LC/MS ANALYSIS OF GLUCURONIDES OF CURCUMINOIDS AND THEIR REDUCTIVE METABOLITES

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INTRODUCTION

Curcuminoids are the yellow-colored constituents of the rhizome of *Curcuma longa*, commonly called turmeric, and have recently gained much interest due to their putative anti-inflammatory and anti-carcinogenic activities. They include curcumin (CUR), mono-demethoxy-CUR and bis-demethoxy-CUR (Fig. 1). Their major phase I metabolites are the hexahydro-derivatives, and the major pathway in phase II metabolism is the formation of glucuronides.

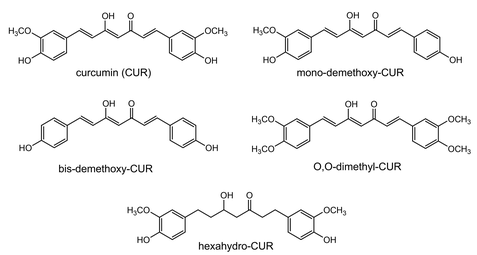

Fig. 1 Chemical structures of curcuminoids and of hexahydro-CUR

Previous studies in rat hepatic microsomes fortified with UDPGA have shown that each of the three curcuminoids formed one glucuronide that was lipophilic and unstable (type A glucuronide), whereas human liver microsomes gave rise to the same type A glucuronide but also small amounts of a second glucuronide that was much more hydrophilic and stable (type B glucuronide). The hexahydro-curcuminoids formed only hydrophilic and stable type B glucuronides with hepatic microsomes from rats and humans. The different behaviour of type A and B glucuronides suggests a different location of the glucuronic acid moiety, which may be bound either to a phenolic or to the enolic hydroxyl group (Fig. 2). O,O-Dimethyl-CUR (Fig. 1), which lacks phenolic groups, gave rise to a type A glucuronide, showing that the enolic hydroxyl group is prone to glucuronidation.
The aim of the present study was to corroborate the postulated structures of the type A and B glucuronides (Fig. 2) by LC/MS analysis.

EXPERIMENTAL PROCEDURES

Chromatography:
Agilent 1100 HPLC system (quaternary gradient pump, diode-array detector, thermostatted column compartment and autosampler) was used. Separation was performed on Phenomenex Synergy Hydro column (4.6x250 mm) with Phenomenex guard cartridge (4.0x3.0 mm) using a gradient of water and acetonitrile (both containing 0.05% acetic acid). The flow rate was 1 ml/min, the eluent was monitored at 425, 370, 280 and 250 nm.

HPLC-MS and MS^n conditions:
Analyses were performed in negative ESI mode using an Agilent MSD-Trap-SL ion trap mass spectrometer. The flow rate of the nitrogen drying gas was 10 L/min, the nebulizer pressure was set at 60 psi, the drying temperature was maintained at 200°C, the capillary voltage was 4500 V and the capillary current was 18.3 nA. MS^n experiments were performed in AutoMS(n) mode. The first scan was a full MS scan, and then one or two precursor ions were selected, isolated and selectively fragmented in the ion trap.

RESULTS

Analysis of the glucuronides of various curcuminoids and their hexahydro-metabolites with HPLC-ESI-MS/MS performed in the negative ionization mode with CAD daughter ion spectra gave the following results:

(1) Each glucuronide contains only one glucuronic acid, i.e. it represents a monoglucuronide.
(2) Type A and type B glucuronides of CUR (Fig. 3 and 4) and of the other parent curcuminoids have virtually identical daughter ion spectra, dominated by the loss of the glucuronic acid moiety.
(3) In contrast, the daughter ion spectra of the glucuronides of hexahydro-CUR (Fig. 5) and hexahydro-bis-demethoxy-CUR (data not shown), both of which are type B glucuronides, are dominated by the loss of water, suggesting a free aliphatic hydroxyl group.
(4) No loss of water is observed in the daughter ion spectrum of the glucuronide of hexahydro-O,O-dimethyl-CUR.

CONCLUSION

The fragmentation patterns of the daughter ion spectra of the various glucuronides of curcuminoids and their hexahydro-metabolites support the notion that the type A glucuronides carry the glucuronic acid at the enolic and the type B glucuronides at a phenolic hydroxyl group.

ACKNOWLEDGEMENT

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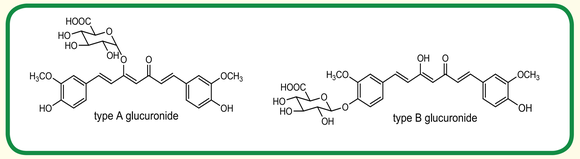

Fig. 2 Postulated chemical structures of the type A and B glucuronides of curcuminoids

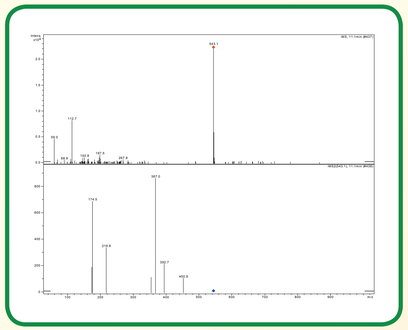

Fig. 3 Daughter ion spectrum of CUR glucuronide type A

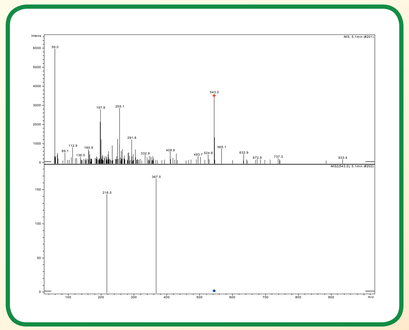

Fig. 4 Daughter ion spectrum of CUR glucuronide type B

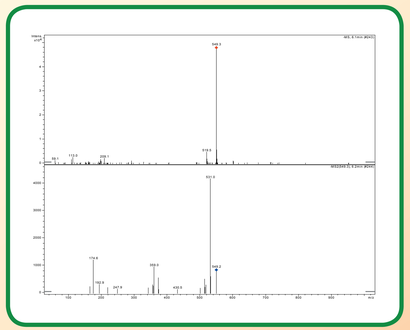

Fig. 5 Daughter ion spectrum of hexahydro-CUR glucuronide