significant antiestrogenic activity *in vivo*. Thus, the *cis*-configuration and *gem*-dichloro substitution appear to be important for antiestrogenic activity in the cyclopropyl series since neither the *trans*-isomer (I) nor the reduced compound (IX) was antiestrogenic in this study.

An examination of receptor binding activity in Fig. 3 and Table I indicates that various structural modifications significantly alter estrogen binding activity. The structure–activity relationships revealed by this series of cyclopropyl analogs are:

1. Replacement of hydroxyl groups with *O*-methyl groups decreases receptor binding activity (compare III with VIII and IV with VII). The importance of free hydroxyl groups for the production of estrogenic activity was demonstrated with other stilbenediol derivatives (16, 17).
2. The absence of *trans*-ethyl groups decreases receptor binding activity (compare VI with VII).
3. The *trans*-configuration produces a slightly greater binding affinity than the *cis*-configuration (compare I with II and IX with X).
4. The *gem*-dichloro substitution generally increases receptor binding activity (compare I with X, IV with VIII, and II with IX). However, the *gem*-dichloro substitution together with *p*-methoxy substitution decreases binding affinity (compare III with VII), perhaps due to a steric interaction of these bulky groups that interfere with access to the receptor site.

Receptor binding activity paralleled *in vivo* estrogenic activity in this series of compounds, except VIII, which displayed the most potent estrogenic activity *in vivo* and the second greatest receptor affinity *in vitro*. This result may be explained by the fact that receptor binding and the initiation of an estrogenic response are two separate events and have been dissociated in the *in vitro* receptor binding assay due to the isolation of the receptor complex. Korenman (13) demonstrated previously that the relative binding affinity of steroidal estrogens paralleled uterotropic activity.

It is difficult to make structure–activity conclusions based on this limited series of analogs. Other members of the cyclopropyl series are being synthesized and evaluated for biological activity. However, the information obtained from this study will guide the design of other members of this series. It appears from the results that the *gem*-dichloro analogs with *cis*-phenyl rings hold the greatest promise for antiestrogenic activity.

This study also indicates that the cyclopropyl analogs of stilbene and stilbenediol may become useful in the treatment of estrogen-dependent tumors and as potential antifertility agents. Antifertility agents with fewer side effects and more effective antineoplastic therapy for breast and uterine cancer clearly are needed. However, synthesis of the complete series of cyclopropyl analogs and thorough evaluation of these compounds will be required. Thus, the design, development, and biological evaluation of these potential therapeutic agents are in progress.

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First-Pass Metabolism of Ethinyl Estradiol in Dogs and Rats

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Abstract □ The contraceptive steroid ethinyl estradiol was extensively metabolized when given orally in solution to dogs. It was thought at first that metabolism occurred exclusively in the liver. However, use of standard equations to predict the oral bioavailability of drugs known to be metabolized by hepatic first pass resulted in significantly higher values than those obtained experimentally. To rationalize the data and to determine whether ethinyl estradiol also is metabolized in the gut wall during absorption, metabolism in rats was studied. The drug was administered in solution intraduodenally, intraportally, and intravenously as a bolus and by first-order infusion. The results indicate that, in rats, 40% of the drug is metabolized by the gut wall and 79% of the drug in the portal blood is metabolized by the liver after intraduodenal administration.

Keyphrases □ Ethinyl estradiol—first-pass metabolism in dogs and rats, gut wall metabolism in rats □ Bioavailability—ethinyl estradiol, first-pass metabolism in rats and dogs, gut wall metabolism in rats □ Metabolism—ethinyl estradiol in rats and dogs, first-pass metabolism and possible gut wall metabolism

The natural contraceptive steroids such as progesterone and estradiol are not effective orally due to their extensive metabolism in the GI tract during absorption and to hepatic first-pass metabolism (1, 2). Although introduction of the ethinyl group at the 17α-position renders the compound stable toward metabolic attack by 17α-hydroxylase, ethinyl estradiol, like estradiol, contains a phenolic hydroxyl group at the 3-position that is subject to sulfate and

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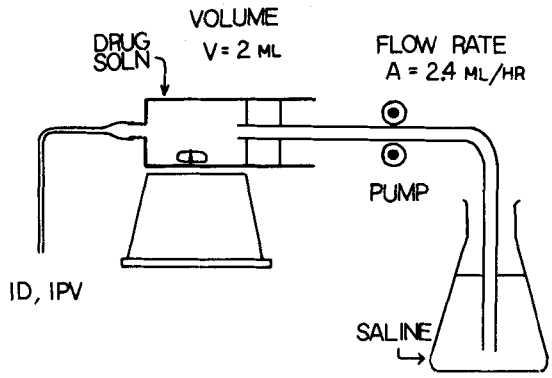

Figure 1—*Apparatus used to administer first-order infusion intraportally and intraduodenally.*

glucuronide conjugation in both humans and animals (3, 4).

In this study, the extent of the GI and hepatic first-pass metabolism of ethinyl estradiol in dogs and rats was investigated.

EXPERIMENTAL

Materials—Radiolabeled ethinyl [6,7-^{3}H(N)]estradiol[1] (57.2 Ci/mmole) and nonradiolabeled ethinyl estradiol[2] were used as supplied. All other solvents and reagents were analytical reagent grade.

Dog Experiment—Three female beagle dogs, ~10 kg, were used. Prior to drug administration, the dogs were fasted overnight with water *ad libitum*. Then, 35 μg of ethinyl estradiol/dog (100 μCi/dog) was administered on separate occasions as an intravenous solution (dissolved in 1 ml of 5% ethanol–saline) and an oral solution (dissolved in 5 ml of 1% ethanol–water). The oral administration was followed immediately with 45 ml of water.

After administration, 3 ml of blood was withdrawn from the cubital vein periodically. After centrifugation, 0.1 ml of plasma was analyzed for total radioactivity, and 0.5 ml of plasma was analyzed for unchanged ethinyl estradiol according to the method described later. Urine was collected for up to 48 hr after administration, and 0.1-ml urine samples were analyzed for total radioactivity and unchanged steroid.

Rat Experiment—Male Sprague–Dawley rats, ~270 g, were anesthetized with pentobarbital sodium (50 mg/kg). For intraduodenal and intraportal administrations, the abdomen was opened through a midline incision. A polyethylene tube[3] was attached to one end of the shank of a 23-gauge needle[4], the other end of which was inserted directly into the duodenum or the hepatic portal vein and fixed with an adhesive agent[5]. The tube was extended to the exterior of the animal prior to closure of the abdomen with sutures. To collect periodic blood samples, the femoral artery was cannulated with a polyethylene tube (PE-50) attached to a syringe filled with heparinized saline (100 units/ml).

For bolus administration, 3.5 μg of ethinyl estradiol/kg was administered in 0.1 ml of 5% ethanol–saline into the femoral vein, the hepatic portal vein, or the duodenum. First-order infusions of the drug (3.5 μg/kg), simulating oral absorption, were given *via* the hepatic portal vein and the duodenum at a rate constant of 1.2 hr^{-1} ($t_{1/2}$ = 0.58 hr) over 4 hr (Fig. 1) according to the method of Ronfeld and Benet (5). After drug administration, 0.2-ml samples of blood were removed periodically from the femoral artery and analyzed for unchanged steroid.

Assays for Total Radioactivity and Unchanged Ethinyl Estradiol—The total radioactive steroid in the plasma and urine was measured by diluting the samples directly with 10 ml of a commercial scintillant[6] and counting the radioactivity using a liquid scintillation spectrometer[7].

Unchanged ethinyl estradiol in plasma and urine was determined as follows. One milliliter of 0.01 *N* HCl was added to the sample, and the mixture was extracted with 6 ml of benzene–heptane (2:3 v/v). Five milliliters of the organic phase was transferred to the scintillation vial and evaporated to dryness with a nitrogen stream. The radioactivity was determined according to the procedure for the total radioactive steroid.

To confirm that only unchanged steroid was measured, the benzene–heptane extracts were concentrated by evaporation with a nitrogen stream and spotted on TLC silica gel HF precoated plates[8]. After development in three solvent systems (benzene–ethanol, 9:1 v/v; benzene–ethyl acetate, 4:1 v/v; and cyclohexane–acetone, 4:1 v/v), the plates were air dried and divided into 1-cm sections. Each section was scraped into 10 ml of scintillation cocktail and counted.

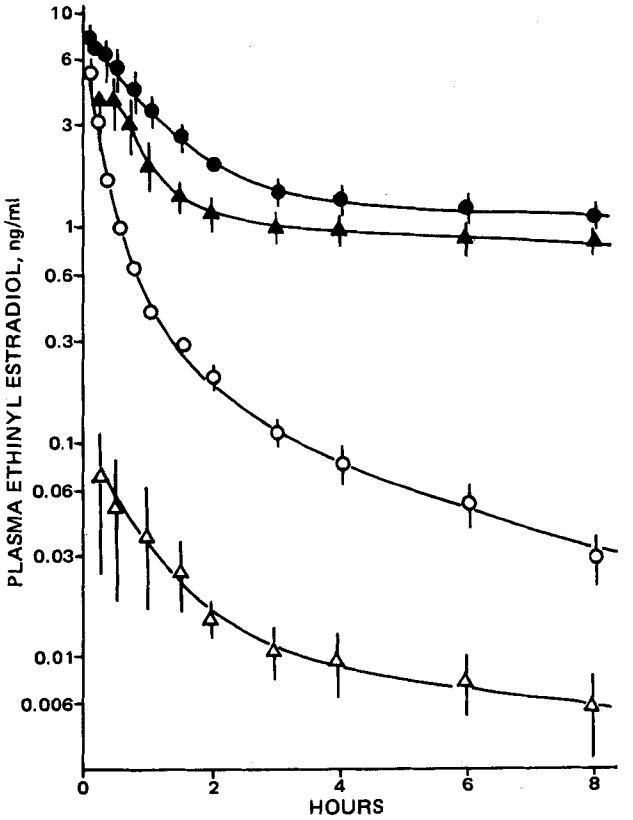

Figure 2—*Mean plasma levels of total activity and unchanged ethinyl estradiol for intravenous and oral solutions. Key:* ● *and* ▲, *total activity of intravenous and oral solutions, respectively; and* ○ *and* △, *unchanged ethinyl estradiol as intravenous and oral solutions, respectively.*

RESULTS AND DISCUSSION

Plasma samples extracted with benzene–heptane after intravenous and oral administration of ^{3}H-labeled ethinyl estradiol to rats and dogs resulted in a single spot corresponding to that of ethinyl estradiol in all TLC systems used. This finding indicates that benzene–heptane extracts only the unchanged fraction of ethinyl estradiol.

Plasma levels, as a function of time, of the total radioactivity and unchanged ethinyl estradiol after intravenous and oral administration of ethinyl estradiol solution to dogs is shown in Fig. 2. The plasma level of unchanged steroid following oral administration was significantly lower than that following intravenous administration, although the plasma level of total radioactivity following either administration was nearly the same. These data strongly indicate that ethinyl estradiol, given orally, is metabolized extensively during or after absorption in dogs. The half-lives of the initial and terminal phases of the plasma level curve of unchanged steroid after the intravenous administration were graphically estimated to be about 15 and 180 min, respectively.

Table I shows the urinary recovery of total radioactivity and unchanged ethinyl estradiol after intravenous and oral administration to dogs. The percent urinary recovery of total radioactivity and unchanged steroid after both routes of administration were almost the same. These urinary recovery data and the plasma total radioactivity data shown in Fig. 2

[1] New England Nuclear, Boston, Mass.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] Clay Adams, Parsippany, N.J.
[4] Jelco Laboratories, Raritan, N.J.
[5] Super Glue-3, Woodhill Permatex, Cleveland, Ohio.
[6] Insta-Gel, Packard Instrument Co., Downers Grove, Ill.
[7] Model 3255 Tri-Carb, Packard Instrument Co., Downers Grove, Ill.
[8] Analtech Inc., Newark, Del.

Table I—Urinary Recovery (Percent of Dose) of Total Radioactivity and Unchanged Ethinyl Estradiol after Intravenous and Oral Adminstration in Three Beagle Dogs [a]

Dog	Intravenous		Oral	
	Total	Unchanged	Total	Unchanged
IJ96	18.5	0.54	21.2	0.32
NJ06	22.3	0.38	17.6	0.21
JO96	24.0	0.63	22.0	0.40
Mean	21.6	0.51	20.2	0.31
±*SE*	1.6	0.07	1.3	0.05

[a] The urine collection period was 0–48 hr, and the dose was 35 μg/dog (100 μCi/dog).

suggest that the drug is completely absorbed from the GI tract. Based on the recovery of radioactivity, ethinyl estradiol was shown by Reed *et al.* (6) to be rapidly and completely absorbed from the GI tract.

For a drug that is completely absorbed and eliminated exclusively by hepatic metabolism, oral bioavailability can be predicted using the equation derived by Rowland (7):

$$\theta = 1 - \frac{f_m D_{iv}}{V_{bl}[H/K_p + (1 - H)]AUC_{iv}} \quad \text{(Eq. 1)}$$

where f_m is the fraction of the dose metabolized in the liver on intravenous administration, V_{bl} is the liver blood flow, H is the hematocrit, K_p is the apparent partition coefficient of the drug between plasma and erythrocytes, D_{iv} is the dose, and AUC_{iv} is the area under the curve for intravenous administration. This equation has been applied with reasonable success to predict the oral bioavailability of propranolol (8) and nortriptyline (9) in humans.

The fraction of unchanged steroid in the urine after intravenous administration was very small (0.5% of the dose). Therefore, the renal clearance was almost negligible. In one study in dogs, the drug was completely absorbed after oral administration. If it is assumed that ethinyl estradiol is metabolized exclusively by the liver, then $f_m = 1$, and Eq. 1 can be used to predict the bioavailability of the drug after oral administration (7). Since H is known to be 0.4 and K_p (for ethinyl estradiol) was determined experimentally in these laboratories to be >500, H/K_p is almost zero. By using the value of V_{bl} in dogs of 51 ml/min/kg (10), the predicted oral bioavailability was calculated and compared to the experimental values.

Table II shows the area under the curve for the unchanged drug after intravenous and oral administration of ethinyl estradiol to dogs and the comparison of the experimentally determined and predicted values. The observed availability (0.075) was much lower than the predicted value (0.222). Such a difference could not be attributed to incomplete absorption of ethinyl estradiol from the GI tract since the total plasma radioactivity and urinary recovery data from the two routes of administration were almost the same. Since the predicted availability was calculated based on the assumption that drug metabolism occurred only in the liver, the differences between the predicted and observed availability after oral administration may be due to metabolism of the drug on its passage through the gut wall, which Eq. 1 does not take into account.

To estimate the extent of gut wall and liver metabolism of ethinyl estradiol, studies were undertaken with rats. Solutions of the drug were administered as an intraportal, intraduodenal, or intravenous bolus. To simulate oral absorption, the drug was also administered intraportally and intraduodenally by first-order infusion.

Figures 3 and 4 show the mean blood levels of unchanged ethinyl estradiol after bolus and first-order infusion, respectively. The drug blood levels after intraduodenal administration were significantly lower than those after intraportal or intravenous administration.

The bioavailability following intraportal and intraduodenal administration can be compared to intravenous administration using the following relationships (11, 12):

$$\frac{AUC_{ipv}}{AUC_{iv}} = 1 - F_l \quad \text{(Eq. 2)}$$

$$\frac{AUC_{id}}{AUC_{iv}} = (1 - F_l)(1 - F_g) \quad \text{(Eq. 3)}$$

where AUC_{ipv}, AUC_{id}, and AUC_{iv} are the areas under the blood level curves when the drug is given intraportally, intraduodenally, and intravenously, respectively, and F_g and F_l are the fractions of the drug metabolized in the gut wall and the liver, respectively.

Table II—Area under Curve Values after Intravenous and Oral Administration of Ethinyl Estradiol and Oral Bioavailability in Three Beagle Dogs [a]

Dog	*AUC*, (ng hr)/ml		Bioavailability	
	Intravenous	Oral	Observed [b]	Predicted [c]
IJ96	2.554	0.310	0.121	0.253
NJ06	2.594	0.106	0.041	0.183
JO96	2.254	0.147	0.065	0.231
Mean	2.467	0.188	0.075	0.222
±*SE*	0.107	0.063	0.024	0.021

[a] The dose was 35 μg/dog (100 μCi/dog). [b] Observed bioavailability was obtained from AUC_{po}/AUC_{iv}. [c] Predicted bioavailability calculated from Eq. 1.

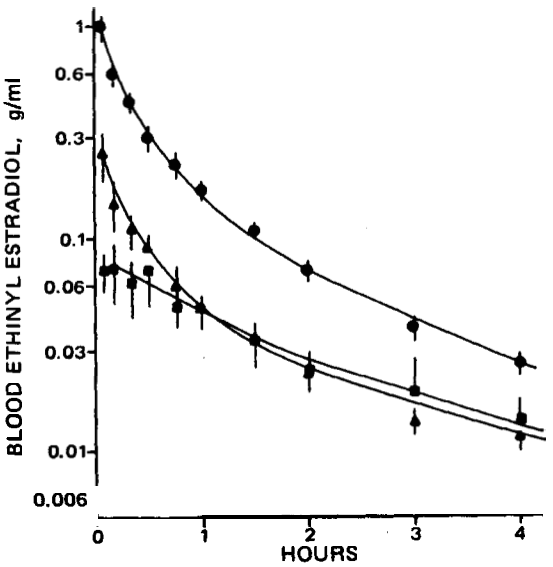

Figure 3—*Mean blood levels of unchanged ethinyl estradiol after bolus intravenous (●), bolus intraportal (▲), and bolus intraduodenal (■) administration of 3.5 μg/kg in rats.*

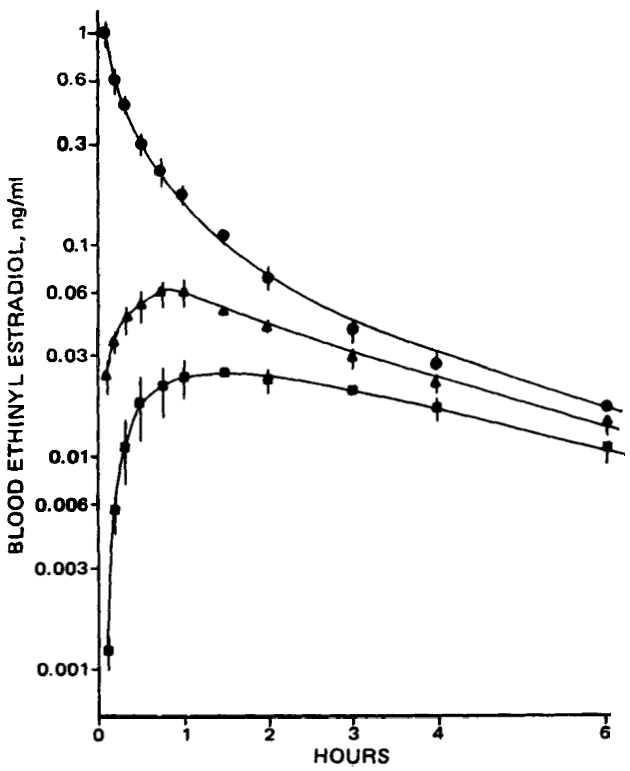

Figure 4—*Mean blood levels of unchanged ethinyl estradiol after bolus intravenous (●), first-order intraportal (▲), and first-order intraduodenal (■) administration of 3.5 μg/kg in rats.*

Table III—AUC, F_g, and F_l Values Obtained from Intraduodenal, Intraportal, and Intravenous Administration in Rats [a]

Route	AUC_{id}	AUC_{ipv}	AUC_{iv}	Intraduodenal/Intravenous	Intraportal/Intravenous	F_g	F_l
First-order infusion [b]	9.0 ± 1.2	15.0 ± 1.1	–	0.129	0.215	0.399	0.785
Bolus	10.8 ± 2.0	13.0 ± 2.9	69.6 ± 6.4	0.155	0.186	0.169	0.814

[a] The ethinyl estradiol dose was 3.5 μg/kg (100 μCi/kg), and the AUC values are expressed in nanogram minutes per milliliter from zero time to infinity and are the mean ± SE (n = 3). [b] K = 1.2 hr^{-1}, $t_{1/2}$ = 0.58 hr.

From Eq. 2:

$$F_l = 1 - \frac{AUC_{ipv}}{AUC_{iv}} \quad \text{(Eq. 4)}$$

Substitution of Eqs. 2 and 4 into Eq. 3 yields:

$$F_g = 1 - \frac{AUC_{id}AUC_{iv}}{AUC_{iv}AUC_{ipv}} = 1 - \frac{AUC_{id}}{AUC_{ipv}} \quad \text{(Eq. 5)}$$

Table III shows the AUC, F_g, and F_l values obtained from intraduodenal, intraportal, and intravenous administration of ethinyl estradiol in rats at a dose of 3.5 μg/kg. The results indicate that 40% of the drug was metabolized by the gut wall (F_g) and that 79% of the drug in the portal blood was metabolized by the liver (F_l) before reaching the systemic circulation after first-order infusion. The F_l value after bolus administration was almost the same as that after first-order infusion, whereas the F_g value was significantly different. The difference could be due to the saturation of the metabolizing enzyme in the gut wall after bolus intraduodenal administration.

The results of this study indicate that, in the rat, the oral contraceptive steroid, ethinyl estradiol, is extensively metabolized in both the gut wall and the liver. In view of this finding, differences between the predicted and observed availability after oral administration of the drug to dogs also may be due to metabolism of the drug in the gut wall.

To compare the F_g and the F_l values in rats with those in dogs, the F_g and F_l values in dogs were estimated by:

$$F_l = 1 - \text{(predicted bioavailability)} \quad \text{(Eq. 6)}$$

$$F_g = 1 - \frac{\text{(observed bioavailability)}}{\text{(predicted bioavailability)}} \quad \text{(Eq. 7)}$$

The F_l value in dogs was 0.78, which was close to that found in rats (0.78–0.81). The F_g value in dogs was 0.66, which was significantly greater than that found in rats (0.17 from a bolus administration and 0.40 from a first-order infusion).

The difference in the F_g value between the two animal species may be due to factors such as stomach emptying, rate of absorption, and the amount of enzymes in the GI tract.

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Terpenoids Biotransformation in Mammals III: Biotransformation of α-Pinene, β-Pinene, Pinane, 3-Carene, Carane, Myrcene, and *p*-Cymene in Rabbits

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Received December 24, 1979, from the **Hiroshima Institute of Technology, Itsukaichi, Hiroshima 738, Japan*, the ‡*Institute of Pharmacognosy, Tokushima-Bunri University, Tokushima 770, Japan*, and the §*Faculty of Integrated Arts and Sciences, Hiroshima University, Hiroshima 730, Japan.* Accepted for publication September 25, 1980.

Abstract □ The biotransformation of (+)-, (−)-, and (±)-α-pinenes, (−)-β-pinene (nopinene), (−)-*cis*-pinane, (+)-3-carene, (−)-*cis*-carane, myrcene, and *p*-cymene in rabbits was investigated. The major metabolites were as follows: (−)-*trans*-verbenol from (+)-, (−)-, and (±)-α-pinenes; (−)-10-pinanol and (−)-1-*p*-menthene-7,8-diol from (−)-β-pinene; (−)-α-terpineol and (−)-*trans*-sobrerol from (−)-*cis*-pinane; (−)-*m*-mentha-4,6-dien-8-ol, 3-caren-9-ol, (−)-3-carene-9-carboxylic acid, and 3-carene-9,10-dicarboxylic acid from (+)-3-carene; carane-9,10-dicarboxylic acid from (−)-*cis*-carane; and myrcene-3(10)-glycol, myrcene-1,2-glycol, uroterpenol, and *p*-cymene-9-carboxylic acid from *p*-cymene. These metabolisms include allylic oxidation, epoxidation, stereoselective *gem*-dimethyl hydroxylation and its oxidation, cleavage of a conjugated double bond by epoxidation, and regioselective oxidation, some of which are not found usually in chemical reactions, and due to which various new compounds were determined. This biotransformation of the monoterpene hydrocarbons gave some insect pheromones in high yield.

Keyphrases □ Biotransformation—neutral and acidic metabolites of α-pinene, β-pinene, pinane, 3-carene, carane, myrcene, and *p*-cymene in rabbits □ Metabolites, neutral and acidic—biotransformation of α-pinene, β-pinene, pinane, 3-carene, carane, myrcene, and *p*-cymene in rabbits □ Terpenoids—α-pinene, β-pinene, pinane, 3-carene, carane, myrcene, and *p*-cymene, neutral and acidic metabolites, biotransformation in rabbits

In many countries, plants with mono-, di-, or sesquiterpenoids are used as folk medicine. Medicinal plants with essential oils can be found in many pharmacopeias. The pharmaceutical activities of mono- and sesquiterpenoids were reviewed (1), and it was reported that turpentine oil containing α-pinene, β-pinene, 3-carene, and myrcene is

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