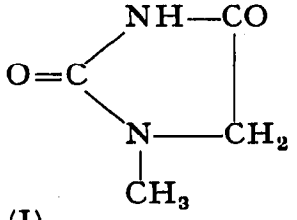
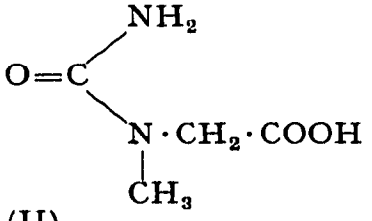
CHROM. 5001

Paper chromatography of 3-methylhydantoic acid and 1-methylhydantoin, possible intermediates of microbial degradation of creatine and creatinine

As is generally known, creatine and creatinine are normal constituents of meat. 1-Methylhydantoin (I) is a product of the bacterial desimidation[1,2] of creatinine. The formation of this substance in whale meat, which was caused by the deterioration of freshness of the meat, has been reported in the previous paper[3]. 3-Methylhydantoic acid (II) is a possible intermediate of creatine decomposition, but its microbial formation has not been reported so far.



(I)



(II)

To further study the freshness of meat[3], either a paper chromatographic (PC) or a thin-layer chromatographic (TLC) technique, which permits the complete separation of the above four compounds and causes a specific color formation, is needed. The present paper describes a PC technique which is applicable.

Materials

3-Methylhydantoic acid was synthesized by the method of GAEBLER[4] (Found: C, 36.16; H, 6.22; N, 21.41. Calculated for $C_4H_8O_3N_2$: C, 36.36; H, 6.10; N, 21.20). It melted at 142.5° with decomposition. The 1-methylhydantoin which had been isolated from deteriorated whale meat and identified in the previous work[3] was used as a material for the present experiments. Creatine and creatinine were purchased from commercial sources. These materials were paper chromatographically pure. Appropriate quantities of these materials were dissolved in water and chromatographed by the procedure described below. All solvents used were of JIS special grade.

Procedure

PC was carried out by the one-dimensional ascending technique, using Toyo-Roshi No. 51 paper which is similar to Whatman No. 1 and one of the following three solvent systems: (a) *n*-butanol–pyridine–water (20:30:15); (b) *n*-butanol–pyridine–water (20:30:20); and (c) isopropanol–pyridine–water (20:30:15). After the solvent front had travelled 12–23 cm from the starting line, the sheet was thoroughly air-dried at room temperature and then heated for 1 h in an oven at 110°. The sheet was then sprayed with Jaffé's reagent which was prepared by mixing a 1.3% ethanolic solution of picric acid with 1/5 volume of 10% NaOH (refs. 5 and 6).

Results and discussion

Of a wide variety of solvent systems tested, the three systems mentioned above gave the most satisfactory results (Fig. 1).

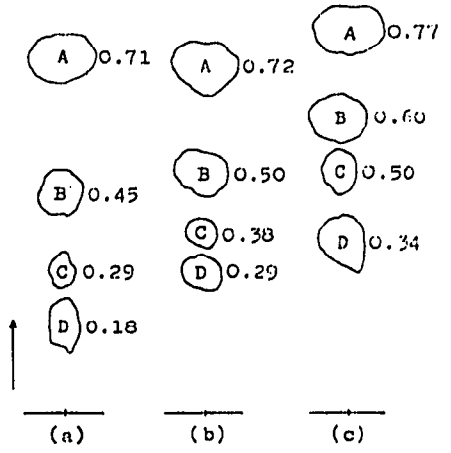


Fig. 1. Resolution patterns obtained with solvent systems a, b and c. A = 1-methylhydantoin; B = creatinine; C = 3-methylhydantoic acid; D = creatine. The numeral close to each spot shows the R_F value of the spot.

Creatinine and 1-methylhydantoin[4] give positive reactions with Jaffé's reagent, but creatine and 3-methylhydantoic acid do not. It is possible, however, to convert creatine to creatinine on paper chromatograms by heating the chromatograms at 110° before spraying[5,6]. A preliminary experiment showed that 3-methylhydantoic acid, similarly as creatine, could be converted to 1-methylhydantoin by heating the chromatograms for 1 h at 110°. The above four substances which had been heated on chromatograms at 110°, therefore, all reacted with the reagent and appeared as orange spots against a yellow background. Creatine and creatinine appeared immediately after spraying, but methylhydantoic acid and methylhydantoin appeared very slowly, often 10–20 min later. Such behavior of the last two is significant for their identification. The color intensities of the spots of the last two reached their maxima usually after the sprayed sheets had been kept in the dark overnight.

In water solution, methylhydantoic acid changed to methylhydantoin spontaneously. The change was already appreciable 2 days after solution and almost complete after about a month, as ascertained by PC. Sample solutions containing methylhydantoic acid, therefore, should be prepared just before spotting.

Acetoacetic and pyruvic acids, which may occur in meat, also give weakly positive Jaffé reactions. Acetoacetic acid, however, is readily destroyed by heating the chromatograms at 110° and does not interfere with the detection of the four substances. Pyruvic acid, as described in the previous paper[3], was not found in extracts from whale meat of different degrees of freshness when the extracts were examined by silica gel TLC with Jaffé's reagent. It seems likely, therefore, that when meat extracts are examined for the above four substances by the present technique, it is practically unnecessary to take account of the presence of the two acids.

Methylhydantoin can also be detected by the chlorine–starch method[7] and by the ferricyanide–nitroprusside method[1]. These methods, however, are not suitable for the present purpose because they also reveal many other substances interfering with the detection of the four substances.

Creatine was added to an aqueous extract solution prepared from whale meat and its aerobic and anaerobic decompositions were surveyed by using the present technique. The formation of 1-methylhydantoin from creatine was observed in an anaerobic experiment, but that of 3-methylhydantoic acid has not been ascertained yet. A detailed report on this subject will be published elsewhere.

Faculty of Pharmaceutical Sciences, TADASHI NAKAI
Science University of Tokyo, SHIZUO UCHIJIMA
Ichigayafunagawara-machi, Shinjuku-ku, Tokyo (Japan) MIDORI KOYAMA

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A rapid chromatographic procedure for the detection of some diuretics in pharmaceuticals and biological fluids

Although a few procedures for the determination of non-mercurial diuretic drugs have been developed by either paper chromatography, UV or visible spectroscopy, most methods lack rapidity, sensitivity and specificity[1–5].

PILSBURY AND JACKSON[2] described a procedure for the extraction and the identification of some thiadiazines in tablets and biological fluids by paper chromatography and UV spectroscopy. They used 1,2-naphthoquinone-4-sulphonate as detecting agent, while NEIDLEIN *et al.*[6] coloured several diuretics with sodium pentacyanoaminoferrate. By using thin-layer chromatography (TLC), DUCHÊNE AND LAPIÈRE[7] identified some diuretics by UV (254 nm).

In order to evaluate a rapid method for the screening of some of these drugs in pharmaceuticals and in urine from patients under medication, we developed a new TLC technique and a new extraction method.

Experimental

Apparatus and reagents. The following material was used.

(1) Desaga TLC set and chromatoplates.

(2) Precoated plates: Merck Alufolien Silica Gel F_{254}, 20 × 20 cm, layer thickness 0.25 mm; Macherey, Nagel & Co. MN-Polyamide TLC 6 UV_{254}, 20 × 20 cm; Macherey Nagel & Co. MN-Polyamide TLC 11 UV_{254}, 20 × 20 cm.