
VARIOUS
TECHNOLOGIES

New Chemical Markers Based on Phthaleins

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Abstract—New chemical markers based on mixtures of individual phthaleins were developed. These markers are characterized by high level of secrecy in use, good transferability to contacting persons, enhanced retention on the objects marked, and reliable identification of phthaleins by expert investigation. The experimental studies of the markers obtained show that the synthesized mixture of three phthalein homologs contains the previously unknown phthalein with unsymmetrical phenolic substituents, o-cresolphthalein [3-(3'-methyl-4'-hydroxyphenyl)-3-(4"-hydroxyphenyl)phthalide], which decreases the probability of the marker falsification. Quantum-chemical calculations of the reaction of the o-cresolphthalein synthesis show that the overall reaction is characterized by small positive changes in the enthalpy and Gibbs free energy, and the second and third steps occur with negative changes in the Gibbs energy. The optimum structure of the transition state was calculated.

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Substances whose marking properties are latent for people who do not have the required information on such marking and on methods for revealing it form a special group of markers. This group of markers is practically important and is widely used by state institutions for protecting financial, economical, and defense systems and in law enforcement operations. It also helps commercial companies to protect their products, documents, and technologies from falsifications, imitations, and distortions. A chemical marker is a definite substance (or a mixture of substances) specially added to a certain material or substance or applied onto the surface of an item to impart to it distinctive features (properties) ensuring its subsequent identification. Such marking can have several purposes, which largely determine both the type and properties of the marker itself and the procedure of its identification. Classification of the existing groups of chemical markers, fields of their application, and methods for identification in the marked objects are discussed in detail in [1].

The most widely used chemical markers are luminophores, substances that do not absorb radiation in the visible range, i.e., are colorless under natural illumination, but are capable to emit visible light under UV irradiation. As a rule, this is a small group of organic compounds that emit blue, green, yellow, or red light under UV irradiation. In this case, the luminophore acts as a converter of a higher-energy invisible UV light into a visible light with lower energy of the quanta. The main application of luminophores in law enforcement operations is secret marking of items subjected to criminal infringement: bank notes, documents, stolen items, and also of package of materials and substances and of the surface of items whose turnover is controlled by the state (weapons, ammunition, drugs, strategic materials, etc.). This allows not only the marked item to be easily detected, but also the very fact of contact of a person or item with the marked object to be proved owing to mechanical transfer of a part of the chemical marker at direct contact. Most frequently, identification of luminescent markers involves only visual confirmation

under the action of a UV radiation source. This feature has both advantages (simplicity, quickness, and cheapness of identification) and drawbacks (high probability of erroneous identification, relative ease of detection and imitation).

Markers revealed by chemical treatment find growing use today. The detection (visualization) of this type of markers is based on the fact that the marking, invisible under daylight, is developed (treated) with a solution of a colorless developer to obtain a color image. The examples are colorless pH indicators (phenolphthalein, cresolphthalein, thymolphthalein) becoming red, lilac, and blue in alkaline medium. If necessary, the developed image can be decolorized by treatment with a weak acid. These markers are used as doping additives to mixed luminescent markers in law enforcement operations, and also as components of indicator mixtures for marking the items of criminal infringements. These complicated marker formulations allow enhancement of the marking reliability, taking into account the fact that the UV radiation sources came into wide and free use recently (portable, bank, medical UV lamps; UV light sources in entertainment clubs, discotheques, etc.) and can be used by criminals for detecting simple luminescent markers. One more advantage of chemical markers based on pH indicators is that they can be detected in low concentrations by methods of thin-layer chromatography and spectroscopy.

This study was aimed at preparing new chemical markers for secret marking of substances, materials, and items with high secrecy of use, good transferability to contacting persons, enhanced retention of the marker on marked objects, and reliable identification of phthaleins in expert investigations.

EXPERIMENTAL

In our experiments, we used analytically pure grade phenol, phthalic anhydride, and sodium hydroxide; the other chemicals were of pure grade. When preparing the first marker, a mechanical mixture of phenolphthalein (PP), *o*-cresolphthalein (CP), thymolphthalein (TP), fluorescein (resorcinolphthalein, RP), silica gel, and citric or oxalic acid was treated with a solution of an adhesion-active polymer, low-oxidized atactic polypropylene [2].

Oxidized atactic polypropylene is produced by OOO Ataktika (Tomsk) on a semicommercial installation by oxidation of the polymer melt with atmospheric oxygen

at 180–250°C. To prepare the marker, we used the polymer of grade OAPP-N, corresponding in properties to TU (Technical Specification) 2211-00-02069318–04, Oxidized Atactic Polypropylene. OAPP-N has the viscosity-average molecular mass of $(28–30) \times 10^3$ and contains hydroxy and carbonyl groups (0.27–0.31 mol %). Commercial OAPP-N contains up to 3.0% powdered talc and up to 20% impurities of the isotactic polypropylene fraction. These components are insoluble in hydrocarbon solvents.

The commercial product was purified by extraction with benzene at 25°C, followed by settling of the insoluble precipitate and filtration. After evaporation of the filtrate to dryness, purified OAPP-N was obtained. It was used for preparing the chemical marker. The yield of purified OAPP-N was 80–90 wt %. OAPP-N is readily soluble in hexane and benzene, whereas phthaleins are insoluble in these solvents. A 10–15% solution of OAPP-N has low viscosity, and powdered phthaleins are readily dispersed in it [3].

Fluorescein present in the marker at a concentration of 0.1–1.0 wt % shows no luminescence in the solid state under visible and UV light with the wavelengths $\lambda < 360$ nm and $\lambda > 380$ nm, which ensures increased secrecy of the object marking.

Introduction of OAPP-N in an amount of 5.0 to 15.0% ensures fixation of phthaleins on the marked objects (bank notes, polyethylene packaging, clothes, hand palms, etc.) for a long time without affecting their properties. The polymer film is hydrophobic, which additionally protects the particles from water and from agglutination.

Citric or oxalic acid acts as a stabilizer preserving the weakly acidic medium upon occasional contact with moisture (e.g., saliva or moist fingers when counting bank notes), i.e., the marker color will not change in this case.

Silica gel acts as filler and moisture absorbent from humid air, which is important at long-term storage of the marker. In the presence of silica gel, the marker powder undergoes no caking and clotting, preserving finely dispersed consistence. In marking, fine silica gel particles favor uniform distribution of the marker over the object surface.

The above mixture of four phthaleins enhances the reliability of identification and the secrecy of use, because synthetic, medical, industrial, cosmetic, and household materials do not contain a similar mixture. The suggested mixture of four phthaleins cannot occasionally

get on the object being marked. The phthaleins are used separately as purgative, antiseptic, and antihelminth agents in medicine; they are also used in perfumery and as indicators in laboratories and in industry [4].

The chemical marker was prepared as follows. A 100-g portion of the marker was prepared from the base mixture of phthaleins (57.0 g) and functional components (43.0 g). The base mixture of phthaleins, containing 45.0 g of phenolphthalein, 5.9 g of *o*-cresolphthalein, 5.6 g of thymolphthalein, and 0.5 g of fluorescein, was mixed in the dry form, loaded into a laboratory mill, and milled for 5 min. To prepare a 12% OAPP-N solution, 13.5 g of OAPP-N was loaded into a heated glass reactor with a stirrer, 151.0 mL (99.7 g) of *n*-hexane was added, and the mixture was stirred for 30 min at 50–60°C until the polypropylene dissolved completely. The powdered base mixture of phthaleins (57.0 g) was gradually added to the solution, the suspension temperature was elevated to 68.7°C, and the major fraction of hexane (100 mL) was distilled off. The wet powder was unloaded from the reactor onto a sheet of thick white paper, distributed as a 5-mm-thick layer, and allowed to completely dry at room temperature (25°C). The powder was intermittently mixed with a putty knife to break down phthalein clots. The dry powder of phthaleins does not adhere to the putty knife and does not have the hexane odor. The base mixture of phthaleins with the polymer deposited onto the particle surface was loaded into a homogenizer, 26.0 g of KSMG grade silica gel preliminarily ground in a mortar and 3.5 g of citric acid were added, and the dry mixture was homogenized for 5 min with vigorous stirring. The prepared chemical marker was charged in 10-g portions into small polyethylene bags. The finely dispersed powder of the marker has gray color, is friable, and can be readily distributed over the surface without particle aggregation.

The second marker was prepared by synthesis of three phthalein homologs including PP, CP, and previously unknown phthalein with unsymmetrical phenolic substituents, *o*-cresolphthalein (CPP) [3-(3'-methyl-4'-hydroxyphenyl)-3-(4''-hydroxyphenyl)phthalide]. The reaction was performed as follows [5].

First, we prepared the catalyst based on anhydrous ZnCl₂. ZnCl₂ was melted on a metal spoon over an alcohol burner flame. The melt was heated until bubbles ceased to evolve (≈10 min), after which it was cooled, and the cake was ground in a mortar. The condensation was performed in two steps using nitrobenzene as solvent. First, 4.9 g (0.052 mol) of phenol was added to 18.6 mL (0.18 mol)

of nitrobenzene in a metal crucible, after which 5.6 g (0.052 mol) of *o*-cresol was added, and the mixture was heated to 50–60°C and stirred until the phenols dissolved completely. Then, 16.5 g (0.11 mol) of phthalic anhydride was added, the mixture was stirred, and 14.2 g (0.104 mol) of anhydrous powdered ZnCl₂ was added. In the second step, the temperature was raised to 120–130°C, and the mixture was heated for 4 h with intermittent stirring with a putty knife. The following reactant ratio was used in the synthesis: phthalic anhydride : phenol : ZnCl₂ : *o*-cresol = 2.15 : 1.0 : 2.0 : 1.0 ; phenol : nitrobenzene = 1.0 : 3.4. The product yield after purification was 34.0% (5.9 g). The product contained (wt %) 15.0 PP, 34.7 CP, and 50.3 CPP.

The phthaleins were isolated from the reaction mixture after distilling off nitrobenzene in a vacuum. The solid residue was ground and boiled in a porcelain cup with 100 mL of distilled water acidified with 5 mL of concentrated HCl. The solution was filtered, and the precipitate was washed with water. The washed precipitate was dissolved in a warm 5% NaOH solution. The resulting dark red solution was filtered to remove the insoluble precipitate. The red filtrate was acidified with glacial acetic acid added in 1-mL portions and then with six drops of hydrochloric acid to obtain an acidic solution. In the process, the solution color changed from dark red to light brown. The solution was left overnight for precipitation. The precipitate was filtered off and dried.

The reaction product was purified as follows. The product was dissolved in approximately 50 mL of alcohol, and the solution was refluxed with activated charcoal. The hot solution was filtered, and the charcoal was washed with hot alcohol. After cooling, the solution was diluted with an eightfold amount of distilled water, filtered, partially evaporated in a porcelain cup, and cooled. The precipitated product was filtered off, dried, and weighed. The reaction product is light brown. The synthesized mixture of three homologs was used for preparing 9.1 g of a chemical marker of the following composition (wt %): PP 9.7 (0.9 g), CP 22.6 (2.0 g), silica gel 20.0 (1.8 g), citric acid 3.0 (0.3 g), OAPP-N 12.0 (1.1 g), and CPP 32.7 (3.0 g). The marker obtained, consisting of a mixture of three phthalein homologs including CPP, allows more reliable marking, and its falsification (copying) becomes less probable; also, the marker formula is simpler.

Quantum-chemical calculations were performed using standard GAUSSIAN'09 package [6]. Theoretical studies were made using the density functional theory (DFT). The calculations were performed by the B3LYP hybrid

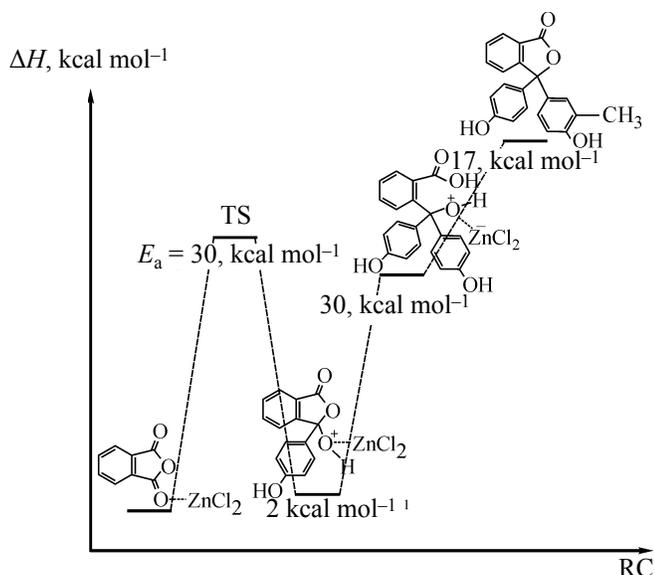


Fig. 1. Enthalpy diagram of the reaction of CPP synthesis. (ΔH) Enthalpy change and (RC) reaction coordinate.

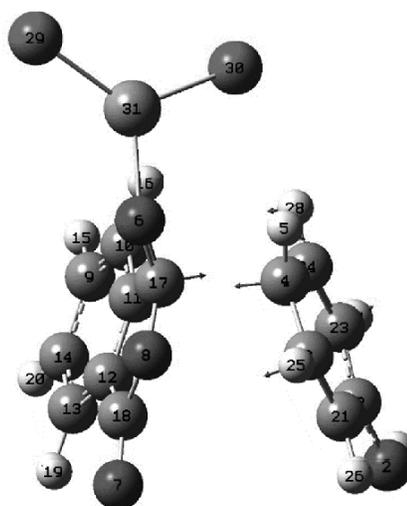


Fig. 2. Optimized structure of the transition state. Oxygen atoms are black, and carbon atoms are gray.

density functional method with Becke exchange functional (B3) [7] and Lee–Yang–Parr correlation functional (LYP) [8]. The 6-31G(d) full-electron basis set was used for all the atoms. The molecular geometries were fully optimized, and the stationary character of the structures was confirmed by the absence of imaginary vibration frequencies. The energies of the calculated compounds were corrected taking into account the zero-point vibration energy (ZPVE) and were brought to standard conditions (298.15 K, 1 atm) using the thermal correction to the enthalpy and free energy. The calculated thermodynamic functions of the reaction of the CPP synthesis are given in Table 1, and the energy diagram, in Fig. 1.

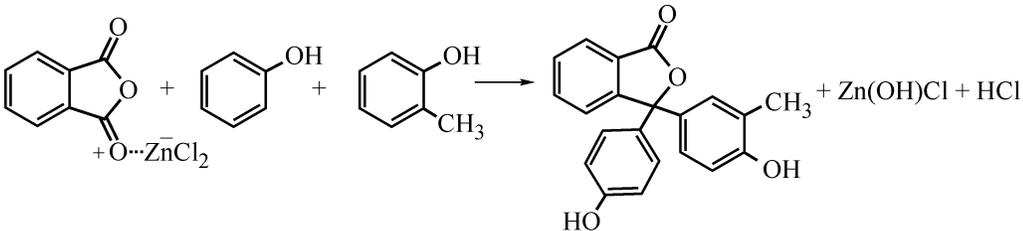
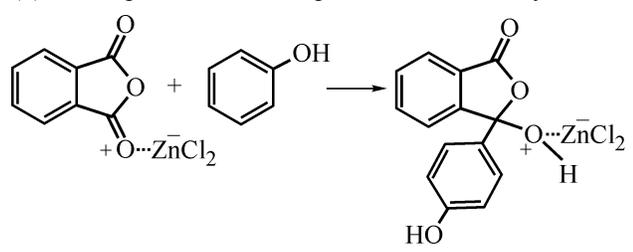
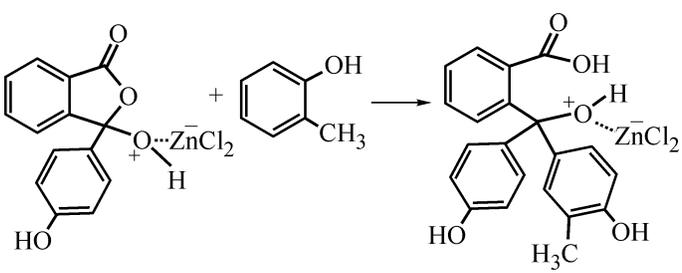
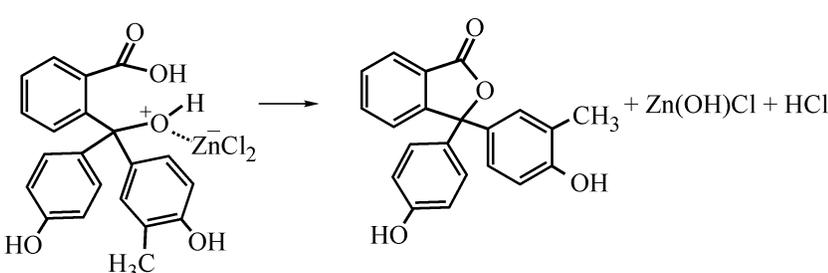
Table 1 and Fig. 1 show that the overall reaction is characterized by small positive changes in the enthalpy and Gibbs free energy. The second and third steps of the reaction are characterized by the negative Gibbs energy. Such pattern is typical of reactions occurring on heating. The formation of a donor–acceptor complex between phthalic anhydride and zinc chloride is accompanied by slight elongation of the C=O bond in the anhydride (from 1.199 to 1.221 Å), by a decrease in its Wiberg index (from 1.82 to 1.58), and by the shift of the electron pair of the C=O π bond toward the oxygen atom. The positive charge on the carbon atom of this bond increases from +0.815 to +0.886, enhancing its electrophilic activity in the reaction with phenol. This reaction mainly occurs at the *p*-position of phenol via the transition state (Fig. 2) optimized using

the Berny algorithm and the STQN (Synchronous Transit-Guided Quasi-Newton) method [9].

In the transition state, the C₄ atom deviates from the benzene ring plane, and the C₄–H₅ bond becomes weaker and slightly longer. The π -electron pair of the benzene ring of phenol is transferred to the newly formed C₄–C₁₇ bond (bond length 1.798 Å, Wiberg index 0.63). In phthalic anhydride, the C₁₇ atom and the O₈ and O₆ atoms bonded to it also deviate from the plane, and the bonds between carbon and oxygen become longer (O₆–C₁₇ bond length 1.284 Å, Wiberg index 1.26; O₈–C₁₇ bond length 1.469 Å, Wiberg index 0.82). The transition character of the structure obtained is proved by the presence of one negative vibration frequency (–165 cm^{–1}) corresponding to the bending vibration that leads to shortening of the C₄–C₁₇ bond and to elongation of the O₆–C₁₇ and O₈–C₁₇ bonds in the anhydride. The activation energy of this transition state from the donor–acceptor complex and phenol is 37 kcal mol^{–1}.

The chemical markers obtained are used for marking various objects: paper, polyethylene packaging, fabrics, hand palms, explosives, “spice” psychotropic mixtures, household devices, bank notes, etc. The marker is applied onto these objects with pincers and a wool tampon. The excess amount of the applied solid marker is removed by air jet with slight tapping. Visual examination of the marked objects shows that their appearance has not changed, and the marker application is revealed neither

Table 1. Thermodynamic functions of reaction steps

Reaction scheme	Thermodynamic functions, kcal mol ⁻¹
<p>Overall reaction</p> 	$\Delta G = 4$ $\Delta H = 50$
<p>(1) Nucleophilic addition of phenol to the carbonyl carbon atom</p> 	$\Delta G = 14$ $\Delta H = 2$
<p>(2) Nucleophilic substitution at the carbon atom with ring opening</p> 	$\Delta G = -5$ $\Delta H = 30$
<p>(3) Elimination with cyclization and formation of the final product</p> 	$\Delta G = -6$ $\Delta H = 17$

visually nor by touch with sensitive skin of hand fingers. Control examination of the objects under UV light with a wavelength of 365 nm confirms that the marker has been applied in sufficient amount. The marker does not fall off at intense folding of bank notes, packaging, and fabric.

The marker sample is taken from the objects with a wool tampon wetted with ethanol, after which the tampon is squeezed, extracted with methanol, and the alcoholic solution is evaporated to dryness in an air stream at room temperature. The residue is dissolved in 200 μL

Table 2. Results of TLC analysis of the chemical marker

Phthalein	Relative retention index (R_f)	Color of chromatographic band		
		UV, 365 nm	1% alkali	marquis reagent
RP	0.43	Yellow-green luminescence	Yellow	Yellow
CP	0.48	None	Purple	Orange
PP	0.57	"	Crimson	Red
TP	0.66	"	Blue	Purple
CPP	0.52	"	Lilac	Red

of methanol. To perform a qualitative reaction, 1 μ L of a methanol solution of phthaleins is applied onto filter paper, and the spot is wetted with a 1.0% alkali solution. The spot becomes blue, which confirms the presence of phthaleins.

Physicochemical expertise for the presence of the marker is performed by thin-layer chromatography (TLC). A 0.5- μ L portion of the methanol solution of the phthaleins is applied onto the start line of two Sorbfil PTSKh-P-A chromatographic plates of size 10 \times 10 cm. The plates are eluted with the toluene–methanol–acetic acid (10 : 2 : 1) eluent to a height of 90 mm from the start line, after which the plates are dried in a stream

of warm air. The chromatographic band of fluorescein (yellow color under daylight) in the chromatograms is detected by the intense yellow-green luminescence under UV light (365 nm). The chromatographic bands of the other phthaleins on the first plate are detected by its treatment with Marquis reagent (Formalin–sulfuric acid, volume ratio 9 : 1), and on the second plate, by its immersion into a bath with a 1% aqueous solution of KOH or NaOH. The chromatograms of the investigation objects are compared with respect to the color, shape, and R_f of the revealed chromatographic bands (Table 2). The presence of the set of chromatographic bands observed in the chromatogram of the reference sample of the chemical marker used is noted.

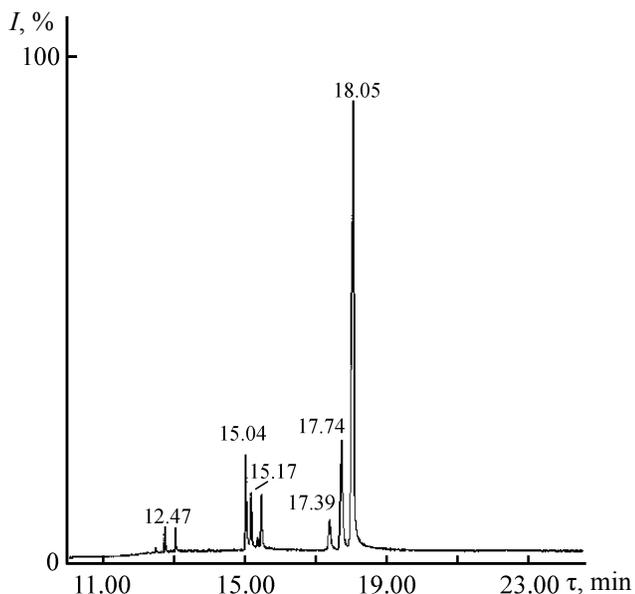


Fig. 3. Chromatogram of the mixture of phthaleins. (I) Peak intensity and (τ) retention time. Relative retention time, min: PP 15.04, CPP 17.74, and CP 18.05.

Complete coincidence of the chromatograms of the tested samples with that of the reference sample of the chemical marker used indicates that the marker is present on the object. High sensitivity of the chemical marker based on phthalein allows reliable experimental detection of the marker on the objects in an amount of 1.0×10^{-6} g. The marker on the objects is detected by analytical reactions with an alkali (quick method) and by TLC after twofold cleaning with a brush wetted with water; the marker is detected on the objects after washing them with warm water with soap. From hand fingers and palms, the marker is removed only after three- to fivefold washing with warm soap-containing water with a brush. Enhanced retention of the marker on the marked objects is ensured by high adhesion properties of OAPP-N deposited onto the surface of phthalein particles. The resistance of the marker to aqueous detergent solutions is enhanced owing to hydrophobization of the surface of phthalein particles with the polymer film, i.e., the phthaleins undergo dressing. Isolation of pure CPP from the mixture of the three homologs is difficult; they are

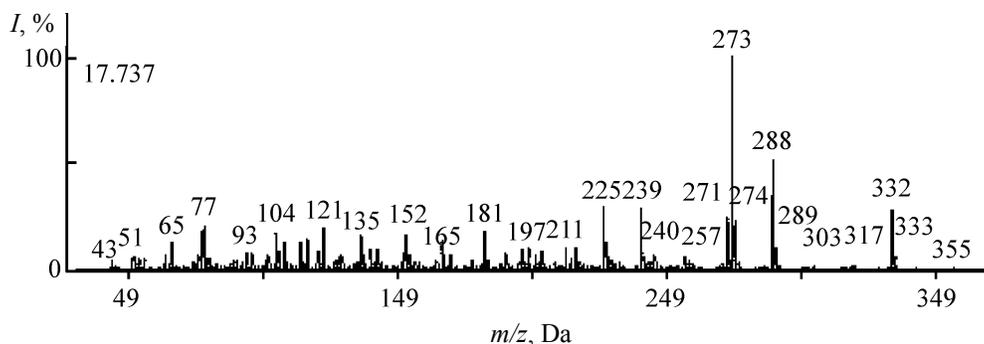


Fig. 4. Mass spectrum of cresolphenolphthalein and main characteristic fragmentation pathways of the molecular ion. (*I*) Peak intensity and (*m/z*) ion weight.

separated only in analytical amounts by thin-layer and gas–liquid chromatography.

The samples were examined by gas chromatography–mass spectrometry under the following conditions: Agilent Technologies 6850 chromatograph with Agilent Technologies 5975C mass-selective detector; HP-5MS capillary chromatographic column 30 m long, 0.25 mm i.d., coated with 0.25- μm layer of the stationary phase; injector temperature 250°C; detector interface temperature 280°C; column heating schedule: 50°C, 2.0 min; heating at a rate of 10 deg min^{-1} to 280°C; 280°C, 15 min; carrier gas helium, flow rate 1.0 mL min^{-1} ; ionization by electron impact (energy 70 eV); data recording and processing with Enhanced Productivity MSD ChemStation software. Qualitative analysis of the phthaleins was performed by the relative retention time of the chromatographic peaks (Fig. 3). RP decomposes in the course of chromatographic analysis.

The chromatographic peaks were identified using NTST 2.0 electronic library of mass spectra (created July 14, 2008). In the chromatogram of the reaction product, there are three fully resolved peaks with relative retention times of 32.78, 33.50, and 34.15 min, corresponding to PP (m/z 318), CPP (m/z 332), and CP (m/z 346). The mass spectra of PP and CP fully coincide with those from the library; the compound with m/z 332 was not found in the electronic library of the mass spectra, containing 200 000 compounds. Analysis of the fragmentation pattern of the molecular peak with m/z 332 confirmed the structure of CPP. In the mass spectrum of this compound, there is a strong peak of molecular ion at m/z 332 and peaks of fragment ions with m/z 288, 273, 239, 225, 195, 181. The molecular ion of CPP undergoes fragmentation via three main pathways (Fig. 4): (1) loss of the CO_2 molecule,

followed by the loss of the methyl radical; (2) loss of the phenoxy radical, followed by the loss of the CO_2 molecule; (3) loss of the *o*-cresol radical, followed by the loss of the CO_2 molecule.

Such fragmentation pathways of the molecular ion are typical of phthaleins [10].

The new chemical markers based on phthaleins were tested by the Expert Criminological Administration of the Department of Special and Criminological Provision of the Federal Drug Control Service of the Russian Federation with the aim of determining the possibilities of using them in the activity of expert criminological sections. The markers were recommended for practical use by drug control institutions.

CONCLUSIONS

(1) New chemical markers based on mixtures of individual phthaleins were developed. These markers are characterized by high level of secrecy in use, good transferability to contacting persons, enhanced retention on the marked objects, and reliable identification of phthaleins by expert investigation.

(2) The synthesized mixture of three phthalein homologs contains previously unknown phthalein with unsymmetrical phenolic substituents, *o*-cresolphenolphthalein [3-(3'-methyl-4'-hydroxyphenyl)-3-(4'-hydroxyphenyl)phthalide], which makes the marker falsification less probable.

(3) Quantum-chemical calculations of the reaction of *o*-cresolphenolphthalein synthesis show that the overall reaction is characterized by small positive changes in the enthalpy and Gibbs free energy, with the second and third step characterized by the negative

Gibbs energy. The optimum structure of the transition state was calculated.

(4) Positive expert conclusion of the Federal Drug Control Service of the Russian Federation with the recommendation for practical use by drug control institutions was received.

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