossible, such problem is more in line with the structure elucidation and can be very complicated. An important group is the characterization of the products of known chemical reactions when the solution is simplified due to the information not only on the chemical nature of the initial substrates and reagents, but also on the mechanism of the process. When preparative isolation of individual products from complex reaction mixture is impossible, then the main way of identifying them is just the use of the chromatography-mass spectral method.

The interpretation of combined mass spectrometric and chromatographic data for the identification of previously unknown compounds can be carried out in various ways, but below we discuss only those proved to be the most effective for these compounds. The products of chemical reactions may be rigidly defined by the nature of the initial substrates and mechanisms of the processes. In such cases, it is almost possible to predict their structures. This feature is widely used in the practice of derivatization of target analytes, where the preparative isolation of the reaction products is impossible and the reactions themselves are considered as a way to prove the structure of the products formed [1, 21].

The identification of a small number of products (2–3) in the simplest cases may not require precise estimates of *RI*, since it is enough to predict the relative order of chromatographic elution. To solve these problems, several algorithms are known, based for instance on an evaluation of the intramolecular vibrational and rotational energies by the method of the molecular dynamics [22]. Another method involves obtaining estimates of normal boiling points (T_b) using the ACD software, since the order of elution of isomers in nonpolar phases unambiguously corresponds to an increase in T_b [32]. In a general case the estimation of *RI* should be carried out using, for instance, an additive scheme.

The most convenient for the evaluation of chromatographic *RI* values turned to be not the traditional methods of calculation involving the use of a set of the retention indices increments (ΔRI) or their modified versions. The operations of computing the ΔRI values and their subsequent application can be combined, which is equivalent to assembling the target structures from simpler molecules by their addition and subtraction. This method assumes selection of the maximally close structural analog of target compounds and combining them with the molecules containing the missing structural fragments and subtracting the superposition of overlapping elements.

This algorithm corresponds to the following general scheme. If for the formation of the target structure ABCD are got the predecessors ABC and BCD, so that ABC +BCD −BC ABC + BCD – BC → ABCD, then $RI \approx$ (ABXΔ) RI(ABC) + RI(BCD) – RI(BC).

In other words, similar fragments of the molecular structures of selected precursors should be cut off to provide the desired stoichiometry of the operation. The estimates of standard deviations of calculation results (s_{RI}) based on the standard deviations of the source data can be obtained using the well-known relation:

$$s_{RI(\mathrm{ABCD})} \approx [s^2_{RI(\mathrm{ABC})} + s^2_{RI(\mathrm{BCD})} + s^2_{RI(\mathrm{BC})}]^{1/2}. \quad (3)$$

The main advantages of this method of the *RI* estimation, besides eliminating the necessity to precalculate the increments, is its structural clarity and the possibility of varying the chosen analogs, ways of the assembly of the target structures, and the direct use of the reference *RI* values. This approach was first proposed and used to evaluate the *RI* values of 839 congeners of polychlorinated hydroxybiphenyls [42], expanded over 211 structural isomers of nonylphenol [52] and the products of free radical chlorination of cyclohexane [62], which in the case of $n_{Cl} \geq 2$ included

not only the structural isomers, but also diastereomers. At the optimal choice the scheme of the formation of the target structure the relatively high accuracy of the estimates is confirmed in all cases. The maximum accuracy of the *RI* estimates is achieved when the precursor molecules comprise all the features of the structures of the target compounds that affect their gas chromatography retention parameters.

The principal stages of the interpretation of the results of gas chromatography-mass spectrometry analysis of previously uncharacterized products of known reactions can be formalized as follows:

(1)) The estimation of the potential number and nature of the possible products of the considered reactions or mixtures of natural components (the formulation of the hypothesis about the composition of the analyzed samples);

(2) Checking in the databases of mass-spectrometric and chromatographic reference data for the presence of compounds with the intended chemical nature. In the event of insufficient information go to step 4;

(3) The identification of the components of the samples using known algorithms of the mass spectrometric library search. In the case of incertainty of the answers go to step 5;

(4) The identification of the unidentified components and isomers with indistinguishable mass spectra;

(5) Depending on the complexity of the samples, the calculation of retention indices of the assumed products or estimating the order of elution (for isomers);

(6) Joint interpretation of mass spectrometric and chromatographic data for compounds at the lack of the reference values of considered analytical parameters.

The sequence of these stages can be varied, but the final step anyway is comprising the information in a single logically consistent answer. It is not surprising finally that the most difficult and time-consuming step in the chromatography-mass spectrometry analysis are not instrumental operations, but the interpretation of the results.

To detail these general statesments it is reasonable to illustrate specific examples of identification of the condensation products of acetone and a few simple aromatic carbonyl compounds, arranged by the increasing complexity of the resulting reaction mixture.

Example 1. Establishing the structure of two products of *acetone* and *acetophenone* condensation (1:1), with the molecular formula $C_3H_6O + C_8H_8O – H_2O = C_{11}H_{12}O$, and the mass 58 + 120 – 18 = 160, for which the obtained *RI* values are 1362 and 1396. Other possible condensation products (acetone + acetone and acetophenone + acetophenone) can be excluded from consideration by their molecular weights that are not consistent with the obtained number, and by the chromatographic parameters. For example, the *RI* of 1,3-diphenyl-2-butene-1-one ($C_{16}H_{14}O$, dipnone) is much higher (1970±6), a component with this *RI* value is not found. The number of theoretically expected products of different structure in this case is equal to two: (*Z*,*E*)-isomers of 4-phenyl-3-penten-2-one **VII** and 3-methyl-1-phenyl-2-butene-1-one **VIII**, that is, corresponds to the number of detected components. Table 1 shows the experimental spectra of components of the reaction mixture, and also indicates the presence or absence of the mass spectra of the expected products in the database [3]. The probable cause of the discrepancy of mass spectrum of compound **VIII** [3] with the spectra of both components could be that the former was recorded for the mixture of the products isolated preparatively from the reaction mixture. Thus, the standard use of reference mass spectra cannot only lead to the failure at the identification of the analytes, but also can mislead the researcher. Attempts to interpret the mass spectra on the basis of general patterns of fragmentation [272] for conjugate structures are complicated by the possibility of skeletal rearrangements of the molecular ions. For example, the presence in the spectrum of the signal $(m/z)^{100} = 105$ can be explained both by the presence in the molecule of benzoyl fragment **VIII**, and the possibility of 1,3-migration of phenyl group **VII**, and therefore cannot be interpreted unambiguously. Most informative signal in the spectrum of compound **VII** is the peak at *m*/*z* 83 [$M – C_6H_5$] corresponding to the acyl fragment $(CH_3)_2C{=}CHCO$, but for a choice of one structure among two alternative structures it is not sufficient to consider a signal of only one mass spectrum.

Formally, for the identification of only two known products of a known reaction it is enough to predict the order of their chromatographic elution. However, in this example, the use of molecular dynamics methods [22] is undesirable, as they insatisfactorily describe molecules with isomeric conjugation systems. In such cases it is more correct to obtain estimates of normal boiling points (T_b) using the ACD software [23]. For

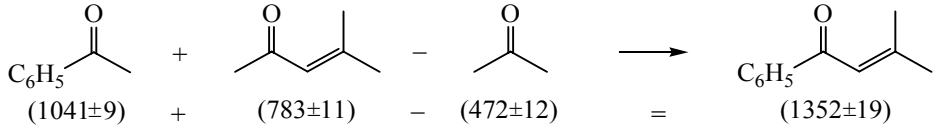
Table 1. Mass spectra and retention indices of the condensation products of acetone and acetophenone (to *Example 1*)

Mass spectrum [$m/z \geq 50$ ($I_{rel} \geq 3\%$)]	RI_{exp}	Existence in the database [3]	RI_{calc}
3-Methyl-1-phenyl-2-butene-1-one (**VII**, 71%)[a]			
161(7), 160(64) $[M]^+$, 159(100) [M – H], 146(9), 145(97), 144(17), 142(10), 141(21), 132(5), 131(21), 130(5), 128(9), 127(17), 120(5), 118(4), 117(29), 116(7), 115(29), 106(4), 105(46), 103(4), 91(11), 89(5), 84(5), 83(63) [$M - C_6H_5$], 82(5), 79(4), 78(3), 77(77), 76(7), 75(6), 74(6), 70(3), 67(4), 65(9), 63(8), 61(3), 57(5), 55(63), 54(9), 53(21), 52(6), 51(58), 50(22)	1362	–	1352±9
4-Phenyl-3-penten-2-one (**VIII**, 29%)			
160(4) $[M]^+$, 159(4), 131(3), 120(20), 106(9), 105(100) [C_6H_5CO], 91(5), 78(9), 77(37), 65(3), 63(3), 59(12), 58(4), 56(5), 53(3), 51(15), 50(7)	1396	+[b]	1378±11(*E*) 1393±14(*Z*)

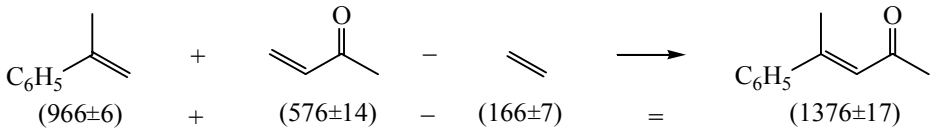
[a] Here and hereinafter (in Tables 2 and 3) after the number of compound is shown in parentheses the estimation of its relative content in the reaction mixture. [b] The mass spectrum of 4-phenyl-3-penten-2-one (**VIII**) in the database [3] [160 (79) $[M]^+$ 159 (100) 145 (36), 117 (95), 116 (20), 115 (71), 91 (25), 55 (15), 43 (41), 39 (12)] differs significantly from that given here and, therefore, cannot be used for identification (see comments in the text).

isomers **VII** and **VIII** we obtain 263.9±10 and 243.2±13°C, respectively, therefore, 3-methyl-1-phenyl-2-butene-1-one **VIII** should be eluted first with the RI = 1362. The ACD software cannot differentiate (*Z*)- and (*E*)-isomers, so the geometry of the second component, 4-phenyl-3-penten-2-one **VII**, remains uncertain in this method of data interpretation.

If the objective is to obtain the absolute rather than relative retention characteristics, it is necessary to estimate the RI value using additive schemes. For 3-methyl-1-phenyl-2-butene-1-one **VIII** at least two ways of assembling the target structure of the molecules can be offered characterized by the RI values of the simpler structural analogues:



Averaging the results gives an estimate 1352±9, which agrees with the RI of the first isomer (1362). For the second potential product 4-phenyl-3-penten-2-one **VII** the similar pattern of assembly is as follows:

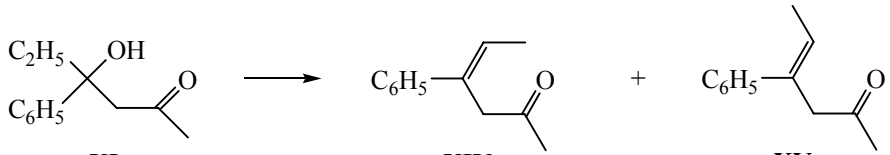


The average value of the estimates *RI* (1383±9) corresponds to the experimental value of the second component (1396). However, based on these additive estimates it is hardly possible to say that for the second product the structure of (*Z*)-isomer is more likely and, therefore, the question of its stereochemistry in this case is better to leave unspecified.

The summary of results is presented in Table 1. From the consideration of even this single example a simple rule follows regarding the order of chromatographic elution of isomeric condensation products of alkylaromatic (ArCOR) and aliphatic (RCOR') carbonyl compounds. The minimum retention parameters of the two structural isomers have the products obtained at the interaction of the carbonyl group RCOR' and methylene fragment in the ArCOR molecule. If this pattern is correct, then it should be observed for the condensation products and other carbonyl compounds that can be used for its verification. For example, acetaldehyde and acetophenone are precursors of 1-phenyl-2-butene-1-one (**IX**) and 3-phenyl-2-butenal (**X**), with the *RI* 1192 (experimental value) and ~1265 (estimate from database [3] by scheme [17]), respectively, which is consistent with the proposed rule and allows us to simplify the interpretation of results in all such cases.

The following example relates to a similar reaction, but the number of isomeric products increases to four.

*Example 2***.** Establishing structures of four isomeric acetone and propiophenone condensation products (1:1) with the molecular formula $C_3H_6O + C_9H_{10}O - H_2O = C_{12}H_{14}O$, and the mass 58 + 134 – 18 = 174 , *RI* are 1383, 1414, 1435, and 1452 (Table 2). Two of them, with $(m/z)^{100}$ = 173 [*M* – H] (nos. 2 and 3 in the order of elution) are the homologs of two natural α,β-unsaturated carbonyl compounds considered in the preceeding example, namely, 3,4-dimethyl-1-phenyl-2-buten-1-one (**XII**) and (*Z*,*E*)-isomers of 4-phenyl-3-hexen-2-one (**XIII**). The other two (nos. 1 and 4) are characterized by the similar abnormal mass spectra, $(m/z)^{100}$ = 43 [CH_3CO] that differ significantly from those of the first pair of isomers. The lower intensity signals of their molecular ions points to shorter conjugation systems. It may be assumed that these two anomalous products result from the alternative way of water cleavage from the ketoalcohol **XI** formed intermediately, and they are (*E*)- and (*Z*)-isomers of 4-phenyl-4-hexen-2-one (**XIV**, **XV**). The presence in their molecules of unconjugated CH_3CO fragments results in the predominance in the mass spectra of the signals $(m/z)^{100}$ = 43.



XI **XIV** (*E*)-isomer **XV** (*Z*)-isomer

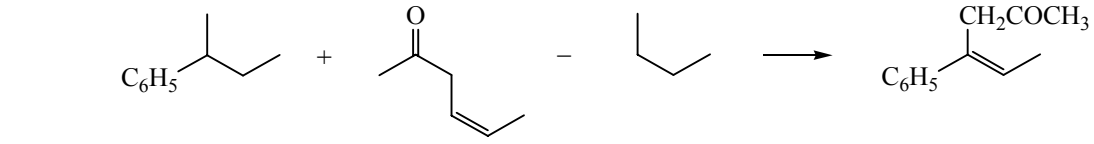
Currently there are no methods of the theoretical prediction of the order of chromatographic elution of π-diastereomers, but the considered additive scheme allows the estimation of *RI* of such structures. However, the seemingly natural simplest version of assembling the target structure starting from (1-methyl-1-propenyl)-benzene (*E*)- and (*Z*)-isomers and acetone is unacceptable because it leads to highly overestimated *RI* values:

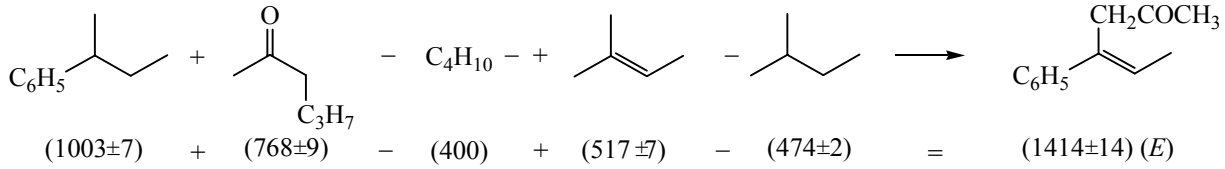
The elucidation of the reasons of this discrepancy shows that the presence in the molecules **XIV** and **XV** of the bulky acetonyl group at the double C=C bond is important because it leads to a violation of its conjugation with the phenyl fragment. The optimization of the geometry of the isomers **XIV** and **XV** by the MM+ method gives an estimate of the dihedral angles θ[C^5–C^4–C(Ar)^1–C(Ar)^2] ~60° (*E*-isomer) and 71° (*Z*-isomer), which corresponds to a decrease in the degree of conjugation of the fragments Ph and C=C (proportional to $\cos^2\theta$) from the maximal value of unity to 0.25 and 0.11, respectively. Consequently,

Table 2. Mass spectra, retention indices and evaluation of normal boiling points of the condensation products of acetone and propiophenone (to *Example 2*)

Mass spectrum [$m/z \geq 40$ ($I \geq 3\%$)]	RI_{exp}	Existence in database [3]	RI_{calc} or T_b
(*E*)-4-phenyl-4-hexen-2-one (**XIV**, 17–23%)			
175(1), 174(12) $[M]^+$, 159(13), 132(4), 131(17), 129(5), 128(4), 117(7), 116(5), 115(8), 91(18), 77(4), 65(3), 53(3), 51(4), 43(100) [CH_3CO]	1383	–	1395±13
2,3-Dimethyl-1-phenyl-2-butene-1-one (**XII**, 8–9%)			
175(8), 174(69) $[M]^+$, 173(100), 160(7), 159(57), 158(4), 155(2), 153(3), 146(4), 145(29), 144 (11), 141(10), 132(3), 131(22), 130(4), 129(20), 128(11), 127(5), 117(10), 116(18), 115(24), 105(3), 103(7), 102(6), 92(5), 91(59), 77(14), 76(4), 75(3), 65(8), 64(6), 63(8), 56(3), 53(12), 51(17), 50(6), 43(84), 41(5)	1414	–	263.7±13
(*Z*, *E*)-4-phenyl-3-hexen-2-one (**XIII**, 25–29%)			
175(7), 174(60) $[M]^+$, 173(100), 160(9), 159(57), 158(8), 150(7), 148(5), 146(9), 145(26), 144 (13), 141(7), 135(5), 134(5), 132(6), 131(35), 129(15), 128(10), 127(7), 121(4), 119(4), 117 (10), 116(19), 115(27), 106(5), 105(26) [C_6H_5CO], 103(6), 102(11), 100(4), 97(7), 92(6), 91 (61), 89(5), 86(3), 83(5), 79(12), 78(5), 77(31), 63(11), 62(4), 56(5), 55(3), 53(12), 51(23), 50 (12), 43(92), 41(18)	1435	–	280.7±10
(*Z*)-4-phenyl-4-hexen-2-one (**XV**, 39–49%)			
175(2), 174(13) $[M]^+$, 159(12), 132(4), 131(15), 129(5), 128(3), 117(7), 116(4), 115(7), 91(16), 77(20), 53(4), 51(5), 43(100) [CH_3CO]	1452	–	1417±14

when assessing the *RI* for the structure **XII** we have to choose as a precursor an alkenylarene with the conjugated Ar and C=C fragments, while the double bond $C^4=C^5$ should be included in the target structure as a non-conjugated. For example, the following slightly more complex versions of the additive evaluation of *RI* can be implemented to satisfy these requirements:





The average *RI* value obtained is 1395, which is acceptably consistent with the experimental value 1383. To estimate the *RI* of the (*Z*)-**XV** the difference in the *RI* of (*Z*)- and (*E*)-(1-methyl-1-propenyl) benzenes [1116 – (1094±5)] = (22±5) can be taken into account which gives approximately (1395±13) + (22±5) ≈ (1417±14). The value obtained is less consistent with the experimental *RI* value (1452), but enough to predict the order of chromatographic elution of (*Z*) and (*E*)-isomers, (*E*) < (*Z*).

As noted above, two other products of this reaction naturally correspond to the theoretically expected 3,4-dimethyl-1-phenyl-2-butene-1-one (**XII**) and (*Z*,*E*)-

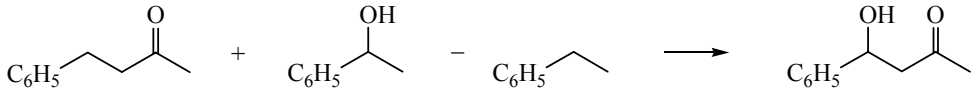
isomers of 4-phenyl-3-hexen-2-one (**XIII**). Given the results of Example 1, for these isomers instead of interpretation of similar mass spectra and obtaining additive estimates of *RI*, one can either use the above stated rule or compare estimates of their normal boiling points obtained using the ACD software: 263.7±13°C (**XII**) and 280.7±10°C (**XIII**). Thus, the order of elution is **XII** < **XIII**.

Table 2 lists the mass spectral data of all four components of the reaction mixture, neither of which is presented in the database [3].

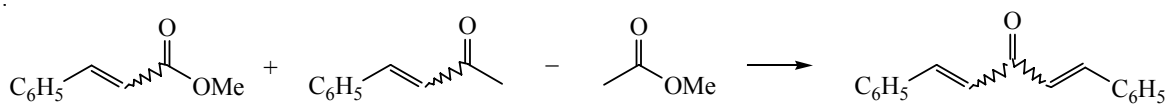
This example shows that the discussed version of the additive schemes is almost the only possible way to obtain estimates for the *RI* of (*E*)- and (*Z*)-isomers of organic compounds π-diastereomerş which should be regarded as one of its major advantages. The following examples further illustrate this possibility by considering the condensation products of acetone and benzaldehyde, although, of course, it requires a separate special discussion. Earlier in the case of products of chlorination of cyclohexane it was shown that this version of the additive scheme is applicable to estimation of the *RI* of σ-diastereomers [26, 28].

Example 3. Identify the five products of acetone and benzaldehyde condensation.. The first one (**XVI**) corresponds to the expected product with the stoichiometry (1:1) with the molecular formula $C_3H_6O + C_7H_6O - H_2O = C_{10}H_{10}O$ and the molecular mass 58 + 106 – 18 = 146. The molecular mass of the second one is 58 + 106 = 164, which corresponds to the formula $C_3H_6O + C_7H_6O = C_{10}H_{12}O_2$ (**XVII**), and the following three are isomeric condensation products (1:2), $C_3H_6O + 2C_7H_6O - 2H_2O = C_{17}H_{14}O$ with molecular masses of 58 + 2·106 – 2·18 = 234 (**XVIII–XX**). It should be noted that among them, the prevailing isomer is **XIX**, whereas the relative amounts of **XVIII** and **XX** do not exceed 1%. In addition, this reaction mixture contains a significant amount of the condensation product of three molecules of benzaldehyde with two molecules of acetone, $3C_6H_5O + 2CH_3COCH_3 - 3H_2O = C_{27}H_{24}O_2$ (**XXI**, blurred peak with the retention time 49.1 min), presumably with the structure of 1,7-diphenyl-5-(2-phenylethenyl)-1,6-heptadien-3-on-5-ol. Table 3 contains the mass spectra of all the products.

The first of these products can be unequivocally identified as 4-phenyl-3-butene-2-one (**XVI**) [most likely (*E*)-isomer] by comparison with the known mass spectrum [3] so that the estimation of its *RI* is not formally required. The poor compliance of the experimental (1374) and published (1346 [29]) values of *RI* does not prevent this conclusion. The poor compliance is caused by the use of a column with polydimethylsiloxane stationary phase HP-5 MS with 5% of phenyl groups and the strong dependence of the *RI* of conjugated compounds such as **XVI** even on a minor variations in the polarity of stationary phase. A component with *RI* 1404 is the primary condensation product of acetone and benzaldehyde, 4-hydroxy-4-phenyl-2-butanone (**XVII**). The database [3] does not include its mass spectrum, but it can be attributed unambiguously. In addition, its *RI* can be estimated in accordance with the following additive scheme:



The three products **XVIII–XX** with identical mass spectra represent three possible (*Z*,*E*)-isomer of 1,5-diphenyl-1,4-pentadien-3-one {the mass spectra of (*Z*,*Z*) and (*E*,*E*)-isomers [3] are known to be almost identical}. but their unambiguous assignment in the absence of reference samples is a rather difficult task. The problem consists primarily in the fact that for many classes of compounds the *RI* values of (*Z*)- and (*E*)-isomers are not very reliable, and often this is caused just by the ambiguity of correlating the structures of isomers with the chromatographic data. To apply the considered additive scheme one must choose among the accessible reference *RI* values those that characterize precisely the structural fragments (*Z*)- and (*E*)-C_6H_5–CH=CH–CO–. The most detailed and reliable are the experimental data for the esters of isomeric cinnamic acids, e.g., methyl-(*Z*)- and -(*E*)-cynnamates: *RI* are equal to 1310±11 and 1372±11, respectively [3̃] Using these data we can suggest the version of assembling the structures of compounds **XVIII–XX** shown below. As another predecessor, we use 4-phenyl-3-butene-2-one identified as a product of the same reaction to which has been attributed the structure of the (*E*)-isomer **XVI**:



(1372±11) (*E*) + (1374) (*E*) – (510±5) = (2236±12) (*E*,*E*), RI_{exp} 2244

(1310±11) (*Z*) + (1374) (*E*) – (510±5) = (2174±12) (*E*,*Z*), RI_{exp} 2185

Since the difference in *RI* of (*E*)- and (*Z*)-isomers is 62 index units, the estimate of *RI* of (1*Z*,4*Z*)-1,5-diphenyl-1,4-pentadiene-3-one (**XVIII**) is 2112±16, which agrees well with the value of RI_{exp} 2122. As a result, it is possible not only to predict the sequence of elution of isomers, (*Z*,*Z*) < (*Z*,*E*) < (*E*,*E*), but also to achieve a satisfactory agreement between experimental and theoretically predicted *RI* values.

Of course, a detailed analysis of the problems and features of gas chromatography-mass spectrometric identification of previously characterized products of

Table 3. Mass spectra and retention indices of the condensation products of acetone and benzaldehyde (to *Example 3*)

Mass spectrum [$m/z \geq 40$ ($I \geq 3\%$)]	RI_{exp}	Existence in database [3]	RI_{calc}
(*E*)-4-Phenyl-3-butene-2-one (**XVI**, 27%)			
147(6), 146(57) $[M]^+$, 145(66), 132(9), 131(89), 115(4), 104(8), 103(100), 102(15), 78(5), 77(45), 76(8), 75(5), 74(6), 65(5), 63(9), 62(4), 58(5), 52(8), 51(43), 50(14), 43(37)	1374	+	–[a]
4-Hydroxy-4-phenyl-2-butanone (**XVII**, 4%)			
165(3), 164(19) $[M]^+$, 149(5), 147(4), 146(30), 145(13), 131(18), 108(4), 107(51) [C_6H_5CHOH], 106(40), 105(40), 104(11), 103(21), 102(3), 91(5), 80(5), 79(68), 78(20), 77(68), 58(29), 53(4), 52(9), 51(34), 50(16), 44(3), 43(100) [CH_3CO], 41(4)	1404	–	1418±3
(1*Z*,4*Z*)-1,5-diphenyl-1,4-pentadiene-3-one (**XVIII**, ~0.1%)[b]			
235(6), 234(32) $[M]^+$, 233(49), 146(24), 133(14), 131(19), 107(10), 106(13), 105(7), 104(100), 103(49), 92(12), 91(24), 89(10), 85(8), 79(11), 78(18), 77(33), 76(9), 75(2), 69(9), 63(9), 53(7), 51(30), 50(5), 45(9), 43(7), 41(6), 40(3)	2122	+	2150±5
((1*E*,4*Z*)-1,5-diphenyl-1,4-pentadiene-3-one (**XIX**, 40%)			
235(18), 234(97) $[M]^+$, 233(100), 217(2), 215(5), 206(6), 205(15), 204(5), 203(7), 202(5), 192(2), 191(13), 190(6), 189(4), 179(3), 178(4), 165(4), 157(5), 156(13), 132(5), 131(46), 129(9), 128(27), 116(5), 115(9), 107(2), 105(2), 104(12), 103(72), 102(24), 101(8), 95(3), 92(3), 91(36), 89(9), 78(8), 77(71), 76(11), 75(6), 74(4), 63(7), 62(2), 53(3), 52(6), 51(35), 50(11)	2185	–	2210±5
(1*E*,4*E*)-1,5-diphenyl-1,4-pentadiene-3-one (**XX**, ~0.6%)[b]			
235(20), 234(36) $[M]^+$, 217(4), 187(5), 170(3), 165(3), 161(3), 160(5), 159(4), 157(3), 156(3), 148(2), 147(5), 146(29), 145(14), 133(4), 132(7), 131(100), 128(3), 118(2), 117(8), 116(4), 115(5), 105(3), 104(14), 103(44), 102(6), 91(12), 89(3), 79(2), 78(7), 77(29), 76(5), 75(2), 74(2), 51(10), 50(5), 43(72)	2244	+	2270±5
$3C_6H_5O + 2CH_3COCH_3 - 3H_2O = C_{27}H_{24}O_2$ (**XXI**, 28%)			
380(4) $[M]^+$, 265(5), 275(7), 257(4), 249(6), 248(15), 247(13), 235(7), 234(6) [M – $C_6H_5CH{=}CH{-}C(OH){=}CH_2$], 233(5), 157(15), 146(4) [M – 234], 144(5), 132(10), 131(100) [$C_6H_5CH{=}CHCO$], 129(15), 129(5), 117(6), 115(10), 104(10), 103(45), 91(16), 78(6), 77(24), 51(6)	–[c]	–	–

[a] Component is uniquely identified by mass spectrum, estimation of *RI* is not required. [b] According to [3], in the mass spectra of isomeric 1,5-diphenyl-1,4-pentadiene-3-ones the maximum peaks belong to the molecular ions, while $I(233) < I(M)$. The observed discrepancies are caused by low content (<1%) of (*Z*,*Z*)- and (*E*, *E*)-isomers. [c] Blurred peak with retention time 49.41 min.

condensation of carbonyl compounds is difficult to carry out in one journal publication. However, the consideration of even a limited number of examples of the condensation of acetone and the simplest aromatic carbonyl compounds illustrates the effectiveness of joint interpretation of mass spectrometric and chromatographic data. To solve such problems the hypotheses should be created about the possible composition of the analyzed samples, including the products of the known organic reactions. One of the most effective ways to interpret the chromatographic parameters is the use of additive schemes for evaluation of retention indices, which allows revealing the structures of the regio- and stereoisomers when the interpretation of the mass spectral information only does not lead to an unambiguous answer.

EXPERIMENTAL

For the synthesis of condensation products, in 2 ml glass ampules was placed 400 μl of an individual carbonyl compound or a binary mixture in the ratio 1:1 (acetone, benzaldehyde, acetophenone, propiophenone) and 10 mg of dry NaOH was added. The ampules were sealed and heated to 70ºC for 2 h. Then to the reaction mixtures 1 ml of diethyl ether was added and 1 μl of solution was taken for analysis.

The chromatography-mass spectrometry analysis was performed on a Shimadzu QP5000 instrument with a quadrupole mass analyzer and a column HP-5 MS of 25 m long and 0.20 mm internal diameter (film thickness of stationary phase 0.33 μm). The mode of temperature programming was heating from 40 to 280ºC at a rate 10 deg min^{-1}, the flow rate of carrier gas (helium) 1.2 ml min^{-1}. The flow separation at the injection 1:50. Evaporator temperature 250ºC, interface temperature 280ºC. To determine the gas-chromatographic retention indices a mixture of *n*-alkanes C_6–C_{20} was used (Supelco, cat. no. 500631), the standard deviation of *RI* was about 1 index unit. The mass spectra were recorded with the standard electron impact ionization energy 70 eV in the scanning mode of the total ion current (2 scans s^{-1}) in the range *m*/*z* 45–700. The data processing was performed using the GCMS Solution 2.60 and AMDIS 2.66 softwares. To estimate the factors of matching the mass spectra with the results of the library search was used the NIST Mass-Spectral Search Program v.2.0 software. All the reference values of retention indices determined on WCOT columns with standard nonpolar phases under linear temperature programming (from the database of NIST/EPA/NIH) were treated statistically, presenting them as $RI \pm s_{RI}$. Standard deviations of calculated *RI* values (*S*) were estimated with the relation $S = (\Sigma s_i^2)^{0.5}$, where s_i is standard *RI* deviation of all the selected structural analogs.

The relative amounts of the condensation products was estimated from the peak areas using internal normalization method without considering the differences in detector sensitivity to different isomers. The eestimates of the normal boiling points of isomers were obtained using the ACD software. The optimization of molecular geometry was performed using MM+ program within the HyperChem (version 6.0) software.

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