Journal of Chromatography, 574 (1992) 283–292
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6198

Determination of yohimbine and its two hydroxylated metabolites in humans by high-performance liquid chromatography and mass spectral analysis

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(First received July 22nd, 1991; revised manuscript received October 23rd, 1991)

ABSTRACT

The existence of at least two metabolites of yohimbine (YO) in humans is demonstrated. Combined high-performance liquid chromatographic (HPLC), NMR and mass spectral analyses permitted them to be identified as hydroxylated metabolites at the C-10 and C-11 positions. A normal-phase HPLC method allowing the simultaneous determination of YO and its main metabolite, 11-hydroxyyohimbine (11-OHYO), in biological samples is described. This assay was performed using a LiChrosorb Si 60 column and a mobile phase consisting of 0.02 *M* sodium acetate (pH 5)–methanol (5:95, v/v) at a flow-rate of 1 ml/min. Detection was achieved by a fluorimetric method (excitation at 280 nm and emission at 320 nm). The extraction yields of YO, 10-OHYO and 11-OHYO from plasma were 91.8, 45.3 and 17.8%, respectively, and their respective within-day reproducibilities were 3.8, 1.4 and 5.9%. The between-day reproducibility for YO at the concentrations of 1 and 10 ng/ml were 8.9 and 6.4%, respectively. The accuracy of the method for YO at concentrations of 1 and 10 ng/ml were 5.1 and 2.3%, respectively. The limits of determination of YO, 10-OHYO and 11-OHYO were 0.1, 0.5 and 1 ng/ml, respectively. The method was used in bioavailability study of YO following oral and intravenous administration in humans.

INTRODUCTION

Yohimbine, methyl 17α-hydroxyyohimban-16α-carboxylate, is an indole alkaloid obtained from various botanical sources. It is a selective α_2-adrenoreceptor antagonist agent, and is an accepted chemical probe for differentiating α-receptor subtypes [1]. Owing to the widespread localization of α_2-adrenoreceptors [2], it has a variety of effects, most of which are autonomic and psychic effects [1]. These effects, related to an increased sympathetic neurotransmitter release may be of clinical interest. An increase in the release of norepinephrine in the brain probably results from an inhibition of the presynaptic α_2-adrenoreceptor [3].

Yohimbine may be of value in the treatment of erectile failure. It may be of interest also in the management of orthostatic hypotension due to autonomic failure [4] or when tricyclic antidepressants are administered [5]. In addition, yohimbine displays behavioral effects resulting from the importance of α_2-adrenoreceptors in brain function. It meets most of the requirements to obtain an ideal model of anxiety and is currently the best pharmacological model molecule

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of anxiety [6]. More recently, yohimbine was shown to display potent lipid-mobilizing properties related to an activation of the adrenergic system [7].

Few pharmacokinetic studies of yohimbine, especially in humans, have been carried out. The results of the last two studies conducted in humans [8,9] indicate that yohimbine is rapidly eliminated from plasma ($t_{1/2}\beta \approx 0.6$ h), has a low renal excretion rate (lower than 1%), has a high plasma clearance and its oral bioavailability is low and variable (about 30%). These data suggest that the low bioavailability of yohimbine may results from a first-pass effect. However, the metabolism of yohimbine in humans is unknown.

Further, the clinical use of yohimbine [10] in the management of impotence shows a delayed action which is inconsistent with its short apparent plasma half-life. Therefore, yohimbine may have a longer half-life (γ elimination phase) that has not yet been detected, on account of either a lack of sensitivity of the analytical methods applied or too short a blood collection period, or its activity may reside in possible metabolite(s) that can display a biological half-life longer than that of the parent drug. Hence a further insight into yohimbine pharmacokinetics and especially a knowledge of its metabolic pathway may be of interest. Such study requires the development of new analytical methods.

The most recent analytical methods allowing the evaluation of yohimbine in biological samples are high-performance liquid chromatographic (HPLC) methods, none of which has permitted any metabolite to be detected. They use either normal-phase [11] or reversed-phase [12–17] packing materials. Detections is achieved by electrochemical [12,13,15,16], spectrophotometric [14] or fluorimetric [11,17] methods. In this paper we describe normal-phase HPLC which allows the determination in human biological samples of yohimbine and two of its metabolites that we identified as 10- and 11-hydroxyyohimbine.

EXPERIMENTAL

Chemicals

Yohimbine (YO) was supplied by Laboratoire Houdé (Paris, France). 10-Hydroxyyohimbine (10-OHYO) was prepared by chemical synthesis from natural alkaloids [18]. Characterization of this standard was assessed using UV, mass (MS), ^{13}C NMR and ^{1}H NMR spectrometry. As the synthesis of 11-hydroxyyohimbine (11-OHYO) is much more difficult than that of 10-OHYO, only a very small amount of it was available, allowing only limited characterization [18].

β-Glucuronidase type H 1 and IX A, arylsulphatase type VI and eserine were purchased from Sigma (St. Louis, MO, USA). All solvents and reagents were of analytical-reagent grade.

NMR methods

High-field ^{1}H NMR and ^{13}C NMR were recorded on an Bruker AC 300 spectrometer (Bruker Instrument, Billerica, MA, USA). Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as the internal standard. The ^{1}H NMR spectra were measured with an acquisition time of 2.227 s, a pulse of 4 μs and a spinning rate of 25 Hz.

Direct insertion mass spectral analysis

Mass spectral analysis with electron impact ionization was carried out with an Incos 50 mass spectrometer (Finnigan MAT, Orsay, France) utilizing a Data General data system and MSDS applications software. Electron impact mass spectral conditions utilized a source temperature at 150°C, a source pressure at 1.066 Pa, an electron energy of 70 eV and an emission current of 750 μA. A 1-μl volume of sample residue, dissolved in methanol, was evaporated and then vaporized with the programming temperature probe (40–400°C at 60°C/min). Identification of the compounds was performed in the full scanning mode (low mass 40, high mass 450 and effective scan time 0.5 s) and comparison of the mass spectra obtained from our synthetized standards. Mass calibration was performed with perfluoro-

tributylamine (Fluorocarbon 43, Finnigan MAT).

HPLC analysis

The chromatographic system consisted of a Waters (Milford, MA, USA) Model 6000A pump equipped with a Waters WISP 710 B automatic injector, an LDC Milton Roy (Riviera Beach, FL, USA) Spectromonitor 3100 variable-wavelength detector set at 280 nm or a Schoeffel (Waters-France, Paris, France) GM 970 monochromator spectrophotofluorimeter (excitation at 280 nm, emission above 320 nm and cell temperature 15°C) and a Delsi (Suresnes, France) Enica 21 integrator.

The analytical chromatographic columns were Merck (Darmstadt, Germany) LiChroCART columns (250 mm × 4 mm I.D.) packed with LiChrosorb Si 60 or LiChrosorb RP-8 (7 μm particle size) or Waters μBondapak CN (10 μm particle size).

Analyses on the LiChrosorb Si 60 column were performed at 30°C with 0.02 *M* sodium acetate (pH 5)–methanol (5:95, v/v) as mobile phase at a flow-rate of 1 ml/min. Analyses on the μBondapak CN or LiChrosorb RP-8 column were performed using acetonitrile–0.01 *M* sodium dihydrogenphosphate (pH 4). (30:70, v/v) as mobile phase, maintained at 30°C, at a flow-rate of 1 ml/min. Analysis using the LiChrosorb Si 60 column with fluorimetric detection was performed during the pharmacokinetic study.

Sample preparation

Two techniques of sample preparation were developed. The first technique was used to isolate, from pooled human urine samples, putative metabolites of YO in order to analyse them further, either by direct insertion MS, gas chromatography–MS or ^{1}H NMR. The second technique, adapted fromt hat reported by Owen *et al.* [8], allowed the isolation and determination of YO and its main metabolite from plasma or urine samples by HPLC following administration of YO in humans.

First technique. Ten aliquots (100 ml each) of a pooled human urine sample were alkalinized (pH 10) with concentrated sodium hydroxide solution and then passed through Sep-Pak C_{18} cartridges (Waters) packed in methanol and then in water. Each cartridge was first eluted with water (20 ml), then with methanol–water (30:70, v/v) (10 ml) and finally with methanol (5 ml). The methanol eluate obtained was evaporated to dryness under vacuum. After this extraction step, a purification step was performed by HPLC using a Merck LiChrosorb Si 60 column (250 mm × 25 mm I.D.) with 0.01 *M* sodium acetate (pH 5)–methanol (3:97, v/v) as mobile phase, maintained at 20°C, at a flow-rate of 5 ml/min. Fluorimetric detection was applied.

Second technique. To each biological sample (2 ml of plasma or 0.5 ml of urine with or without enzymic hydrolysis) were added 5 μg/ml eserine chlorhydrate (internal standard) solution (100 μl), 0.5 *M*, dissodium phosphate solution (pH 11) (1 ml for plasma and 250 μl for urine samples) and chloroform (2 ml). Biological samples were diluted for analysis within the dosing range when required. The tube was shaken for 5 min and then centrifuged at 5000 *g* for 5 min. A 1.5-ml aliquot of the organic phase was transferred to a polyethylene conical tube. The organic phase was evaporated under a gentle stream of nitrogen at 40°C. The residue was dissolved in 100 μl of methanol–ethanol (85:15, v/v) and 80-μl samples were injected.

Urine enzymic hydrolysis was performed as follows: to three 0.5-ml urine samples were added 0.5 ml of 0.1 *M* sodium acetate buffer (pH 5.0), followed by 0.25 ml of β-glucuronidase type H I solution (6000 U/ml, 0.2% NaCl, or 0.5 ml of 0.1 *M* sodium Tris–HCl (pH 6.8) and in a second stage 0.25 ml of β-glucuronidase type IX A solution (10 000 U/ml, 0.2% NaCl), or 0.5 ml of 0.1 *M* sodium Tris–HCl buffer (pH 7.1) and then 0.25 ml of arylsulphatase type VI solution [5 U/ml, Tris buffer (pH 7.1)]. The mixtures obtained were allowed to stand at 37°C for 8 h.

Pharmacokinetic application

For a bioavailability study of yohimbine in twelve healthy subjects, the following protocol was applied: the subjects, who had given in-

formed consent, received an 8-mg oral dose of YO hydrochloride and a 5-mg intravenous bolus dose of YO hydrochloride on two occasions 1 week apart. Venous blood was drawn into heparinized Vacutainer tubes (Becton Dickinson, Oxnard, CA, USA) immediately before drug administration and then at 2, 5, 10, 15, 30 and 45 min and 1, 1.5, 2, 3, 4, 6 and 8 h following intravenous administration and at 15, 30 and 45 min and 1, 2, 4, 6, 8, 12 and 24 h following oral administration. The urine output during the next 24 h was collected, 0–8 h and 8–24 h following intravenous administration and 0–12 h and 12–24 h following oral administration. Blood samples were centrifuged immediately after collection. Plasma and urine samples were stored at −20°C until assayed.

Total plasma concentration *versus* time curves for YO and 11-OHYO identified in each sample were fitted with the non-linear least-squares regression computer program SIPHAR [19] (Simed, Créteil, France).

RESULTS AND DISCUSSION

Identification of yohimbine metabolites

Liquid chromatographic analysis of human biological samples allowed the detection of two unknown compounds, *i.e.*, putative yohimbine metabolites. Their chromatographic behaviour, checked on three different chromatographic packings (one normal-phase and two reversed-phase packings), was homogeneous and close to that of YO. Both compounds appeared in urine samples, but only one of them could be detected in plasma samples. With regard to their relative concentrations, the compound present in both urine and plasma samples was labelled as a major metabolite (M) and the compound that was detected only in urine as a minor metabolite (m).

The chromatographic behaviour of these compounds indicated that they were more hydrophilic than YO (Table I). As YO is likely to undergo a conjugation metabolic pathway, they were first investigated as possible conjugates. However, comparison of their HPLC profiles before and after enzymic hydrolysis with β-glucuronidase and arylsulphatase revealed that these compounds were not conjugates of YO.

The major metabolite (M) was then isolated from urine samples. Following isolation and purification, the metabolite-containing residue was analysed by MS. This experiment indicated that this compound was probably a hydroxylated metabolite on the aromatic ring, or that possibly hydroxylation of the nitrogen atom N-1 had occurred. The mass/charge (m/z) and relative intensity (%) of the typical ion fragments of YO were 353 (100%), 184 (17.1%), 169 (32.5%) and 156 (15.1%). The m/z and relative intensity (%) of the typical ion fragments of metabolite M were 369 (100%), 200 (22.0%), 185 (41.5%) and 172

TABLE I

CHROMATOGRAPHIC DATA

Conditions as described under Experimental. k' = Capacity factor; α = separation factor.

Compound	Normal-phase packing		Reversed-phase packing			
	LiChrosorb Si 60		LiChrosorb RP-8		μBondapak CN	
	k'	α	k'	α	k'	α
Yohimbine	2.65		2.76		1.64	
10-Hydroxyyohimbine	2.89	1.20	0.87	1.13	0.83	1.05
11-Hydroxyyohimbine	3.46		0.98		0.87	
Metabolite m	2.74	1.24	0.88	1.11	0.81	1.07
Metabolite M	3.39		0.98		0.87	

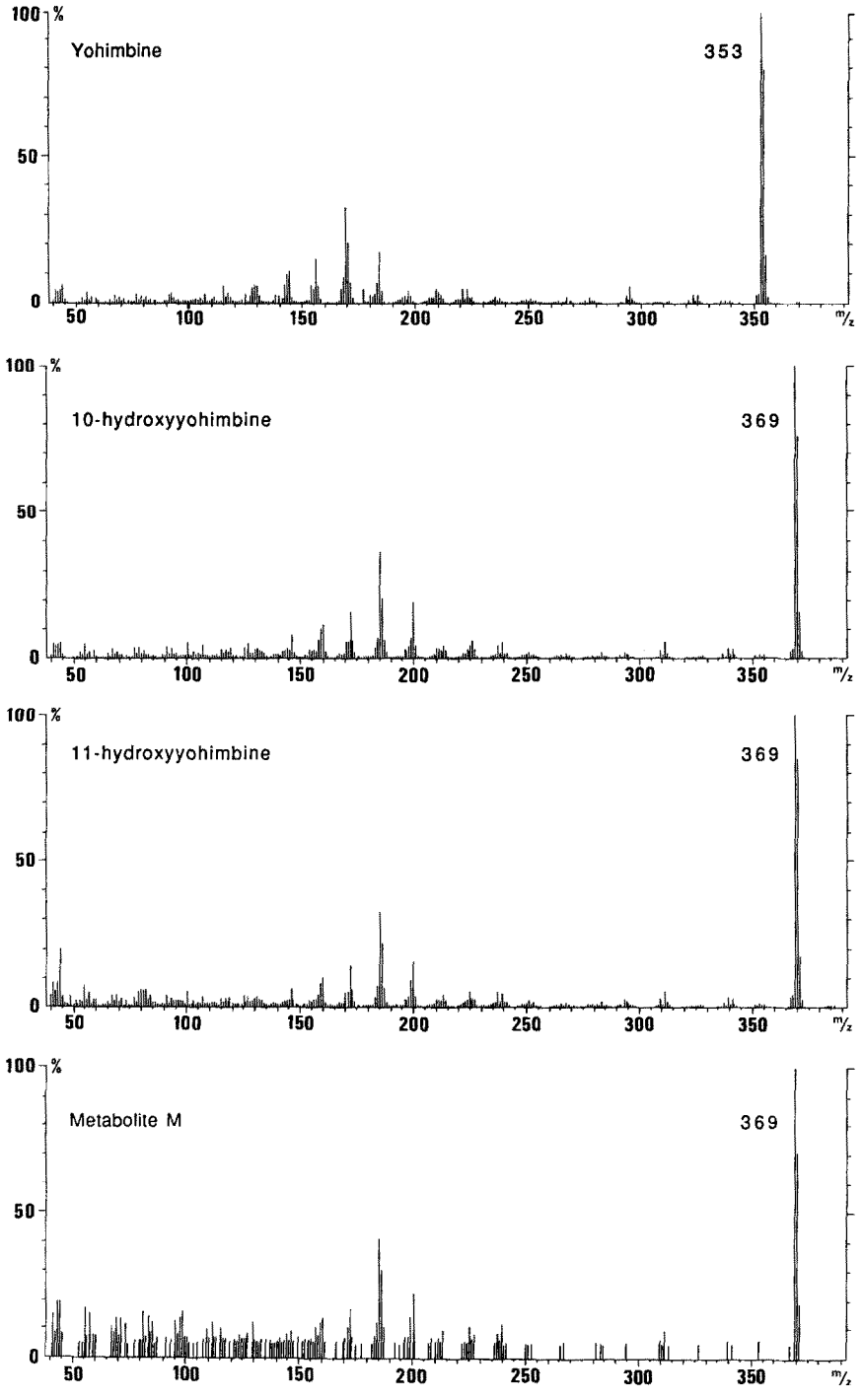

Fig. 1. Direct insertion electron impact mass spectra of authentic standards of yohimbine, 10-hydroxyyohimbine, 11-hydroxyyohimbine and its main metabolite M extracted from a urine sample from a subject who had been given an oral dose of yohimbine. Conditions as described under Experimental. The hydroxylated metabolite M was subsequently identified as 11-hydroxyyohimbine.

(17.0%). Comparison of the mass spectra and analysis of the mass data for YO and metabolite M contained in a urine sample indicated that M was probably a metabolite of YO hydroxylated on the aromatic ring (Fig. 1 and Table II).

As hydroxylation on the aromatic ring at the *para* position with respect to the nitrogen atom is likely to be a metabolic pathway, the synthesis of an authentic standard of 10-OHYO (Fig. 2) was initially achieved. Further, compared with the other possibilities, *i.e.* hydroxylation at the C-9, C-11 or C-12 position, hydroxylation of YO at the C-10 position is more easily feasible by chemical methods [18]. The structure and purity of the authentic 10-OHYO were assessed by UV and mass specrometry, ^{1}H NMR and ^{13}C NMR analysis. Comparison of the spectrometric data of the 10-OHYO main ion fragments with those of metabolite M confirmed that metabolite M was a hydroxylated metabolite (Table II).

However, normal- and reversed-phase HPLC of the metabolites isolated from the urine sample and of the authentic 10-OHYO indicated that 10-OHYO had chromatographic characteristics similar to those of metabolite m. Hence metabolite M, which was the major metabolite detected in both urine and plasma, was assumed to be isomeric with 10-OHYO, as revealed by the mass spectra (Fig. 1, Table II).

The NMR spectrum (300 MHz) of metabolite M was measured in $C^{2}HCl_{3}$ to which was added a small amount of $C^{2}H_{3}O^{2}H$ in order to ensure complete dissolution (2400 spectra collected). The aromatic part of this spectrum displayed a system of three protons (A, B and C) whose coupling constants were J_{AB} = 8.8 Hz and J_{AC} = 1.8 Hz. This indicated that this compound was a metabolite of yohimbine, hydroxylated either at the C-10 or C-11 position.

This part of the spectrum was clearly different from the corresponding part on the spectrum of authentic 10-OHYO. However, as the solvent conditions used for this experiment were different, it was not possible to conclude that there was a difference between these two compounds, and further investigations were required.

Therefore, this metabolite was mixed with an identical amount (0.2 mg) of authentic 10-OHYO, and NMR spectra of the mixture were recorded (6000 spectra collected). Under these conditions, both compounds undergo the same solvent effects. The aromatic part of this spectrum displayed two series of signals, one of which was related to the authentic 10-OHYO and the

TABLE II

MASS SPECTROMETRIC DATA OF AUTHENTIC STANDARDS OF YOHIMBINE, 10-HYDROXYYOHIMBINE, 11-HYDROXYYOHIMBINE AND OF THE MAIN METABOLITE M EXTRACTED FROM A URINE SAMPLE OF A SUBJECT WHO HAD BEEN GIVEN AN ORAL DOSE OF YOHIMBINE

Conditions as described under Experimental. The hydroxylated metabolite M was subsequently identified as 11-hydroxyyohimbine. M_r = Relative molecular mass.

m/z (relative intensity, %)			
Yohimbine (M_r 354)	10-Hydroxyyohimbine (M_r 370)	11-Hydroxyyohimbine (M_r 370)	Metabolite M (M_r 370)
355 (16.4)	371 (15.9)	371 (17.3)	371 (18.9)
354 (79.5)	370 (76.4)	370 (84.9)	370 (71.1)
353 (100)	369 (100)	369 (100)	369 (100)
184 (17.1)	200 (19.3)	200 (15.7)	200 (22.0)
170 (20.2)	186 (20.4)	186 (21.7)	186 (30.2)
169 (32.5)	185 (36.4)	185 (32.5)	185 (41.5)
156 (15.1)	172 (15.9)	172 (14.2)	172 (17.0)

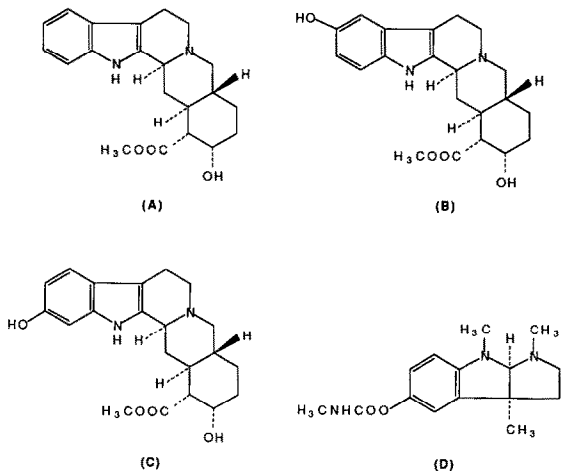

Fig. 2. Structural formulae of (A) yohimbine (M_r 354), (B) 10-hydroxyyohimbine (M_r 370), (C) 11-hydroxyyohimbine (M_r 370) and (D) eserine (internal standard).

other, which was not superposable, could be related to the unknown metabolite (Fig. 3). On the other hand, both the methyl of the carboxymethyl group at the C-16 position and the hydrogen at the C-17 position gave a single signal, at 3.79 and 4.23 ppm, respectively. These data confirmed that this hydroxylated isomer of yohimbine is different from 10-OHYO. The pattern of the aromatic protons of this main metabolite, present in plasma, on the ^{1}H NMR spectrum suggested that it was a hydroxylated metabolite of yohimbine at the C-11 position (11-OHYO, Fig. 2). Final identification of metabolite M was achieved with an authentic sample of 11-OHYO that was synthetized on purpose. HPLC showed that metabolite M and 11-OHYO had the same chromatographic characteristics in both the normal- and reversed-phase systems (Table I).

Such a metabolic pathway, *i.e.*, hydroxylation at the *meta* position with regard to the nitrogen atom of an indole structrure, is exceptional. Further, these data indicated that this metabolite had not undergone any epimerization either at the C-16 or at the C-17 position, because if that had been the case, signals related to the methyl of the carboxymethyl group at the C-16 position and to the hydrogen at the C-17 position would have

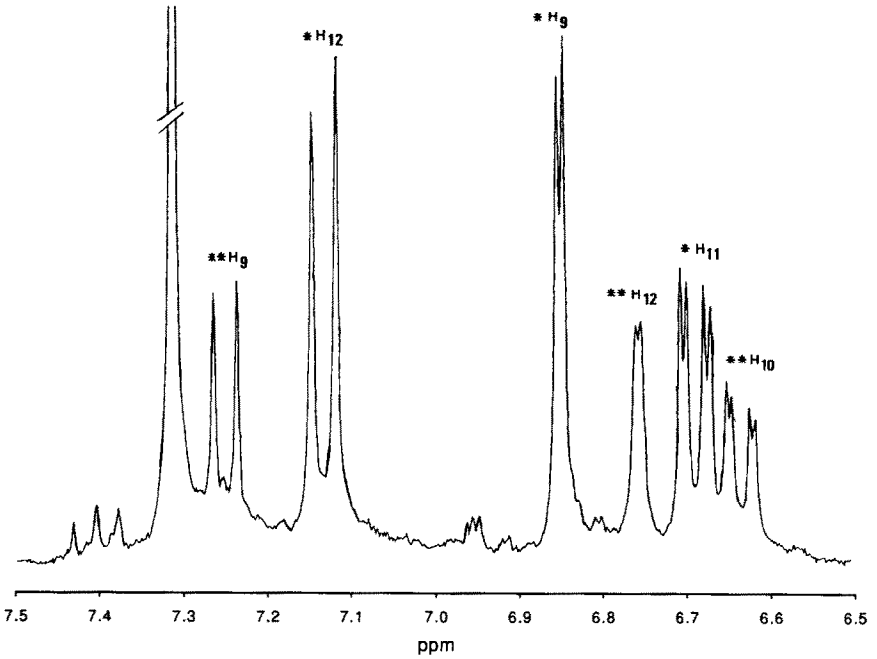

Fig. 3. Aromatic part of the ^{1}H NMR spectrum of a mixture of authentic 10-hydroxyyohimbine and metabolite M extracted from a urine sample from a subject who had been given an oral dose of yohimbine. Conditions as described under Experimental. The methyl of the carboxymethyl group at the C-16 position and the hydrogen at the C-17 position gave a unique signal. Single asterisks denote the protons of 10-hydroxyyohimbine and double asterisks those of metabolite M.

been split up. These findings are interesting because the study of structure–activity relationships of yohimbine and some of its related analogues has highlighted the influence of the conformations of the carboxymethyl group and of the hydroxyl group on the determination of their selectivity for α_1 and α_2 adrenoreceptor blocking activities [20].

Chromatographic studies

The isolation and identification of indole alkaloids have commonly been investigated by silica gel liquid chromatography owing to its selectivity for fine differences in chemical structure, especially the variety of functional groups and the stereoisomeric features of such compounds. The nitrogen atoms contained in the quinolizidine and indole nuclei play an important role in the adsorption phenomena of alkaloids. Further, the steric environment of these active amino groups may weaken their adsorptivity [21]. Among the other functional groups in alkaloids, the hydroxyl group has been known to have the most potent active function in adsorption chromatography. In addition, the nature of the solvent systems may significantly affect the retention characteristics of such compounds [21].

In this study, the liquid chromatographic separation of YO and two of its metabolites was achieved using different systems. In the first system a normal-phase packing (silica) and a reversed-phase solvent were used, and in the second, two chromatographic columns of different polarity (CN and C_8) and reversed-phase solvents were used. Owing to the mobile phase used with the LiChrosorb Si 60 column, the underlying mechanism of the chromatographic behaviour combined adsorption and partition chromatography.

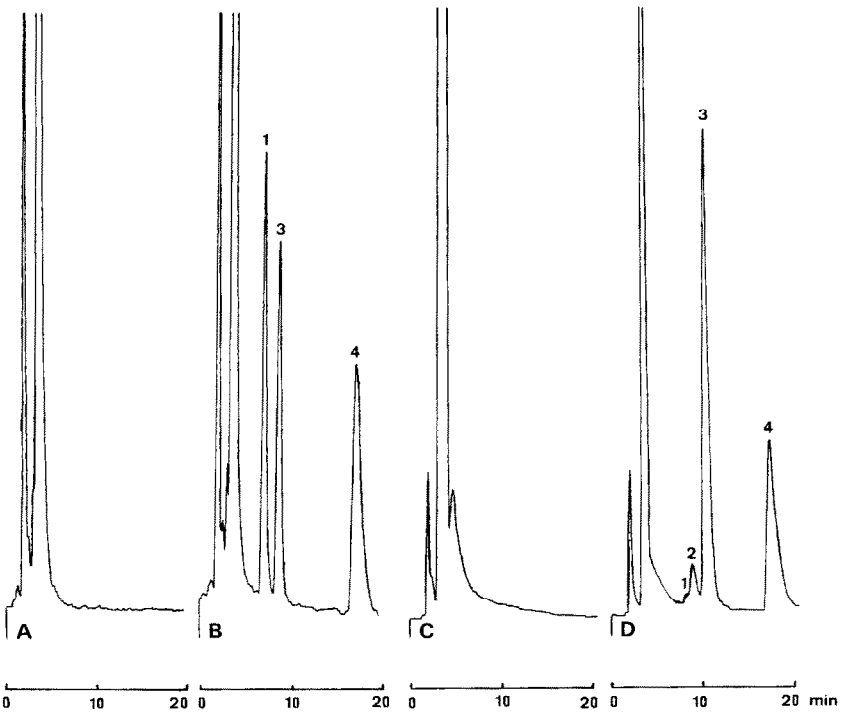

Fig. 4. Chromatograms of extracted biological samples. (A) Drug-free plasma sample; (B) plasma sample (dilution 1:5) obtained 1 h after administration of an 8-mg oral dose of yohimbine hydrochloride in a subject (yohimbine 5.9 ng/ml and 11-hydroxyyohimbine 94.2 ng/ml); (C) drug-free urine sample; (D) urine sample (dilution 1:3) obtained following administration of an 8-mg oral dose of yohimbine hydrochloride in a subject (yohimbine and 10-hydroxyyohimbine not measured, 11-hydroxyyohimbine 127 ng/ml). Peaks: (1) yohimbine; (2) 10-hydroxyyohimbine; (3) 11-hydroxyyohimbine; (4) eserine (internal standard).

Based on the chromatographic data in Table I, the former system proved to be the more interesting. Whereas the order of elution of the hydroxylated metabolites of YO was the same with both systems, the elution of YO was different. Typical chromatograms are shown in Fig. 4.

Quality controls

The fluorescence characteristics of both metabolites were determined, in comparison with YO, in the mobile phase using the detection conditions mentioned above. The fluorescence intensity of 10-OHYO and 11-OHYO was 1.98 and 3.77 lower than that of YO, respectively.

Drug-free human plasma samples (2 ml) were spiked with YO at 10 ng/ml and with eserine (internal standard) at a concentration of 100 ng/ml. Each sample was handled according to the normal-phase HPLC method described and compared with a standard solution. The extraction yield of YO, at a plasma concentration of 10 ng/ml, was 91.8 ± 2.5% (mean ± S.D., n = 10). The extraction yield of eserine was 77.7 ± 2.0% (mean ± S.D., n = 10). As authentic 11-OHYO, the main metabolite in plasma, is available in only very small amounts so far, minimum quality controls, *i.e.*, extraction yield and within-day reproducibility, were determined. The extraction yield of 11-OHYO at a plasma concentration of 100 ng/ml was 17.8 ± 0.8% (mean ± S.D., n = 10). Extraction yield data are presented in Table III. Improvement in the extraction yield of this hydroxylated metabolite may result from the use of more polar solvents such as ethyl acetate instead of chloroform. However their use led to less clean extracts, resulting in troublesome chromatographic interferences. The extraction yield of 10-OHYO at a plasma concentration of 50 ng/ml was 45.3 ± 1.6% (mean ± S.D., n = 10).

The linearity of the method was checked for YO, 10-OHYO and 11-OHYO within the concentration ranges 0.1–10, 0.5–50 and 1–100 ng/ml, respectively. Calibration graphs were constructed by plotting peak-area ratios of analyte to internal standard against concentration. The equations of the calibration graphs was fitted by linear least-squares regression without weighting. The correlation coefficients (r^2) for YO, 10-OHYO and 11-OHYO were 0.995, 0.998 and 0.998, respectively.

The limits of determination of YO, 10-OHYO and 11-OHYO were 0.1, 0.5 and 1 ng/ml, respectively.

The within-day reproducibilities for YO, 10-OHYO and 11-OHYO at concentrations of 10, 50 and 100 ng/ml (n = 10) were 3.8, 1.4 and 5.9%, respectively. The between-day reproducibilities for YO at concentrations of 1 and 10 ng/ml were 8.9% (n = 15) and 6.4% (n = 14), respectively. The accuracies of the method for YO at the concentrations of 1 and 10 ng/ml were 5.1% (n = 15) and 2.3% (n = 14), respectively. These data overall indicated the suitability of the method for pharmacokinetic studies of YO.

Pharmacokinetic application

Total plasma concentrations *versus* time curves for YO and 11-OHYO following intravenous (5 mg) and oral (8 mg) administration of YO hydrochloride in a subject are shown in Fig. 5.

TABLE III

EXTRACTION YIELDS

Conditions as described under Experimental.

Compound	Concentration (ng/ml)	Yield (%)	Relative standard deviation (n = 10) (%)
Yohimbine	10	91.8	2.7
10-Hydroxyyohimbine	50	45.3	3.6
11-Hydroxyyohimbine	100	17.8	4.7
Eserine	10	77.7	2.5

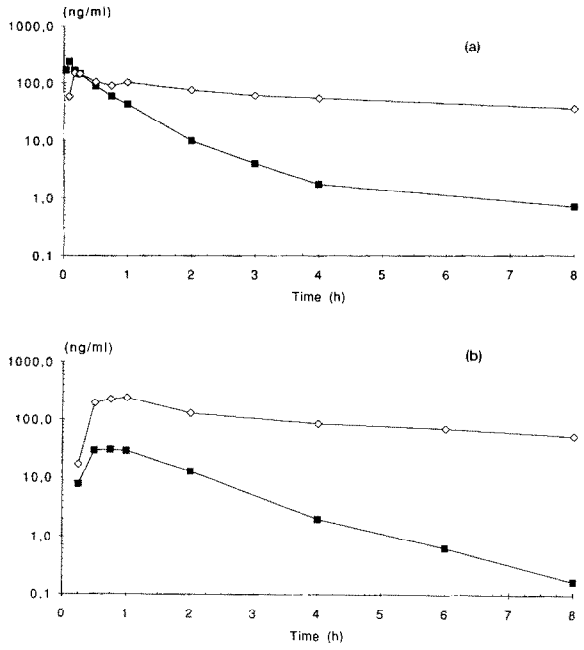

Fig. 5. Total plasma concentration-time curves of (■) yohimbine and (◇) 11-hydroxyyohimbine following intravenous (top, 5 mg) and oral (bottom, 8 mg) administration of yohimbine hydrochloride in a subject.

The distribution half-life (11.3 min^{-1}), the elimination half-life (55.5 min^{-1}) following intravenous administration and the systemic bioavailability (30%) of YO determined in this preliminary pharmacokinetic study are in agreement with data in the literature [8,9], However, the main contribution of this study resides in the discovery of two metabolites of YO. One of these metabolites, 11-OHYO, which is largely present in plasma, exhibits a longer elimination half-life than the parent drug, 6.0 h *versus* 1 h, respectively. This may explain the discrepancy noted between previous pharmacokinetic data and the therapeutic effects and supports the hypothesis of a first-pass effect and subsequent low oral bioavailability after hepatic oxidative metabolism.

In conclusion, the results presented here show that YO has at least two metabolites that we have identified as metabolites hydroxylated at the C-10 and the C-11 positions. As far as we know, these are the first data relating to the metabolism of YO in humans. These results will allow a better understanding of the pharmacokinetics and probably the pharmacodynamics of YO. The normal-phase HPLC method described here allows the simultaneous evaluation of YO and its main metabolite in plasma, *i.e.*, 11-OHYO. A complete pharmacokinetic study of YO be reported later.

ACKNOWLEDGEMENT

We are grateful to Laboratoire Houdé (Paris, France) for material support.

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