THE POLAROGRAPHIC AND VOLTAMMETRIC DETERMINATION OF 1-(3'-CARBAMOYLPHENYL)-3,3-DIMETHYLTRIAZENE*

Jiří BAREK[a], Jana KUBÍČKOVÁ[a], Viktor MEJSTŘÍK[b] and Jiří ZIMA[a]

[a] *UNESCO Laboratory of the Environmental Electrochemistry, Department of Analytical Chemistry, Charles University, 128 40 Prague 2*

[b] *Toxicological Department, Research Institute of Organic Synthesis, 533 51 Pardubice-Rybitví*

Received December 12, 1991
Accepted March 20, 1992

A study was made of the polarographic reduction of the title triazene, a mechanism was proposed for this process and optimal conditions were found for its analytical application using tast polarography in the range $1 \cdot 10^{-4} - 2 \cdot 10^{-6}$ mol l^{-1}, differential pulse polarography in the range $1 \cdot 10^{-4}$ to $2 \cdot 10^{-7}$ mol l^{-1} and fast scan differential pulse voltammetry at a hanging mercury drop electrode in the range $1 \cdot 10^{-4} - 2 \cdot 10^{-7}$ mol l^{-1}. The sensitivity of the latter technique was increased through adsorptive accumulation of the test substance on the surface of the working electrode, permitting determination in the concentration range $1 \cdot 10^{-7} - 2 \cdot 10^{-9}$ mol l^{-1}.

In the framework of a systematic study of the use of modern polarographic and voltammetric methods for the analysis of chemical carcinogens, previous communications[1,2] have been devoted to the 2'-carbamoyl and 4'-carbamoyl derivatives of 1-phenyl-3,3-dimethyltriazene. As a continuation of this work, the present communication describes a study of the polarographic behaviour of the structurally related 1-(3'-carbamoylphenyl)-3,3-dimethyltriazene, which is suspected of chemical carcinogeneity[3] and is also a potential chemical carcinostatic[4,5]. The polarographic behaviour of this substance is mentioned in refs[6,7] and the polarographic behaviour of a number of other triazenes is discussed in detail in the previous communication[1]. So far, no detailed study has been carried out either of the mechanism of the polarographic reduction of the test substance or of the possibility of utilizing modern polarographic and voltammetric techniques such as differential pulse polarography (DPP) at a classical dropping mercury electrode (DME) or fast scan differential pulse voltammetry (FS DPV) at a hanging mercury drop electrode (HMDE) for its determination.

* Part XXII in the series Analysis of Chemical Carcinogens; Part XXI: Collect. Czech. Chem. Commun. *57*, 2248 (1992).

EXPERIMENTAL

Reagents

The stock solution of 1-(3′-carbamoylphenyl)-3,3-dimethyltriazene (c = 1 . 10^{-3} mol l^{-1}) was prepared by dissolving an exactly weighed amount of the solid substance (Research Institute of Organic Synthesis, Pardubice-Rybitví) in 1 l of p.a. methanol. The method for preparation and controlling the purity of the substance is described in ref.[8]. More dilute solutions were prepared by precise dilution of the stock solution with methanol. It followed from a spectrophotometric study of the stability of this solution, with measurement of the absorbance at 288 nm, where this substance exhibits an absorption maximum (ε = 1.515 . 10^3 mol^{-1} l cm^{-1}), that solutions with concentrations of 1 . 10^{-3} and 1 . 10^{-4} mol l^{-1} are stable in the dark for at least 3 months, with no change in absorbance within experimental error. Solutions with a concentration of 1 . 10^{-5} mol l^{-1} are stable for at least 1 week, after which a statistically significant decrease in their absorbance was observed. Solutions with even lower concentrations were prepared fresh daily. The remaining chemicals were of p.a. purity (Lachema, Brno), and water was doubly distilled in a quartz apparatus. The methanol employed was stored only in glass vessels, as contact with polyethylene led to extraction of substances that interfered in the determination of the lowest concentrations of the test substance.

Apparatus

A PA 4 polarographic analyser was employed together with an XY 4106 recorder (both from Laboratorní přístroje, Prague). Measurements were carried out using a three-electrode arrangement with a platinum foil auxiliary electrode and saturated silver chloride reference electrode, to which all the potential values are referred.

The parameters of the classical DME used in tast and DP polarography, the HMDE used in FS DPV and of the PA 4 analyser used to measure the polarographic and voltammetric curves were the same as in an earlier paper[9].

The coulometric and spectrophotometric measurements were carried out using the instruments described in ref.[10]. The actual pH of the Britton–Robinson buffer–methanol mixture (1 : 1) was measured using a PHM 62 pH meter (Radiometer, Copenhagen) with glass and saturated calomel electrodes. Calibration was carried out using acetate, borate and phosphate buffers in 50% (v/v) methanol[11,12].

Procedures

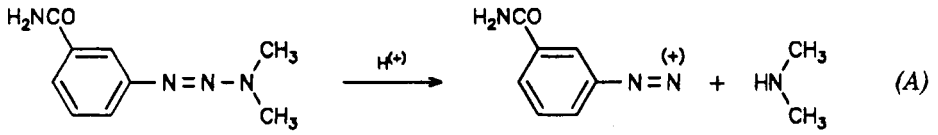
The calibration curves were measured in triplicate and evaluated by linear regression using the least squares method. The determination limit was calculated as ten times the standard deviation for determination of the analyte with a concentration corresponding to the lowest point on the given calibration dependence[13]. The procedure in coulometric measurements was analogous to that in an earlier communication[10], except that a Britton–Robinson buffer–methanol mixture (1 : 1) was used.

RESULTS AND DISCUSSION

STUDY OF THE MECHANISM OF THE POLAROGRAPHIC REDUCTION OF 1-(3′-CARBAMOYLPHENYL)-3,3-DIMETHYLTRIAZENE

Table I describes the effect of the pH on the polarographic behaviour of the test substance, where it can be seen that the dependence of the half-wave potential in tast polarography or the peak potential in DPP on the pH is asymptotic. The decrease in the

height of the wave or peak at pH < 4.9 can be explained in terms of protolysis of the test substance according to Eq. (*A*).



(*A*)

In contrast to the 2'-carbamoyl derivative, studied in ref.[1] which can be stabilized by intramolecular cyclization, the transient product of the reduction of 1-(3'-carbamoylphenyl)-3,3-dimethyltriazene, a diazonium compound, is further decomposed to form a polarographically inactive product. This assumption was also confirmed by a spectrophotometric study of the decomposition of the test triazene in medium with pH 2.8 (see Fig. 1), where the absorption maximum at 288 nm, assigned to the triazene functional group[6], gradually disappears. It followed from the tast polarographic study of this decomposition at pH 2.8 that this is a first-order reaction with respect to the concentration of the test triazene (linear dependence of the logarithm of the limiting current on time) with a reaction half-time of 30 min at 20 °C. All the tast polarograms

TABLE I
The effect of the pH on the tast and DP polarographic curves of 1-(3'-carbamoylphenyl)-3,3-dimethyltriazene (c = 1 . 10^{-4} mol l^{-1}) in Britton–Robinson buffer–methanol medium (1 : 1)

pH	$E_{1/2}$, mV	I_{lim}, μA	α[a], mV	E_p, mV	I_p, μA
2.8	-730	0.85	63.8	-705	1.41
4.0	-880	1.08	62.4	-840	1.60
4.9	-945	1.22	63.3	-910	1.80
5.9	-985	1.17	58.3	-960	1.85
7.1	-1 050	1.13	60.9	-1 010	1.83
8.1	-1 095	1.04	–[b]	-1 045	1.55
8.8	-1 100	0.63	–[b]	-1 065	0.73
9.4[c]	-1 125	0.26	–[b]	-1 070	0.33
10.3[c]	-1 120	0.06	–[b]	-1 075	0.04
11.2[c]	–[d]	–[d]	–[d]	–[d]	–[d]
12.1[c]	–[d]	–[d]	–[d]	–[d]	–[d]

[a] Slope of the logarithmic analysis. [b] Logarithmic analysis was not carried out because of the poorly developed waves. [c] A further wave or peak appears at these pH values at about -1 700 mV; these are difficult to evaluate as they coincide with the decomposition of the background electrolyte. [d] The more positive wave or peak completely disappears at these pH values.

measurred during the decomposition at this pH consisted of only a single wave, corresponding to the initial triazene. In contrast, in medium at pH 1.3, in a mixture of 0.1M-HCl–CH_3OH (1 : 1) another wave appears in the region around –0.2 V, apparently corresponding to the temporarily formed arene diazonium ion (see Fig. 2). At pH > 5, no decrease was observed in the absorbance at 288 nm with time and the UV spectrum of the test substance did not change in the whole test interval pH 5 – 12, indicating that the test triazene is present in an unprotonated form in solution under these conditions.

The effect of the pH on the polarographic behaviour of the test triazene can be explained by the concept of surface protonation prior to the actual reduction[14]. In the framework of this concept, the substance diffuses to the surface of the mercury electrode, is adsorbed and then protonated; the protonated form then undergoes reduction. As the pH increases, the rate of the prior protonation decreases, leading to a decrease in the wave height. The concept of surface protonation explains the observed decrease in the limiting current with increasing potential, as explained in detail in a previous paper[1] and also the fact that limiting current is diffusion-controlled at pH 5.9 and completely

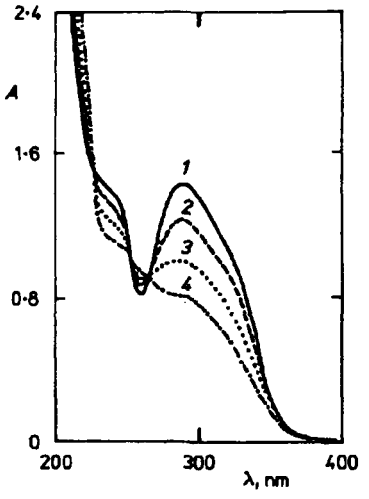


FIG. 1
The absorption spectra of the protonated 1-(3'-carbamoylphenyl)-3,3-dimethyltriazene (c = 1 . 10^{-4} mol l^{-1}) in Britton–Robinson buffer–methanol medium (1 : 1) at pH 2.8 at times 0 (*1*), 20 (*2*), 40 (*3*) and 60 (*4*) min after preparation of the solution

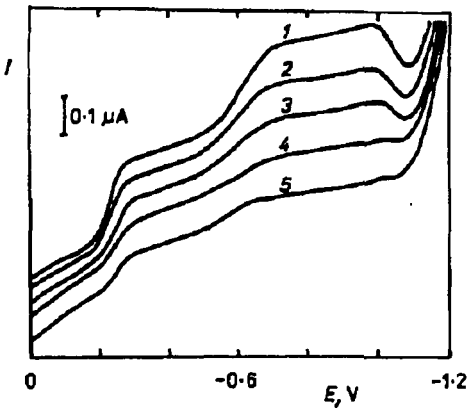
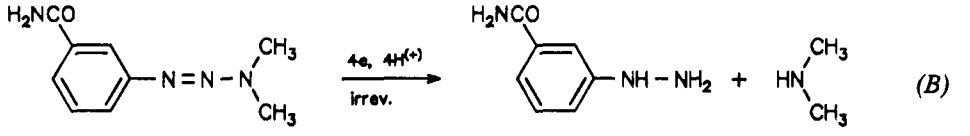


FIG. 2
Tast polarograms of the protonated 1-(3'-carbamoylphenyl)-3,3-dimethyltriazene (c = 1 . 10^{-4} mol l^{-1}) in 0.1M-HCl–CH_3OH medium (1 : 1) at pH 1.3 at times 10 (*1*), 20 (*2*), 30 (*3*), 45 (*4*) and 60 (*5*) min after preparation of the solution

kinetically controlled at pH 9.4 (see Fig. 3). The peak observed in alkaline medium, whose position is pH-independent, apparently corresponds to direct polarographic reduction of the unprotonated molecule of 1-(3′-carbamoylphenyl)-3,3-dimethyltriazene. This wave is difficult to evaluate in mixed water–methanol medium as it coincides with the decomposition of the base electrolyte. On the other hand, these waves are well developed in 0.01M-LiOH medium in anhydrous methanol ($E_{1/2}$ = −1.675 V) and their height is diffusion-controlled, confirmed by the linear dependence of the peak height in DC polarography on the square root of the mercury reservoir height.

It follows from cyclic voltammetry at the HMDE that this is an irreversible process (see Fig. 4), also explaining the observed shift in the half-wave potential with decreasing concentration to more positive values by about 15 mV on a change in the concentration of the test substance by one order. The anodic peak at about 0 V, which forms an almost reversible pair with the cathodic peak present only after the second cycle, was assigned to the 3′-carbamoyl phenylhydrazine formed in the reduction (see Fig. 4).

It was found by potentiostatic coulometry at a large-surface mercury electrode that, in a Britton–Robinson buffer–methanol (1 : 1) medium at pH 5.9, 1-(3′-carbamoylphenyl)-3,3-dimethyltriazene exchanges a total of 4 electrons at a constant potential of −1 095 mV vs SCE.



(B)

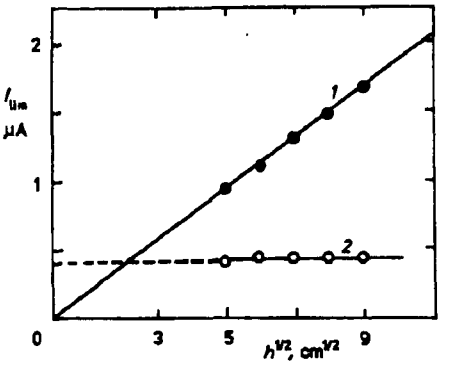


FIG. 3
The variation of the limiting current of 1-(3′-carbamoylphenyl)-3,3-dimethyltriazene (c = 1 . 10^{-4} mol l^{-1}) in DC polarography with the square root of the mercury reservoir height in Britton–Robinson buffer–methanol medium (1 : 1) at pH 5.9 (*1*) and 9.4 (*2*)

On the basis of analogy with the polarographic behaviour of unsubstituted 1-phenyl-3,3-dimethyltriazene[15] and its 2'-carbamoyl[1] and 4'-carbamoyl[2] derivatives, it can be assumed that 1-(3'-carbamoylphenyl)-3,3-dimethyltriazene also undergoes a four-electron irreversible reduction at pH < 7, according to Eq. (*B*):

ANALYTICAL UTILIZATION OF THE POLAROGRAPHIC REDUCTION OF 1-(3'-CARBAMOYLPHENYL)-3,3-DIMETHYLTRIAZENE

Tast Polarography and Differential Pulse Polarography at a Dropping Mercury Electrode

From an analytical point of view, a Britton–Robinson buffer–methanol (1 : 1) medium at pH 5.9 is optimal, and yields the highest waves or peaks (see Figs 5 and 6). Simultaneously, solutions polarographed in this medium are sufficiently stable (the height of the wave or peak does not change after 60 min within experimental error), in contrast to acidic solutions, where acid hydrolysis leads to a decrease in the wave height after 30 or 60 min by 30 and 60%, respectively, at pH 2.8 and by 5 and 10%, respectively, at pH 4.0. At pH 5.9, the dependence of the wave or peak height on the concentration of the test substance was linear in the range $1 \cdot 10^{-4} - 2 \cdot 10^{-6}$ mol l^{-1} for tast polarography and $1 \cdot 10^{-4} - 2 \cdot 10^{-7}$ mol l^{-1} for DPP. The peak height was measured from

TABLE II
Parameters of the calibration straight lines and determination limits of 1-(3'-carbamoylphenyl)-3,3-dimethyltriazene

Method	*c* mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	Correlation coefficient	Determination limit, mol l^{-1}
Tast[a]	$(1-10) \cdot 10^{-5}$	11.9	21.5	0.9988	–
	$(2-10) \cdot 10^{-6}$	11.0	2.0	0.9985	$1.8 \cdot 10^{-6}$
DPP[a]	$(1-10) \cdot 10^{-5}$	18.8	-21.0	0.9996	–
	$(1-10) \cdot 10^{-6}$	18.1	1.8	0.9999	–
	$(2-10) \cdot 10^{-7}$	16.0	-0.1	0.9980	$1.9 \cdot 10^{-7}$
FS DPV[a]	$(1-10) \cdot 10^{-5}$	8.4	21.0	0.9999	–
	$(1-10) \cdot 10^{-6}$	8.0	6.3	0.9991	–
	$(2-10) \cdot 10^{-7}$	8.8	0.5	0.9989	$2.2 \cdot 10^{-7}$
FS DPV[b]	$(1-10) \cdot 10^{-8}$	121	0.3	0.9925	–
	$(2-10) \cdot 10^{-9}$	141	-0.07	0.9910	$3.6 \cdot 10^{-9}$

Britton–Robinson buffer–methanol medium: [a] 1 : 1, pH 5.9; [b] 99 : 1, pH 5.1, adsorptive accumulation for 60 s in stirred solution, recorded 10 s after termination of stirring.

the line connecting the minima on both sides of the peak. The parameters of these dependences calculated by linear regression using the least squares method and the calculated detection limits are given in Table II.

Fast Scan Differential Pulse Voltammetry at a Hanging Mercury Drop Electrode

The effect of the pH on the position and height of the FS DPV peaks of the test substance can be seen in Table III, from which it is apparent that the highest peaks are obtained in medium with pH of about 6. The decrease in the peak height at higher pH values is connected with decreasing wave height in tast polarography, while the decrease in the peak height at lower pH values is connected with the hydrolysis of the test substance in acid medium. The dependence of E_p on the pH for FS DPV has the same character as for DPP and can be interpreted in the same manner. In medium with pH 5.9, the dependence of the peak height on the analyte concentration is linear in the range $1 \cdot 10^{-4} - 2 \cdot 10^{-7}$ mol l^{-1}. The parameters of this dependence and the determination limits are given in Table II. For comparison, an attempt was made to carry out the FS DPV determination of the test substance in 0.01M-LiOH medium in anhydrous methanol, yielding a relatively well-developed peak (E_p = −1.515 V). The calibration curves are linear in the range $1 \cdot 10^{-4} - 2 \cdot 10^{-6}$ mol l^{-1}, but their slope (5.9 mA mol^{-1} l) is less than in mixed water–methanol medium, leading to a higher determination limit

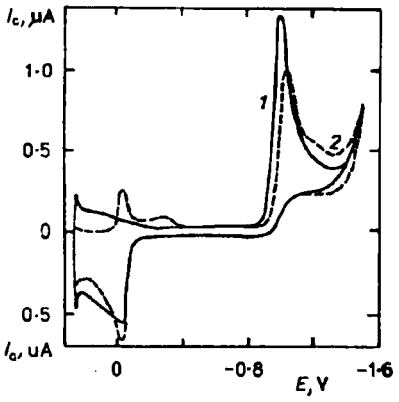


FIG. 4
Cyclic voltammogram of 1-(3'-carbamoylphenyl)-3,3-dimethyltriazene (c = 1 . 10^{-4} mol l^{-1}) in Britton–Robinson buffer–methanol medium (1 : 1), pH 5.9 at a polarization rate of 100 mV s^{-1}. Cycle: 1 (*1*); 2 (*2*)

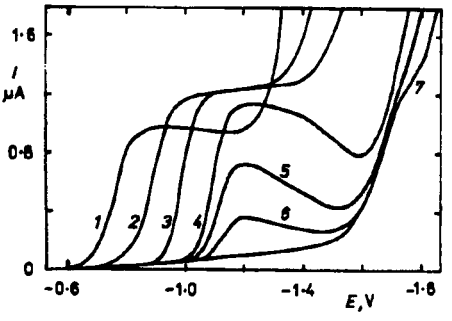


FIG. 5
Tast polarograms of 1-(3'-carbamoylphenyl)-3,3-dimethyltriazene (c = 1 . 10^{-4} mol l^{-1}) in Britton–Robinson buffer–methanol medium (1 : 1), pH: 2.8 (*1*), 4.0 (*2*), 5.9 (*3*), 8.1 (*4*), 8.8 (*5*), 9.4 (*6*) and 11.2 (*7*)

($2.8 \cdot 10^{-6}$ mol l^{-1}). The determination cannot be carried out at lower concentrations because of the high residual current value connected with the position of the peak close to the potential of decomposition of the background electrolyte.

It can be seen in Fig. 7 that the height of the FS DPV peak increases with the time elapsed between the formation of the drop and the recording of the voltammetric curve, as a consequence of the adsorptive accumulation of the test substance on the surface of the HMDE. It can also be seen from Fig. 7 that this increase becomes greater as the

TABLE III
The effect of the pH on the position (E_p) and height (I_p) of the FS DPV peaks of 1-(3'-carbamoylphenyl)-3,3-dimethyltriazene ($c = 1 \cdot 10^{-5}$ mol l^{-1}) in Britton–Robinson buffer–methanol medium (1 : 1)

pH	E_p, mV	I_p, nA	pH [a]	E_p, mV	I_p, nA
2.8	-660	65	8.1	-1 005	80
4.0	-775	72	8.8	-1 030	60
4.9	-850	77	9.4	-1 050	32
5.9	-905	88	10.3	-1 060	8
7.0	-960	85	11.2	–[b]	–[b]

[a] At pH > 8, a slight peak appears at about -1 400 mV, but coincides with the decomposition of the background electrolyte; [b] at pH > 11, the pH-dependent peak disappears.

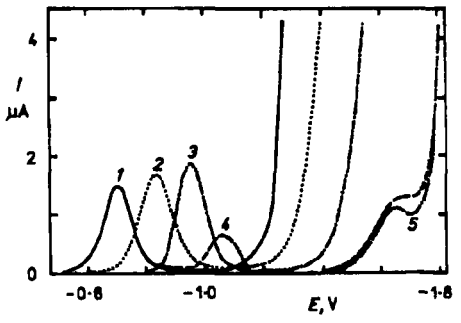


FIG. 6
DP polarograms of 1-(3'-carbamoylphenyl)-3,3-dimethyltriazene ($c = 1 \cdot 10^{-4}$ mol l^{-1}) in Britton–Robinson buffer–methanol medium (1 : 1), pH: 2.8 (*1*), 4.0 (*2*), 5.9 (*3*), 8.8 (*4*) and 11.2 (*5*)

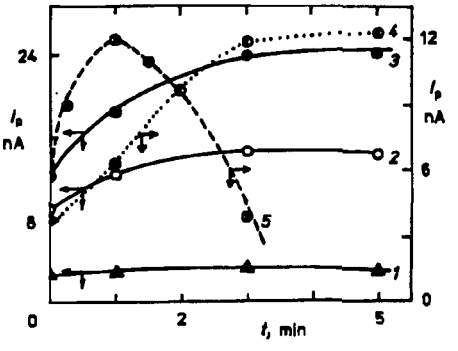


FIG. 7
Dependence of the height of the FS DPV peak of 1-(3'-carbamoylphenyl)-3,3-dimethyltriazene (*1*) on the accumulation time in medium containing 50% (*1*), 10% (*2*) and 2% (*3*) methanol (v/v) in unstirred solution ($c(1) = 2 \cdot 10^{-7}$ mol l^{-1}) and in medium containing 1% methanol ($c(1) = 1 \cdot 10^{-7}$ mol l^{-1}) in unstirred (*4*) and stirred (*5*) medium

methanol content decreases in the test solution, which can be explained both by the higher solubility of the analyte in methanol and also by the competitive adsorption of the molecules of this solvent on the surface of the HMDE. It is also apparent that this increase is favoured by stirring. The observed decrease in the height of the FS DPV peak during accumulation in stirred solution with an accumulation time longer than 60 s is connected with the accumulation of impurities in the background electrolyte producing deformation of the peak of the test substance. The dependence of the peak height on the analyte concentration in Britton–Robinson buffer–methanol medium (9 : 1) at pH 5.1 for 60-second accumulation in stirred solution is linear in the range $1 \cdot 10^{-7} - 2 \cdot 10^{-9}$ mol l^{-1}. The parameters of the calibration straight lines and the determination limits are given in Table II.

REFERENCES

1. Barek J., Toubar S., Zima J.: Collect. Czech. Chem. Commun. *56*, 2073 (1991).
2. Barek J., Mejstřík V., Toubar S., Zima J.: Collect. Czech. Chem. Commun. *57*, 1230 (1992).
3. Burchenal J. H., Carter S. K.: Cancer (Philadelphia) *30*, 1636 (1972); Chem. Abstr. *78*, 37811b (1973).
4. Schmidt F. A., Hutchinson D. J.: Cancer Res. *34*, 1917 (1974).
5. Matrka M., Zvěřina V., Marhold J.: Cesk. Farm. *24*, 262 (1975).
6. Matrka M., Rambousek V., Držková L., Zvěřina V.: Cesk. Farm. *27*, 299 (1978).
7. Matrka M., Rambousek V., Zvěřina V.: Cesk. Farm. *27*, 346 (1978).
8. Matrka M., Rambousek V., Remeš M., Zvěřina V.: Cesk. Farm. *27*, 70 (1978).
9. Barek J., Kubíčková J., Mejstřík V., Petira O., Zima J.: Collect. Czech. Chem. Commun. *55*, 204 (1990).
10. Barek J., Haladová-Bláhová H., Zima J.: Collect. Czech. Chem. Commun. *54*, 1538 (1989).
11. Paabo M., Robinson R. A., Bates R. G.: J. Am. Chem. Soc. *87*, 415 (1965).
12. Kozáková E., Czéfelvayová B.: Chem. Zvesti *34*, 610 (1980).
13. Beyermann K.: *Organic Trace Analysis*, p. 45. Ellis Horwood, Chichester 1984.
14. Mairanovskii S. G.: Talanta *12*, 1299 (1965).
15. Kazemifard G., Moatar F., Reisch J.: Acta Pharm. Iugoslav. *28*, 151 (1978).

Translated by M. Štulíková.