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Microsomal metabolism of dictamnine: identification of metabolites and evaluation of their mutagenicity in *Salmonella typhimurium*

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Incubation of the furoquinoline alkaloid dictamnine with microsomal fractions from phenobarbital-induced rat liver resulted in a metabolite mixture from which demethyldictamnine and dictamnic acid were identified by a GC-MS technique. The identity of the two compounds was confirmed by synthesis. Other metabolites were characterized by their mass spectra only. The pattern of the metabolites suggested a possible pathway of metabolism *in vitro*. We assume that the metabolism of dictamnine is analogous to the metabolism of the related 8-methoxypsoralen and takes place via an unstable epoxide and subsequent oxidative opening of the furan ring. Direct evidence for the formation of an epoxide was, however, not obtained. The identified compounds as well as some putative metabolites were shown to be non-mutagenic in *Salmonella typhimurium* TA98 except for 8-hydroxydictamnine, which, however, was not detected in the metabolite mixture.

Introduction

The furoquinoline alkaloid dictamnine is very common within the family Rutaceae. It is the main alkaloid in the roots of *Dictamnus albus* and responsible for the mutagenicity of extracts derived from the crude drug (Mizuta and Kanamori, 1985). Dictamnine was also reported to be a phototoxic and photomutagenic compound (Pfyffer *et al*., 1982; Schimmer and Kühne, 1991). It participates in the severe skin phototoxicity of the plant (Schempp *et al*., 1996).

Dictamnine mutagenicity has been studied in detail using *Salmonella typhimurium* TA98, TA100, TA1535 and TA1538. The results were reported in several papers (Paulini *et al*., 1987, 1989; Häfele and Schimmer, 1988). A relatively strong activity was detected in experiments with S9 mix from rat liver. The highest number of revertants was induced by microsomes from phenobarbital-induced liver (Häfele and Schimmer, 1988). From inhibitor experiments the authors concluded that dictamnine was activated by cytochrome P450. In view of other results, dictamnine *N*-oxide was discussed as a possible ultimate mutagen.

On the other hand, the photobiological activity of dictamnine was shown to be connected with the reactive furan double bond, which reacts covalently with DNA under UV-A irradiation conditions (Pfyffer *et al*., 1982). It seemed worthwhile to test the possibility that the furan ring could also be involved in metabolic activation, forming an epoxide structure as the active intermediate instead of the *N*-oxide.

Metabolites resulting from incubation with rat liver microsomes were analysed by a GC-MS technique and tested for mutagenicity. This paper describes the identification of demethyldictamnine and dictamnic acid. The structures of other metabolites were proposed from their mass spectra. The identified metabolites and some related compounds were tested for mutagenicity in *S.typhimurium* TA 98.

Materials and methods

General

^{1}H-NMR. ^{1}H-NMR were run at 360 and 90 MHz, respectively, in DMSO-d_6, $CDCl_3$ and CD_3OD. The internal standard was TMS.

TLC was performed on pre-coated HPTLC plates (silica gel 60 F_{254}) using the solvent system toluene/EtOAc/formic acid (5:4:1). Detection: UV, anisaldehyde, Dragendorffs reagent.

Gas chromatography. For the reference compounds a Vega 2 model with a DB5 column (25 µm film thickness) was used. The temperature programme was 80–280°C, 6°C/min. For metabolites a DB1-30W column was employed, with a temperature program of 150–300°C, 6°C/min.

Gas chromatographic profiles. The retention indices (RI) of the individual metabolites were determined with a series of alkanes (C_{18}–C_{24}) as standards. The RI of dictamnine was 1881.5. Only those peaks were analysed which were formed after incubation of dictamnine with microsomes but not in control incubations (dictamnine without microsomes or dictamnine plus inactivated microsomes).

Mass spectrometry. The EI ionization spectra were recorded with a Finnigan model 4500 and MAT 8930. The ionization energy was 70 eV and the source temperature was 120°C.

N-Methyl-*N*-trimethylsilyl trifluoroacetamide (MSTFA reagent) was purchased from Macherey-Nagel (Düren, Germany).

Reference compounds

Dictamnine. Dictamnine was isolated from *Dictamni radix* (Müggenburg, Hamburg, Germany). Dried powdered plant material was extracted with petroleum ether (boiling point 30–60°C) for 48 h in a Soxhlet apparatus. The extracts were collected and concentrated under reduced pressure. The concentrate was extracted exhaustively with 3 N HCl. The aqueous acidic solution was alkalized with NaOH, adjusted to pH 10 and then extracted with CH_2Cl_2. The organic phases were collected and evaporated under reduced pressure. The residue was suspended in a small amount of acetone, centrifuged and the clear supernatant was chromatographed on a Sephadex LH20 column with acetone as eluent. The acetone fractions were chromatographed on silica gel plates. Fractions which showed blue fluorescent spots were collected and dried. For further purification the residue was again chromatographed on a Sephadex LH20 with MeOH/water (99:1) as the eluent. This resulted in the isolation of dictamnine (43 mg/kg crude drug) and of robustine (0.96 mg/kg). The identity and purity of dictamnine was confirmed by GC-MS analysis. The data were in agreement with the literature (Paulini *et al*., 1989).

8-Hydroxydictamnine (robustine). The extraction and isolation procedures have been described in the dictamnine section. The UV, MS and ^{1}H-NMR data and the melting point are in agreement with the literature (Chang *et al*., 1976). MS data of the MSTFA derivative: *m/z* 287 (M^+), 272 (100), 257, 242, 227, 214, 201, 186, 156, 136, 100, 73, 45.

7-Hydroxydictamnine (confusameline). The compound was a generous gift from Prof. F.Tillequin (Université René Descartes, Paris, France). UV and MS data and melting point agree with the literature (Johns *et al*., 1968). ^{1}H-NMR δ (p.p.m.), 8.19 (1 H, d, H-5, J = 9 Hz), 7.57 (1 H, d, H-2, J = 3 Hz), 7.07 (1 H, dd, H-6, J_1 = 9 Hz, J_2 = 2.5 Hz), 7.05 (1 H, d, H-3, J = 3 Hz), 7.31 (1 H, d, H-8, J = 2.5 Hz), 5.35 (1 H, s, OH), 4.44 (3H, s, OMe-4). MS data of the MSTFA derivative, *m/z* 287 (M^+, 100), 272, 257, 242, 228, 200, 186, 149, 136, 102, 73, 45.

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Demethyldictamnine. Fifty milligrams of dictamnine were dissolved in 3 ml HBr + HOAc (1 + 1) and heated under reflux while being stirred for 1 h (bath temperature 110°C). The reaction mixture was diluted with 20 ml of distilled water. Then the solution was made alkaline with aqueous sodium hydroxide (6 N) and extracted with diethylether to remove traces of unreacted dictamnine. After acidification with 3 N HCl the reaction product was extracted with diethylether. On removal of the solvent *in vacuo* a white solid was obtained. The solid was purified by column chromatography (Sephadex LH20, MeOH) yielding 18.5 mg (36%) of demethyldictamnine, melting point 244°C. UV (MeOH) λ_{max} = 238, 256, 330, 340 nm; UV (MeOH + HCl) λ_{max} = 238, 310, 326, 338 nm; UV (MeOH + NaOH) λ_{max} = 208, 258, 336, 350 nm. ^{1}H-NMR (90 MHz, in DMSO) δ (p.p.m.), 10.61 (1 H, br, s, NH), 8.26 (1 H, m, H-5), 7.39 – 7.65 (3 H, m, H-2, H-6 or H-7, H-8), 7.15 (1 H, ddd, H-7 or H-6, J_1 = 5 Hz, J_2 = 4 Hz, J_3 = 1.4 Hz), 6.97 (1 H, d, H-3, J = 1.8 Hz). MS, *m/z* (%) 186 (9), 185 (100, M^+) 156 (10), 129 (31), 128 (32), 102 (15), 101 (9), 92 (7), 77 (7), 76 (11), 75 (7). MS (MSTFA derivative) *m/z*, 257 (100, M^+), 242, 226, 214, 184, 140, 113, 73, 45.

Dictamnic acid. Dictamnic acid was synthesized after the method described by Monkovic *et al*. (1967). Yield 20.8% (5.2 mg), melting point 255°C. UV (MeOH) λ_{max} = 229, 275, 277, 314 nm. ^{1}H-NMR (360 MHz, CD_3OD) δ (p.p.m.), 8.03 (1 H, br, d, H-5, J = 8.5 Hz), 7.65 (1 H, dd, H-6 or H-7, J_1 = J_2 = 7.5 Hz, J_3 = 1.5 Hz), 7.36 (1 H, br, d, H-8, J = 8.5 Hz), 7.33 (1 H, ddd, H-7 or H-6, J_1 = J_2 = 8 Hz, J_3 = 1 Hz), 4.22 (1H, s, OMe-4). MS of the MSTFA derivative, *m/z* 363 (M^+), 348, 332, 318, 304, 274, 256, 246 (100), 200, 159, 147, 133, 73, 45.

Dictamnine N-oxide. The *N*-oxide was synthesized by analogy to the method published by Craig and Purushothaman (1970). Yield 15% (7.5 mg), melting point 90°C (decomposition). UV (MeOH) λ_{max} = 222, 252, 355 nm. UV (MeOH + HCl) λ_{max} = 210, 243, 335 nm. ^{1}H-NMR (360 MHz, $CDCl_3$) δ (p.p.m.), 8.63 (1 H, d, H-5, J = 8.5 Hz), 8.41 (1 H, br, d, H-8, J = 8.5 Hz), 8.04 (1 H, d, H-2, J = 3 Hz), 7.97 (1 H, ddd, H-6 or H-7, J_1 = J_2 = 8 Hz, J_3 = 1.5 Hz), 7.69 (1 H, ddd, H-7 or H-6, J_1 = J_2 = 7.5 Hz, J_3 = 1.5 Hz), 7.61 (1 H, d, H-3, J = 3 Hz), 4.56 (3H, s, OMe-4). MS, *m/z* (%) 215 (M^+, 3), 199 (100), 185 (13), 184 (60), 156 (32), 128 (22), 101 (13), 76 (14).

Mutagenicity experiments

Chemicals. Phenobarbital (PB) was obtained from Bayer (Leverkusen, Germany), 3-methylcholanthrene (MC), β-naphthoflavone (BNF), clofibrate (CLO), corn oil and 2-aminoanthracene (2-AA) were obtained from Sigma-Chemie (Deisenhofen, Germany). NADP and isocitrate dehydrogenase came from Boehringer (Mannheim, Germany). The other chemicals were purchased from E.Merck (Darmstadt, Germany).

Assay. The assays were performed by the plate incorporation method (Maron and Ames, 1983) with *S.typhimurium* TA98.

S9 fraction. Liver S9 fractions were prepared from male Wistar rats (200–300 g body wt) which had been treated i.p. daily with PB (70 mg/kg), MC (25 mg/kg), BNF (80 mg/kg) or CLO (400 mg/kg) for 3 days. The control group received only corn oil (0.5 ml) and was regarded as uninduced (UN). Four days after the first injection the animals were killed by cervical dislocation. The animals were fasted for 24 h prior to killing. S9 fractions were prepared by a 20 min centrifugation at 9000 *g*. The protein contents of the S9 fractions were 30 (PB), 30 (MC), 32.2 (UN), 30.8 (BNF) and 37.9 (CLO) mg/ml, respectively, and was adjusted to 30 mg/ml. The cytochrome P450 levels were 0.37 ± 0.01 (PB), 0.15 ± 0.005 (MC), 0.11 ± 0.005 (UN), 0.14 ± 0.005 (BNF) and 0.19 ± 0.005 (CLO) nmol/mg protein, respectively.

S9 mix. One millilitre of the standard activation mixture contained 0.1 ml of S9 fraction, 0.1 ml of 70 mM $MgSO_4$, 0.1 ml of 40 mM NADP, 0.1 ml of 200 mM D,L-isocitrate and 0.6 ml of 100 mM phosphate buffer (pH 7.4).

Microsomal fraction. A part of the S9 fraction was centrifuged for 1 h at 100 000 *g*. The supernatant was used as a cytosolic fraction (26 mg protein/ml). The pellet was resuspended in phosphate buffer and again centrifuged. The resulting pellet was resuspended in phosphate buffer and analysed for its protein and cytochrome P450 content. The data on the cytochrome P450 content (nmol/mg protein) were as follows: 0.84 ± 0.04 (UN); 2.09 ± 0.13 (PB); 1.41 ± 0.02 (MC); 1.54 ± 0.09 (CLO); 1.24 ± 0.03 (BNF).

Microsomal mix. Microsomal fraction (0.1 ml) and isocitrate dehydrogenase (0.05 ml) were added to the standard activation mixture (see S9 mix) for microsomal activation.

Data evaluation. The protein content was measured by the method of Lowry *et al*. (1951). The cytochrome P450 content was estimated by the method of Omura and Sato (1964). The values presented above were the mean of eight individual determinations of a pooled fraction from four rat livers. The data presented in Tables I and II are the mean of eight plates ± SD from two separate experiments each performed with quadruplicate plates. The interexperimental variation was controlled using the *F*-test (P = 0.05), which established a homogeneity of variance.

Toxicity. Toxicity was evaluated by microscopic control of the background lawn.

Microsomal incubations and preparation of the metabolite fraction. PB-induced microsomes showed stronger activation capacities for dictamnine in TA98 than uninduced or MC-, BNF- and CLO-induced fractions (Table I). Therefore, the *in vitro* experiments were performed with microsomal fractions obtained from PB-induced rat liver, which were adjusted to 10 mg protein/ml and 2 nmol cytochrome P450/mg protein. Dictamnine (5 mg, dissolved in DMSO) was incubated with 100 ml microsomal mix (10 ml microsomal fraction) for 1 h at 37°C. Incubations were terminated by the addition of 100 ml of ice-cold acetone. The acetone was removed and the residue was frozen in liquid nitrogen. The frozen mixture was stored dry at –20°C and dissolved in water. The water extract was chromatographed on a RP18 column using successively water, water/methanol (4:6) and methanol as eluents. The three fractions were evaporated to dryness and MSTFA added for derivatization. The derivatized metabolites were then analysed by a GC-MS technique.

Results

Häfele and Schimmer (1988) reported that dictamnine can be activated best with microsomes from PB-induced rat liver. The results presented here confirm this tendency by comparing the effect based on the number of revertants. A statistically significant effect of PB-derived microsomes was observed only in experiments with 0.1 nmol cytochrome P450/plate. With higher cytochrome doses no significant differences between PB-, MC- and BNF-derived microsomes were obtained (Table I). By taking all the results into consideration, PB-induced microsomes were used to study the microsomal metabolism of dictamnine. Liver microsomes from PB-pretreated rats were incubated with dictamnine. Figure 1 shows a typical chromatogram of extracts obtained after incubation with dictamnine.

Microsomal metabolism resulted in the formation of various peaks eluting with the same retention times as the reference compounds. The peaks were analysed by mass spectra. The spectra were compared with the fragmentation pattern of the reference compound.

Figure 2 shows the EI spectrum of the demethyldictamnine peak derived from dictamnine along with that of authentic demethyldictamnine. The two spectra are strikingly similar, with *m/z* 257 as the molecular ion peak of the MSTFA derivative and intense fragments at *m/z* 242 and 214; *m/z* 73 was identified as a fragment of the MSTFA reagent. A second metabolite was identified as dictamnic acid. The mass spectrum of the derivatized substance showed a molecular ion peak at *m/z* 363 and fragments at *m/z* 348 [M-CH_3], 332 [M-OCH_3], 318 [M-3CH_3], 246 [M-COOTMS] and 274 [M-OTMS]. The authentic MSTFA derivative showed a very similar pattern of fragments. A further metabolite, M287, was identified as a hydroxydictamnine, but the position of the hydroxyl group could not be deduced from the data. In the mass spectrum a prominent M^+ peak at *m/z* 287 and a major peak at *m/z* 272 [M-CH_3] were observed. Two other hydroxy derivatives were analysed by MS for comparison. 8-Hydroxydictamnine (robustine) showed similar peaks at *m/z* 272 and 257 but a minor molecular ion peak at m/z 287. 7-Hydroxydictamnine (confusameline) showed major peaks at *m/z* 287 and 272 but

Table I. Metabolic activation capacity of the various microsomal fractions (indicator *Salmonella typhimurium* TA98)

Substrate	Cyt-P 450 content (nmol/plate)	Revertants/plate (±SD)[a]				
		UN microsomes	PB microsomes	MC microsomes	BNF microsomes	CLO microsomes
Dictamnine (250 nmol)	0.1	200 ± 15	314 ± 17[b]	238 ± 17	247 ± 22	97 ± 7
	0.25	624 ± 43	950 ± 51	844 ± 68	868 ± 41	246 ± 15
	0.50	960 ± 89	1586 ± 89	1381 ± 42	1432 ± 109	414 ± 32
2-AA (1 µg/plate)	0.50	4568 ± 210	1761 ± 97	1665 ± 61	1339 ± 46	1658 ± 125

[a]Spontaneous number of revertants were from 17 to 37/plate.
[b]Significantly different (*t*-test): BNF microsomes, $P < 0.05$; MC microsomes, $P < 0.01$; UN and CLO microsomes, $P < 0.001$.

Table II. Mutagenicity of dictamnine and its putative metabolites *in vitro* in *Salmonella typhimurium* TA98

Dose (nmol/plate)	Revertants/plate (±SD)[a]											
	Dictamnine		Demetyldictamnine		Dictamnic acid		8-Hydroxydictamnine		7-Hydroxydictamnine		Dictamnine *N*-oxide	
	–S9	+S9	–S9	+S9	–S9	+S9	–S9	+S9	–S9	+S9	–S9	+S9
0[b]	24 ± 4	26 ± 5	22 ± 3	38 ± 6	22 ± 4	37 ± 5	21 ± 6	26 ± 3	20 ± 4	26 ± 3	24 ± 4	26 ± 5
5	21 ± 2	85 ± 14	23 ± 5	32 ± 6	31 ± 5	37 ± 2	22 ± 4	188 ± 15	19 ± 4	30 ± 4	17 ± 2	28 ± 3
25	31 ± 4	298 ± 28	25 ± 7	32 ± 5	27 ± 6	34 ± 5		744 ± 82			16 ± 3	32 ± 8
50	27 ± 4	548 ± 45	24 ± 7	32 ± 6	31 ± 4	37 ± 5	26 ± 2	1101 ± 75	27 ± 6	24 ± 1	19 ± 4	32 ± 7
100	28 ± 5	1051 ± 83	23 ± 6	38 ± 6	28 ± 4	42 ± 7	34 ± 2	t[c]	24 ± 4	37 ± 5	20 ± 5	37 ± 5
250	26 ± 5	1557 ± 67	20 ± 1	38 ± 9	32 ± 3	38 ± 5		t	20 ± 2	57 ± 12	18 ± 6	46 ± 4
500	51 ± 3	1863 ± 93[c]	20 ± 5	46 ± 6	28 ± 3	40 ± 3	40 ± 9	t	2 ± 2[c]	10 ± 10[c]	20 ± 7	56 ± 4[c]

[a]Data are the mean of eight plates from two separate experiments each performed with quadruplicate plates.
[b]Solvent control, DMSO (50 µl).
[c]Toxic.

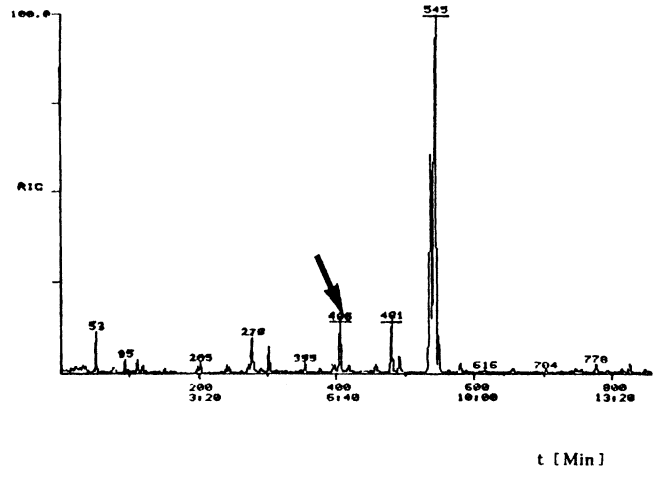

Fig. 1. Typical gas chromatogram of extracts of dictamnine incubated with microsomes. Metabolite M257 at relative retention time 406 min is indicated. This corresponds to a RI of 1945.2 (see Material and Methods).

its retention time was significantly higher than that of the metabolite (RI = 2266.3 versus 2213.0). The other metabolites (M451, 465, 349, 363b and 377) were characterized only by their mass spectra, since no reference compounds were available.

Metabolite M451 was a major product of the *in vitro* incubation of dictamnine. It showed only a weak signal of the molecular ion at *m*/*z* 451. The fragment *m*/*z* 348 showed an intense peak and may be explained by the loss of $CH_2OSi(CH_3)_3$; a minor peak at *m*/*z* 260 was possibly due to the loss of a further 88 mass units [$Si(CH_3)_4$], followed by formation of a heterocyclic ring. Fragments *m*/*z* 147 and 73 were derived from the MSTFA reagent. Metabolite M465 showed

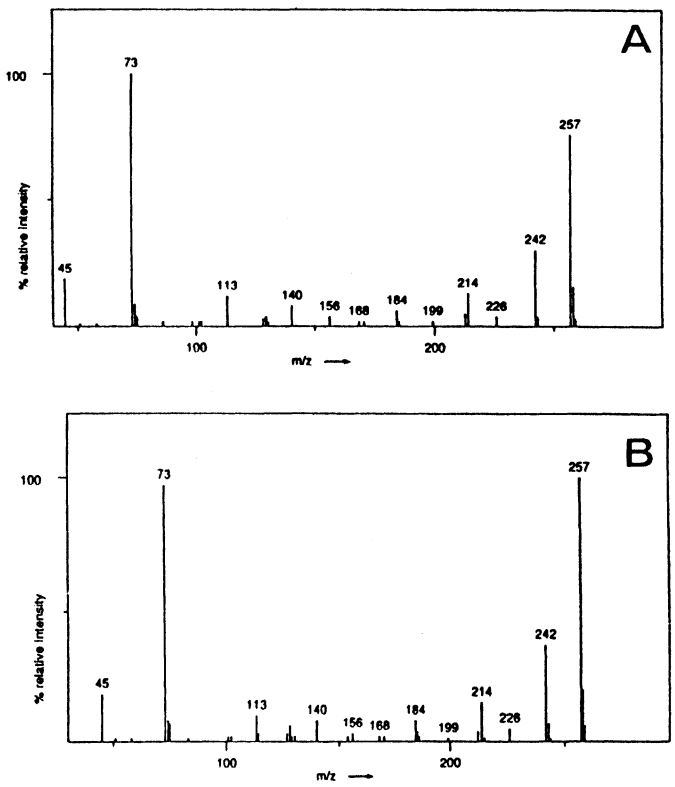

Fig. 2. EI ionization mass spectra of (**A**) demethyldictamnine, the MSTFA derivative from an extract of dictamnine incubated with microsomes, and (**B**) the MSTFA derivative of authentic demethyldictamnine.

a fragmentation pattern similar to M451 but with no detectable molecular ion peak. Characteristic peaks were shown at *m/z* 348 (M-COOTMS) and 260 (analogous to M451). M349 showed a fragmentation pattern with *m/z* 349 (M^+), 334 (M-CH_3), 318 (M-OCH_3), 304 (M-3CH_3), 260 (M-OTMS), 246 (M-CH_2OTMS), 244, 230, 147, 133 and 73. M363b showed a molecular ion at *m/z* 363, but was characterized by a different fragmentation pattern and different retention time compared with dictamnic acid. The major peaks were at *m/z* 348 (M-CH_3), 318 (M-3CH_3), 260 (M-OTMS), 246, 244, 232, 230, 147, 103, 73 and 45. Metabolite M377 was identified only from the urine of rats treated orally with dictamnine. The MS showed a weak molecular ion at *m/z* 377 and major peaks at *m/z* 362 (M-CH_3), 348, 334, 318, 260 (M-COOTMS), 246 (M-CH_2COOTMS), 244, 230, 200, 156, 147, 73 and 45. Although this metabolite was not detected in microsomal metabolism, it fitted well in the degradation scheme proposed for dictamnine.

Some identified and putative metabolites were synthesized and evaluated for their mutagenicity in *S.typhimurium* TA98. The *N*-oxide was included because this compound has been discussed as the eventual ultimate mutagen enzymatically formed by microsome mix. None of these compounds were capable of inducing revertants, apart from 8-hydroxydictamnine, whose presence in the metabolite mixture could not be verified (Table II). Thus, the *N*-oxide and the other compounds had to be excluded as possible ultimate mutagens.

Discussion

From our results with the various microsomal preparations it is evident that PB ist the most active inducer of the enzymes which metabolize dictamnine into a mutagen. This also becomes clear when we compare the activity on the basis of the cytochrome P450 content. Since it is known that PB preferentially induces cytochromes P450 of the 2B subfamily (McManus and McKinnon, 1991), one of these enzyme forms may be particularly involved in the bioactivation of dictamnine.

Among the metabolites separated by GC and identified by MS, neither dictamnine *N*-oxide nor dictamnine 2,3-oxide were detected. To test the possibility that the *N*-oxide acted as the ultimate mutagen but was not detected because of its instability, we synthesized the compound and examined it for its mutagenicity. The negative result clearly demonstrated the inability of the *N*-oxide to function as the mutagenic intermediate.

On the other hand, formation of an epoxide as an intermediate seemed to be a possible alternative, since the furyl double bond represents a crucial site for chemical reactions. This was also indicated by the inactivity found in the corresponding 2,3-dihydro derivative (Klier, 1993; data available on request). The postulated epoxide, however, could not be isolated nor synthesized, possibly due to its short-lived nature. However, the formation of furan epoxides is a common and biologically relevant phenomenon, which often leads to mutagenicity (Essigmann *et al.*, 1977; Baertschi *et al.*, 1988; Adam *et al.*, 1990, 1991). The most convincing example came from metabolism experiments with 8-methoxypsoralen. *In vivo* this furocoumarin undergoes *O*-demethylation, hydroxylation and oxidative cleavage of the furan ring. The metabolism has been postulated to take place via the 2,3-epoxide (Kolis *et al.*, 1979; Mays *et al.*, 1987, 1989). Experiments with rat liver microsomes were, however, not successful. This was possibly

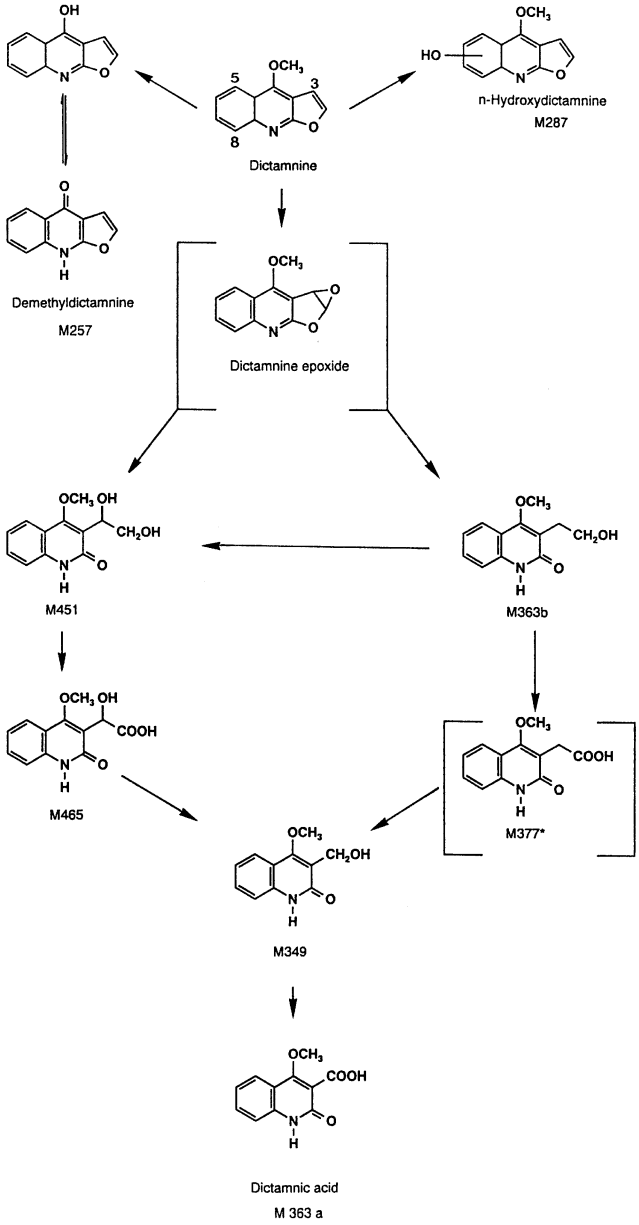

Fig. 3. Proposed pathway of metabolism of dictamnine *in vitro*. M377 was detected in urine from rats treated orally with dictamnine. M365 was detected after microsomal incubation, but not in urine samples.

caused by covalent binding of metabolites to microsomal proteins (Mays *et al.*, 1987).

The similarity of dictamnine and 8-methoxypsoralen in chemical structure and reactivity and the pattern of metabolites led us to postulate an analogous pathway of metabolism from our results (Figure 3). The demethyl and the hydroxy derivatives were formed in this, but the quinoline derivatives dominated as a consequence of oxidative opening of the furan ring. A few metabolites in the pathway were only identified tentatively by their mass spectra and have not yet been confirmed by synthesis. Nevertheless, there is a certain plausibility to the pathway proposed analogous to 8-methoxypsoralen. Although this pattern is consistent with the idea that the metabolism of dictamnine is via a furan epoxide, no direct evidence was found that such an intermediate has been formed and acted as the ultimate mutagen. Therefore, we cannot

exclude the possibility that an unidentified compound, e.g. the hydroxy derivative, is involved in dictamnine mutagenicity.

M377 was not detected *in vitro*, but this may be explained by the finding that microsomal metabolites were formed to a lesser extent than the metabolites isolated from *in vivo* experiments (Klier, 1993). Assuming that the furan ring serves as the reactive site, we must ask why some metabolites with an intact furan ring cannot be activated by microsomes or S9 mix. In the case of the demethyl derivative the lack of metabolic activation may be explained by the finding that this compound can exist in two tautomeric forms, namely 4-hydroxyfuroquinoline and furoquinolone-4. This is indicated by a signal in the ^{1}H-NMR which splits at room temperature (Klier, 1993). The quinolone structure may have been favoured under our treatment conditions. This agrees with the result that isodictamnine, another quinolone derivative, was shown to be inactive in TA98 ± S9 mix (Klier, 1993).

It is more difficult to explain the different activities of the two hydroxy derivatives. Whereas 8-hydroxydictamnine (robustine) showed strong mutagenicity with S9 mix, the 7-hydroxy derivative and other furoquinolines substituted with an hydroxy group at C_7 were inactive in TA98 (Klier, 1993; this work). As the position of the hydroxy group in M287 is not known at present, far-reaching conclusions on its activity cannot be drawn. However, it seems very unlikely that 8-hydroxydictamnine is the ultimate mutagen because of its stability and its absence in the metabolite mixture.

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