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Studies on the Oxidation of 5*H*-*N*-Substituted Dibenz[*b*,*f*]azepines. III.[1] Oxidative Metabolism by Rat Liver Microsomes[2]

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The metabolism of various 5*H*-*N*-substituted dibenz[*b*,*f*]azepines (**1a**—**e**) by rat liver microsomal fractions was investigated. 5*H*-*N*-Acyldibenz[*b*,*f*]azepines (**1a**—**c**) were metabolized to 5*H*-*N*-acyldibenz[b,*f*]azepine 10,11-oxides (**2a**—**c**), while 5*H*-*N*-alkyldibenz[*b*,*f*]azepines (**1d**, **e**) were metabolized to *N*-alkyl-9(10*H*)-acridones (**5d**, **e**). 5*H*-*N*-Acyl-10,11-dihydro-*trans*-10,11-dihydroxydibenz[*b*,*f*]azepine (**3b**) was additionally detected as a metabolite of 5*H*-*N*-acyldibenz[*b*,*f*]azepine 10,11-oxide (**2b**).

The conditions for the enzymatic formation of these metabolites are discussed.

Keywords——5*H*-*N*-substituted dibenz[*b*,*f*]azepine; 5*H*-*N*-acyl-10,11-dihydrodibenz[*b*,*f*]azepine 10,11-oxide; 5*H*-*N*-acyl-10,11-dihydro-10,11-dihydroxydibenz[*b*,*f*]azepine; *N*-alkyl-9(10*H*)-acridone; epoxidation; rat liver microsome; monooxygenase; epoxide hydratase; oxidative metabolism; microsomal cytochrome P-450

There are some biologically significant metabolites that arise even from minor metabolic pathways, and this has become an important area of study in recent years. Many 5*H*-dibenz[*b*,*f*]azepine derivatives having pharmacological activities (opipramol, dehydroimipramine, carbamazepine, *etc.*) are known. However, as regards the oxidative metabolism of the 5*H*-dibenz[*b*,*f*]azepine ring, investigation has been limited to the case of carbamazepine. That is, Frigerio *et al.* have reported that carbamazepine 10,11-oxide was detected as a metabolite in urine after the oral administration of carbamazepine,[4] and this epoxide possessed the same degree of pharmacological activity as carbamazepine.[5] This finding suggests that oxidation of the 10,11-double bond may be an important metabolic reaction of 5*H*-dibenz[*b*,*f*]azepine ring.

In the course of our chemical studies on the oxidation of 5*H*-dibenz[*b*,*f*]azepine derivatives, we found that different types of oxidation products were afforded corresponding to the differences of *N*-substituted group in the oxidation with *m*-chloroperbenzoic acid (*m*-CPBA).[1] Namely, in the case of the oxidation of 5*H*-*N*-acyldibenz[*b*,*f*]azepine derivatives, the 10,11-epoxides were obtained as a main product, but in the case of the oxidation of 5*H*-*N*-alkyldibenz[*b*,*f*]azepine derivatives, *N*-alkyl-9(10*H*)-acridones were obtained instead of the epoxides. Thus, we carried out the present study to clarify the oxidative metabolism of the 5*H*-dibenz[*b*,*f*]azepine ring carrying various *N*-substituted groups.

Materials and Methods

Materials——5*H*-*N*-Acetyl- (**1a**), 5*H*-*N*-benzoyl- (**1b**) and 5*H*-*N*-carboethoxydibenz[*b*,*f*]azepine (**1c**), 5*H*-*N*-propyl- (**1d**) and 5*H*-*N*-benzyldibenz[*b*,*f*]azepine (**1e**), 5*H*-*N*-acetyl- (**2a**), 5*H*-*N*-benzoyl- (**2b**) and 5*H*-*N*-carboethoxydibenz[*b*,*f*]azepine 10,11-oxide (**2c**), 5*H*-*N*-benzoyl-10,11-dihydro-*trans*-10,11-dihydroxydibenz[*b*,*f*]-azepine (**3b**), and *N*-propyl- (**5d**) and *N*-benzyl-9(10*H*)-acridone (**5e**) were synthesized by the methods described previously.[1b] 5*H*-Dibenz[*b*,*f*]azepine (**4d**) was purchased from Aldrich Chemical Co. 5*H*-*N*-Benzoyl-10,11-dihydro-*cis*-10,11-dihydroxydibenz[*b*,*f*]azepine (**4b**) was synthesized from 5*H*-*N*-benzoyldibenz[*b*,*f*]azepine (**1b**)

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with OsO_4 in the manner described by Cook and Schoental.[6] Nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate (G6P) and G6P dehydrogenase (Grade II) were purchased from Boehringer Mannheim Co.

Preparation of Liver Microsomal Fraction from Rat——Male Wistar strain rats (body weight 150—200 g), fasted for one day befofe being killed, were used. A 10 vol. homogenate in 0.25 M sucrose solution was prepared from the livers by a standard procedure. Microsomes were obtained from the post mitochondrial supernatant fraction (centrifuged at $15000 \times g$) of the homogenate by centrifugation at $105000 \times g$ for 2 h and resuspended in 0.2 M phosphate buffer (pH 7.5). Protein content of the suspension, determined by the method of Lowry *et al.*,[7] was 2 mg/ml.

Incubation Conditions——Incubation mixtures (3 ml) contained $MgCl_2$ (5 mM), nicotinamide (10 mM), G6P dehydrogenase (3.5 units), NADP (0.3 mM), microsomal suspension (1 ml), 0.2 M phosphate buffer (pH 7.5), and substrate (1—0.05 mM) dissolved in 0.3 ml of acetone. Incubation was carried out under aerobic conditions at 37 °C for 1 h with gentle shaking.

Extraction Procedure——The incubation mixtures were extracted three times with 10 ml of ethyl acetate. The extracts were dried over Na_2SO_4 and evaporated to dryness under reduced pressure and the residues were dissolved in methylene chloride or methanol, then subjected to thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) or gas chromatography-mass fragmentography (GC-MF).

TLC——TLC was carried out on aluminum sheets (5 × 20 cm) coated with Silica gel $60F_{254}$ (0.2 mm thickness). The solvent systems used are listed in Table I. Substrates and metabolites were visualized under ultraviolet (UV) light at 254 nm.

HPLC——HPLC were obtained by using the following instruments with a 254 nm fixed-wavelength detector: Waters 6000A pump, Shimadzu SIL-1A sample injector and Waters M-440 UV detector. All chromatography was done at ambient temperature, and the other conditions of analysis are given in the figure legends.

GC-MF——A gas chromatograph-mass spectrometer (Shimadzu LKB 9000) was used under the following conditions: energy of ionization beam, 20 eV; ion source temperature, 290 °C; trap current, 60 μA. Sample introduction was carried out by a gas-liquid chromatograph procedure utilizing a glass column (1 ml long and 3 mm i.d.) packed with 1.5% OV-17 (column temperature, 260 °C).

Results and Discussion

Identification of Microsomal Metabolites of 5*H*-*N*-Acyldibenz[*b*,*f*]azepines (1a—c)

The ethyl acetate extracts of the incubation mixture of 5*H*-*N*-acyldibenz[*b*,*f*]azepines (**1a**—**c**) with rat liver microsomal fractions were analyzed by TLC and HPLC. The analysis by TLC showed one product (metabolite **2a**—**c**) other than the unchanged compound (**1a**—**c**) in each case. The separation in solvent system A or B and the *Rf* values as compared with those of authentic 5*H*-*N*-acyl-10,11-dihydrodibenz[*b*,*f*]azepine 10,11-oxides (**2a**—**c**) confirmed the identities of the products.

The metabolites (**2a**—**c**) were also identified by HPLC. Figures 1, 2, and 3 showed the profiles of the extracts of the incubation mixtures of **1a**—**c** separated by HPLC. The peaks (metabolites **2a**—**c**) corresponded to those of authentic **2a**—**c**, which were completely separated from the unchanged compounds (**1a**—**c**).

These chromatograms clearly indicate that the oxidation of the 10,11-double bond to form the 10,11-epoxide is a common metabolic pathway in the metabolism of 5*H*-*N*-acyldibenz[*b*,*f*]azepines (Chart 1).

Table I. *Rf* Values of 5*H*-*N*-Acyldibenz[*b*,*f*]azepines (**1a**—**c**) and Their Metabolites on TLC

	Compound	*Rf*[a]	Compound	*Rf*[b]	Compound	*Rf*[b]
Metabolites	**1a**	0.41	**1b**	0.51	**1c**	0.57
	2a	0.32	**2b**	0.35	**2c**	0.41

a) Solvent system A: methylene chloride : methanol = 20 : 1. *b*) Solvent system B: methylene chloride only.

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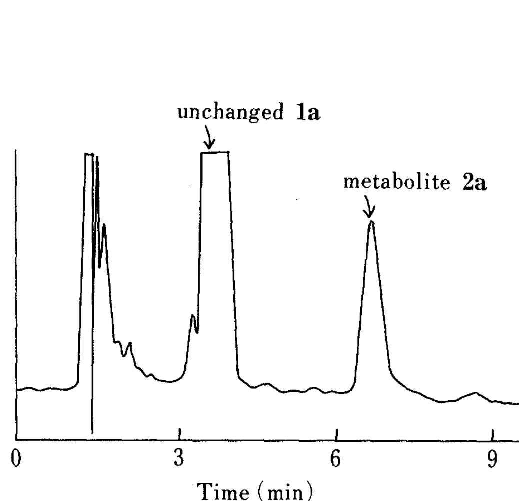


Fig. 1. HPLC of the Ethyl Acetate Extract of 5*H*-*N*-Acetyldibenz[*b*,*f*]azepine (**1a**) Incubated with Rat Liver Microsomal Fractions

Column, Wakogel LC-10H (300 mm × 4.3 mm); mobile phase, 67% methylene chloride in *n*-hexane (v/v); flow rate, 3.0 ml/min.

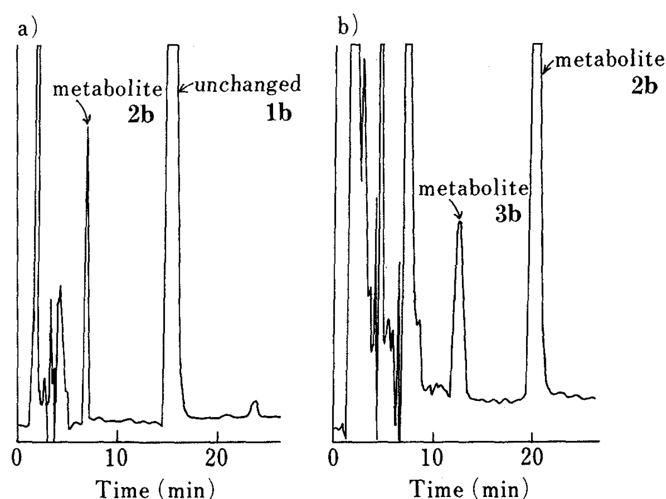


Fig. 2. HPLC of the Ethyl Acetate Extract of 5*H*-*N*-Benzoyldibenz[*b*,*f*]azepine (**1b**) Incubated with Rat Liver Microsomal Fractions

Column, Lichrosorb RP-18 (5 μm) (150 mm × 4.3 mm); mobile phase, a) 60% methanol in water (v/v), b) 50% methanol in water (v/v); flow rate, 2.0 ml/min.

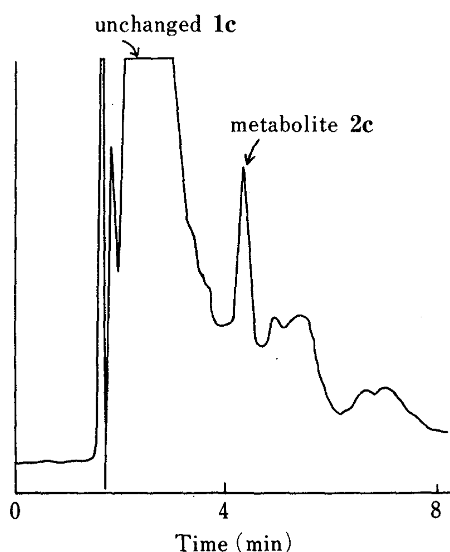
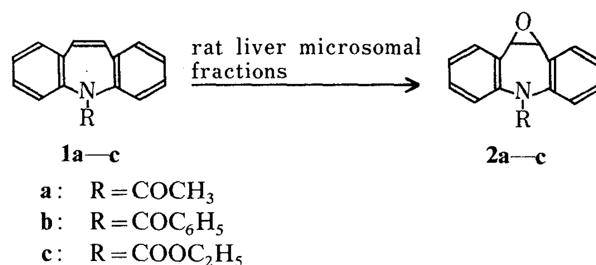


Fig. 3. HPLC of the Ethyl Acetate Extract of 5*H*-*N*-Carboethoxydibenz[*b*,*f*]azepine (**1c**) Incubated with Rat Liver Microsomal Fractions

Column, Wakogel LC-10H (300 mm × 4.3 mm); mobile phase, 67% methylene chloride in *n*-hexane (v/v); flow rate, 2.5 ml/min.



a: $R = COCH_3$
b: $R = COC_6H_5$
c: $R = COOC_2H_5$

Chart 1

In order to establish suitable conditions for the biotransformation of 5*H*-*N*-acyldibenz[*b*,*f*]azepines to their 10,11-epoxides, **1b** was incubated under various experimental conditions. The results are summarized in Table II. When **1b** was incubated with the complete system, the biotransformation ratio of **1b** to **2b** was 18%, and unchanged **1b** amounted to about 80%. When **1b** was incubated with microsomes which had been inactivated by heating, or incubated in the absence of the NADPH-generating system, very little formation of **2b** was observed. Thus, the presence of the complete microsomal system is necessary. The formation of **2b** was not observed when the substrate was incubated with cofactors alone, indicating that

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Table II. Incubation of 5*H*-*N*-Benzoyldibenz[*b*,*f*]azepine (**1b**) with Rat Liver Microsomal Fractions under Various Experimental Conditions

Experimental conditions	Epoxide (**2b**) formation (%)
Microsomes + cofactors (complete system)	100
Boiled microsomes[a] + cofactors	3
Microsomes + cofactors − NADPH generating system	3
Cofactors only	0
CO treated microsomes[b] + cofactors	21

a) Boiled microsomes were prepared by heating microsomal suspensions at 80 °C for 5 min. *b*) Microsomal suspension was treated with CO gas for 10 min before incubation.

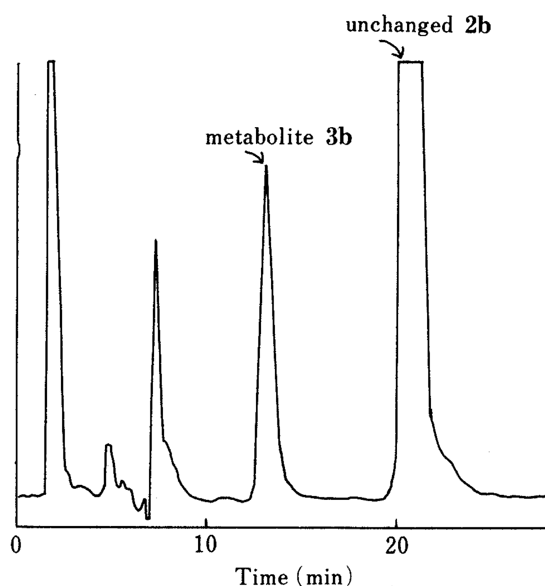

Fig. 4. HPLC of the Ethyl Acetate Extract of 5*H*-*N*-Benzoyldibenz[*b*,*f*]azepine 10,11-Oxide (**2b**) Incubated with Rat Liver Microsomal Fractions

Column, Lichrosorb RP-18 (5 μm) (150 mm × 4.3 mm); mobile phase, 50% methanol in water (v/v); flow rate, 2.0 ml/min.

Table III. Incubation of 5*H*-*N*-Benzoyldibenz[*b*,*f*]azepine 10,11-Oxide (**2b**) with Rat Liver Microsomal Fractions under Various Experimental Conditions

Experimental conditions	Dihydrodiol (**3b**) formation (%)
Microsomes + cofactors (complete system)	100
Boiled microsomes[a] + cofactors	23
Microsomes + cofactors − NADPH generating system	75
Cofactors only	0
Microsomes + cofactors + phenanthrene 9,10-oxide (10 mM)	5—20

a) Boiled microsomes were prepared by heating microsomal suspensions at 80 °C for 5 min.

the formation of **2b** was not a chemical artifact. Moreover, when **1b** was incubated with microsomes which had been treated with carbon monoxide, this biotransformation was depressed by about 80% compared with that in the complete system. These phenomena implicate cytochrome P-450 in this biological epoxidation.

As shown in Fig. 2b, another minor metabolite (**3b**) was detected. This metabolite was determined to be the *trans*-10,11-dihydrodiol by a comparison of the retention time (13.5 min) with that of an authentic sample. The formation of the *cis*-dihydrodiol isomer (**4b**; retention time, 9.0 min) was not detected in this experiment. As **3b** was also detected when **2b** was incubated with rat liver microsomal fractions (Fig. 4), the biotransformation of the epoxide to the dihydrodiol was investigated under various incubation conditions to clarify the possible

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involvement of epoxide hydratase. These results were summarized in Table III.

When **2b** was incubated with the complete system, **3b** was detected in about 8% conversion ratio together with unchanged **2b**. When **2b** was incubated with microsomes which were inactivated by heating, diol formation was depressed. These results proved that the presence of the complete microsomal system was necessary. In the absence of the NADPH-generating system, the diol formation was not greatly depressed, whereas addition of phenathrene 9,10-oxide (an epoxide hydratase inhibitor) to the complete system drastically depressed the diol formation. These results suggest that the epoxide is transformed to the *trans*-dihydrodiol by microsomal epoxide hydratase.

Identification of Microsomal Metabolites of 5*H*-*N*-Alkyldibenz[*b*,*f*]azepines (1d and 1e)

Analysis of the ethyl acetate extract of the incubation mixture of 5*H*-*N*-propyldibenz[*b*,*f*]azepine (**1d**) by HPLC showed two products (metabolites **4d** and **5d**), in addition to the unchanged compound. The metabolite **4d** (Fig. 5a) was identical with *N*-dealkylated 5*H*-dibenz[*b*,*f*]azepine (**4d**) based on the retention time in HPLC as compared with that of an authentic sample. The other peak (metabolite **5d**) in Fig. 5b is a minor metabolite; the retention time (5.0 min) of **5d** was identical with that of authentic *N*-propyl-9(10*H*)-acridone (**5d**).

In order to confirm the biotransformation of 5*H*-*N*-propyldibenz[*b*,*f*]azepine (**1d**) to *N*-propyl-9(10*H*)-acridone (**5d**), GC-MF was utilized. By the use of the peaks at *m*/*e* 237 (M^+) and 208 ($M^+ - 19$), *N*-propyl-9(10*H*)-acridone (**5d**) was detected at the retention time of 2.6 min, corresponding to that of authentic **5d**.

In the same manner, *N*-benzyl-9(10*H*)-acridone (**5e**) was detected as a metabolite of 5*H*-*N*-benzyldibenz[*b*,*f*]azepine (**1e**) by HPLC as shown in Fig. 6. By the use of the peak at *m*/*e* 285 (M^+) in GC-MF, **5e** was detected at the retention time of 4.6 min.

Though the conversion ratios from **1d** and **1e** to **5d** and **5e** are only 0.24% and 0.12%, respectively, these data suggest that the biotransformation of 5*H*-*N*-alkyldibenz[*b*,*f*]azepines

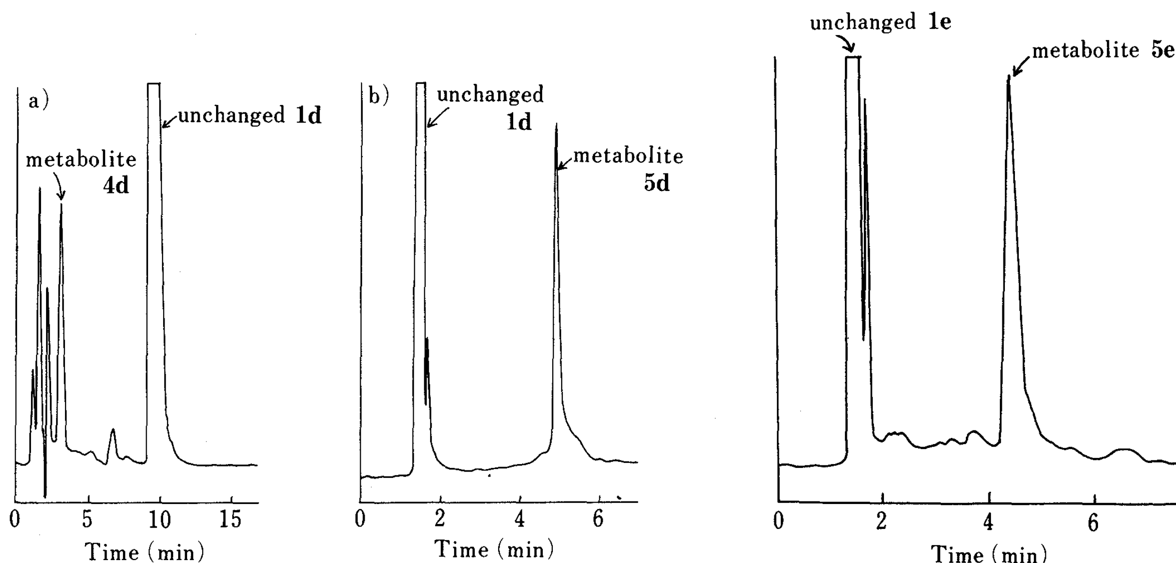

Fig. 5. HPLC of the Ethyl Acetate Extract of 5*H*-*N*-Propyldibenz[*b*,*f*]azepine (**1d**) Incubated with Rat Liver Microsomal Fractions

a) Column, Lichrosorb RP-18 (5 μm) (150 mm × 4.3 mm); mobile phase, methanol–water–aq. ammonia (80 : 2 : 1); flow rate, 2.0 ml/min.
b) Column, Wakogel LC-10H (300 mm × 4.3 mm); mobile phase, 33% methylene chloride in *n*-hexane (v/v); flow rate, 3.0 ml/min.

Fig. 6. HPLC of the Ethyl Acetate Extract of 5*H*-*N*-Benzyldibenz[*b*,*f*]azepine (**1e**) Incubated with Rat Liver Microsomal Fractions

Column, Wakogel LC-10H (300 mm × 4.3 mm); mobile phase, 33% methylene chloride in *n*-hexane (v/v); flow rate, 3.0 ml/min.

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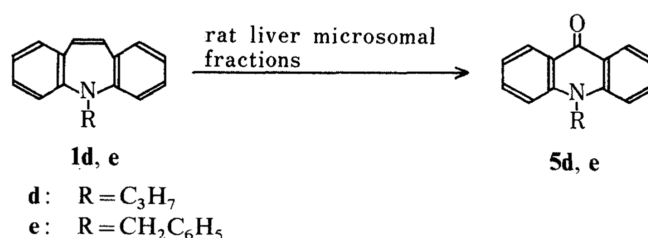

Chart 2

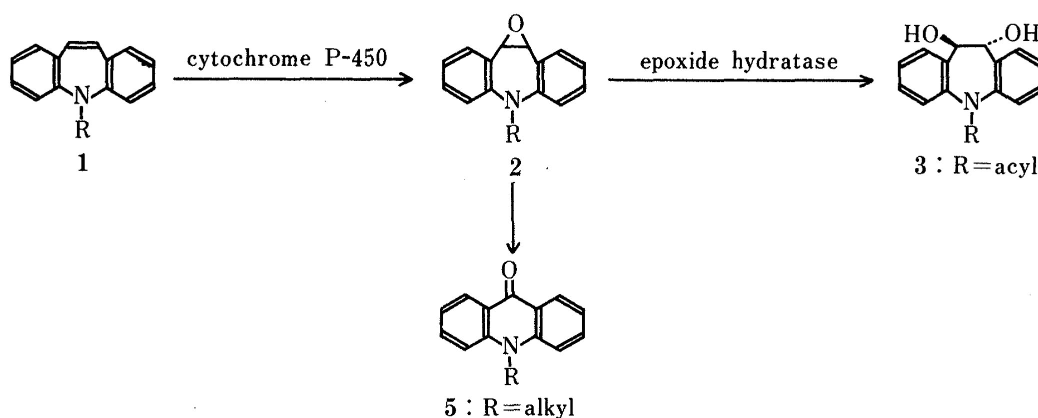

Chart 3

to N-alkyl-9(10H)-acridones is a common metabolic pathway for the metabolism of 5H-dibenz[b,f]azepines which have N-alkyl groups (Chart 2).

In the light of our previous results[1] that the unstable 10,11-epoxides were obtained by the chemical oxidation of 5H-N-alkyl-10,11-disubstituted-dibenz[b,f]azepines with m-CPBA, and that their azepine rings were readily contracted to afford acridone derivatives under mild conditions, the following pathways are proposed for the biological oxidation of 5H-dibenz[b,f]azepines (Chart 3). 1) Both 5H-N-alkyl- and 5H-N-acyldibenz[b,f]azepines (**1**) are equally oxidized to 10,11-epoxides (**2**) by microsomal cytochrome P-450. 2) While the metabolically formed N-acylepoxides are stable and are hydrated to *trans*-dihydrodiols (**3**) by microsomal epoxide hydratase, N-alkylepoxides, not so far detected as metabolites, are immediately converted to 9(10H)-acridone derivatives (**5**) because of the instability of the oxirane ring.

As regards the metabolism of drugs having the dibenz[b,f]azepine skeleton, the epoxide has been reported as a metabolite of carbamazepine,[4] while acridone derivatives have not been reported yet as metabolites of dibenz[b,f]azepine derivatives having N-alkyl substituent groups. We found that different metabolites were obtained, depending on the nature of the N-substituent groups. These biological findings are consistent with the results of chemical studies.[1] It is particularly interesting that N-alkyl-9(10H)-acridones, presumably formed *via* epoxides, were found. The mechanism of formation of N-alkyl-9(10H)-acridones and the pharmacological significance[8] of the different metabolic fates of 5H-dibenz[b,f]azepines are now under investigation.

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