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Mass spectral characterization of urinary metabolites of D,L-kawain

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ABSTRACT

The urinary metabolism of D,L-kawain was studied in humans after an oral dose of 200 mg. Ten metabolites of kawain could be identified by gas chromatography–mass spectrometry with electron impact and chemical ionization. The main metabolic pathways were hydroxylation of the phenyl ring, reduction of the 7,8-double bond, hydroxylation of the lactone ring with subsequent dehydration and opening of the lactone ring. The metabolites were mainly excreted in the form of their conjugates.

INTRODUCTION

D,L-Kawain is a synthetic drug which was originally isolated in the form of its (+)-isomer from *Piper methysticum* [1–7]. Extracts of the root and stem of this shrub are used as a folk medicine and as a ceremonial and social drink in the South Pacific. D,L-Kawain has a distinct anxiolytic and, in parenteral application, also analgesic and muscle-relaxing activity [4,8–13]. It is marketed under the trade name Neuronika® (Klinge Pharma, Munich, F.R.G.).

A single oral dose of 200 mg of D,L-kawain resulted in a maximum plasma concentration of 18 ng/ml. A distribution half-life of 3–5 h and an elimination half-life of 9 h have been determined [4]. The maximum effect of the drug on the encephalogram parallels peak plasma levels of kawain [14].

The metabolism of D,L-kawain has been studied in rats after intravenous and oral administration [15]. *p*-Hydroxybenzoic acid, hippuric acid, 4-hydroxy-6-phenyl-5-hexen-2-one, 4-hydroxy-6-hydroxyphenyl-5-hexen-2-one, 12-hydroxy-dihydrokawain, two isomers of hydroxykawain and 12-hydroxy-5,6-dehydrokawain could be identified, and there are further unidentified metabolites. Little is known about the human metabolism of kawain. 12-Hydroxykawain has been detected in plasma [4]. It has an elimination half-life of 29 h [4].

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In a case of kawain overdose, a screening procedure using gas chromatography–mass spectrometry (GC–MS) revealed several previously unknown metabolites of kawain [16]. This finding prompted us to study the urinary metabolism of kawain after a therapeutic dose.

EXPERIMENTAL

Clinical studies

Urine was collected for 24 h from five healthy volunteers after an oral dose of 200 mg of D,L-kawain (Neuronika). The urine samples were stored at −20°C prior to analysis.

Reagents and chemicals

Pure samples of kawain and its derivatives were generously provided by Professor Dr. R. Hänsel, Institut für Pharmakognosie, Freie Universität Berlin (Berlin, F.R.G.). All reagents, of analytical-reagent grade or better, were purchased from commercial sources and used without further purification.

GC–MS of urine samples

Urine samples were pooled and a 20-ml sample was extracted at pH 7.5 with diethyl ether (Nanograde; Mallinckrodt, St. Louis, MO, U.S.A.). The organic solvent was removed with a stream of dry nitrogen. The residue was dissolved in 100 μl of methanol and a 1–3 μl aliquot was used for GC–MS. Urine was extracted similarly after incubation (37°C) with 0.5 ml of glucuronidase (12 U/ml)–sulphatase (60 U/ml) (Merck, Darmstadt, F.R.G.) at pH 5.5 for 30 min. Extracts were analysed directly or after methylation with diazomethane.

Mass spectra were run on a Model 4021 gas chromatograph–mass spectrometer with an Incos data system (Finnigan, San José, CA, U.S.A.). For GC a fused-silica capillary column (SE-54, 25 m × 0.32 mm I.D., 0.3 μm film thickness) (Macherey-Nagel, Düren, F.R.G.) was used with an injection port temperature of 280°C, splitless injection and a column temperature programme of 75–300°C at 15°C/min. The carrier gas was helium at a flow-rate of 1.7 ml/min. The column was coupled directly to the mass spectrometer. The ion source pressure was $4 \cdot 10^{-5}$ Pa in the electron-impact (EI) mode and $3 \cdot 10^{-3}$ Pa in the chemical ionization (CI) mode, using methane. The ion source temperature was 220°C. The multiplier voltage was 1200 V.

All samples were run in the EI (70 eV) and in the CI (30 eV) mode. Structure elucidation of kawain derivatives was based on reference mass spectra, determination of the molecular ion by CI, fragmentation pattern and formation of the corresponding derivatives after methylation of the extracts.

The chemical stability of kawain was studied under *in vitro* conditions. An aqueous solution of kawain (10 μg/ml) was adjusted to pH 12 and kept at 80°C for 30 min. In addition, an aqueous solution of Kawain (10 μg/ml) was refluxed

with concentrated hydrochloric acid for 30 min. The reaction mixtures were adjusted to pH 7–7.5 and extracted twice with diethyl ether. The extracts were analysed by GC–MS.

RESULTS

The molecular ions of kawain and its metabolites were identified by chemical ionization with methane, which yielded the typical $[M+41]^+$, $[M+29]^+$ and

TABLE I

RETENTION TIMES AND MASS SPECTRA OF KAWAIN AND ITS METABOLITES AND DERIVATIVES

Compound	Retention time (s)	m/z[a] (intensity, %)
I	610	M^+ 230 (27), 202 (32), 186 (3), 171 (4), 135 (4), 131 (9), 115 (7), 104 (22), 98 (95), 91 (40), 77 (18), *68 (100)*
IIa (methyl ester)	684	M^+ 244 (22), 186 (6), *185 (100)*, 170 (21), 169 (3), 153 (2), 152 (11), 151 (10), 141 (6), 115 (21), 91 (3), 77 (3)
III	627	*M^+ 228 (100)*, 200 (24), 185 (11), 157 (36), 140 (43), 131 (12), 129 (16), 128 (16), 114 (18), 103 (23), 77 (40), 69 (48)
IV	683	M^+ 246 (19), 217 (8), 202 (8), 188 (3), 161 (6), 140 (42), 133 (12), 114 (14), *107 (100)*, 98 (11), 91 (14), 77 (28)
IVa (methylated)	615	M^+ 260 (55), 216 (3), 201 (2), 195 (3), 185 (4), 161 (29), 135 (9), *134 (100)*, 133 (9), 131 (13), 121 (93), 119 (18), 69 (25), 68 (37)
V	663	M^+ 246 (10), 204 (4), 189 (3), 161 (4), 133 (18), 115 (16), *114 (100)*, 107 (10), 105 (10), 91 (10), 86 (56), 77 (14), 56 (92)
VI	739	*M^+ 244 (100)*, 229 (3), 212 (2), 201 (11), 173 (16), 162 (8), 160 (12), 135 (12), 134 (13), 133 (7), 132 (11), 131 (35), 121 (18), 115 (8), 103 (12), 69 (52)
VIa (methylated)	702	*M^+ 258 (100)*, 230 (30), 215 (11), 202 (6), 187 (32), 159 (13), 151 (13), 115 (30), 103 (6), 77 (12), 69 (28)
VII	695	M^+ 248 (26), 216 (6), 189 (6), 171 (3), 142 (13), 133 (70), 127 (32), *107 (100)*, 98 (13), 94 (12), 77 (20)
VIIa (methylated)	640	M^+ 262 (27), 230 (3), 203 (2), 200 (3), 187 (3), 163 (3), 147 (93), 134 (11), *121 (100)*, 108 (6), 91 (11), 77 (12)
VIIIa (methylated)	627	M^+ 260 (9), 215 (2), 203 (2), 201 (2), 134 (6), 122 (8), *121 (100)*, 114 (11), 91 (10), 77 (11), 69 (13), 55 (14)
IX	414	M^+ 190 (22), 172 (11), 133 (28), 132 (30), 131 (29), 129 (8), 128 (7), 121 (10), 115 (22), 105 (23), *104 (100)*, 99 (23), 77 (32), 55 (95)
X	550	*M^+ 188 (100)*, 187 (28), 173 (55), 159 (4), 147 (8), 145 (42), 131 (8), 127 (40), 115 (30), 91 (90)
XI	379	M^+ 174 (48), 159 (4), 141 (47), 121 (3), 117 (28), 104 (12), 92 (32), *91 (100)*, 77 (16), 65 (21)

[a] M^+ = molecular ion; the base peak (100%) is given in italics.

$[M+1]^+$ ions. Direct analysis of urine extracts revealed eight different metabolites of kawain (III–VII, IX–XI) in addition to the unchanged drug (I). Five methylated derivatives of the metabolites (IIa, IVa, VIa, VIIa and VIIIa) could be identified after methylation. The mass spectra of kawain and its metabolites and derivatives are summarized in Table I. The exact positions of the hydroxyl and O-methyl functions of IV, VI–VIII, IVa and VIa–VIIIa could not be determined from the mass spectra.

Analysis of the urine extracts with and without enzymatic conjugate cleavage revealed that most of the metabolites were excreted in the form of their conjugates. The main metabolite was IV. Only trace amounts of VIIIa could be detected. In addition to the metabolites listed in Table I, hippuric acid and *p*-hydroxybenzoic acid were identified in urine extracts. However, as these substances are also physiological metabolites, their identification could not be attributed unequivocally to kawain administration.

The metabolites of kawain detected in a case of kawain overdose were identical with those found after a therapeutic dose to volunteers.

Under alkaline *in vitro* conditions, kawain completely decomposed to cinnamaldehyde, cinnamylacetone (IX) and a decarboxylation product of kawa acid (II). After acid hydrolysis, only cinnamaldehyde was detected.

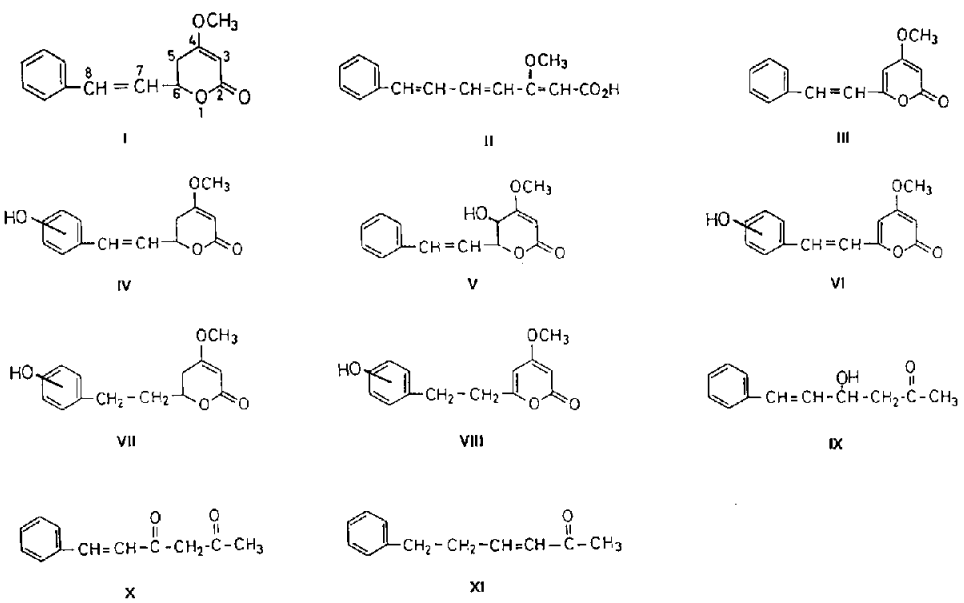

Fig. 1. Structure of kawain and its metabolites.

DISCUSSION

The structures of kawain and its metabolites are depicted in Fig. 1. The main metabolite pathway of kawain is hydroxylation of the aromatic ring. In addition, ring opening of the lactone ring, hydroxylation of the lactone ring and subsequent dehydration and reduction of the 7,8-double bond are observed. Components IX, X and XI were degradation products of kawa acid (II). Some of the metabolites, such as VI, VII and VIII, were formed by a combination of the different metabolic steps. The bulk of the kawain dose was excreted in the form of conjugates. Compound VIII could only be detected in form of its methylated derivative VIIIa. The reason might be that VIII is only formed in trace amounts. The main metabolite was IV. The human metabolism of kawain is similar to that in rats. However, 4-hydroxy-6-hydroxyphenyl-5-hexen-2-one could not be detected in humans. In humans, however, II, III, VIII, X and XI could be identified in addition.

The *in vitro* experiments indicated that kawain is rather unstable under extreme alkaline and acidic conditions.

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