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Short Communications

Studies on Keto Acids of Normal and Cancerous Tissues of Rabbit

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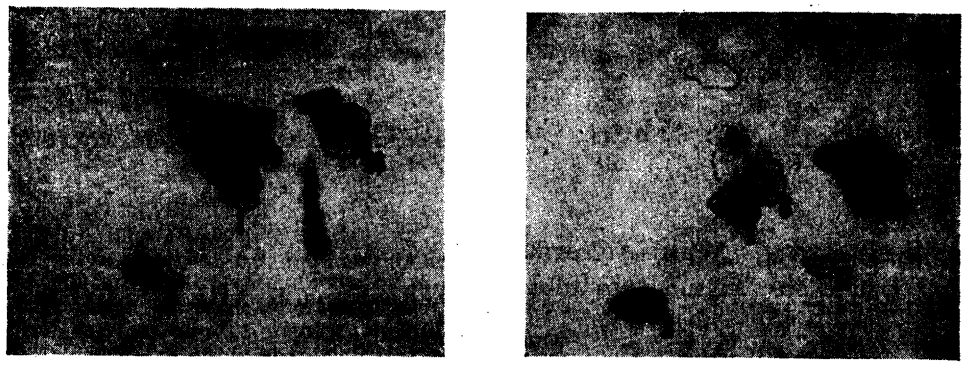
Virtanen and Alfthan [1-3] have previously determined the keto-acids in plants by reducing their 2,4-dinitrophenylhydrazones with tin in an alcoholic solution of hydrochloric acid to the corresponding amino acids, which were then determined paperchromatographically. The present authors have now applied the same method for examining animal tissues. Kulonen [4] determined the keto-acids of urine by a similar method using as reducing agent aluminium amalgam. He could show the presence of pyruvic, glyoxalic and α-ketoglutaric acids, the method, however, giving very unsatisfactory yields with dicarboxylic acids. The serine, which he could recognize on his chromatograms, he explained to be an artefact from aldol-condensation products of pyruvic acid [5]. Serine has also been found by the present authors among the reduction products of the keto acid 2,4-dinitrophenylhydrazones from both plant [1-3] and animal tissues, but under these reaction conditions no aldol condensation can be thought to occur. The pure 2,4-dinitrophenylhydrazones of pyruvic, aspartic and glutamic acids were also reduced but the only artefacts which were found were some alanine from oxaloacetic acid (about 3 %) and sometimes a very faint spot of γ-aminobutyric acid from α-ketoglutaric acid.

Adams' platinum oxide catalyst was also tried as reducing agent and, when the reduction was carried out at room temperature and 1 atm. pressure, somewhat better yields were obtained in the cases of the dicarboxylic acids than was found by tin reduction (about 50 % from α-ketoglutaric and 35 % from oxaloacetic acid). When starting from pure 2,4-dinitrophenylhydrazones (1 mg/ml in abs. ethanol) and slightly increasing the amount of the catalyst the reduction time was reduced from 2—3 hours to about 3 minutes and the solution did not darken at all when standing in air after reduction. The yield in these cases was as high as about 80—90 % from α-ketoglutaric and 50—60 % from oxaloacetic acid. From the former no γ-aminobutyric acid could be detected in these conditions and from the latter only about 2 % of alanine. The yields from other synthetical 2,4-dinitrophenylhydrazones examined were similar, irrespective of the reduction method used, varying from 15 to 50 %.

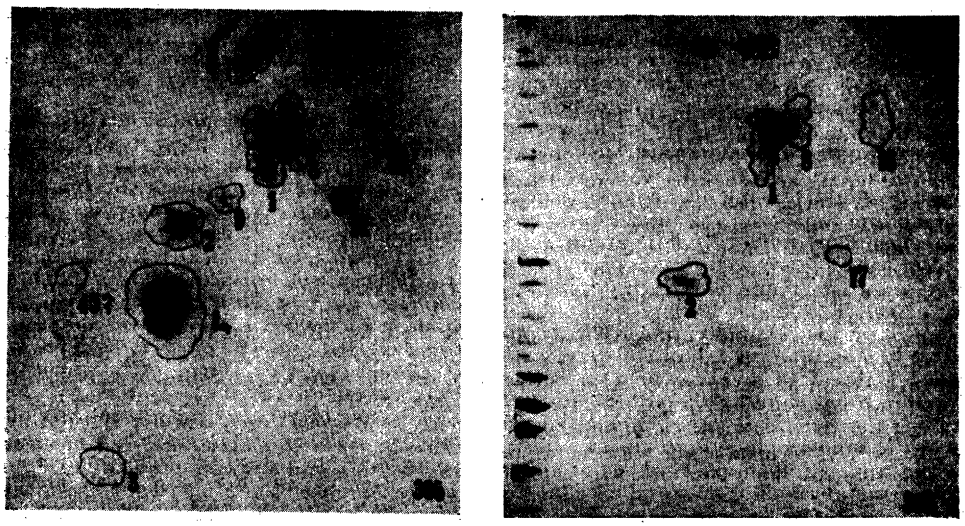
However, when attempting to apply the catalytical reduction to animal tissues, the catalyst was rapidly inactivated to a considerable extent. From muscle and kidney the yields were much lower than after reduction with tin, and from liver extracts no amino acids could be obtained by catalytical reduction. Therefore we found the reduction by tin as the best hitherto known method for this purpose.

The cancer was developed by treatment of the right ear of the rabbit with a 0.3 % solution of 9,10-dimethyl-1,2-benzanthracene in acetone every second day using 15 to 20 drops each time. The left ear was used as a control and quite healthy rabbits were examined too. Some determinations were also made on kidney and liver. In every case large amounts of pyruvic acid and somewhat smaller amounts of glyoxylic, hydroxypyruvic, oxaloacetic and α-ketoglutaric acids were found. In most cases no notable differences between normal and cancerous tissues could be found. In those cases where differences occurred they seemed accidental. No regular differences between normal and cancerous tissues could thus be observed on the basis of the material used. Le Page [6] has found somewhat higher amounts of pyruvic and α-ketoglutaric acid in cancerous tissues than in normal ones.



A B

Fig. 1. Two-dimensional chromatograms of amino acids formed by reduction of keto acid 2,4-dinitrophenylhydrazones from normal (A) and cancerous (B) ears of a rabbit. 1 = gly, 2 = ala, 8 = ser, 16 = asp, 17 = glu.



A B

Fig. 2. Two-dimensional chromatograms of amino acids formed by reduction of keto acid 2,4-dinitrophenylhydrazones from normal (A) and cancerous (B) ears of a rabbit. L = unknown amino acid, 9 = threo, 3 = val.

In one case, a remarkable amount of glyoxalic acid was found in the normal ear, but in the cancerous ear only normal a concentration of this acid, and instead a very high concentration of oxaloacetic acid (Fig. 1, A and B). In another case a high concentration of an unknown keto-acid was found in the normal ear, which could not be found in the cancerous ear (Fig. 2, A and B). The ninhydrin reaction of the corresponding amino acid was at first similar to that of tyrosine, the colour rapidly changing to normal violet. In a few other cases very faint spots of this substance were

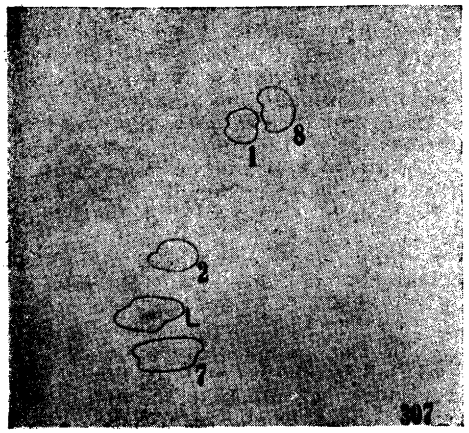


Fig. 3. Same as Fig. 2A, but tyrosine (spot 7) is added to the chromatogram.

found too. The amino acid is certainly not identical with tyrosine (Fig. 3).

In kidney about the same amounts of keto-acids were found as in muscle (calculated on fresh wt.), but in liver only 1/8 of this quantity. In all cases hydroxypyruvic acid was found, as mentioned earlier. Thus this acid is now for the first time shown to exist free in animal tissues. However Sprinson and Chargaff [7] noticed that hydroxypyruvic acid is formed in slices of rat kidney as an oxidative deamination product of serine.

In some cases small spots of threonine (from β-hydroxy α-ketobutyric acid) and tyrosine (from phenylpyruvic acid) were found, but the existence of the corresponding keto-acids in nature could, however, not be proved because of the poor material at our disposal.

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*iso*Thiocyanates XIII. Methyl *iso*-Thiocyanate, a New Naturally Occurring Mustard Oil, Present as Glucoside (Glucocapparin) in *Capparidaceae*

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In a previous communication of this series [1] attention was given to a thiourea of extraordinarily low R_{Ph}-value (0.03) [2], derivable from the volatile *iso*thiocyanate fraction of certain seed samples. The mustard oil was tentatively regarded as methyl *iso*thiocyanate which had not previously been encountered in Nature. This suspicion has now been confirmed by the isolation of methylthiourea from the ammonia-treated distillate of extracts of seeds of *Cleome spinosa* Jacq. (*C. pungens* Willd.) after enzymatic hydrolysis.

The older literature [3] reports the use of various members of *Capparidaceae* as spices and remedies in folk medicine. These applications have been partly attributed to the contents of volatile constituents of unknown structure. In a previous paper [1] the species *Cleome arabica* L. and *Gynandropsis gynandra* (L.) Briq. were listed as sources of the presumed methyl *iso*thiocyanate, the latter being particularly rich in its contents. Methanolic extracts of various species of *Capparidaceae* have now been submitted to paperchromatographic investigation of their content of *iso*thiocyanate glucosides, essentially by the method of Schultz and Gmelin [4,5]. The results are schematically reproduced in Fig. 1. It should be noted that two glucosides with widely differing R_F-values appear