Gas chromatographic and IR spectroscopic characteristics of tri- and tetramethylcyclohexenyl butenyl ketones

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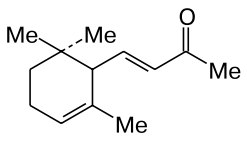
Infrared spectroscopic characteristics and gas chromatographic retention indices of tri- and tetramethylcyclohexenyl butenyl ketones were determined at different temperatures of analysis. Thermodynamic characteristics of sorption on the apolar stationary phase were calculated. The sorption characteristics of tri- and tetramethylcyclohexenyl butenyl ketones show that under conditions of capillary gas chromatography these compounds are retained due to the dispersive energy. It was shown by FTIR spectroscopy that the formation of conjugation systems of double bonds is responsible for the higher retention of the β-isomers compared with that of the α-isomers. The shortening of the distance between the carbonyl and trimethylcyclohexenyl groups was found to be accompanied by a decrease in the retention of the isomeric compounds.

Key words: capillary gas chromatography, damascenone, α- and β-damascones, α- and β-ionones, α-irone, retention indices, FTIR spectra.

Methyl-substituted cyclohexenyl butenyl ketones are contained in essential oils of Bulgarian rose, tea, and iris roots.[1,2] These compounds, which were also found in concentrates of volatiles of raspberries, gooseberries, peach, apricot, and other fruits,[3,4] play the key role in fragrance formation of fruits and berries and are widely used in the development of perfume compounds and food aromatizers. Therefore, these compounds require identification in complicated mixtures. The most important characteristic for the identification of these compounds are gas chromatographic (GC) retention indices (RI) on columns of different polarities. Retention indices of not easily available and isomeric compounds are calculated from structure—sorption correlations, and the study of the latter makes it possible to extent the range of the known retention indices.



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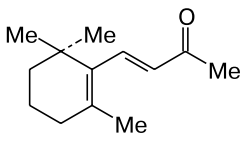

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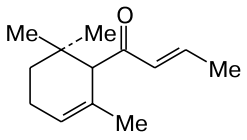

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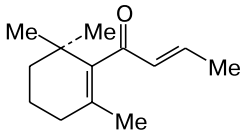

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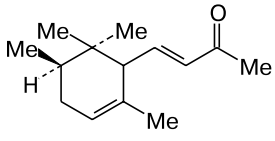

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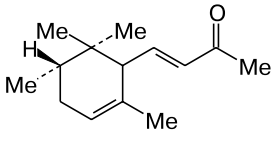

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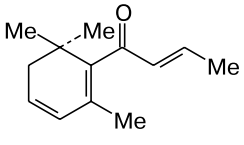

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The purpose of this work is to study the influence of the structure of cyclohexenyl butenyl ketones **1**—**7** on their sorption parameters and IR spectra and to estimate their interaction with the nonpolar stationary phase thermodynamically.

Experimental

Gas chromatographic analysis of 4-(2,6,6-trimethyl-2-cyclohexenyl)-3-buten-2-one (α-ionone, **1**), 4-(2,6,6-trimethyl-1-cyclohexenyl)-3-buten-2-one (β-ionone, **2**), 4-(2,6,6-trimethyl-2-cyclohexenyl)-2-buten-4-one (α-damascone, **3**), 4-(2,6,6-trimethyl-1-cyclohexenyl)-2-buten-4-one (β-damascone, **4**), *cis*-4-(2,5,6,6-tetramethyl-2-cyclohexenyl)-3-buten-2-one (*cis*-α-irone, **5**), *trans*-4-(2,5,6,6-tetramethyl-2-cyclohexenyl)-3-buten-2-one (*trans*-α-irone, **6**), and 4-(2,6,6-tri-

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methyl-1,3-cyclohexadienyl)-2-buten-4-one (damascenone, **7**) was carried out on a Micromat-412 chromatograph (Finland) using a fused silica capillary column with the SPB-1 nonpolar stationary phase (37 m × 0.32 mm, layer thickness of the phase d_f = 0.25 μm, Supelco, USA). The temperature of the flame-ionization detector and injector was 250 °C. The analysis was carried out in the isothermic run at temperatures 132, 143, 152, and 162 °C and in the temperature-programmed run from 100 to 250 °C with a linear rate of 8 °C min^{-1}. The pressure of the carrier gas (helium) at the inlet of the column was 0.7 atm, the split ratio was 1 : 50, the volume of the sample was 1 μL, and mixtures of a 1% solution of the studied compounds in EtOH and *n*-alkanes in pentane were used. To calculate isothermic retention indices, *n*-alkanes C_{13}—C_{16} were used. The hold up time was determined from the retention of methane that was introduced into the column simultaneously with a mixture of the compounds under study and hydrocarbons. Retention indices were calculated using the Kovats formula[5] as an average of five to seven measurements. Indices for programming the temperature of analysis (I_{pr}) were calculated using the van den Dool—Kratz formula[6] from the retention times of the substance to be analyzed and *n*-alkanes. The standard deviation for the determination of retention indices did not exceed ±1 i.u.

The standard molar Gibbs energy of sorption (SMGES) of the analyzed compounds was calculated from the formula

$$\Delta G^\circ = -2.3RT\log(k\beta), \quad (1)$$

where R = 8.3143 J mol^{-1} deg^{-1} is the universal gas constant; T/K is the temperature of analysis; $k = (t - t_0)/t_0$ is the retention factor; t is the retention time of the substance; t_0 is the residence time of methane in the column; and β is the phase ratio. The β values were calculated from the equation[7]

$$\beta = (d_c - 2d_f)^2/[4d_f(d_c - d_f)], \quad (2)$$

where d_c is the inner diameter of the column, and d_f is the layer thickness of the stationary phase.

The standard deviation of the ΔG° values calculated using formula (1) from five measurements was not higher than ±0.050 kJ mol^{-1}.

The enthalpy (ΔH°) and entropy (ΔS°) terms of SMGES were calculated by the solution of the system of equations (3) and using the ΔG° values obtained experimentally from the linear portions of log$k$ *vs.* $1/T$ plots

$$\Delta G^\circ_1 = \Delta H^\circ - T_1\Delta S^\circ,$$
$$\Delta G^\circ_2 = \Delta H^\circ - T_2\Delta S^\circ. \quad (3)$$

IR spectra of cyclohexenyl butenyl ketones **1**—**7** were obtained on an Avatar 360 E.S.P. FTIR spectrometer (Thermo Nicolet, USA) in the 400—4000 cm^{-1} spectral region with a resolution of 4 cm^{-1} and 16 scans. All compounds were analyzed in the transmitted light using the "crashed drop" procedure of sample preparation between two KRS-5 windows (thallium bromide and iodide). The results were processed using the software for IR spectroscopy (Thermo Nicolet OMNIC v.6.0). Intensities of peaks were calculated from the ratio of absorbances of the peaks to the absorbance of the most intense peak accepted as 100%.

Results and Discussion

The compounds under study, *viz.*, α- and β-damascones (**3** and **4**) and α- and β-ionones (**1** and **2**), are structural isomers (see above). In these compounds the position of the double bond in the ring and in the butenyl substituent and the position of the carbonyl group change. A molecule of α-irone (**5**, **6**), unlike that of α-ionone **1**, contains another Me substituent. The variation in structures results in differences in the sorption characteristics of the substances.

The retention indices (RI) of the compounds studied at different temperatures are presented in Table 1 along with the indices obtained in the temperature-programmed run. The latters can be used for the identification of the studied compounds.

The retention indices of the analyzed compounds depend on the position and number of double bonds in the cyclohexenyl ring, on the position of the carbonyl group relatively to this ring, and on the number of Me substituents. The influence of the position of the carbonyl group can be estimated by the comparison of the RI values of the α- or β-isomers. For example, α- and β-ionones **1** and **2** are retained more strongly than α- and β-damascones **3** and **4**, because the displacement of the O atom from the ring strongly increases the retention. It is possible that the carbonyl group in damascone molecules interacts weakly with the Me substituents of the cyclohexenyl ring, whereas this interaction is unlike in molecules of isomeric ionones.

The retention indices of α-irone and α-ionone can be compared to estimate the contribution of the Me group in position 5 of the α-irone molecule to the retention. The difference in RI is 83—85 i.u. for *trans*-α-irone **6** and 104—107 i.u. for *cis*-isomer **5**. The additional Me group in the α-irone molecule is situated in the *ortho*-position toward one of the geminal Me groups in positions 6, which are present in both isomers. As shown

Table 1. Retention indices (RI) of the compounds studied at different temperatures of analysis from 132 to 162 °C

Compound	RI at different temperatures of analysis				
	132	143	152	162	I_{pr}*
Damascenone (**7**)	1366	1371	1375	1377	1369
α-Damascone (**3**)	1378	1384	1386	1392	1380
β-Damascone (**4**)	1395	1400	1406	1408	1401
α-Ionone (**1**)	1408	1413	1418	1420	1413
β-Ionone (**2**)	1462	1467	1472	1474	1470
trans-α-Irone (**6**)	1491	1496	1502	1504	1501
cis-α-Irone (**5**)	1512	1518	1525	1527	1525

* The indices were measured in the temperature-programmed run.

previously for planar aromatic rings of the dimethyl derivatives of benzene and the nitrogen-containing heterocycles,[8—11] such a mutual arrangement of the Me groups results in the appearance of the *ortho*-effect increasing the retention of the *ortho*-isomers compared to the *meta*- and *para*-isomers. Probably, in the case of the nonplanar cycle of the *cis*-α-irone **5** molecule, the incipient *ortho*-effect increases the retention index of this compound. The *ortho*-effect value is 21—24 i.u. (see Table 1). Its virtual independence of the temperature of analysis confirms the dispersive character of interactions between the sorbate molecules and the stationary liquid phase. This effect is not manifested in the *trans*-isomer because the Me substituents are remote from each other. Therefore, the value of 83—85 i.u., which is the difference in RI of *trans*-α-irone **6** and α-ionone **1**, can be considered as the contribution from the "independent" Me group to the RI value. Since natural irone is a mixture of isomers,[1,2] the difference in retentions of the α-irone *cis*- and *trans*-isomers allows their separation and identification.

The data obtained by IR spectroscopy (Fig. 1) indicate that the Me groups in the studied compounds depend on each other. Analysis of bands in the IR spectra in the 2820—3030 cm^{-1} region corresponding to the absorption of the Me groups[7] showed that the Me groups in the α- and β-isomers of ionone or damascones are sensitive to the position of the double bond in the cyclohexenyl ring. The transfer of the double bond from position 2 to position 1 in cyclohexenyl of isomeric ionones shifts the wave numbers from 2914 to 2932 cm^{-1}. Similar distinctions were found in the IR spectra of α- and β-damascones. The IR spectra for the α-ionone—α-damascone or β-ionone—β-damascone pairs exhibit almost the same wave numbers and intensities of absorption bands of the Me groups in this spectral region. The closeness of the wave numbers (2961 and 2962 cm^{-1}) indicates a weak influence of the carbonyl group on two Me groups in position 6 of cyclohexenyl in molecules of α- and β-damascones **3** and **4**. In the IR spectrum of α-irone this band is the highest in intensity and its insignificant shift to 2966 cm^{-1} is caused by the introduction of the fourth Me group into the cycle. The absorption region of the Me groups in the IR spectrum of damascenone **7** looks analogously to the IR spectrum of α-ionone **1** and α-damascone **3** but the intensity of the absorption bands is lower.

Considerable distinctions in the IR spectra of the substances under study were found in the regions of wave numbers from 1700 to 1600 cm^{-1} corresponding to the absorption of the carbonyl group and conjugated double bonds. The IR spectrum of each compound in this region have three main bands, and these bands differ insignificantly for α- and β-ionone and α-irone. The most

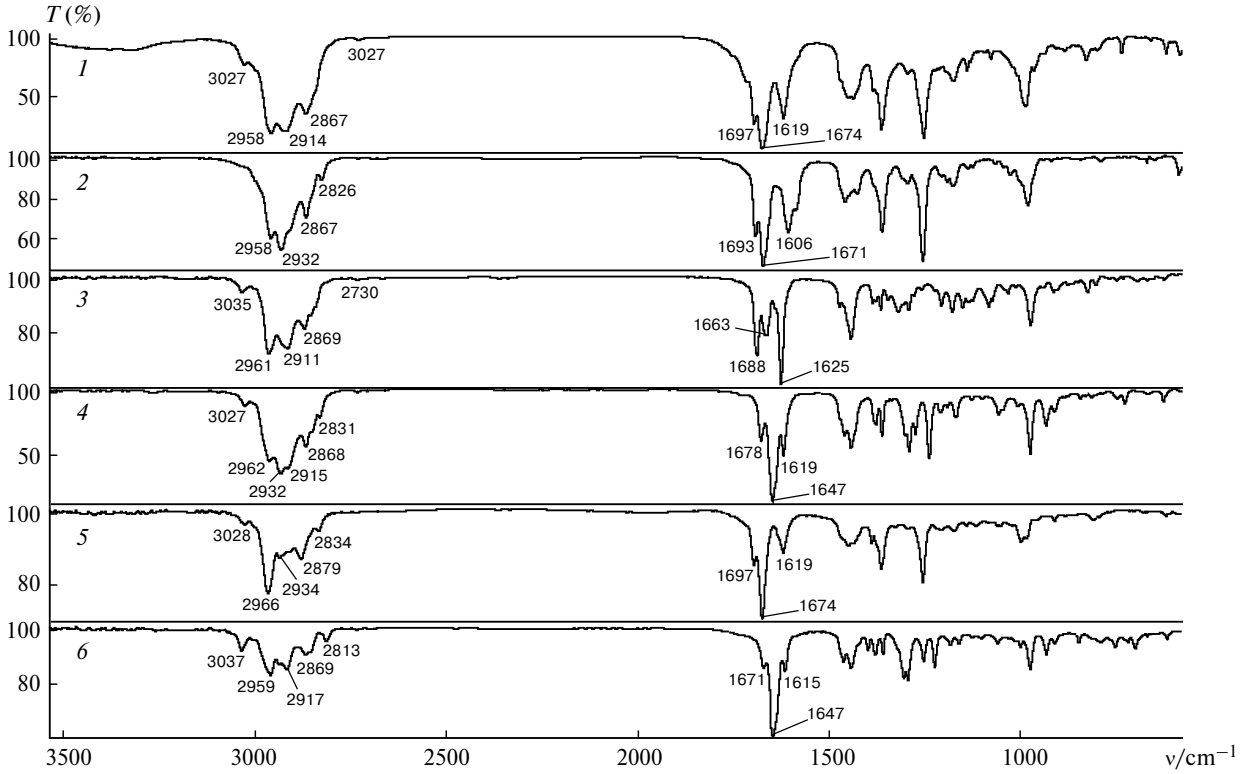

Fig. 1. IR spectra of tri- and tetramethyl cyclohexenyl butenyl ketones: *1*, α-ionone **1**; *2*, β-ionone **2**; *3*, α-damascone **3**; *4*, β-damascone **4**; *5*, *cis*-α-irone **5** and *trans*-α-irone **6**; and *6*, damascenone **7**.

intense band at 1671—1674 cm^{-1} is attributed to the absorption of the carbonyl group. Due to the conjugation of the double bonds in the ring, substituent, and carbonyl group, the absorption band of the carbonyl group in the spectrum of β-ionone **2** is insignificantly shifted (by 3 cm^{-1}), and the absorption band of the double bond has a greater shift of 13 cm^{-1}. As a result, the retention index of β-ionone **2** is by 54—57 i.u. higher than that of α-ionone **1**. A similar effect has previously been observed for the carbonyl derivatives of furan and thiophene.[12]

On going from isomeric ionones to damascones, the carbonyl group migrates to the ring, and its absorption band is shifted in the spectrum of α-damascone **3** to 1663 cm^{-1} and in the spectrum of β-damascone **4** to 1678 cm^{-1}. The intensity of this band is lower than that in the spectrum of ionones. The IR spectra of the pair of β-damascone **4** and damascenone **7** are close to each other and differ substantially from the IR spectra of other compounds. The intensity of the absorption band of the carbonyl group (ν = 1671 and 1678 cm^{-1}) for this pair of substances is much lower than the intensity of the band at 1647 cm^{-1} ascribed to absorption of the conjugated C=C bonds in ketones. This band, which is present only in the spectra of β-damascone **4** and damascenone **7**, is due, most likely, to vibrations of the conjugated system including the C=C double bonds in the cycle and in the side chain and the carbonyl group.

Conjugation results in the situation that β-damascone **4** is retained for a longer time than α-damascone **3**, but the difference in RI is lower than that for α- and β-ionones **1** and **2** (16—20 i.u.). This slight difference is related, most likely, to the fact that the retention of β-damascone **3** is effected by two opposite effects: conjugation that increases retention and the possible interaction of the Me groups of the ring and carbonyl group (shielding effect) that decreases the retention of the substance.

The double bonds of the cyclodiene ring and the C=O group can also conjugate in a molecule of damascenone **7**. However, in this case, the RI is by 29—31 i.u. lower than that for β-damascone and by 11—15 i.e. lower than the RI of α-damascone **3**, whose molecule does not exhibit double bond conjugation at all. It is quite probable that the lower retention of damascenone is related to a more flattened configuration of the cyclohexadiene ring compared to the cyclohexene rings of α- and β-damascones **3** and **4**. Such a distinction in the configurations of molecules of damascenone and damascones allows, evidently, their complete separation and identification in complicated mixtures by the GC method.

The distinctions found for the retention of the compounds allow the calculation of retention indices of difficultly available isomeric compounds related to this group. For example, the difference in RI between α-irone and α-ionone (88 i.u.) characterizes the contribution from the Me group in position 5 in the α-irone molecule. In this case, the index of β-irone should be by 88 i.u. higher than that of β-ionone, *i.e.*, to be 1558 i.u. At the same time, the difference in RI for α- and β-ionones, which characterizes the transfer of the double bond in cyclohexenyl from position 1 to position 2, is 57 i.u. By analogy, the RI of α- and β-irones should differ by this value, *i.e.*, the RI of β-irone calculated by the other scheme is also 1558 i.u. This RI can be used in analysis of a complicated mixture for the conventional identification of β-irone.

The chromatographic retention of substances is determined by the energy of intermolecular interactions of analyzed substances with the stationary phase. The SMGES (ΔG°) is a quantitative characteristic of these interactions, which for the compounds studied on the nonpolar phase are brought about by the dispersion forces. Adsorption of substances on the gas—stationary liquid phase and stationary liquid phase—fused silica column wall interfaces can contribute to ΔG°. These terms are usually small, and the main contribution to the retention of substances is made by their interaction with the stationary liquid phase. The temperature plot of the ΔG° values can provide an additional information on the specific features of the GC behavior and allows one to calculate the enthalpy and entropy of sorption of analyzed compounds. In the temperature interval studied, the ΔG° values decrease virtually linearly with the temperature of analysis for all compounds (Table 2).

Damascenone **7** is characterized by the lowest SMGES value, and the highest SMGES belongs to *cis*-α-irone **5**. The influence of the position of the O atom of the C=O group relatively to the cyclohexene ring, which has been mentioned above, can be estimated by the comparison of the sorption energies of β-ionone **2** and β-damascone **4**. The difference in ΔG° values of these compounds ranges from 1.09 kJ mol^{-1} at 162 °C to 1.18 kJ mol^{-1} at 132 °C, *i.e.*, it shows a weak dependence on the temperature of analysis. The contribution of the Me group to ΔG° for *trans*-α-irone lies in the interval from 1.38 to 1.48 kJ mol^{-1}, and that for the *cis*-isomer ranges from 1.76 to 1.85 kJ mol^{-1} in the same temperature interval. Therefore, the difference in contributions to ΔG° from the *cis*- and *trans*-Me groups in the α-irone molecule arising due to the *ortho*-effect remains unchanged regardless of the temperature of analysis, which confirms the dispersion type of intermolecular interactions occurring between the analyzed substances and the stationary liquid phase.

The influence of conjugation of the double bonds of the cyclohexenyl ring and C=O group on the sorption energy can be estimated by comparison of the ΔG° values for α- and β-ionones. The difference between them is 0.89—0.96 kJ mol^{-1} and also depends slightly on the temperature of analysis.

Table 2. Differential molar free energy of sorption ($\Delta G°$) at temperatures of analysis from 132 to 162 °C, enthalpy (ΔH), and entropy of sorption (ΔS) of compounds under study

Compound	$-\Delta G°$/kJ mol^{-1} at different temperatures of analysis				$-\Delta H$	$-T\Delta S$ (152 °C)	$-\Delta S$ /J mol^{-1} K^{-1}
					kJ mol^{-1}		
	132	143	152	162			
Damascenone (**7**)	22.82	22.12	21.55	20.96	48.57	27.02	63.5
α-Damascone (**3**)	23.10	22.45	21.80	21.16	49.81	28.01	65.9
β-Damascone (**4**)	23.36	22.64	22.07	21.46	49.34	27.27	64.1
α-Ionone (**1**)	23.58	22.86	22.28	21.66	49.89	27.61	64.9
β-Ionone (**2**)	24.54	23.79	23.17	22.55	51.81	28.64	67.3
trans-α-Irone (**6**)	25.06	24.30	23.68	23.04	52.78	29.10	68.4
cis-α-Irone (**5**)	25.43	24.67	24.05	23.42	52.98	28.93	68.0

We also estimated the contributions of the enthalpy and entropy components to the SMGES value (see Table 2). An increase in the enthalpy of sorption of compounds analyzed is proportional, in most cases, to an increase in $\Delta G°$, while the entropy changes insignificantly. However, even an insignificant increase in entropy decreases the free energy of sorption. For example, $\Delta H°$ of α-damascone **3** is higher than $\Delta H°$ for β-damascone **4**; however, its $\Delta S°$ is also higher, and, as a result, the SMGES value decreases (see Table 2). Due to this, α-damascone **3** is eluted from the column before β-damascone **4**. The difference in $\Delta H°$ values of the α-irone *cis*- and *trans*-isomers is 0.20 kJ mol^{-1} but SMGES for these isomers differ by 0.37 kJ mol^{-1} (see Table 2) and, hence, the isomers can be completely separated upon GC analysis.

It is of interest to compare the contributions from the enthalpy ($\Delta H°$) and entropy ($T\Delta S°$) terms of SMGES. At 152 °C the entropy contributions $T\Delta S°$ for all studied compounds are 54.6—56.0% of the $\Delta H°$ values, *i.e.*, they are close to each other (see Table 2). Evidently, retention of cyclohexyl butenyl ketones on the nonpolar stationary phase is mainly determined by the enthalpy factor.

Thus, the obtained sorption and IR spectroscopic characteristics of tri- and tetramethylcyclohexenyl butenyl ketones indicate that these compounds are retained in capillary GC due to the dispersion energy. The double bond in position 1 of the cyclohexenyl ring increases the retention of β-isomers compared to α-isomers due to the formation of the conjugated system of double bonds. The approach of the carbonyl group to trimethylcyclohexenyl is accompanied by a decrease in the retention of the isomeric compounds. The structural distinctions in the isomers resulting in the conjugation effects and the influence of the Me substituents make it possible to separate the isomers, and the experimental and calculated RI values form a basis for their identification.

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