2. Methods

2.1. Differential Scanning Calorimetry

DSC curves were recorded on a Perkin Elmer (Pyris 1, USA) equipment. The instrument was calibrated versus temperature and furnace with two standards (indium and zinc). Aluminium closed pans were used. An amount of 4–5.0 mg was weighed. A first heating run was performed from 273 K to 358 K at 10 K · min^{-1}. This step was followed by an isothermal 5-min step to avoid incomplete melting. Two cooling rates were tested: brutal quenching to room temperature (200 K · min^{-1}), or relatively slow cooling to room temperature (10 K · min^{-1}). A second heating run was then carried out at 10 K · min^{-1}. Mean ΔH_F values of the melting endotherms were determined from replicate experiments. 95% confidence intervals are given in parenthesis.

2.2 X-ray Diffraction study

X-ray diffraction studies were carried out to characterize fenofibrate modifications. A Philips PW 1730 diffractometer was used as X-ray generator of Cu Kα radiation (λ = 1.541838 Å). The experimental X-ray powder patterns were recorded on a PH 8203 instrument. The goniometer supply was a PW 1373 and the channel controle a PW 1390. The data were collected in the continuous scan mode using a step size of 0.01 2θ. The range scanned was 2θ to 30 . X-ray diffraction was also used to determine the physical stability of the metastable modification II. This form was stored for 90 days. X-ray analyses were performed and diffractograms were recorded at regular intervals.

Acknowledgements: The authors gratefully acknowledge the financial support of the Italian MURST – "Fondi 40% Progetto Nazionale Tecnologie Farmaceutiche".

References

1 Hancock, B. C.; Zografi, G.: J. Pharm. Sci. **86**, 1 (1997)
2 Bauer-Brandl, A.: Int. J. Pharm. **140**, 195 (1996)
3 Reynolds, J. E. F. (Ed.): Martindale, The Extra Pharmacopoeia. 31. Ed. P. 1307, Royal Pharmaceutical Society, London 1996
4 Shoji, R.; Watanabe, T.; Tashiro, S.; Shi, S.: Iyakuhin Kenkyu **26**, 386 (1995)

Received October 19, 1999
Accepted Febuary 26, 2000

Piera Di Martino
Department of Chemical Sciences
University of Camerino
Via S. Agostino 1,
62032 Camerino
Italy
dimartin@camserv.unicam.it

Department of Phytochemistry[1], Shenyang Pharmaceutical University, and Department of Traditional Chinese Medicine[2], Beijing Medical University, P.R. China

Metabolites of baicalein in human urine

Y. J. Zhou [1], Q. M. Che [2], S. X. Xu [1] and Q. S. Sun[1]

Baicalein is the aglycone of baicalin, which is one of the major constituents present in Traditional Chinese Medicine Radix Scutellariae. The crude drug and baicalin are widely used for the treatment of various inflammations including hepatitis. We have recently studied the metabolism of baicalin in human urine after oral administration and obtained some valuable results. In order to clarify the metabolic pathway of baicalin in human, further investigation of its metabolism in humans led to the identification of three main metabolites in urine after oral administration (Table).

Reversed-Phase HPLC of a sample from human urine showed three distinct peaks in the chromatogram due to the metabolites of baicalein, which were designated as M1, M2 and M3 in the order of increasing polarity (Fig. A).

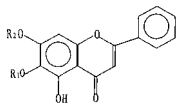
In the UV spectrum of M1, maximal absorptions were seen at 268.8 nm and 316.3 nm, which are characteristic of the flavone skeleton. By comparing the UV spectrum and the HPLC profile with an authentic sample, M1 was identified as baicalein-6-*O*-β-D-glucopyranuroside (Fig. B).

In the UV spectrum of M2, maximal absorptions were seen at 273.6 nm and 311.5 nm, which are characteristic of the flavone skeleton. The spectra were similar to those of 6-methyl-baicalein-7-*O*-β-D-glucopyranuroside [1]. These findings prompted us to synthesize the 6-methyl-baicalein-7-*O*-β-D-glucopyranuroside from baicalin (see experimental). The UV spectrum and HPLC profile of the synthetic compound were in agreement with M2 of the sample. (Fig. C). Thus, M2 was identified as 6-methyl-baicalein-7-*O*-β-D-glucopyranuroside.

In the UV spectrum of M3, maximal absorptions appeared at 278.3 nm and 316.3 nm, which are characteristic of a flavone skeleton and similar to those of baicalin. On the other hand, the chromatographic profile of M3 was in agreement with that of baicalin (Fig. D). Also, the UV spectrum and the HPLC profile of the sample kept unchangeable except for peak height enhancing of M1 as some baicalin was added to the sample. Thus, M3 was identified as baicalein7-*O*-β-D-glucopyranuroside (baicalin).

In the present study, three main metabolites of baicalein in human urine were identified to be in agreement with those of baicalin found in a previous study, which confirmed the

Table: Structures of the metabolites and derivative



	R_1	R_2
M_1	GlcUA	H
M_2	Methyl	GlcUA
M_{2a}	Methyl	GlcUA methyl ester
M_3	H	GlcUA

GlcUA: β-glucopyranuronosyl

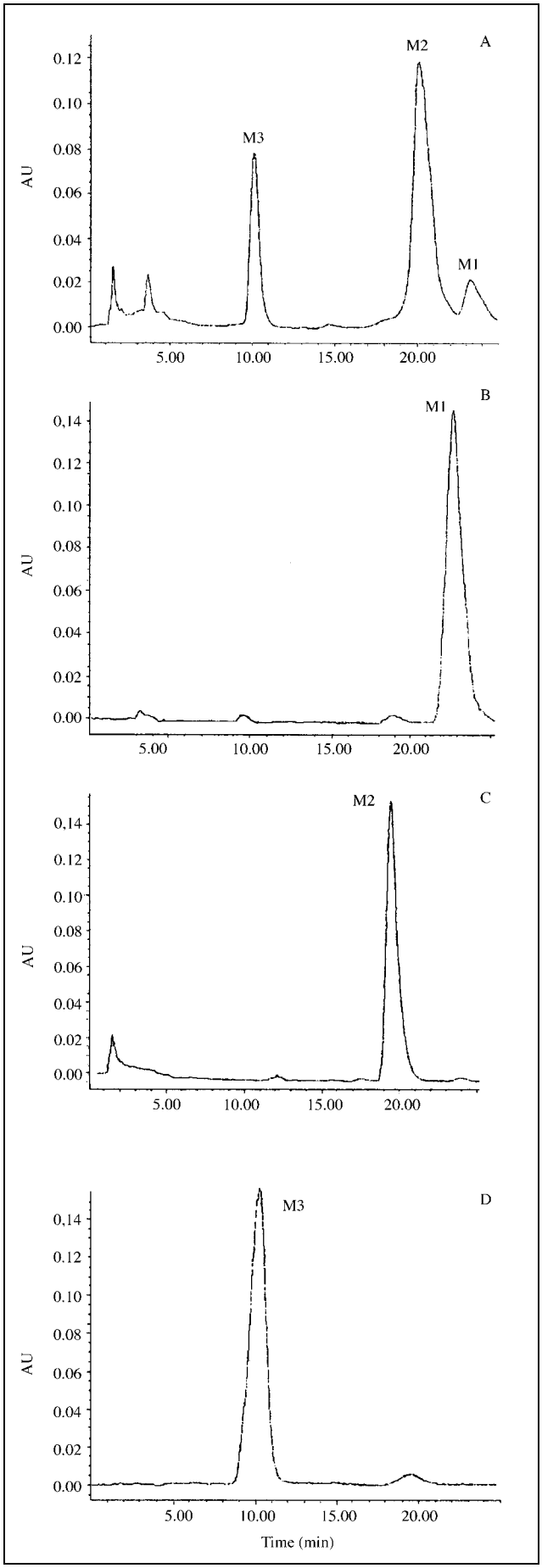


Fig.: HPLC profiles for urine sample (A) and reference samples of M1 (B), M2 (C) and M3 (D).

following presumption: some portion of baicalin underwent microbial hydrolysis in the gastrointestinal tract prior to absorption. Additional studies, e.g. examinations of human plasma concentration and evaluation for antianaphylactic and anti-inflammatory activities of the metabolites are in progress.

Experimental

D_{101} Macro-resin was provided by Tianjing Nankai University Resin Factory. SiO_2 was from Qingdao Marine chemical Factory. Sephadex LH-20 was purchased from Pharmcia Fine Chemicals Co., Ltd.

Baicalin was isolated from Scutellariae Radix according to the method of Takido *et al.* [2–3]. Baicalein was received by hydrolysis of baicalin with 10% H_2SO_4 in EtOH. ^{1}H NMR spectra were recorded on a VXR-300 spectrometer. TMS was used as internal standard. HPLC was carried out on a Waters Liquid Chromatograph equipped with two model 510 pumps and Waters 996 photodiode array detector. Acetonitrile was of chromatographical, all the other reagents were of analytical grade. HPLC conditions: for searching for and characterizing the metabolites, a 4 μm Octadecyl Silica (ODS) column (Nova-Pak® C_{18}, 60 A°, 3.9 × 150 mm) and a mobile phase of acetonitrile – 0.08M formic acid (20 : 80) were used. The flow rate was 1 ml/min and column eluate was monitored at 315.6 nm.

Synthesis of M_2: a mixture of baicalin (500 mg in 1 ml DMF), Me_2SO_4 (6 ml) and K_2CO_3 (6 g) in dry Me_2CO (60 ml) was heated under reflux for 3h. The mixture was filtered, and then filtrate was evaporated in vacuum. The residue was subjected to Sephadex LH-20 CC eluting with methanol, to give light yellow needles of compound M_{2a}. It was identified by comparing its ^{1}H NMR spectrum with that of baicalin. ^{1}H NMR (DMSO-d_6) of M2a : δ = 3.77 (3 H, s, C6−OCH_3), 3.67 (3 H, s, C6″−OCH_3), 5.38 (1 H, d, J = 7.0 Hz, anomeric H of C7−O-glucuronic acid), 7.07 (1 H, s, C3-H), 7.14 (1 H, 3, C8−H), 7.6 (3 H, m, C3′, 4′, 5′-H) and 8.1 (2 H, m, C2′, 6′-H). Partial alkaline hydrolysis of M2a with 3% sodium hydroxide for 30 min tensulted in the desired product M2.

The urine of ten healthy male volunteers (20 to 45 years old) with a body weight from 60 to 80 kg, was collected 2–18 h after taking 1.0 g of baicalein orally. The urine (18 l) was concentrated to 0.5 l, adjusted to pH 4.0–5.0, then filtered. The filtrate was subjected to D_{101} CC (5 × 60 cm), eluted with 1.0 l H_2O, 1.0 l 10% EtOH and 1.0 l 50% EtOH. The 50% EtOH fraction was concentrated and loaded on a sephadex LH-20 CC (2 × 50 cm), eluted with 0.5 l of H_2O to get 30 fractions, which were detected by SiO_2 TLC ($CHCl_3$/MeOH/H_2O, 65 : 45 : 12). Fractions 19–25 showed flavonoid characteristics by spraying with $FeCl_3$ reagent and the seven fractions were combined to obtain the sample containing metabolites of baicalein for HPLC analysis.

References

1 Abe, K.; Inove, O.; and Yumioka, E.: Chem. Pharm. Bull., **38**, 208 (1990)
2 Takido, M.; Aimi, M.; Takahashi, S.; Yamauchi, S.; Torii, H.; Doi, S.: Yakugaku Zasshi, **95**, 108 (1975)
3 Tomimori, T; Miyaichi, Y.; Kizu, H.: Yakugaku Zasshi **102**, 388 (1982)

Received November 15, 1999
Accepted March 15, 2000

Prof. Sui-Xu Xu
Dept. of Phyto chemistry
Shenyang Pharmaceutical University
103 Wehua Road, Shenhe District
Shenyang, 110015
P. R. CHINA
ykxxsx@mail.sy.in.cn