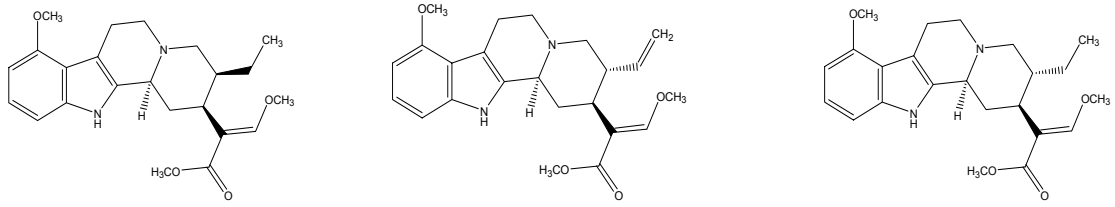
Seksan Kaewklum, Morakot Kaewklum, Rungkan Pootrakronchai, Usa Tassana,
Prapin Wilairat, Tongtavuch Anukarahanonta,

DETECTION OF MITRAGYNINE AND ITS METABOLITE IN URINE FOLLOWING INGESTION OF LEAVES OF *Mitragyna speciosa* Korth

National Doping Control Centre, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand

Introduction

The leaves of *Mitragyna speciosa* Korth.(Rubiaceae), a tree indigenous to Thailand and other Southeast countries (Thai name "kratom"), produce a narcotic-like action when smoked, chewed or taken as an infusion drink [1,2]. The leaves have been used by local people to combat fatigue [1, 2]. The use of this plant has been banned in Thailand since 1943 because of its narcotic effect. Figure 1 shows the structure of the main alkaloids [3] found in the leaves.



(1) **(2)** **(3)**

Fig.1.The chemical structure of mitragynine **(1)**, paynantheine **(2)** and speciogynine **(3)**

Experimental

1. We have extracted and analyzed excretion urine from a person who had ingested the "Kratom" leaves using the normal screening procedure for volatile nitrogen-containing compounds (Procedure 1B) [4] and total steroids (Procedure 4B) [5].
2. GC-MS measurements of pure compounds of the alkaloids were also carried out using the same chromatographic procedures to confirm the structures of the compounds.

Results and Discussion

Figures 2 and 3 show the TIC and mass spectra from excretion urine. The three constituent alkaloids compounds **(1)**, **(2)** and **(3)** are clearly observed in Procedure 1B (liquid-liquid extraction without derivatisation), although only the peak for mitragynine is observed in Procedure 4B. Figure 2 (Procedure 1B) shows only one metabolite peak (Metabolite 3), whereas the chromatogram in Figure 3 (procedure 4B) shows 3 metabolites. Metabolites 1 and 3 (Figure 3) are identified as the O-TMS derivatives of mitragynine desmethyl ester and 9-hydroxycorynantheidine, respectively. Their structures were confirmed by comparison with the O-TMS derivatives of the pure compounds, chromatographed under the same condition. The single metabolite peak (Metabolite 3) in Figure 2 was also confirmed as 9-hydroxycorynantheidine. Metabolite 1, containing a carboxylic acid group, would not be extracted into the organic phase and thus not detected by Procedure 1. The structure of Metabolite 2 is proposed, on the basis of its mass spectrum, to be the bis O-TMS derivative of 9-hydroxycorynantheidine desmethyl ester. Unfortunately a pure compound is as yet unavailable for confirmation. The relative retention time (RRT) of compounds **(1)**, **(2)**, **(3)** and Metabolite 3 in Figure 2 (Procedure 1B) are 2.424, 2.437, 2.455 and 2.471 (ISTD diphenylamine). The RRT of compound **(1)**, Metabolites 1, 2 and 3 in Figure 3 (Procedure 4B) are 1.289, 1.301, 1.307 and 1.312 (ISTD 17 α-methyltestosterone), respectively.

Conclusion

Use of leaves of *mitragyna speciosa* Korth can be easily detected since mitragynine is excreted in the urine, as well as other alkaloid constituents. Three metabolites have been observed and the structure of two of the metabolites identified by comparison with pure compounds.

References

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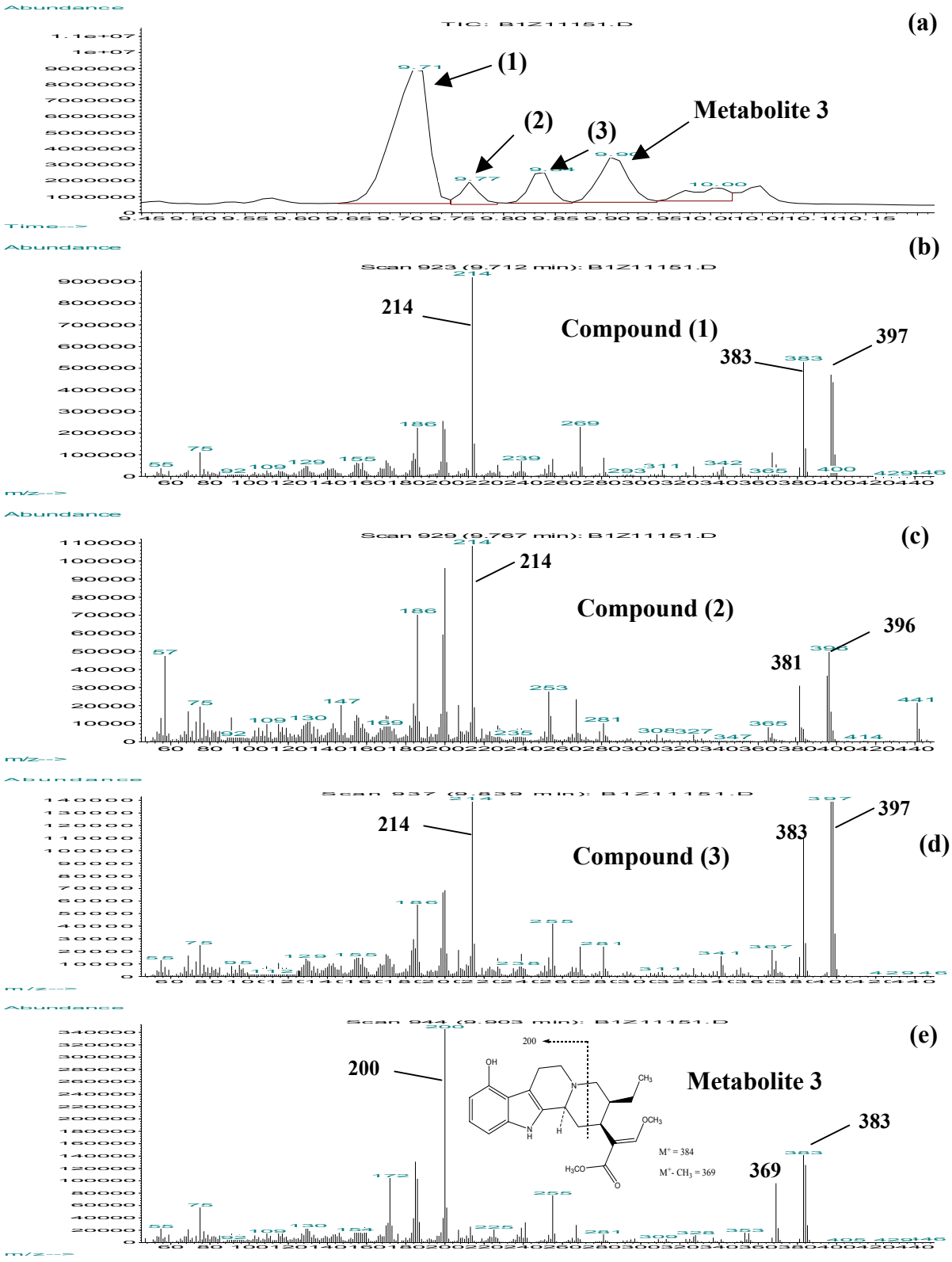

Fig. 2 TIC and mass spectra of excretion urine using the procedure 1B

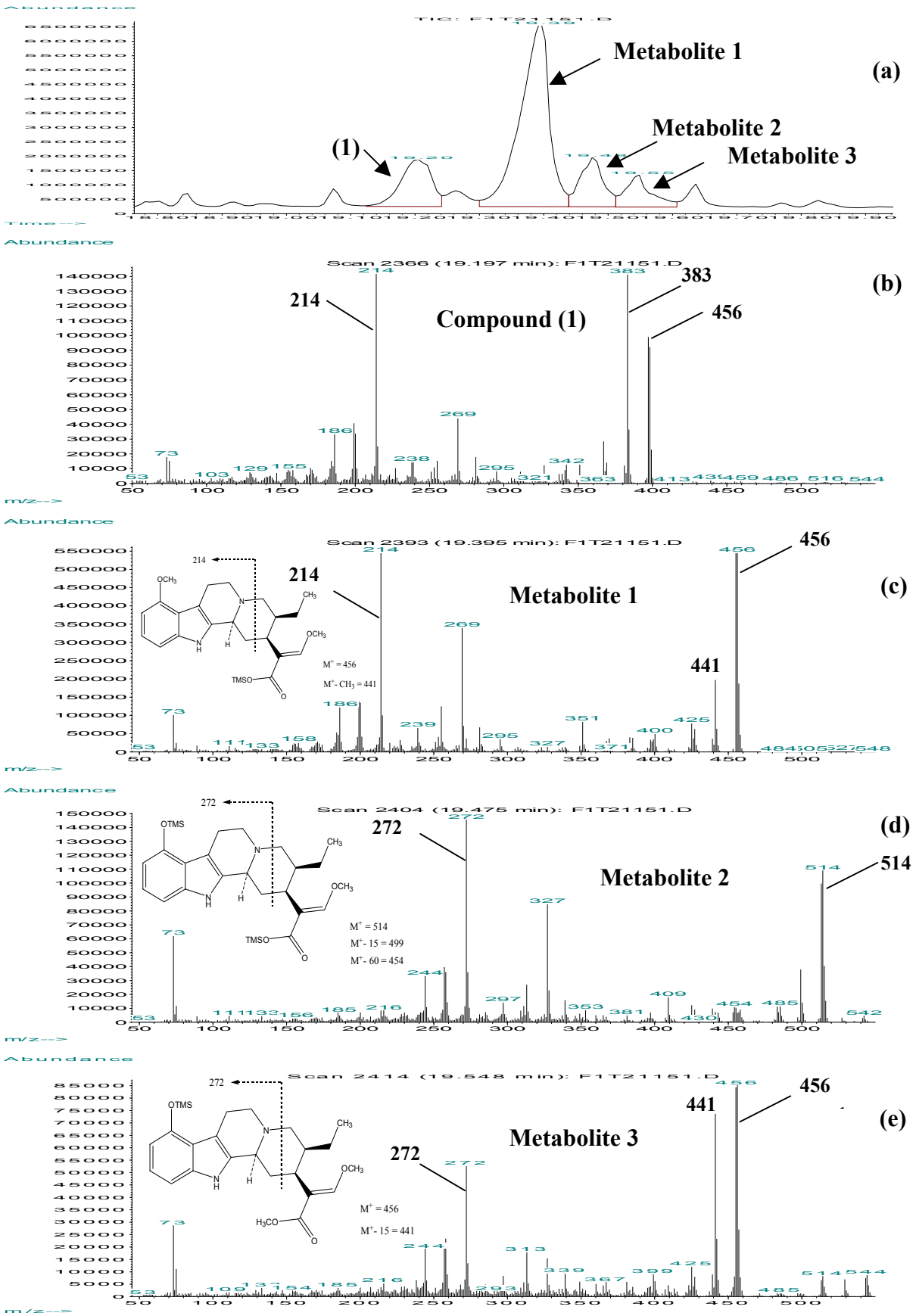

Fig. 3 TIC and mass spectra of excretion urine using the procedure 4B. The structure for Metabolite 2 has not been confirmed with a pure compound.