Journal of Steroid Biochemistry, Vol. 10, pp. 471 to 473
Pergamon Press Ltd 1979. Printed in Great Britain

SHORT COMMUNICATION

INTERFERENCE OF TIAPROFENIC ACID IN ZIMMERMANN REACTION

K. NAHOUL, L. DEHENNIN and R. SCHOLLER
Fondation de Recherche en Hormonologie, 26, Bd Brune 75014 Paris, 67/77, Bd Pasteur 94260 Fresnes France

(*Received* 11 *September* 1978)

SUMMARY

The interference of a new anti-inflammatory drug, tiaprofenic acid (SURGAM®) in the Zimmermann reaction is reported. The efidence of this interference is established by comparison of urinary 17-oxosteroid levels measured by the Zimmermann reaction with those obtained by gas liquid chromatography on capillary column, before, during and after the drug administration to two healthy volunteers. The mechanism by which tiaprofenic acid reacts with m-dinitrobenzene is studied.

INTRODUCTION

It is now well known that several drugs interfere either by themselves or by their metabolites in the fluorometric or the colorimetric hormone determinations in biological fluids [1–3]. Concerning the urinary 17-oxosteroid (17-OS) determination by Zimmermann reaction, a list of interfering drugs has recently been established by Nocke-Finck and Breuer[4], to which has to be added carbamazepine (Tegretol) [5] and cephalotin [6]. We wish to report hereon the interference of a new anti-inflammatory drug, tiaprofenic acid: 2-(5'-benzoyl-thienyl) propionic acid (Roussel) (Fig. 1), commonly named SURGAM® (RU 15060).

During the last six months we have noticed on many occasions very high urinary 17-oxosteroid levels which could not be accounted for clinically. In order to elucidate this puzzling problem the urinary levels of 17-oxosteroids were determined both by Zimmermann reaction and by gas liquid chromatography. The levels obtained by the latter technique were within the normal range. Therefore, an eventual drug interference was very likely, and the subsequent inquiry revealed that these patients were submitted to different drugs including SURGAM®.

MATERIALS AND METHODS

Two healthy adult volunteers, a man and a woman without any clinical evidence of an endocrine disorder received a daily dose of 600 mg of SURGAM® for three consecutive days. Urine was collected every 24 h before, during drug administration and the three days after its withdrawal.

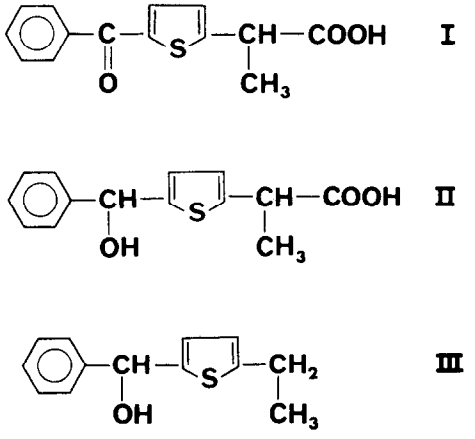

Fig. 1. Tiaprofenic acid (I), its reduced (II) and decarboxylated (III) derivatives.

Total urinary 17-oxosteroids (17-OS) were measured by a modification of the technique of Drekter *et al.*[7]. In this modification, Zimmermann chromogens are extracted by diethyl ether and the absorbance measured at three wavelengths 440, 520 and 600 nm. The correction formula of Allen[8] was applied. 17-Hydroxycorticosteroids (17-OHCS) were estimated by a modification [9] of the Silber and Porter technique [10]. Gas liquid chromatography on a glass capillary column was performed as described previously [11].

Tiaprofenic acid (**I**) and its reduced metabolite (**II**) [-2-(5'-thienylphenylcarbinol) propionic acid] were kindly supplied by Dr Matthieu de Fossey (Roussel Laboratories). Tiaprofenic acid was decarboxylated by refluxing 24 h in ethylene-glycol, containing trace amounts of *p*-toluenesulfonic acid. The crude compound (**III**) (Fig. 1) was submitted to the Zimmerman reaction without further purification.

RESULTS AND DISCUSSION

From the results presented in Fig. 2, it is obvious that interference by SURGAM® occurs as the baseline levels of 11.5 mg/24 h for the man and 7.5 mg for the woman rose to about 100 mg/24 h and even 200 mg/24 h during the treatment. On the contrary, levels obtained by gas liquid chromatography remain quite constant. Moreover, these results suggest a quick elimination of the drug, since two days after the end of the treatment, the 17-OS levels were similar to what they had been before therapy. This finding is in agreement with the pharmacokinetic study of Berlin *et al.*[12].

However, one may wonder if the above-mentioned interference is due to the compound itself or to its principal metabolite (**II**) (Fig. 1) as about half of the compound administered is excreted in the following 24 h urines either free or conjugated to glucuronic acid [12].

To try to answer this question, tiaprofenic acid and its metabolite were dissolved in ethanol and the Zimmermann reaction was performed on an aliquot of these solutions. The purple color characteristic of the Zimmermann Chromogens was only observed with tiaprofenic acid. However the absorption spectrum shows some differences with that of authentic dehydroepiandrosterone since the absorption maximum is located at 550 nm, while for dehydroepiandrosterone it is located at 520 nm (Fig. 3).

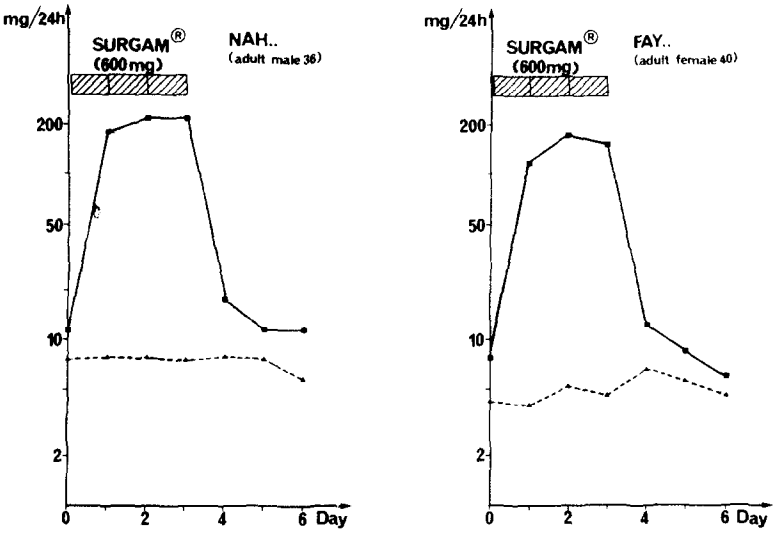

Fig. 2. Urinary 17-oxosteroids levels in two adult volunteers before, during and after SURGAM® administration as determined either by Zimmermann reaction (——) or as the sum of the fractions obtained by gas liquid chromatography (– – –).

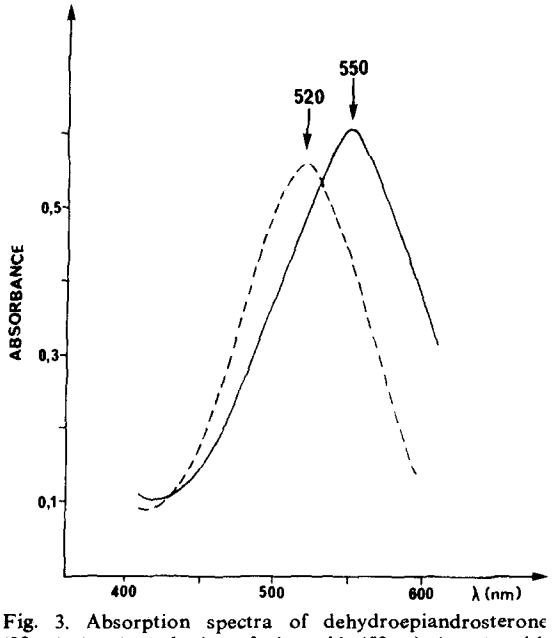

Fig. 3. Absorption spectra of dehydroepiandrosterone (80 μg) (– – –) and tiaprofenic acid (50 μg) (——) with Zimmermann reagent.

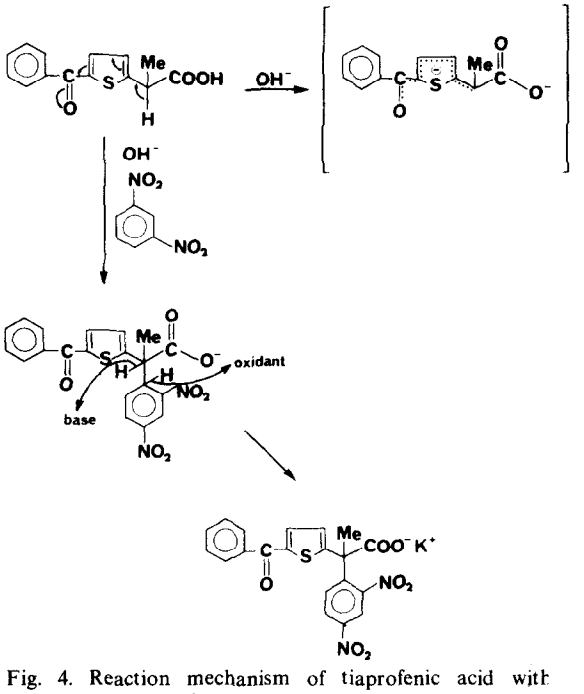

Fig. 4. Reaction mechanism of tiaprofenic acid with Zimmermann reagent.

The mechanism by which tiaprofenic acid gives the Zimmermann reaction was then examined. As neither the reduced nor the decarboxylated derivatives react with m-dinitrobenzene, we came to the conclusion that the ketone group of the tiaprofenic acid can be enolized in alcaline medium according to the reaction mechanism outlined on Fig. 4.

Finally, in the course of this study we have also measured the urinary 17-OHCS, and no interference by SURGAM® occurred. The 17-OHCS levels were similar before, during, and after the administration of SURGAM®.

CONCLUSION

The estimation of 17-oxosteroids in urine by the Zimmermann reaction is an easy to apply and very widespread technique, but many drugs interfere with this reaction, so that the results are often unreliable.

SURGAM® should be added to these interfering compounds, and its administration should be withdrawn if 17-OS have to be assayed by the Zimmermann reaction.

Acknowledgements—We wish to thank Dr Matthieu de Fossey (Roussel Laboratories) for having provided us with tiaprofenic acid and its metabolite, and Miss A. Collinot and P. Salvador for their skilful technical collaboration.

REFERENCES

1. Borushek S. and Gold J. J.: Commonly used medications that interference with routine endocrine laboratory procedures. *Clin. Chem.* **10** (1964) 41–52.

2. Wirth W. A. and Thompson R. L.: The effect of various conditions and substances on the results of laboratory procedures. *Am. J. clin. Pathol.* **35** (1965) 579–590.
3. Lubran M.: The effects of drugs on laboratory values. *Med. Clins N. Am.*, **53** (1969) 211–222.
4. Nocke-Finck L. and Breuer H.: In *Methods of Hormone Analysis*, (edited by H. Breuer, D. Hamel and H. L. Krüskemper), Georg Thieme Verlag, Stuttgart, Wiley, New York (1976) pp. 301–305.
5. Richardson R. A.: Interference in the Zimmermann test for steroids. *Clin. Chim. Acta* **50** (1974) 151–152.
6. King A.: Positive interference by cephalothin with Zimmermann reaction for 17-ketosteroids. *Clin. Chem.* **20** (1974) 401–402.
7. Drekter I. J., Heisler A., Scism G. R., Stern S., Pearson S. and McGavack T. H.: The determination of urinary steroids. I. The preparation of pigment-free extracts and a simplified procedure for the estimation of total 17-ketosteroids. *J. clin. Endocr. Metab.* **12** (1952) 55–85.
8. Allen W. M.: A simple method for analyzing complicated absorption curves, of use in the colorimetric determination of urinary steroids. *J. clin. Endocr. Metab.* **10** (1950) 71–83.
9. Scholler R., Busigny M. et Jayle M.-F.: Méthode de dosage des 17,21-dihydroxy-20-cétostéroïdes (17-OH-corticoïdes). *Archs Soc. fr. Biol. méd.* **2** (1957) 49–52.
10. Silber R. H. and Porter C. C.: Determination of 17,21-dihydroxy-20-ketosteroids in urine and plasma. *J. biol. Chem.* **210** (1954) 923–932.
11. Nahoul K., Salvador P., Fortin M. et Scholler R.: In *Biologie prospective. Organisation des labaratoires* (Edited by G. SIEST), IIIème Colloque de Pont-à-Mousson, 1975, Expansion Scientifique Fran aise, Paris (1975), pp. 453–461.
12. Berlin D., Pottier J. et Raynaud J.-P., Pharmacocinétique de l'acide tiaprofénique. *J. Pharmac.* **6** (1975) 106.