SEPARATION OF THE MAIN COMPONENTS OF RAW ACETONINE BY NORMAL-PHASE LIQUID CHROMATOGRAPHY

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The separation procedure of raw acetonine, that is unstable at the room temperature, has been worked out. Alumina was used as the stationary phase. The mixture of an aliphatic hydrocarbon with an alcohol saturated with the concentrated aqueous solution of ammonium hydroxide was used as the mobile phase. It has been proved that the result of the analysis at the room temperature was not affected by the spontaneous decomposition of the sample provided that the analysis did not exceed 30 min. Raw acetonine was found to contain ten components, four of which were identified.

Catalytic condensation of acetone with ammonia at low temperatures yields acetonine (2,2,4,6,6-pentamethyl-1,1,5,6-tetrahydropyrimidine)[1,2], at room or higher temperatures the main condensation product is triacetoneamine (2,2,6,6-tetramethyl-4-oxopiperidine)[3,4]. Both these compounds are important intermediates in the production of degradation inhibitors of the sterically-protected amine type that are used as additives to plastics. The main condensation products — 2,2,6,6-tetramethyl-4-oxopiperidine (*I*) and 2,2,4,6,6-pentamethyl-1,2,5,6-tetrahydropyrimidine (*II*), acetonine ACE) — are accompanied by various reaction byproducts. It follows from the mechanism of the condensation reaction that *e.g.* 4-methyl-3-penten-2-one (*III*), 2,6-dimethyl-2,5-heptadien-4-one (*IV*), 4-hydroxy-4-methyl-2-pentanone (*V*), 4-amino-4-methyl-2-pentanone (*VI*), may results. The composition and amount of byproducts depend both on the catalyst used and on the reaction conditions. For the production of *I* and *II* a series of various technological procedures was proposed and used, *e.g.* refs[5,6]. The analytical control of these procedures and even the determination of the main components content in raw products have not been successfully mastered. The main reason for this situation is the thermal lability of both these compounds and, particularly, the spontaneous subsequent secondary changes of the reaction mixture composition following the controlled condensation reaction. These subsequent changes of the composition are denoted as degradation processes. So far neither their cause nor course have been sufficiently investigated. It is only known that the degradation is stimulated by higher temperatures, by the presence of water, oxygen[1], and acidic substances.

These properties of the system make the choice of the analytical procedure and its realization very difficult. The determination of the main reaction products by distillation *in vacuo* that is used particularly in the preparation studies of *I* and *II*, is rather suspicious, namely due to the thermal instability of the substances that are to be determined, and also due to the increase of the degradation rate with temperature. Gas chromatography[7] is suitable for the determination of the condensation products with low boiling points. However, the analysis of the basic components with higher molecular weight and with lower volatility presents various problems.

Troubles caused by the thermal lability, basicity, and limited volatility of many condensation products and by the increase of the degradation rate with temperature could be avoided or at least substantially reduced using liquid chromatography. This method has been therefore used for working out the separation procedure of raw acetonine that fulfils the basic requirements arising from the studies of compound *II* synthesis and degradation of the raw product: to separate from the unstable reaction mixture of condensation products at least the most important components from the point of view of practice, *i.e.*, *I* and *II*, so that their content in the sample will not change during the analysis. Raw ACE was chosen for this study as it is often met as an intermediate also in the synthesis of *I* so that it can be considered to be the most important product from the analytical point of view.

EXPERIMENTAL

Laboratory set up was used for the measurements. It consisted of a high-pressure pulse-less pump Varian 8 500 (Varian, U.S.A.), of the spectrophotometric detector Variscan (Varian, U.S.A.), and of the differential refractometer Knauer 2 025/50 (Knauer, F.R.G.). The septum sampling system and the columns were of our own design.

Stainless steel columns with the bed dimensions of 4 × 200 mm were filled using the viscosity version of the high-pressure filtration technique[8]. The mixture of cyclohexanol with heptane (95 : 5) was used as the suspending liquid, the mixture of hydrocarbons served as the pressure liquid. The filling pressure of 25 MPa was maintained with the precision of ±0·3 MPa. The filled columns were stabilized by washing with 250 ml of ethanol. During the analyses of the reaction mixtures the column was kept at a constant temperature of 25 ± 0·2°C. The column filling materials used, summarized in Table I, are commercial products of Lachema, Brno (Czechoslovakia).

TABLE I
Solid phases used

Type	Commercial name	Specific surface $m^2\ g^{-1}$	Average particle size, μm	Other characteristics
Alumina	Alusorb N 075	70	7·5	neutral, low surface
Alumina	Alusorb VN	195	9·0	neutral, high surface
Modified silica gel	Silasorb 600-AP	—	10·0	modified by aminopropyl groups
Silica gel	Silasorb 300	250	10·0	washed by HCl and distilled water

At the beginning of experiments the mobile phase was prepared so that n-hexane was mixed with 2—10% of acetone or isopropanol at the room temperature. Methanol (0·1%), triethylamine (0·1%), water, gaseous ammonia or the saturated aqueous solution of ammonia were added to this mixture. These components of the mobile phase were added either individually or in various combinations. After a series of preliminary experiments the procedure of the mobile-phase preparation was used as follows: into the mixture of basic components (*i.e.*, hexane and isopropanol) the saturated aqueous solution of ammonia was added dropwise, under vigorous stirring. After adding 3—5 drops of the ammonium hydroxide solution exceeding the solubility limit, the mixture was stirred by ultrasound so that a milk-like emulsion resulted. The mobile phase, obtained after the separation of the excess ammonium hydroxide, was used for the measurements.

Raw ACE was prepared by a slightly modified procedure[1] and after sampling into different samples it was kept in closed probes at the temperature of dry carbon dioxide. Every day a fresh sample was used for the measurements. *I* was prepared according to ref.[5]. After a triple recrystallization from cyclohexane its melting point, 35·5°C, was in good agreement with the published value of 35—36°C (*cf.* ref.[3]). *I* was sampled in the form of the acetone solution. Mesityloxide was obtained as a laboratory preparation. Other reagents used for the measurement of the dead volume of the column (n-octane, benzene, cyclohexane), as model solutes (aniline, triethylamine, n-butylamine, ethanol, piperidine, acetone) or those used for the preparation of the mobile phases were products of Lachema, Brno (Czechoslovakia), mostly of the analytical grade. n--Hexane was produced in U.S.S.R.

RESULTS

Raw ACE is sensitive to both oxygen and water[1] and it is unstable in the acidic medium[9]. In the preliminary decision the chromatographic systems containing water as the major component of the mobile phase and substances of the acidic nature as, *e.g.*, non-modified silica gel, have been ruled out. In the first series of experiments the neutral alumina with low surface area and silica gel modified chemically by basic aminopropyl groups were chosen as the stationary phase.

The good solubility of raw ACE in organic solvents offered the possibility to use an aliphatic hydrocarbon as the major component of the mobile phases. Two polar solvents, isopropanol and acetone, were tested for the modification of the mobile--phase elution strength. The effect of the concentration of these modificating components as well as the effect of moderating admixtures (methanol, triethylamine, water, ammonia) to the mobile phase, added either individually or in various combinations, was tested by the separation of injected samples of raw ACE (by the number of zones, their symmetry, retention value, *etc.*) and also by the retention and zone shapes of the model solutes.

Results of the preliminary experiments can be summarized as follows: retention of all components of the raw ACE can be modified by the concentration of the polar organic component of the mobile phase. The highest selectivity of the separation of raw ACE and the best symmetry of the separated zones was achieved using the mobile phase containing — in addition to the hydrocarbon and the polar component

– also both water and ammonia. None of these compounds added to the mobile phase individually or in combination with an organic compound that could substitute water (*e.g.*, methanol) or ammonia (*e.g.*, triethylamine) gave a comparable result. Chromatographic systems with silica gel modified chemically by aminopropyl groups gave consistently worse results than alumina.

The capacity factors of the raw ACE components on alumina with low surface area were too low. All subsequent experiments were therefore made with alumina with high surface area as the stationary phase. In the mobile phase, acetone was replaced by isopropanol in order to reach higher column efficiency and avoid a danger of contingent reaction of acetone with ammonia if the mobile phase is kept in stock. At the content of 2–3% of isopropanol the low-polar components of raw ACE were very well separated (Fig. 1*a*). The increase of the alcohol content in the mobile phase to 7–10% resulted in a much faster elution of the components with highest polarity (Fig. 1*b*). The mobile phase with 4–5% of isopropanol is considered to be the best compromise. At the isopropanol concentration of 4% altogether 10 zones could be recognized in the raw ACE chromatogram using the UV detection. The low-polar components were distinctly separated from each other, the most polar component was washed out within 25 min. With 5% isopropanol the analysis was shorter by one third, the low-polar components remained only partly separated (Fig. 2). The complete elution of all components of the sample using the mobile

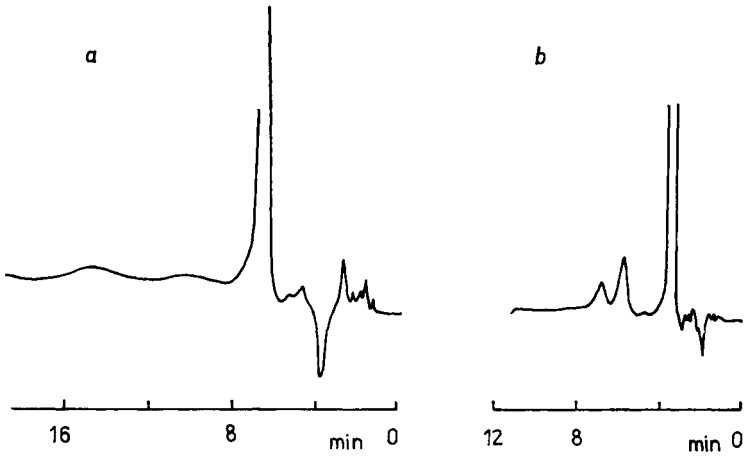

FIG. 1

Effect of the isopropanol content in the mobile phase on the retention of the raw ACE components. Stationary phase: Alusorb VN; mobile phase: n-hexane–isopropanol, saturated with the concentrated aqueous solution of ammonia. Isopropanol content: *a* 3% v/v, *b* 7% v/v. Detection: refractometer Knauer; flow rate of the mobile phase 1 ml min^{-1}

phase containing 4% of isopropanol has been confirmed by experiments using higher concentrations of isopropanol (up to 15%).

The content of water in the mobile phase saturated by the aqueous solution of ammonium hydroxide amounted to 0·5 vol. % max. With regard to the reported sensitivity of raw ACE to water[1] it was necessary to investigate whether, eventually how much, varies the composition of the sample during the analysis. Therefore the effect of the retention time of the sample in the column on the relative size of the separated zones was investigated. To secure the full separation of zones with lowest retention values that were most affected by the changes caused by the decomposition of compound *II*, these measurements were made with the mobile phase containing 3·5% of isopropanol. The duration of the sample contact with the column was prolonged by stopping the flow of the mobile phase. At the column temperature of 25°C the most polar component of the sample was eluted in 30 min. From the data summarized in Table II it can be deduced that even for the last-eluted component contact time of 35 min neither any changes of the number of eluted components nor any detectable changes of the zone peak heights could be observed. Hence, if the chromatographic system with the column temperature of 25°C is chosen so that the total time of the analysis is 30–35 min, the sample distortion during the analysis is negligible.

The mobile phase contains ammonium hydroxide that neutralizes the acid sites on the surface of the solid phase. It should be therefore possible to use also silica gel as the stationary phase. For better comparison of both analytical procedures, *i.e.*, either with alumina or with silica gel as the stationary phase, silica gel with the specific surface area close to that of alumina was chosen (Table I). Silica gel used

TABLE II

Peak heights of several zones in the chromatogram of raw ACE as a function of the sample contact time with the column. Stationary phase: Alusorb VN; mobile phase: isopropanol–hexane 3·5 : 96·5, saturated with the concentrated aqueous solution of ammonium hydroxide; column temperature 25°C

t^a min	Δt^b	h, mm			
		$k = 0{\cdot}3$	$k = 1{\cdot}1$	$k = 1{\cdot}5^c$	$k = 6{\cdot}3$
30	0	—	16·8	54·2	4·7
35	5	—	16·4	53·9	4·6
45	15	6·2	16·2	54·1	9·0
60	30	8·4	16·5	60·2	11·2

[a] t total time of the analysis; [b] Δt delay between the sample injection and the beginning of the analysis; [c] zone with the capacity factor of acetone.

did not come into contact with buffer solutions during its preparation. The remainders of hydrochloric acid from the acid washing after silica gel sieving were washed out by ethanol during the preparation of the column.

The effect of the alcohol concentration in the mobile phase and also the effect of the aqueous solution of ammonium hydroxide on the retention and on the shape of zones of the separated compounds was the same as that when alumina was used. The chromatograms obtained in control analyses with the same mobile phase differed only by the absolute values of capacity factors. The number of separated zones, their shapes, areas, and the elution sequence were the same as in the separation of raw ACE on the column with alumina.

By the comparison of elution times of standards in chromatograms obtained by UV-detection it was possible to identify zones in which *III*, acetone, and *I* were eluted (Figs 2 and 3). The purity and identity of substances in separated zones was not checked by an independent non-chromatographic method.

Zones of standards and zones corresponding to them by retention, separated from raw ACE by the elution with mobile phases containing 4 or less per cent of isopropanol, had the same shape. With the changes of the mobile phase composition all perfectly separated zones behaved as zones of chemical individuals. The same

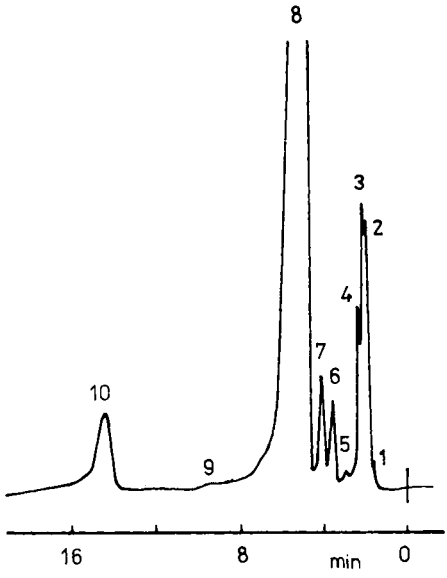

FIG. 2

Separation of raw ACE by the mobile phase with 5% of isopropanol. Stationary phase: Alusorb VN; column temperature 25°C; flow rate 1 ml min^{-1}; injection 1 µl. Detection: Variscan, wavelength 240 nm, range 0—2 AUFS; zone identification: 2 mesityloxide; 5 acetone; 6 *II*; 8 ACE

can be said also about the zones of *II* as the principal component. The chromatographic system under study was examined also for the separation of raw ACE degraded for a long period of time by elevated temperature. A typical result of such analysis is presented in Fig. 3. From the comparison of Figs 2 and 3 it is evident that in the chromatographic system used it could be possible (at the same duration of the analysis) to separate raw ACE into a number of components much higher than 10. With respect to all these arguments it can be assumed that the perfectly separated zones after the injection of raw ACE are zones of pure substances, the identity of which can be found from the comparison of their capacity factors with the capacity factors of standards.

So far the kinetics of the catalyzed condensation of acetone with ammonia is not sufficiently understood. Therefore, neither the number nor the structure of by-products, that can be formed during the condensation, is known. The complete identification of zones is therefore a separate problem that exceeds the scope of this study.

From the structure of standards and model substances and their retentions it

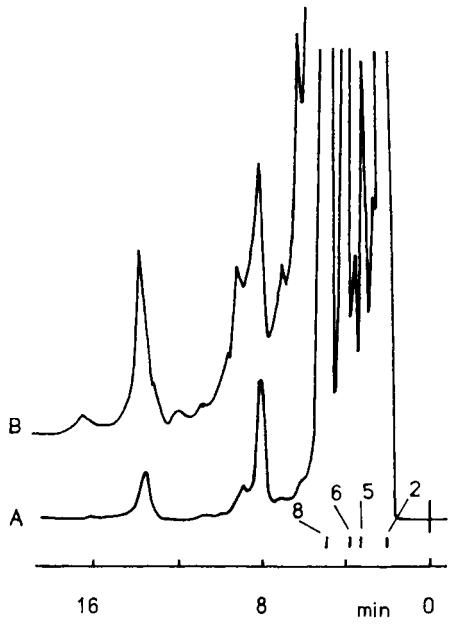

FIG. 3

Chromatogram of raw ACE after thermal degradation. A chromatographic system and other conditions -- see Fig. 2; B repeated analysis of the same sample with the UV detector range 0—0·1 AUFS. Elution times of standards: 2 mesityloxide; 5 acetone; 6 *II*; 8 ACE

turned out that in the chromatographic system used the compounds containing in their molecules only double bonds and keto-groups are eluted first. At the same time compound *III* with a higher number of carbon atoms in the molecule was less retained by the column than acetone (Fig. 2). Compounds containing nitrogen in the molecule were always eluted later. The capacity factors of *I* and *II* show that – for the similar structure of solutes – their retention increases with the number of nitrogen atoms in the molecule.

DISCUSSION

The low stability of *II* at the room temperature is the main source of difficulties with working out the separation of raw ACE that would yield undistorted results. Samples of the volume of 1–2 ml, kept in air and at temperatures of about 20°C, show changes of viscosity and colour already after 6–8 hours. Lower amounts of samples, *e.g.*, a drop on the microsyringe needle, degraded much faster. It was therefore necessary to look for the least severe conditions of the analysis. The results summarized in Table II prove that this basic problem has been solved. In the chromatographic system with ternary mobile phase of the type hydrocarbon–polar organic solvent–aqueous solution of ammonium hydroxide the stationary phase is dynamically modified during the equilibration of the column. A layer of highly polar stationary liquid appears on the adsorbent surface so that a liquid–liquid distribution system is formed[10,11,12]. According to Snyder[10] the ratio of the adsorption energies of an arbitrary component of the mobile phase on alumina and silica gel is a constant. The composition of stagnant layers that are formed at the contact of the given mobile phase with alumina or silica gel must therefore be the same. This explains also the fact that the exchange of silica gel for alumina did not affect the separation selectivity. At the same time from the independence of the elution sequence and separation selectivity on the stationary phase used it follows that adsorption does not participate significantly on the separation of the components of raw acetonine in the chromatographic system used. Therefore, the zone asymmetry and spreading of the basic model compounds and standards should be caused by secondary chemical equilibria of the basic components of the sample[13]. The improvement of the zone shapes observed after the simultaneous addition of water and ammonia or after the addition of the concentrated aqueous solution of ammonium hydroxide leads to the conclusion that ammonium hydroxide is the effective compound that suppresses the secondary chemical equilibria of the basic compounds of the sample.

In connection with the use of the strongly alkaline mobile phase, the long-term stability of the silica gel and alumina beds used seems remarkable. The column with alumina prepared for this study was later used also for the comparative analyses of products after long-term degradation of raw ACE. It preserved its good efficiency even after a half-year period. In column filled with silica gel that was used for about

one month, any deterioration of the efficiency was unmeasurable. The long-term stability of alumina and silica gel beds can be evidently ascribed to the minimum solubility of ionic reaction products in the mobile phases, the main component of which was a hydrocarbon. The chromatographic system described was proposed for the separation of raw ACE. However, the difference between the capacity factors of *II* and *I* is small and both these compounds are eluted approximately in the middle of the chromatographic spectrum. Furthermore, both these compounds are prepared from the same initial raw material under conditions that differ but slightly. It is therefore very probable that many byproducts that are formed in the preparation of *II* and *I* are either identical or very similar from the point of their structure. It can be therefore expected that raw *I* could be separated using the chromatographic system that was found to be suitable for the analysis of raw ACE.

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