

Sensitive determination of kojic acid in foodstuffs using PVP (polyvinylpyrrolidone) modified acetylene black paste electrode

Xiaofeng Yang *, Huajie Zhang

Department of Pharmacy, Wenzhou Medical College, Wenzhou, Zhejiang 325035, PR China

Received 20 April 2006; received in revised form 7 June 2006; accepted 1 July 2006

Abstract

The voltammetric behaviors of kojic acid (KA) were investigated at polyvinylpyrrolidone (cross-linked) modified acetylene black paste electrode (denoted as PVP/ABPE) in 0.1 mol/L, pH 5.0 HAc–NaAc buffer. A well-defined and sensitive oxidation peak appears at 0.84 V. Compared with an unmodified acetylene black paste electrode (ABPE), the PVP/ABPE remarkably enhances the oxidation peak current of kojic acid, indicative of good potential in sensitive determination of kojic acid. After optimization of experimental conditions, a fast and sensitive electrochemical method was developed for the determination of kojic acid. This method exhibits good linearity over the range from 1.0×10^{-6} mol/L to 1.0×10^{-4} mol/L, and the detection limit is as low as 5.0×10^{-7} mol/L. In order to validate the feasibility of this proposed method, it was used for quantitative analysis of kojic acid in some food samples and the results were satisfying.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Kojic acid (KA); Polyvinylpyrrolidone (cross-linked) (PVP); Acetylene black paste electrode (ABPE); Voltammetric determination

1. Introduction

Kojic acid (5-hydroxy-2-(hydroxymethyl)-4-pyrone, see Fig. 1) is a natural product found in fermented food, and is becoming more and more important because of its various effective features, such as antibacterial action, sequestering heavy metal ions which accelerate oxidation, retardation of melanism, and inhibition of the enzyme which deteriorates foodstuffs. In everyday life, it is widely used as a food additive and preservative, as a skin-whitening agent in cosmetics, as a plant growth regulator as well as a chemical intermediate (Chen & Iubo, 2002; Chen & Liu, 2002; Kotani, Ichimoto, Tstsumi, & Fujita, 1976; Saruno, Kato, & Ikeno, 1979; Chen et al., 1991). Otherwise, kojic acid also plays an important role in monitoring fermentation process. However, because the 4-pyrone in kojic acid molecules had aromaticity like benzene, it is not clear

whether kojic acid in all kinds of foodstuffs have side effects on human health. So detection of trace amount of kojic acid is necessary. At the present time, several methods have been reported to determine kojic acid including high-performance liquid chromatography (HPLC) (Huang, Lin, Huang, & Wen, 2004; Kimura, Hirokado, Yasuda, & Nishijima, 2000; Shih, 2001), stopped-flow method (Tanigaki, Obata, & Tokuyama, 1980), electroanalytical method (Vachalkova, Bransova, Brtko, Uher, & Novotny, 1996; Shih & Zen, 1999). To the best of our knowledge, a convenient and direct electrochemical method for determination of kojic acid using cross-linked polyvinylpyrrolidone modified acetylene black paste electrode (PVP/ABPE) was firstly reported here.

PVP is a water-fast polymer and shows strong adsorption character to phenolic compounds, which is attributed to hydrogen bond between imide in the center of polymer and hydroxyl group in phenolic compounds. In this work, the above-discussed excellent properties of PVP were fully utilized to improve the sensitivity of determination of kojic

* Corresponding author.

E-mail address: yang_xiaofeng@163.com (X. Yang).

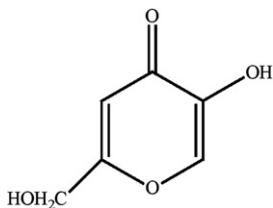


Fig. 1. Chemical structure of kojic acid.

acid. As expected, the oxidation peak current of kojic acid was significantly improved at PVP/ABPE, compared with that at an unmodified ABPE. Based on this, a sensitive and simple electrochemical method was developed for the determining kojic acid. This newly-proposed method possesses following advantages: low cost, high sensitivity and rapid response, which demonstrated with foodstuff samples.

2. Experimental

2.1. Reagents

Cross-linked polyvinylpyrrolidone (PVP) was purchased from Acros Chemical Reagent Co., USA. 1.0×10^{-2} mol/L Kojic acid stock solution was prepared by dissolving kojic acid (Sigma) into water, and then diluted to working solutions with different concentrations. All the kojic acid solutions were stored at 4 °C. All the chemicals were used without further purification and all the solutions were prepared with doubly distilled water.

2.2. Apparatus

All the electrochemical measurements were performed on a CHI 650B electrochemical workstation (Shanghai Chenhua Co., China) in a three-electrode system. The working electrode was a PVP/ABPE. A Pt wire and a saturated calomel electrode (SCE) were used as the counter and reference electrodes, respectively.

High-performance liquid chromatographic determination was carried out on an Agilent 1100 with an UV detector. The kojic acid in foodstuffs was analyzed using a Cosmosil 5 C18-AR-II column. A mixture of 0.05 mol/L KH_2PO_4 buffer solution (pH 2.5) and methanol (99:1, v/v) was used as mobile phase. The UV detector was set at 280 nm.

2.3. Preparation of PVP/ABPE

The PVP/ABPE was prepared by mixing 10.0 mg acetylene black (AB), 4.0 mg PVP and 12.0 μL paraffin oil in a small mortar to form a homogeneous acetylene black mixture. Then the mixture was pressed into the end cavity of a homemade poly (tetrafluoroethylene) (PTFE) cylindrical electrode body (diameter: 2.0 mm), and the electrode surface was polished manually on a piece of weighting paper. It is important that the amount of paraffin oil must be care-

fully controlled because the conductivity of the electrode decreased with excessive paraffin oil, and insufficient paraffin oil was not beneficial to the uniform mix of acetylene black.

2.4. Experimental procedures

A certain volume of 0.1 mol/L HAc–NaAc (pH 5.0) was used as supporting electrolyte in a conventional electrochemical cell. At the beginning of experiment, PVP/ABPE was activated by successive cyclic voltammetric sweeps between 0.40 V and 1.20 V at 100 mV/s to get a stable cyclic voltammograms. After that, a known volume of kojic acid solution was placed into the cell to make up 10.0 mL mixture solution. The accumulation was carried out at open circuit via stirring the solution for 2 min, and then kept quiet for 15 s. Linear sweep voltammograms were recorded from 0.40 V to 1.20 V at a scan rate 100 mV/s. After each measurement, the modified electrode was refreshed by successive cyclic voltammetric sweeps in blank supporting electrolyte (between 0.40 and 1.20 V at 100 mV/s) to get a reproducible electrode surface.

2.5. Preparation of kojic acid sample

Two kinds of sauce and vinegar samples were purchased from a local super market. Kojic acid was extracted from the various commercial foods with 0.05 mol/L KH_2PO_4 buffer solution (pH 2.5). The extract was centrifuged, and then the supernatant fluid was filtered through a 0.45 μm membrane filter.

3. Result and discussion

3.1. Electrochemical responses of kojic acid

Fig. 2 shows the successive cyclic voltammograms of 5.0×10^{-5} mol/L kojic acid at a PVP/ABPE. In 0.1 mol/L HAc–NaAc buffer (pH 5.0), a well-defined oxidation peak

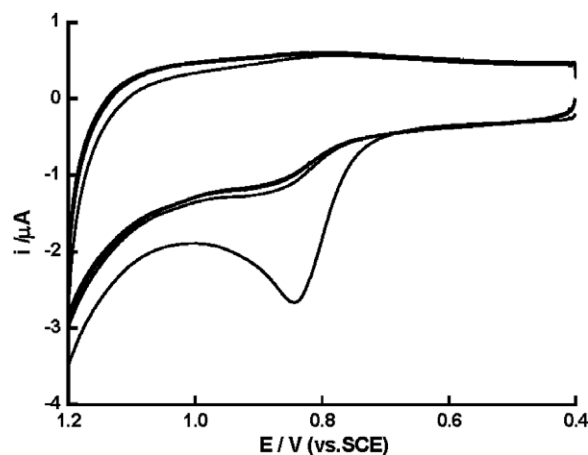


Fig. 2. Cyclic voltammograms of 5.0×10^{-5} mol/L kojic acid at PVP/ABPE in 0.1 mol/L HAc–NaAc buffer (pH 5.0). Scan rate: 100 mV/s.

appears at 0.84 V on the first anodic sweep from 0.40 V to 1.20 V and no corresponding reduction peak is observed in the reverse scan, suggesting that the electrode process of kojic acid was totally irreversible. During the following successive cyclic sweeps, the oxidation peak decreases significantly. It may be caused by the fact that the electron transfer was blocked by the adsorption of reaction products resulting in the decrease of reaction sites on electrode surface.

In order to illustrate good potential of PVP in the sensitive determination of kojic acid, the electrochemical responses of 5.0×10^{-5} mol/L kojic acid were compared at bare ABPE and PVP/ABPE by linear sweep voltammetry (LSV). Curves (a) and (c) depict the LS voltammograms of ABPE and PVP/ABPE in 0.1 mol/L HAC–NaAC buffer (pH 5.0) without kojic acid, and there is no observable oxidation peak. After addition of 5.0×10^{-5} mol/L kojic acid, a well-defined oxidation peak is observed at the unmodified ABPE (curve b) and PVP/ABPE (curve d), respectively. Compared with those at unmodified ABPE (curve b), the oxidation peak current of kojic acid increases greatly at the PVP/ABPE (curve d), and meanwhile, the oxidation peak potential shifts negatively from 1.00 to 0.84 V. The significant peak current enhancement and obvious negative shift of oxidation peak potential reveal that PVP facilitates the electron transfer of kojic acid. PVP, a polymeric sorbent with hydrophobic backbone and heterocyclic groups, exhibits high accumulation efficiency toward kojic acid, therefore, the concentration of kojic acid at PVP/ABPE surface increases remarkably, and the oxidation peak current increases and oxidation overpotential lowers in the presence of PVP (see Fig. 3).

3.2. Choice of supporting electrolytes and solution pH

In this work, the oxidation response of 5.0×10^{-5} mol/L kojic acid in various supporting electrolytes were examined by LSV. The supporting electrolytes include pH 5.0–

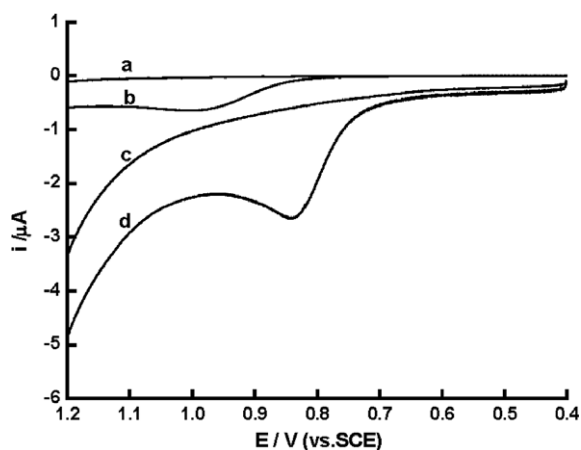


Fig. 3. Linear sweep voltammograms of bare ABPE (a, b) and PVP/ABPE (c, d) in the absence of kojic acid (a, c) and in the presence of 5.0×10^{-5} mol/L kojic acid (b, d). Other conditions are the same as in Fig. 1.

9.0 phosphate buffer, pH 3.5–5.6 HAC–NaAc buffer, pH 1.0–4.0 sodium citrate–hydrochloric acid (HCl) buffer, pH 5.0–7.0 sodium citrate–sodium hydroxide (NaOH) buffer, pH 1.4–4.5 sodium tartrate–tartaric acid buffer, pH 7.6–9.2 borax–hydrochloric acid buffer, pH 9.2–12.3 borax–sodium hydroxide buffer, HClO₄, H₂SO₄, HCl, HAC, NaOH and KCl (each 0.1 mol/L). The results show that higher peak current and better peak shape was obtained in 0.1 mol/L HAC–NaAc buffer solution. Consequently, 0.1 mol/L HAC–NaAc buffer solution was chosen as supporting electrolyte for quantitative analysis of kojic acid.

Additionally, the influences of HAC–NaAc buffer pH on oxidation peak current and peak potential were also researched by LSV, and the results described in Fig. 4. It can be seen that the oxidation peak current of kojic acid almost keeps unchangeable over pH range from 3.5 to 5.6 (curve a). However, the oxidation peak potential shifts negatively with increasing solution pH (curve b), obeying the equation $E_p = 1.1541 - 0.06267 \text{ pH}$ ($R = 0.9953$). The slope of 63 mV/pH suggests that the number of proton and electron involved in the oxidation of kojic acid is equal.

3.3. Effect of mass ratio of AB vs. PVP

The oxidation peak current of kojic acid dependence of the amount of PVP was also examined. As gradual increasing the amount of PVP, the oxidation peak current of kojic acid also gradually increases, attributing to the fact that more PVP exhibits much higher accumulation efficiency to kojic acid. However, the oxidation peak current of kojic acid increases slightly when the mass ratio of AB versus PVP exceeds 10:4, what's more, more PVP will enhance the background current of PVP/ABPE since PVP is electric insulator. Therefore, the best mass ratio of AB/PVP is 10:4 to achieve high sensitivity and better oxidation peak shape.

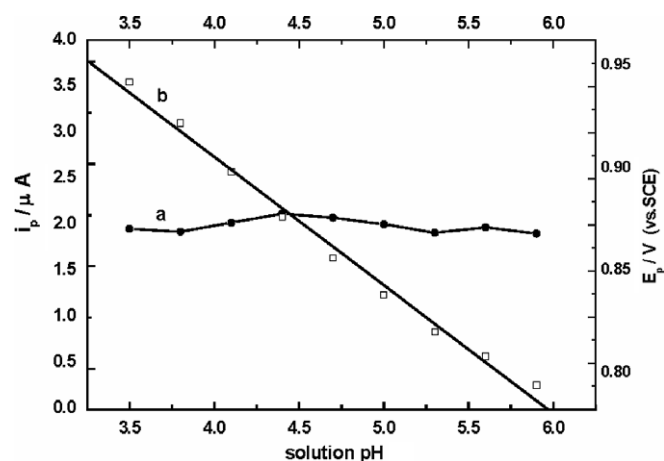


Fig. 4. Dependence of pH on peak current (i_p , curve a) and peak potential (E_p , curve b) of 5.0×10^{-5} mol/L kojic acid at PVP/ABPE. Other conditions are the same as in Fig. 1.

3.4. Effect of accumulation time

Accumulation is a common and effective way to improve the sensitivity in electrochemical determination. Fig. 5 depicts the effect of accumulation time on the oxidation peak current of kojic acid. It is found that the oxidation peak current of kojic acid increases as prolonging accumulation time. However, it increases greatly within the first 2 min and then slowly. It was a typical adsorption character that brought on the saturated adsorption of kojic acid to electrode surface if enough accumulation time was offered. In the current work, the accumulation time was selected as 2 min to acquire higher sensitivity and shorten analysis time.

3.5. Electrode process of kojic acid on PVP/ABPE

The effect of scan rate (v) on the oxidation process of 5.0×10^{-5} mol/L kojic acid at PVP/ABPE in 0.1 mol/L HAc–NaAc buffer (pH 5.0) were examined by LSV. The peak current (i_p) increased linearly with scan rate over the range from 0.025 to 0.30 V/s, which expressed as follows:

$$i_p = 15.7263v + 0.5142 (R = 0.9983) \quad (1)$$

Thus, the electrode process was controlled by adsorption step. This was consistent with the discussion about successive cyclic voltammograms of kojic acid.

Otherwise, the relationship between oxidation peak potential (E_p) and v was investigated. As increasing v , E_p gradually shifts positively described by the following equation:

$$E_p = 0.02415 \ln v + 0.9101 (R = 0.9974) \quad (2)$$

As far as totally irreversible electrode process controlled by adsorption step was concerned, the relationship between E_p and v was determined by following equation (Laviron, 1974):

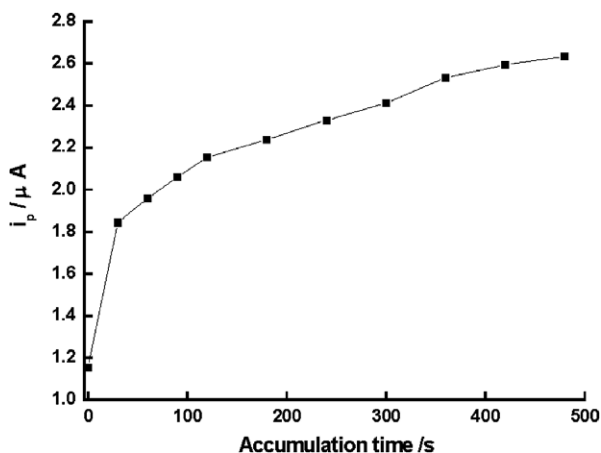


Fig. 5. Effect of accumulation time on peak current of 5.0×10^{-5} mol/L kojic acid at PVP/ABPE. Other conditions are the same as in Fig. 1.

$$E_p = E^{0'} + (RT/\alpha n_a F) \ln(RT k^0/\alpha n_a F) + (RT/\alpha n_a F) \ln v \quad (3)$$

where $E^{0'}$ is formal potential, T is temperature, α is the transfer coefficient and n_a is the number of electron transferred in the rate-determining step, k^0 is the electrochemical rate constant, F is the Faraday constant. The slope $RT/\alpha n_a F$ of 0.02415 indicates that the value of αn_x is 1.03. On the basis of assumption of α was 0.5 for multi-electron transfer process, the value of n_x could be calculated to be 2. Combining with the data from the plot of E_p vs. pH, it can be concluded that the electrochemical oxidation of kojic acid at PVP/ABPE involves total loss of two electrons and two protons. From the molecular structure of kojic acid, the oxidation maybe occurs at hydroxyl group.

3.6. Calibration curve

Under the optimized conditions, the calibration curve for kojic acid at PVP/ABPE was characterized by LSV. In the range from 1.0×10^{-6} mol/L to 1.0×10^{-4} mol/L, the oxidation peak current (i_p) of kojic acid increases linearly with its concentration. The linear regression expresses as following: $i_p = 0.03152 \times 10^6 c + 0.6297$ (i_p in μA , c in mol/L, $R = 0.9987$). This method can detect 5.0×10^{-7} mol/L kojic acid after 2 min accumulation ($S/N = 3$).

The relative standard deviation (RSD) of 3.8% for 12-times parallel detections of 5.0×10^{-5} mol/L kojic acid suggests excellent reproducibility of PVP/ABPE. Additionally, the reproducibility between multiple electrode preparations was estimated by comparing the oxidation peak current of 5.0×10^{-5} mol/L kojic acid. The RSD is 4.6% for 10 PVP/ABPEs, revealing that the method for preparation of PVP/ABPE has good potential applications.

The long-term stability of the PVP/ABPE was tested by measuring the current response at a fixed kojic acid concentration of 5.0×10^{-5} mol/L over a period of three weeks. The PVP/ABPE was used daily and stored in air. After three weeks, the current response only decreases 5.1%, suggesting that PVP/ABPE reported in this work has long-term stability.

3.7. Interferences

The influences of some organic compounds on the oxidation response of 5.0×10^{-5} mol/L kojic acid were evaluated. It is found many common organic compounds do not interfere with the oxidation signal of 5.0×10^{-5} mol/L kojic acid (signal change < 4%), such as 100-fold concentration of glucose, amyllum and dextrin, 50-fold concentration of ascorbic acid (AA), dopamine (DA), uric acid (UA), L-glutamic acid, L-phenylalanine, L-aspartic acid, DL-valine, L-cysteine and L-cystine. However, two-fold concentration of L-tryptophan and five-fold concentration of L-tyrosine had influences on the oxidation of kojic acid (signal change = 10%) due to the fact that their oxidation potentials were similar to that of kojic acid. In addition, effects

Table 1
Determination of kojic acid in sauce and vinegar samples ($n = 5$)

Samples	Detected by HPLC (mg/mL)	Detected by this method (mg/mL)	RSD (%)	Recovery (%)
Sauce A	5.010	5.001	4.9	99.6
Sauce B	4.656	4.687	5.1	101.3
Vinegar A	1.485	1.479	5.0	98.5
Vinegar B	1.509	1.524	5.2	99.2

of many inorganic ions were also tested. 100-fold concentration of Ca^{2+} , Mg^{2+} , SO_4^{2-} , NO_3^- , HCO_3^- and 10-fold concentration of Zn^{2+} did not affect the determination of kojic acid. However, one-fold concentration of Fe^{3+} and Al^{3+} , two-fold concentration of Cu^{2+} make the oxidation current of kojic acid greatly decrease and the signal changes are -25% , -16% and -8% , respectively. This may be caused by the fact that these metallic ions could form complexes with kojic acid and affect the adsorption of kojic acid at electrode surface.

3.8. Determination of kojic acid in foodstuffs

In order to demonstrate its application in practical analysis, the proposed electrochemical method was used to detect kojic acid in sauce and vinegar samples. 1.0 mL of kojic acid sample solution was added into 9.0 mL 0.1 mol/L HAc–NaAc (pH 5.0), and then the electrochemical measurement described in the procedure was performed to determine kojic acid content under the optimum conditions. The concentration of kojic acid was calculated using standard addition method. The results were illustrated in Table 1. The relative standard deviation (RSD) of each sample for five parallel detections was less than 5.2%. Otherwise, the recovered ratio of this method was investigated, and the value is between 98.5% and 101.3%. These experimental data indicate that determination of kojic acid using PVP/ABPE was effective and sensitive. At last, in order to estimate the feasibility, precision and efficiency of this method, high-performance liquid chromatography (HPLC) reported by Huang et al. (2004) was also used to detect kojic acid in samples. It is found that the results obtained by PVP/ABPE are in good agreement with those obtained by HPLC.

4. Conclusion

A sensitive and reliable electrochemical method was proposed for the determination of kojic acid utilizing the

excellent properties of PVP. Compared with an unmodified ABPE, the PVP modified ABPE significantly enhances the oxidation peak current of kojic acid and lowers its oxidation overpotential. Finally, this method was successfully applied to determine kojic acid in foodstuff forms, which verified by HPLC.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (Nos. 30370397 and 60571042).

References

- Chen, Q. X., & Iubo, K. (2002). Kinetics of mushroom tyrosinase inhibition by quercetin. *Journal of Agricultural and Food Chemistry*, 50, 4108.
- Chen, C. S., & Liu, K. J. (2002). Optimisation of kojic acid monolaurate synthesis with lipase PS from *Pseudomonas cepacia*. *Journal of the Science of Food and Agriculture*, 82, 601.
- Chen, J. S., Wei, C., Rolle, R. S., Otwell, W. S., Balaban, M. O., & Marshall, M. R. (1991). Inhibitory effect of kojic acid on some plant and crustacean polyphenol oxidases. *Journal of Agricultural and Food Chemistry*, 39, 1396.
- Huang, S. C., Lin, C. C., Huang, M. C., & Wen, K. C. (2004). Simultaneous determination of magnesium ascorbyl phosphate, ascorbyl glucoside, kojic acid, arbutin and hydroquinone in skin-whitening cosmetics by HPLC. *Journal of Food and Drug Analysis*, 12, 13.
- Kimura, K., Hirokado, M., Yasuda, K., & Nishijima, M. (2000). Determination of kojic acid in various commercial foods by HPLC. *Journal of the Food Hygienic Society of Japan*, 41, 70.
- Kotani, T., Ichimoto, I., Tstsumi, C., & Fujita, T. (1976). Bacteriostatic activities and metal chelation of kojic acid analogues. *Agricultural and Biological Chemistry*, 40, 765.
- Laviron, E. (1974). Adsorption, autoinhibition and autocatalysis in polarography and in linear potential sweep voltammetry. *Journal of Electroanalytical Chemistry*, 52, 355.
- Saruno, R., Kato, F., & Ikeno, T. (1979). Kojic acid: a tyrosinase inhibitor from *Aspergillus albus*. *Agricultural and Biological Chemistry*, 43, 1337.
- Shih, Y. (2001). Simultaneous determination of magnesium L-ascorbyl-2-phosphate and kojic acid cosmetic bleaching products by using a microbore column and ion-pair liquid chromatography. *Journal of AOAC International*, 84, 1045.
- Shih, Y., & Zen, J. M. (1999). Voltammetric determination of kojic acid in cosmetic bleaching products using a disposable screen-printed carbon electrode. *Electroanalysis*, 11, 229.
- Tanigaki, H., Obata, H., & Tokuyama, T. (1980). The determination of kojic acid using the stopped-flow method. *Bulletin of the Chemical Society of Japan*, 53, 3195.
- Vachalkova, A., Bransova, J., Brtko, J., Uher, M., & Novotny, L. (1996). Polarographic behavior of kojic acid and its derivatives, determination of potential carcinogenicity and correlation of these properties with their other attributes. *Neoplasma*, 43, 265.