Journal of Chromatography, 378 (1986) 1—8
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3083

CHANGES IN THE EXCRETION OF ORGANIC ACIDS IN HUMAN URINE AFTER PHYSICAL EXERTION

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(First received November 1st, 1985; revised manuscript received January 13th, 1986)

SUMMARY

The excretion of some acids in urine is subject to a circadian rhythm, if diversified nutrition is offered to the body sufficiently. The effect of physical exertion upon the excretion rates of acids in urine is very similar to that of a zero diet, resulting in an increase of final degradation products and a decrease of metabolic intermediates.

INTRODUCTION

Several years ago we reported that the excretion of citric acid in urine drastically increased after a 90-min cross-country run [1].

Citric acid, excreted in urine, may originate from different sources: one part may be produced in the course of the Krebs cycle from compounds stored in the body, another may originate from nutrition and beverages which are known to contain large amounts of citric acid [2, 3]. If citric acid supplied by food passes through the body unchanged, an increase in urinary excretion must be expected a few hours after the intake of nutrition. During the described physical exertion experiment [1], two items were left unconsidered: the possible changes in excretion rates of citric acid originating from nutrition and the possibility that changes in excretion could be caused by a circadian rhythm. Therefore, a more detailed investigation was necessary. The results of this study are explained in this paper.

EXPERIMENTAL

Reagents and solvents

DEAE-Sephadex A-25 was purchased from Sigma (Munich, F.R.G.),

pyridine, acetic acid, sulphuric acid and ethyl acetate were obtained from Merck (Darmstadt, F.R.G.) and N-methyl-N-trimethylsilyltrifluoracetamide (MSTFA) from Macherey and Nagel (Düren, F.R.G.). The internal standard, 3,3-dimethylglutaric acid, was purchased from Merck. Pyridinium acetate solutions were made freshly each week: pyridine–acetic acid (1:1) in water.

Instruments

Gas chromatograph 1 (used in the course of the anion-exchange method): HRGC Carlo Erba 4160 (Hofheim/Ts., F.R.G.), WCOT glass capillary columns (25 m × 0.3 mm I.D.), OV-101.

Gas chromatograph 2 (used in the course of the ethyl acetate extraction method): Siemens L 42 (Nürnberg, F.R.G.), WCOT glass capillary columns (25 m × 0.3 mm I.D.), OV-101.

Gas chromatograph–mass spectrometer: mass spectrometer Varian MAT 312, combined with a gas chromatograph Varian 3700 (Bremen, F.R.G.).

Procedure

Sample preparation. Urine was sampled in clean polyethylene bottles and frozen at −20° C until use.

Quantitative determination of urinary citric acid. Citric acid was determined according to the procedure of Thompson and Markey [4] with some modifications: DEAE-Sephadex A-25 anion-exchange resin was prepared by soaking 20 g in distilled water for a day. Then the adsorbent was washed with 0.5 *M* hydrochloric acid (500 ml), water (until neutral), 0.5 *M* sodium hydroxide (500 ml) and water (until neutral). The resin was stored in distilled water until use.

After this preparation, the resin was packed into a 22 × 1 cm column to a height of 8 cm and washed with 40 ml of a 0.5 *M* pyridinium acetate solution. Of the amount of urine sampled in 1 h 1% was added to the head of the anion-exchange column. Then, 100 μg (in 10 μl of water) of the internal standard (3,3-dimethylglutaric acid) were added. The sample and the standard were allowed to drain into the resin, 30 ml of water were passed through and the acids were eluted with 18 ml of 1.5 *M* pyridinium acetate. The eluate was collected, frozen and lyophylized (0.5 Torr, −10° C). The residue was dissolved in 3 ml of methanol, transferred to a reacti-vial and the solvent removed by a stream of nitrogen.

The samples were trimethylsilylated for gas chromatographic (GC) analysis by adding 50 μl of pyridine–tetrahydrofuran (50:50) and 250 μl of MSTFA. The solution was kept at 60° C for 15 h. A 0.6-μl aliquot of the solution was injected into the gas chromatograph. Fig. 1 shows the gas chromatogram of the trimethylsilylated acids obtained.

Quantification. The method used was semi-quantitative, because we did not know all of the response factors of the compounds in the gas chromatograph. The relation of peak area or peak height to the peak area or peak height of the internal standard was determined. This value is denoted "unit/standard". This method allowed the relative alterations in excretion to be determined, but not the absolute values. Quantitative determination of urinary propylurofuran acid, pentylurofuran acid, nonanedioic acid and tetrahydrofuran–diacetic acid were worked out as described previously [5].

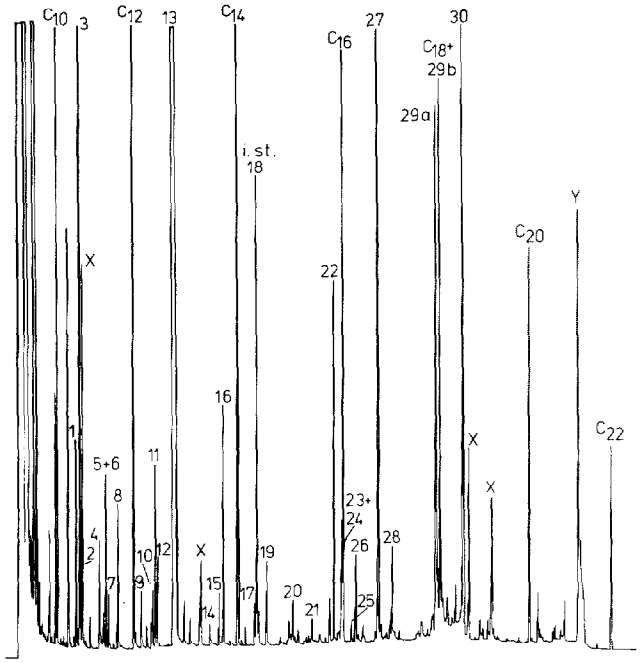


Fig. 1. Glass capillary chromatogram of urinary organic acids separated as their trimethylsilyl derivatives. Peaks: 1 = lactic acid; 2 = 2-hydroxyisobutyric acid; 3 = glycolic acid; 4 = oxalic acid; 5 = 3-hydroxytoluene; 6 = 3-hydroxypropionic acid; 7 = sulphate; 8 = 3-hydroxyisobutyric acid; 9 = 3-hydroxyisovaleric acid; 10 = benzoic acid; 11 = succinic acid; 12 = 4-hydroxybutyric acid; 13 = phosphate; 14 = glyceric acid; 15 = 2,3-dihydroxybutyric acid (*erythro*); 16 = 2,3-dihydroxybutyric acid (*threo*); 17 = 2,4-dihydroxybutyric acid; 18 = 3,3-dimethylglutaric acid (internal standard); 19 = 3,4-dihydroxybutyric acid; 20 = pyroglutamic acid; 21 = 5-hydroxymethyl-2-furoic acid; 22 = 2,3,4-trihydroxybutyric acid (*erythro*); 23 = 2,3,4-trihydroxybutyric acid (*threo*); 24 = 3-hydroxyphenylacetic acid; 25 = 3-hydroxy-3-methylglutaric acid; 26 = 4-hydroxyphenylacetic acid; 27 = tartaric acid; 28 = 3,4,5-trihydroxyvaleric acid; 29a = hippuric acid (TMS); 29b = hippuric acid (DiTMS); 30 = citric acid + isocitric acid, X = unknown compound; Y = uric acid.

Diet. A 25-year-old male was subjected to the following diet for nine days. 09.00 a.m.: 1 cup of coffee with 1 spoon of sugar and 1 roll; 10.30 a.m.: 1 apple; 11.45 a.m.: 1/2 chicken with potato salad, 1/2 litre of beer; 02.30 p.m.: 1 bar of chocolate; 05.00 p.m.: 2 rolls with 100 g of sausage, 1/2 litre of beer; 08.15 p.m.: 1/2 litre of beer and 1/2 litre of lemonade.

RESULTS AND DISCUSSION

It is well known that urinary steroids are excreted in a circadian rhythm [6]. As far as we know, similar behaviour of organic acids was previously unknown because in most investigations 24-h specimens were used [7]. During other experiments we became suspicious that the excretion of organic acids might also be subject to a circadian rhythm. So we studied the excretion of citric acid and other acids during the course of a day for a period of several days.

Excreted acids may originate from food. In order to exclude any changes

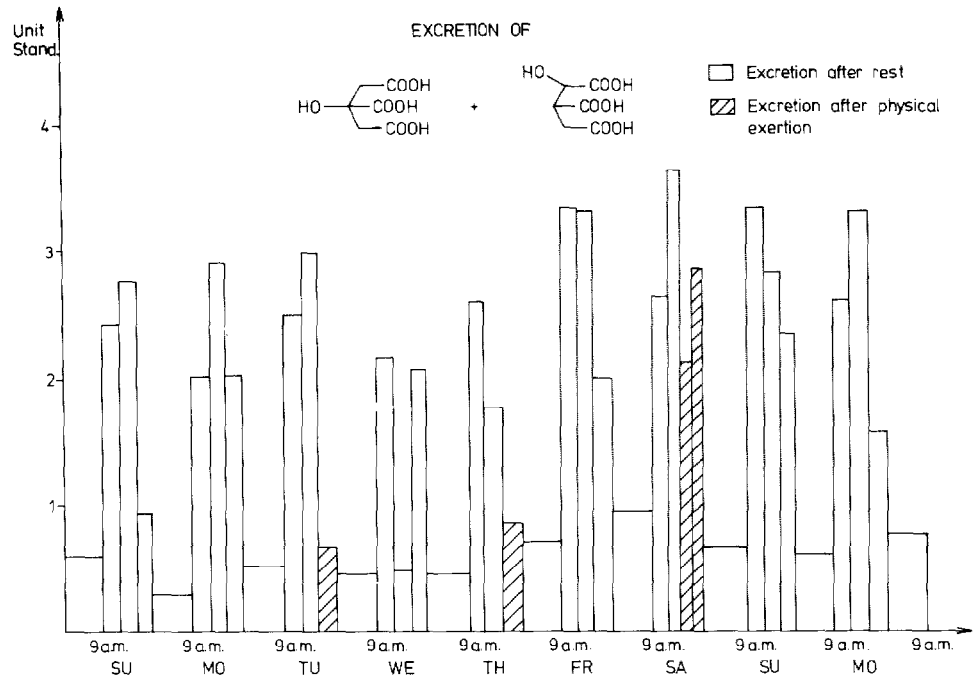


Fig. 2. Excretion of citric acid during a period of nine days by intake of exactly the same food at the same time of the day.

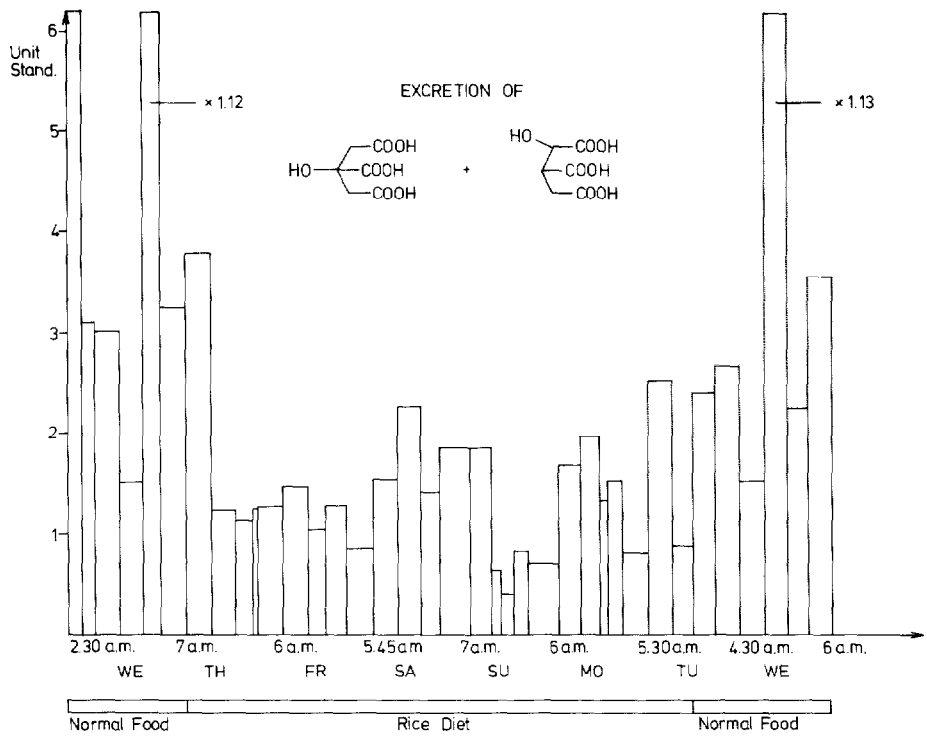
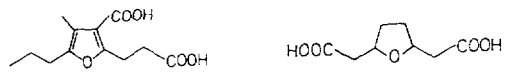


Fig. 3. Excretion of citric acid during a period of six days in the course of a rice diet.

caused by this factor, the same food was eaten at the same time of the day during the nine days when urine was collected. Furthermore, we had to exclude the changes observed in excretion resulting from metabolites of nutrition consumed at a specific time of day. Therefore, the food intake was deferred for 12 h in combination with a change of the day—night rhythm of the test person (work at night, sleep at day time). Considering these experiments (the results of which will be submitted in a following paper), we are able to state that the excretion of citric acid is independent of the consumption of food; most or all of the citric acid consumed must be channelled into the body metabolism. The excreted citric acid is produced in the citric acid cycle of the body; nutrition can contribute to this excretion at most only to a minor extent.

When a sufficiently diversified diet was practised in which all the necessary food (sugar, protein and fat) was supplied to the body in sufficient amounts (see diet), the excretion of citric acid in urine showed a circadian rhythm. The excretion of citric acid is low in resting periods during the night. It increases rapidly from morning till noon with by factor of 4–5 and reaches a maximum at noon. Then the excretion drops rapidly (see Fig. 2). During the



I II

Fig. 4. Structural formulae of propylurofuran acid (I) and tetrahydrofuran acid (II).

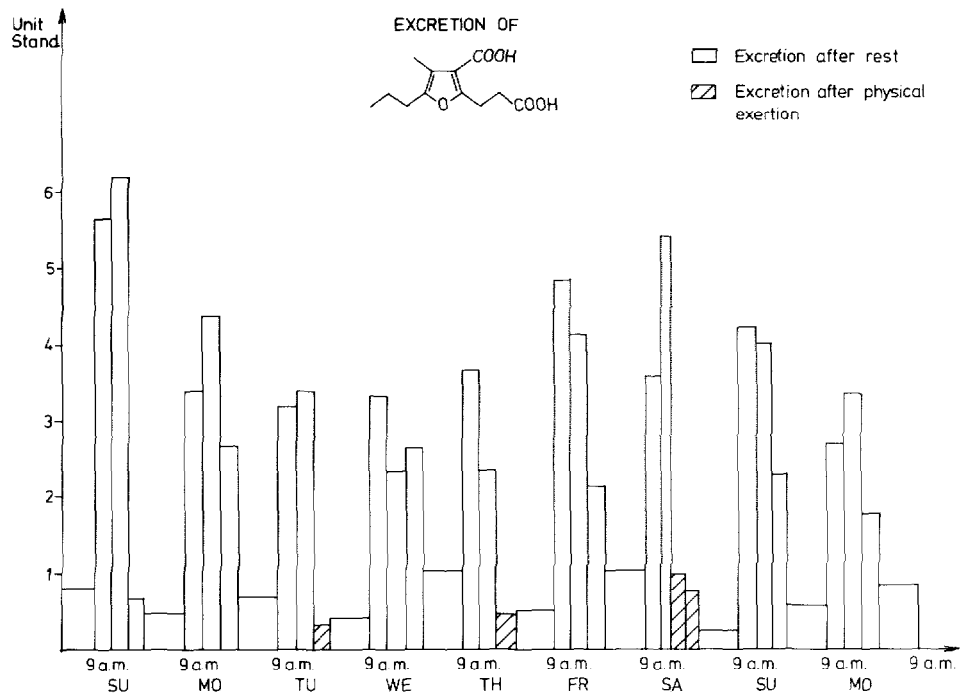


Fig. 5. Excretion of propylurofuran acid during a period of nine days by intake of exactly the same food at the same time of the day.

experiment, physical exertion was performed for 1 h at different times (07.00 h p.m. or 03.00 h p.m.). As demonstrated (Fig. 2), no change in the excretion of citric acid was observed, compared to the days without physical exertion. Therefore, we must correct our previous statement that physical exertion causes an increase in citric acid excretion: the effect observed [1] was caused by the circadian rhythm only.

It is interesting to note that the circadian rhythm cannot be observed in the case of a rice diet (Fig. 3). Obviously rice does not provide enough fatty acids to feed the citric acid cycle sufficiently; therefore only a minimum of citric acid is excreted during this experiment.

As with citric acid, propylurofuran acid (Fig. 4, I) [8] shows a pronounced rhythmic excretion (Fig. 5), which again disappears during a rice diet. In contrast to citric acid, physical exertion considerably changes the excretion of propylurofuran acid (Fig. 5). This is even better illustrated in Fig. 6, showing the average values of excretion at 06.00 p.m. and 10.30 p.m. during a nine-day period.

A similarly diminished excretion of propylurofuran acid was also observed during a zero diet. Therefore, we must conclude that the influence of physical exertion on the metabolism is comparable to a zero diet. This statement is further affirmed by the change in excretion of other acids. For instance, we observed a decrease in the excretion of long-chain dicarboxylic acids, e.g. nonanedioic acid, after physical exertion, and an increase of heptanedioic acid, a final degradation product. Less pronounced is the increase in the excretion of tetrahydrofuran acid (Fig. 4, II) [9] when physical exertion is practised, another acid which is a final degradation product.

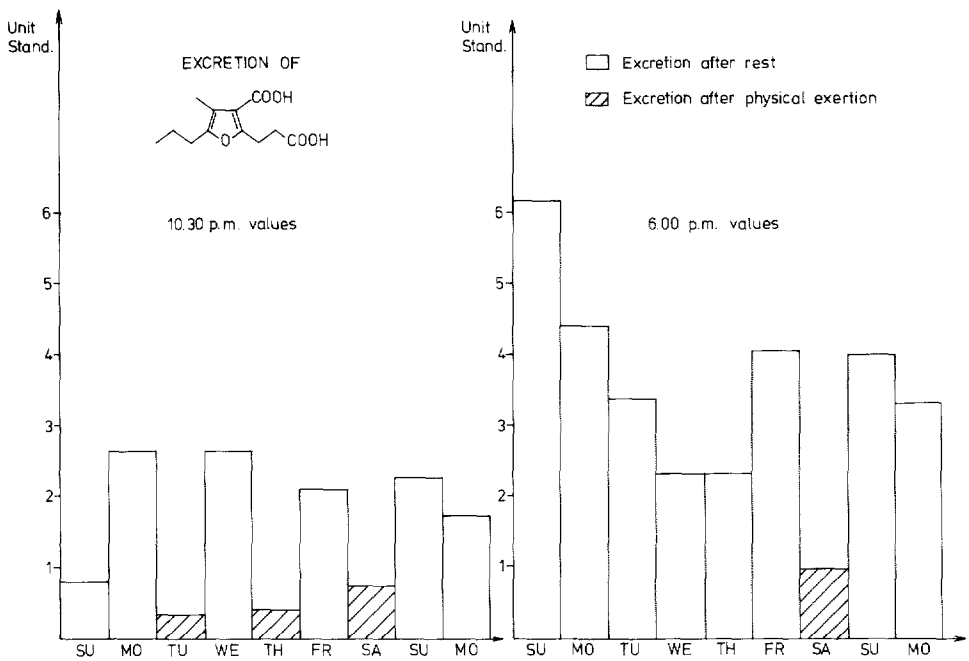
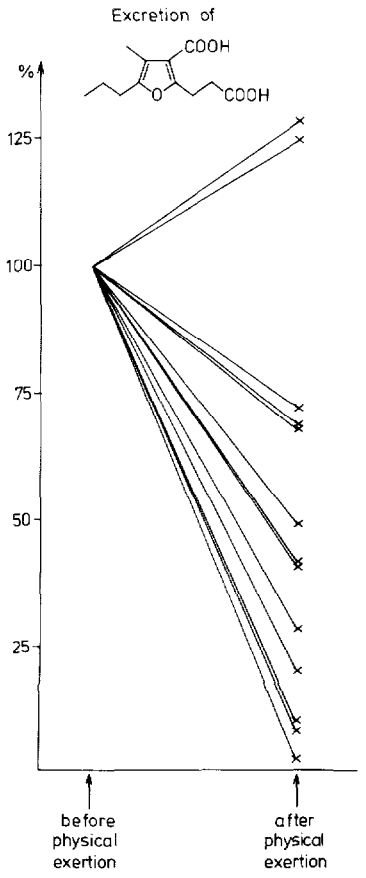


Fig. 6. Excretion of propylurofuran acid comparing the average values at 06.00 p.m. and 10.30 p.m., indicating a decrease after physical exertion.



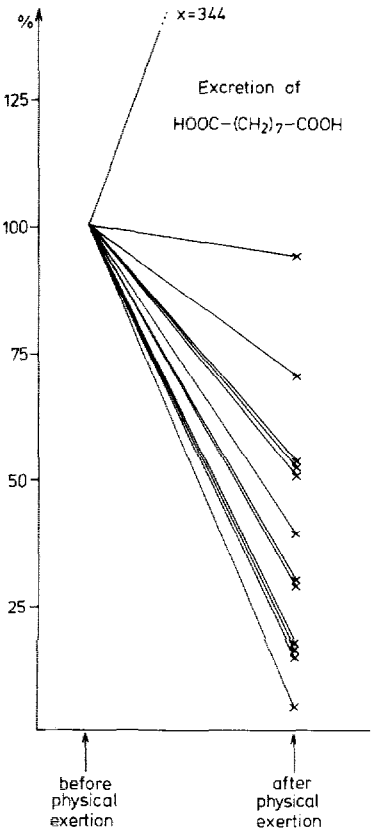


Fig. 7. Excretion of nonanedioic acid before and after physical exertion. The excretion values of the thirteen persons before physical exertion were always taken as 100%, the values after physical exertion show the relative alteration.

Fig. 8. Excretion of propylurofuran acid before and after physical exertion. The excretion values of the thirteen persons before physical exertion were always taken as 100%, the values after physical exertion show the relative alteration.

To prove the results of this metabolic experiment carried out with one person only, urine samples were collected from thirteen persons before and after a strenuous soccer game. They lived on a normal diet. Twelve persons out of thirteen showed a decrease in the excretion of nonanedioic acid after the soccer game (Fig. 7), and eleven out of thirteen also showed a decrease in the excretion of propylurofuran acid (Fig. 8).

The average excretion of propylurofuran acid from all thirteen persons was 3.5 U per standard before the exertion, and only 1.4 U per standard after the exertion, corresponding to a factor of 2.5. No change in the average excretion was observed with pentylurofuran acid. As expected, the excretion of tetrahydrofuran acid (Fig. 4, II) increased in eleven out of thirteen urine samples taken after physical exertion.

CONCLUSION

The excretion of citric acid is not increased by physical exertion as reported previously [1]. Some acids produced in the body, e.g. citric acid and propylurofuran acid, show a circadian rhythm if a diet of sufficient food (fat, carbohydrates, proteins) is supplied. If insufficient amounts of these compounds are consumed, the circadian rhythm disappears. Physical exertion causes an increase in the excretion of final products of a metabolic pathway and a decrease in the excretion of intermediates. So physical exertion has a similar effect on the excretion of acids as a zero diet.

ACKNOWLEDGEMENTS

We thank the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie and Dr. Robert Pfleger-Stiftung in Bamberg for financial support.

REFERENCES

1 G. Spiteller, Pure Appl. Chem., 50 (1978) 205.
2 H. Fricke and S.B. Jensen, Food Process. Ind., 44 (1975) 558.
3 G.I. Fertman and Yu. I. Rakhmanin, Fermentn. Spirt. Promst., 4 (1976) 44.
4 J.A. Thompson and S.P. Markey, Anal. Chem., 47 (1975) 1313.
5 A. Heindl, P. Dietel and G. Spiteller, J. Chromatogr., 377 (1986) 3.
6 J. Aschoff, Arzneim.-Forsch., 28 (1978) 1820.
7 A. Heindl, Thesis, University of Bayreuth, Bayreuth, 1985.
8 M. Spiteller, G. Spiteller and G.-A. Hoyer, Chem. Ber., 113 (1980) 699.
9 O. Rau, W. Kern and G. Spiteller, Liebigs Ann. Chem., (1984) 1504.