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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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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Table I—Orally Administered Monoterpene Hydrocarbons and Yields of Metabolites [a]

Compound	Administered Dose, g	Metabolite, g	
		Neutral	Acidic as Methyl Ester
(+)-α-Pinene	10.0 (560)[b]	0.318	—[c]
(−)-α-Pinene	8.0 (440)	1.423	3.0
(±)-α-Pinene	10.0 (560)	1.199	—[c]
(−)-β-Pinene	12.0 (670)	0.753	1.0
(−)-*cis*-Pinane	18.0 (670)	1.040	0.35
(+)-3-Carene	26.0 (670)	2.159	2.6
(−)-*cis*-Carane	12.0 (670)	1.014	2.0
Myrcene	19.0 (670)	4.00	0.8
p-Cymene	2.0 (670)[d]	0.20	0.20

[a] Six male rabbits were administered one dose (<12 g/six rabbits) or repeated doses (>12 g/six rabbits). [b] The values in parentheses mean the maximum dose in milligrams per kilogram in each case. [c] Not measured. [d] Only one rabbit was used.

irritating to the skin, is used as a rubefacient and a liniment, and has choleretic activity. Other monoterpenoids have expectorant (1,8-cineol, bornyl acetate, and phellandrenes) or diuretic (diosphenol and terpinen-4-ol in juniper oil) properties. In addition, some sesquiterpenoids show anticancer, analeptic, antibiotic, and anthelmintic properties.

Terpenoid biotransformation in mammals was studied on camphor (2, 3), cedrol (4, 5), patchoulol (6), limonene (7, 8), α-pinene (9), β-pinene (9), and 1,8-cineol (10, 11). These biotransformation products gave enzymatically specific metabolites, that is, regioselective or stereoselective hydroxylated, carboxylated, or reduced metabolites. It is expected that these metabolites become the starting materials in organic synthesis. Moreover, biotransformation of terpenoids is expected to give a new method for optical separation of racemic isomers or to give biologically active substances such as pheromones, cosmetic fragrances, and food additives. It is also interesting to compare the metabolism of terpenoids in mammals with that in microorganisms with regard to enzymatic systems.

During the investigation of structure–activity relationships among metabolites and administered terpenoids in mammals (12, 13), the metabolites of naturally occurring monoterpene hydrocarbons, (+)-, (−)-, and (±)-α-pinenes, (−)-β-pinene, (+)-3-carene, myrcene, and *p*-cymene were studied. Two saturated monoterpene hydrocarbons, (−)-*cis*-pinane and (−)-*cis*-carane, were also studied for comparison.

EXPERIMENTAL[1]

Administered Monoterpene Hydrocarbons—The administered monoterpene hydrocarbons are listed in Table I.

[1] A Shimadzu GC-6A gas chromatograph equipped with both a hydrogen flame-ionization detector and a thermal-conductivity detector was used. For the detection of neutral metabolites, the following conditions were used unless stated otherwise: a 0.26-mm × 2-m glass column packed with 5% OV-1 on Diasolid L (60–80 mesh) or 5% Thermon 1000 on Chromosorb W (80–100 mesh, AW-DMCS), an injection temperature of 250°, a column temperature of 100–250°, a program rate of 10°/min, a detector temperature of 250°, and nitrogen gas at 40 ml/min. For preparative purposes, the same size glass column of 5% Thermon 1000 was used under the same conditions. GLC–mass spectrometric analyses were performed at 70 ev using a 2-mm × 3-m column packed with OV-1 or OV-17 (5%) with temperature programmed at 50–250° at 5°/min and helium at 30 ml/min on a Shimadzu LKB 9000B. TLC plates of silica gel 60 (0.25 cm) F-254 were used, and the spots were observed by UV light (254 nm), 50% H_2SO_4 or iodine vapor. The ^{1}H-NMR spectra were recorded with Hitachi R-22 (90 MHz) and Varian T-60 (60 MHz) spectrometers. The ^{13}C-NMR spectra were conducted on a Hitachi R-42 FT spectrometer. IR spectra were obtained with a Hitachi 215 spectrophotometer, and UV spectra were recorded on a Shimadzu US 200S spectrophotometer. The solvents used for spectral determinations were tetramethylsilane–deuterochloroform (^{1}H-NMR of 90 and 60 MHz and ^{13}C-NMR of 90 MHz) and chloroform ([α]$_D$ and IR) unless stated otherwise.

Table II—Optical Rotations and Purities of Verbenols Obtained from Optically Active α-Pinenes

[α]$_D$ of Administered α-Pinenes	Verbenols as Metabolites	
	[α]$_D$	Purity[a], %
+22.0°	±0°	67.2
−30.9°	−31.2°	98.9
±0.0°	−11.3°	67.5

[a] The purities were obtained from the GLC of the neutral metabolites.

Animals and Dosing—Six male albino rabbits[2] (2–3 kg) were starved for 2 days before the experiment. Monoterpene hydrocarbons were suspended in water (100 ml) containing polysorbate 80[3] (0.1 g) and were homogenized well. This solution (20 ml) was administered to each rabbit through a stomach tube followed by water (20 ml). This dose of monoterpene corresponds to 400–700 mg/kg. The animals were housed in individual stainless steel metabolism cages and were allowed rabbit food[4] and water *ad libitum*. The urine was collected daily for 3 days after drug administration and stored at 0–5° until time of analysis.

Extraction and Fractionation of Urinary Metabolites—The urine was centrifuged to remove feces[5] and hairs at 0°, and the supernate was used for the experiments. The urine was adjusted to pH 4.7[6] with acetate buffer and incubated with β-glucuronidase–arylsulfatase (3 ml/1000 ml of the fresh urine) at 37° for 48 hr, followed by continuous ether extraction for 48 hr. The ether extracts were washed with 5% $NaHCO_3$ and 5% NaOH to remove the acidic and phenolic fractions, respectively, and dried (magnesium sulfate). Ether was evaporated under reduced pressure to give neutral metabolites. The yields are shown in Table I.

The neutral metabolites were chromatographed on a column containing 100 g of silicic acid (200 mesh). Elution was started with *n*-hexane, and *n*-hexane–ethyl acetate mixtures (95:5, 90:10, 85:15, 70:30, and 50:50) were used as subsequent eluents. The acidic metabolites were recovered from the sodium bicarbonate layer by acidification with 5% HCl, followed by ether extraction. The ether extracts were esterified with diazomethane in ether or with dimethyl sulfate in the presence of potassium carbonate in anhydrous acetone. These esters of the acidic metabolites also were chromatographed in the same manner as the neutral metabolites.

Determination and Identification of Urinary Metabolites—Purification by silicic acid gave pure metabolites. When necessary, metabolites were isolated by preparative TLC or GLC. Structure determination or identification was based on spectral data and chemical transformations.

RESULTS

Metabolites of (+)-, (−)-, and (±)-α-Pinenes—From each α-pinene, the same three metabolites were identified as follows.

Verbenol and Myrtenol—IR and ^{1}H-NMR spectra of the main urinary metabolites of (−)-α-pinene (I) agreed well with those of (−)-*trans*-verbenol (II), with a 98.9% purity from the GLC peak area of the neutral metabolite (15). All spectral data of the acetylation and oxidation products of this main metabolite also agreed with those of (−)-*trans*-verbenyl acetate and (−)-verbenone, respectively. The yields (purities by GLC) and the optical rotations of the verbenols obtained from (+)-, (−)-, and (±)-α-pinenes are listed in Table II. The minor urinary metabolites of each α-pinene were identified as myrtenol (III) by comparison of R_t (GLC) and R_f (TLC) values with those of an authentic sample prepared from (−)-α-pinene. The yields of III were 14.9, 0.9, and 9.3% by GLC from (+)-, (−)-, and (±)-α-pinenes, respectively.

Myrtenic Acid (IV) as Methyl Ester—As the acidic metabolite from (+)-, (−)-, and (±)-α-pinenes, methyl myrtenate (IV*a*)[7] was isolated in each case; mass spectrum: *m/z* (%) 180 (M^+, $C_{11}H_{16}O_2$, 6), 165 (16), 137 (75), 105 (base), and 93 (81); ^{1}H-NMR[8] (90 MHz): δ 6.80 (m, 1H), 3.71 (s, 3H), 1.33 (s, 3H), and 0.80 (s, 3H) (16).

Metabolites of (−)-β-Pinene—Four neutral and one acidic metabolites were isolated from (−)-β-pinene (V).

[2] Japanese White, Miyamoto Jikken Dobutsu, Hiroshima, Japan.
[3] Tween 80.
[4] Oriental RC-4.
[5] In this experiment, feces were not analyzed.
[6] The pH value for enzymatic hydrolysis by β-glucuronidase was variously reported. In this experiment, pH 4.7 was adopted following the method of LUU Bang *et al.* (4). More recently, pH 6.0 was recommended for the hydrolysis by β-glucuronidase (14).
[7] Compound IV*a* is the methyl ester of IV in Scheme I.
[8] m = multiplet, s = singlet, d = doublet, t = triplet, q = quartet, ss = sharp singlet, bs = broad singlet, and b = broad.

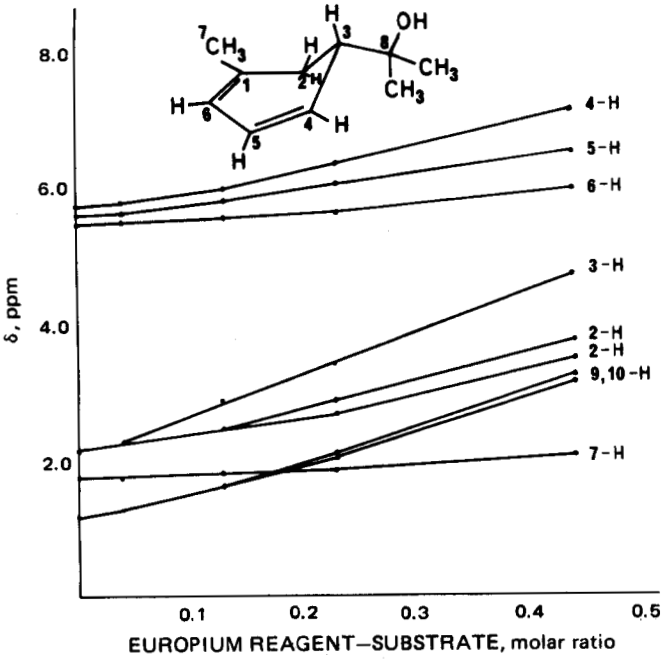


Figure 1—*Lanthanide-induced shifts with europium reagent in the* ^{1}*H-NMR spectrum of* m-*cymene-4,6-dien-8-ol (XXIII).*

(+)-trans-*Pinocarveol (VI) (11%)*[9]—$[\alpha]_D$ +28.2° (*c*, 1.42); mass spectrum: *m/z* (%) 134 ($M^+ - H_2O$, 37), 119 (38), 109 (38), 92 (base), 83 (77), 70 (69), 55 (76), and 41 (50); IR: ν 3580 and 3350 cm^{-1}. These data compared well with the reported values of (+)-*trans*-pinocarveol (17). All spectral data of the derived pinocarvone [$[\alpha]_D$ +28.6° (*c*, 0.35)] were coincident with literature values (18).

(−)-trans-*10-Pinanol (VII) (39%)*—$[\alpha]_D$ −21.4° (*c*, 1.68); mass spectrum: *m/z* (%) 154 (M^+, $C_{10}H_{18}O$, 9), 139 (base), 121 (40), 83 (85), 64 (69), and 55 (33); IR: ν 3400 cm^{-1}; ^{1}H-NMR: δ 3.47 (s, 2H), 1.23 (s, 3H), and 0.88 (s, 3H). These spectral data revealed this alcohol to be (−)-*trans*-10-pinanol (VII) (19, 20). Its acetate showed IR: ν 1720 cm^{-1}; ^{1}H-NMR: δ 4.10 (s, 2H), 2.12 (s, 3H), 1.26 (s, 3H), and 0.96 (s, 3H).

*(−)-1-*p-*Menthene-7,8-diol (VIII) (30%)*—$[\alpha]_D$ −33.3° (*c*, 0.83); mass spectrum: *m/z* (%) 152 ($M^+ - H_2O$, 21), 137 (6), 121 (19), 109 (32), 94 (26), 93 (27), 79 (base), 59 (88), and 43 (32); IR: ν 3650, 3450, 1365, 1230, 1035, and 910 cm^{-1}; ^{1}H-NMR: δ 5.67 (bs, 1H), 3.95 (2H, $w_{1/2}$ = 3 Hz), and 1.20 (s, 6H). The derived monoacetate showed $[\alpha]_D$ − 51.2° (*c*, 5.4); IR: ν 3500, 1720, 1240, 1020, and 920 cm^{-1}; ^{1}H-NMR: δ 5.75 (bs, 1H), 4.48 (s, 2H), 2.08 (s, 3H), and 1.20 (s, 6H). Furthermore, the derived aldehyde of IX showed $[\alpha]_D$ −65.4° (*c*, 0.46); ^{1}H-NMR: δ 11.10 (s, 1H), 6.75 (s, 1H), and 1.27 (s, 6H). The spectral data established this metabolite to be VIII, which was prepared by Sato (21).

(−)-α-Terpineol (IX) (5%)—$[\alpha]_D$ −51.0° (*c*, 1.57); mass spectrum: *m/z* (%) 154 (not appeared, M^+), 136 ($C_{10}H_{16}$, $M^+ - H_2O$, 30), 121 (33), 107 (5), 93 (50), 81 (36), 79 (17), 68 (19), 67 (20), and 59 (base); IR: ν 3400 cm^{-1}; ^{1}H-NMR: δ 5.43 (bs, 1H), 1.67 (bs, 3H), and 1.22 (s, 6H) (22).

As an acidic metabolite of (−)-β-pinene, myrtenic acid (IV) was identified by GLC–mass spectrometry as its methyl ester.

Metabolites of (−)-*cis*-Pinane—In (−)-*cis*-pinane (X), three neutral compounds were isolated as alcohols and four as derivatives. No acidic metabolites were identified from GLC–mass spectrometry.

(−)-α-Terpineol (IX) (43%)—$[\alpha]_D$ −46.3° (*c*, 1.77); mass spectrometry, IR and ^{1}H-NMR spectra, and $[\alpha]_D$ agreed well with those of (−)-α-terpineol biotransformed from (−)-β-pinene.

(−)-trans-*Sobrerol (XIV) (6%)*—$[\alpha]_D$ −68.8° (*c*, 0.8); mass spectrum: *m/z* (%) 152 ($M^+ - H_2O$, $C_{10}H_{16}O$, 7), 137 (11), 119 (18), 109 (base), 94 (69), and 79 (93); IR: ν 3300, 1460, 1440, 1380, 1175, 1150, 1030, and 905 cm^{-1}; ^{1}H-NMR (90 MHz): δ 5.27 (bs, 1H), 3.93 [m, 1H, C*H*(OH)], 1.66 (s, 3H), 1.27 (s, 3H), and 1.21 (s, 3H). The signal at δ 3.93 was replaced by deuterium oxide. These spectral data were easily assigned to XIV.

trans-*Carveol (XV)*—Mass spectrum: *m/z* (%) 152 (M^+, $C_{10}H_{16}O$, 1), 134 ($M^+ - H_2O$, 32), 119 (base), 105 (11), 91 (77), 77 (20), and 65 (12) (23).

In addition to IX, XIV, and XV, one fraction that was difficult to isolate was oxidized with Jones reagent. The preparative GLC of the oxidation products resulted in the isolation of four compounds, (−)-3-pinanone, (−)-4-pinanone, verbenone, and a lactonic compound, which were not found among the original metabolites.

(−)-3-Pinanone—$[\alpha]_D$ −5.42° (*c*, 2.02); mass spectrum: *m/z* (%) 152 (M^+, $C_{10}H_{16}O$, 19), 110 (18), 95 (48), 83 (96), 69 (base), and 55 (92); IR: ν 1705 cm^{-1}; ^{1}H-NMR: δ 1.32 (s, 3H), 1.21 (d, 3H, J = 7 Hz), and 0.89 (s, 3H) (24).

(−)-4-Pinanone—$[\alpha]_D$ −27.9° (*c*, 1.67); mass spectrum: *m/z* (%) 152 (M^+, $C_{10}H_{16}O$, 12), 137 (14), 109 (32), 95 (64), and 83 (base); IR: ν 1705 cm^{-1}; ^{1}H-NMR: δ 1.33 (s, 3H), 1.15 (d, 3H, J = 6.5 Hz), and 1.00 (s, 3H) (24).

Verbenone—Mass spectrum: *m/z* (%) 150 (M^+, 43); IR: ν 1685 and 1622 cm^{-1}; ^{1}H-NMR: δ 5.72 (m, 1H), 2.02 (m, 3H), 1.52 (s, 3H), and 1.03 (s, 3H) (15).

The three alcohols, 3-pinanol (XI), 4-pinanol (XII), and verbenol (XIII), were recognized as neutral metabolites from (−)-*cis*-pinane. The lactonic compound (XVI) gave the following spectra: mass spectrum: *m/z* (%) 184 (M^+, $C_{10}H_{16}O_3$, trace), 169 ($M^+ - CH_3$, 3), 166 ($M^+ - 18$, 19), 151 (11), 127 (11), 123 (8), 111 (27), 98 (35), 83 (11), 82 (15), 81 (7), 69 (9), 68 (10), 55 (17), and 43 (base); IR: ν 1765, 1720, 1380, and 1360 cm^{-1}; ^{1}H-NMR (90 MHz): δ 2.43 (q, 4H, J = 14 and 8 Hz, A_2B_2), 2.16 (4H), 1.46 (s, 3H), and 1.28 (s, 3H). These spectral data suggest the presence of a γ-lactone having a *gem*-dimethyl group. The comparison of these data with the authentic spectra of homoterpenyl methyl ketone (XVI) showed good agreement (25, 26). This lactone might be derived by Jones oxidation from α-terpineol or sobrerol.

Neutral Metabolites of (+)-3-Carene—In (+)-3-carene (XVII), two neutral compounds were isolated, one of which is a new compound. In addition, a third neutral metabolite was identified by comparison with an authentic sample as a front shoulder in the GLC of the main neutral metabolite.

(−)-m-*Mentha-4,6-dien-8-ol (XXIII) (71.8%)*—The IR spectrum of the main metabolite showed the same pattern in 1660 and 1598 cm^{-1} as occidentalol having a *cis*-conjugated diene in the six-membered ring. Other spectral data agreed with those of XXIII, $[\alpha]_D$ −116.1° (*c*, 1.08); mass spectrum: *m/z* (%) 152 (M^+, $C_{10}H_{16}O$, 1), 134 ($M^+ - H_2O$, 40), 119 (base), and 91 (71); IR (liquid film): ν 3400, 3050, 1720, 1660, and 1598 cm^{-1}; ^{1}H-NMR (90 MHz): δ 5.83–5.67 (total 3H), 1.80 (s, 3H), and 1.22 (ss, 6H). A lanthanide-induced shift was also studied to confirm this structure (Fig. 1).

The following three derivatives of XXIII also gave the same spectral data as the reported ones (27):

1. 3,5-Dinitrobenzoate. The dienol (XXIII) (36.2 mg) and 3,5-dinitrobenzoyl chloride (85.6 mg) were dissolved in pyridine (1.0 ml) and allowed to stand overnight at 5°. The reaction mixture was treated in the usual manner, and 3,5-dinitrobenzoate (84.5 mg) was obtained: $[\alpha]_D$ −25.6° (*c*, 2.03); IR (liquid film): ν 3100, 1720, 1622, 1600, and 1540 cm^{-1}.

2. Maleic anhydride adduct. The dienol (XXIII) (61.6 mg) and maleic anhydride (40.4 mg) were dissolved in anhydrous benzene (10 ml), and the reaction mixture was refluxed at 90° for 1.5 hr. The product was obtained from the neutral fraction (51.7 mg), mp 142°, $[\alpha]_D$ −63.3° (*c*, 2.10); IR: ν 3100, 1845, 1775, 1370, 1260, 1050, 880, and 860 cm^{-1}.

3. Acetate. IR (liquid film): ν 1720 and 1240 cm^{-1}.

m-*Cymen-8-ol (XXIV)*—A front shoulder was included in the GLC peak of XXIII, and one set of unshifted protons was observed in the field of aromatic protons in the lanthanide-induced shift experiment of XXIII. On the other hand, 3-carene (XVII) was oxidized according to the method of Dauben *et al.* (28), and XXIV was obtained as one product. The comparison of ^{1}H-NMR data and the GLC retention time indicated the shoulder to be XXIV; mass spectrum: *m/z* (%) 150 (M^+, $C_{10}H_{14}O$, 1), 135 (10), 132 (98), 117 (base), 115 (47), 92 (53), 91 (75), 77 (16), and 65 (30); ^{1}H-NMR: δ 7.40–6.85 (total 4H), 2.35 (s, 3H), and 1.55 (s, 6H) (29).

3-Caren-9-ol (XVIII) (10.5%)—Mass spectrum of the secondary major neutral metabolite of 3-carene showed the molecular ion of $C_{10}H_{16}O$ at *m/z* 152, and its IR spectrum showed a hydroxyl absorption at 3350 cm^{-1}. The ^{1}H-NMR spectrum showed the hydroxymethylene protons at 3.33 and an allylic methyl group at 1.62 ppm. These results indicate that one *gem*-dimethyl group was hydroxylated. This alcohol was easily changed to its acetate with acetic anhydride in pyridine, and it was clear that this alcohol was a primary one.

The determination of which methyl group was metabolized was based on the comparison of the ^{1}H-NMR signals of the *gem*-dimethyl groups

[9] The value in the parentheses means the relative ratio of identified metabolites in the chromatogram of the neutral portions. The ratio of acidic metabolites in GLC cannot be given because the acidic fraction contained many acidic and phenolic metabolites originating from normal urine of rabbits.

Table III—Chemical Shifts (in Hertz) of Methyl Protons of 3-Carene and 3-Caren-9-ol

	3-Carene (30)	3-Caren-9-ol	Difference[a]
C-8 methyl	48	52.8	4.8
C-9 methyl	64	—	11.2

[a] This value means the difference of the chemical shift (in Hertz) of methyl protons between these two compounds.

with those of 3-carene (Table III). In this table, the signal at 52.8 Hz can be reasonably assigned as the C-8 methyl group on the basis of the small difference (4.8 Hz) from the C-8 methyl group of 3-carene (30). As the result, this metabolite was determined to be XVIII, $[\alpha]_D$ −17.9° (*c*, 0.6); mass spectrum: *m/z* (%) 152 (M^+, $C_{10}H_{16}O$, 2), 134 ($M^+ - H_2O$, 18), 121 (25), 119 (38), 108 (21), 105 (24), 93 (47), 91 (76), 78 (base), 77 (41), and 67 (22); IR (liquid film): ν 3350, 1720, 1430, 1370, 1240, 1020, 820, and 780 cm^{-1}; ^{1}H-NMR: δ 5.27 (bs, 1H), 3.33 (s, 2H), 2.17 (OH, 1H), 1.62 (bt, 3H), and 0.88 (s, 3H).

3-Caren-9-ol Acetate—Compound XVIII (61.8 mg) was acetylated with acetic anhydride in pyridine, and an acetate (41.6 mg) was obtained; mass spectrum: *m/z* (%) 194 (M^+, $C_{12}H_{18}O_2$, 7), 150 (10), 134 (50), 132 (45), 119 (base), 105 (35), 93 (48), 92 (36), 91 (36), 79 (33), and 43 (76); IR: ν 1720, 1600, 1450, 1380, 1360, 1240, and 1020 cm^{-1}; ^{1}H-NMR (90 MHz): δ 5.22 (bs, 1H), 3.80 (s, 2H), 2.04 (s, 3H), 1.60 (s, 3H), and 0.84 (s, 3H).

Acidic Metabolites of (+)-3-Carene—The acidic metabolites of 3-carene (XVII) were esterified with diazomethane in ethereal solution; three compounds were isolated, and their structures were determined. In addition, one metabolite was estimated on the basis of the mass spectrum.

3-Carene-9-carboxylic Acid (XIX) as Methyl Ester—The major acidic metabolite showed *m/z* 180 of $C_{11}H_{16}O_2$ as a molecular ion. The presence of one methyl ester group (1720 cm^{-1} and 3.62 ppm) was suggested from its spectrum. The position of the carbomethoxyl group was determined to be C-9 on the basis of the lanthanide-induced shift experiment (Fig. 2). According to the increase of europium reagent, two methine protons (H-1 and H-6) on the cyclopropane ring and the C-9 carbomethoxymethyl group (9-CO_2CH_3) slanted more sharply than the C-8 methyl group on the cyclopropane ring. This result suggests the metabolized methyl group of *gem*-dimethyl orients to an *exo*-configuration against the cyclohexenyl ring. Hence, this methyl ester was determined to be methyl 3-carene-9-carboxylate (XIX*a*). Other signals in ^{1}H-NMR agreed with those of 3-carene, and the conformation of this monoester is shown in Fig. 2. Thus,

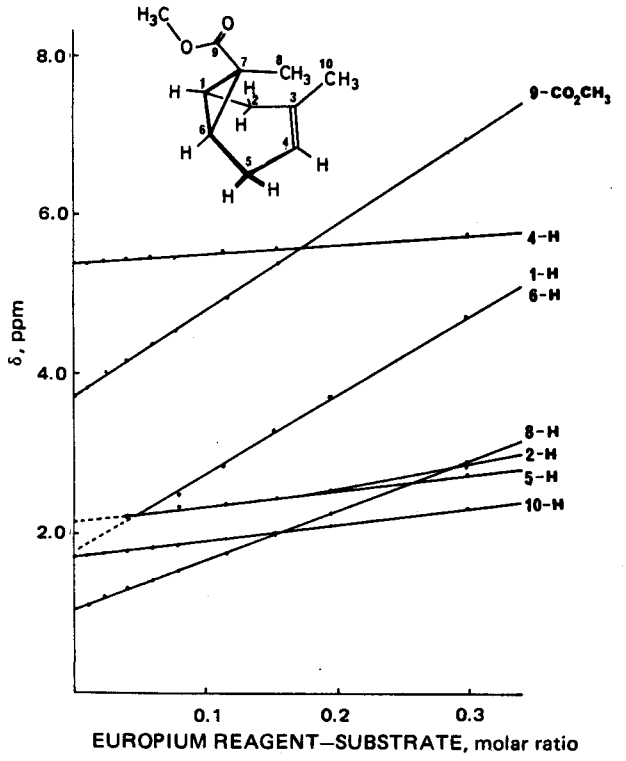


Figure 2—*Lanthanide-induced shifts with europium reagent in the ^{1}H-NMR spectrum of methyl 3-carene-9-carboxylate (XIXa).*

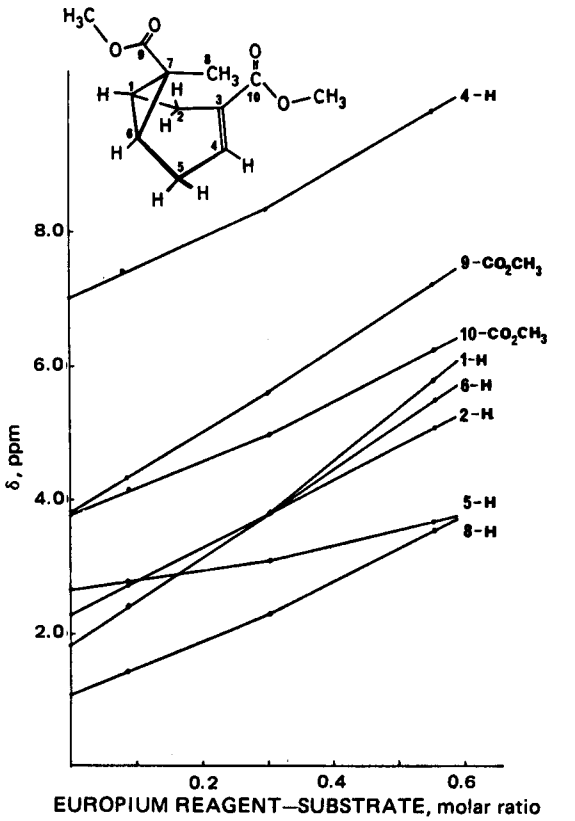


Figure 3—*Lanthanide-induced shifts with europium reagent in the ^{1}H-NMR spectrum of dimethyl 3-carene-9,10-dicarboxylate (XXIIa).*

the formation of a primary alcohol, 3-caren-9-ol (XVIII), is suspected from the acidic metabolite, methyl 3-carene-9-carboxylate (XIX*a*), $[\alpha]_D$ +8.94° (*c*, 2.6); mass spectrum: *m/z* (%) 180 ($C_{11}H_{16}O_2$, 15), 121 (23), 112 (base), 105 (35), and 93 (29); IR: ν 1720, 1260, and 1120 cm^{-1}; ^{1}H-NMR (90 MHz): δ 5.24 (bs, 1H), 3.62 (s, 3H), 2.70–1.60 (6H), 1.60 (s, 3H), and 1.02 (s, 3H).

(−)-3-Carene-9-carboxylic Acid (XIX)—The ester (XIX*a*) (120.5 mg) was hydrolyzed with 1% KOH in ethanol refluxing for 2 hr, and the free acid (XIX) (30.7 mg) was obtained, $[\alpha]_D$ −3.26° (*c*, 4.3); UV: $\lambda_{max}^{ethanol}$ 208 (log ϵ 3.5) nm; IR: ν 3500–2300, 1680, and 1260 cm^{-1}; ^{1}H-NMR: δ 10.95 (b, 1H, COOH), 5.23 (bs, 1H), 1.62 (bs, 3H), 1.03 (s, 3H), and 1.23 and 1.00 (dt, 2H, J = 5 Hz, cyclopropane). Furthermore, XIX*a* was converted into XVIII (10.2 mg) with aluminum lithium hydride (25 mg) in ether. Thus, the structure of this acid was determined.

3-Carene-9,10-dicarboxylic Acid (XXII) as Dimethyl Ester—The secondary major acidic metabolite showed the parent peak at *m/z* 224 of $C_{12}H_{16}O_4$, and the IR spectrum revealed the presence of an ester group at 1720 cm^{-1}. In the ^{1}H-NMR spectrum, in comparison with that of 3-carene, disappearence of the signal of an allylic methyl group and the appearance of new signals at 3.72 and 3.36 ppm assignable to two methyl groups of ester groups were observed. The lanthanide-induced shift in ^{1}H-NMR of this compound is shown in Fig. 3. Increasing amounts of europium reagent caused the shift of one methyl group at 3.72 ppm to the lower field. This shift pattern looks like that of an esteric methyl group of XIX*a*, suggesting the identity of the position of one carboxyl group in XXII with the carboxyl group of XIX*a*. This diester was reduced to an alcohol to give 3-carene-9,10-diol. In the IR spectrum of this diol, the absorption near 3320 cm^{-1} was shifted to 3400 cm^{-1} as the temperature increased. In addition, the absorption at 3525 cm^{-1} disappeared with increasing dilution with carbon tetrachloride.

These results mean that the diol is hydrogen bonded intermolecularly (dimerically) and not intramolecularly. Thus, the relative configuration of two hydroxyl groups in 3-carene-9,10-diol was concluded as being in the *anti*-position in the model. This result indicates that the positions of the carboxylated carbon atoms of 3-carene in rabbits are C-9 and C-10. Consequently, the structure of dimethyl 3-carene-9,10-dicarboxylate (XXII*a*) was established: $[\alpha]_D$ −7.33° (*c*, 2.7); UV: $\lambda_{max}^{ethanol}$ 223 (log ϵ 3.8) nm; mass spectrum: *m/z* (%) 224 (M^+, $C_{12}H_{16}O_4$, 17), 193 (25), 192 (23), 164 (15), 133 (40), 112 (base), and 105 (78); IR: ν 1720, 1680, 1460, 1440,

Table IV—^{13}C-NMR Spectral Data of Metabolites or Derivatives (Parts per Million from Tetramethylsilane)

Metabolite	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	9-CO_2CH_3	10-CO_2CH_3
9,10-Dimethoxy-3-carene	21.07[a] d	21.07 t	128.06 s	137.22 d	18.60 t	20.00[a] d	25.25 s	7.41 q	167.30 s	176.15 s	51.95 q	51.59 q
1,4,4-Trimethylcycloheptan-1-ol	73.19 s	36.51 t	34.74 t	32.93 s	43.30 t	19.26 t	45.07 t	31.55 q	30.43[a] q	30.54[a] q	—	—
Reported values[b]	73.6	37.1	35.3	33.5	44.0	19.8	45.7	31.2	31.1	31.1		
Myrcene-3(10)-glycol	115.30 t	140.88 d	76.35 s	36.90 t	22.07 t	124.31 d	132.29 s	25.73 q	17.71 q	68.95 t	—	—
Linalool reported values[c]	111.6	146.3	72.9	43.0	28.1	125.5	131.0	25.9	17.8	23.2	—	—

[a] Means the alternative value. [b] The values of 1,4,4-trimethylcycloheptan-1-ol in Ref. 33. [c] The values of linalool in Ref. 34.

1340, 1315, 1260, 1198, 1160, 1140, 1095, 1060, 1010, and 890 cm^{-1}; ^{1}H-NMR (90 MHz): δ 6.88 (q, 1H), 3.72 (s, 3H, 9-CO_2CH_3), 3.64 (s, 3H, 10-CO_2CH_3), 1.00 (s, 3H, 8-CCH_3), and 3.0–1.6 (6H). The ^{13}C-NMR spectral data are shown in Table IV, and these values also support the structure of XXII*a*.

3-Carene-9,10-dicarboxylic Acid (XXII)—This diester (XXII*a*) (35 mg) was hydrolyzed in 5% KOH in ethanol with refluxing for 1 hr to give a diacid (XXII) (29 mg), mp 163.5° (ethanol); UV: $\lambda_{max}^{ethanol}$ 290 (log ϵ 4.23) nm; IR: ν 1690 and 1220 cm^{-1}. This diacid was not soluble in deuterochloroform at room temperature.

3-Carene-9,10-diol—The diester (XXII*a*) (33.1 mg) was dissolved in anhydrous ether and stirred with aluminum lithium hydride (15.4 mg) for 1 hr under nitrogen gas at room temperature to give a diol (20.3 mg); mass spectrum: *m/z* (%) 168 (M^+, $C_{10}H_{16}O_2$, 23), 150 (8), 132 (14), 119 (62), 91 (99), 79 (base), and 77 (41); IR: ν 3350, 1710, 1695, 1430, 1265, and 1030 cm^{-1}; ^{1}H-NMR (acetone-d_6): δ 5.53 (bm, 1H), 3.90 (s, 2H, =C-CH_2OH), 3.30 (s, 2H, CH_2OH), 2.83 (two OH), and 0.87 (5H, 8-CH_3 and two cyclopropane protons).

3-Carene-9,10-diol Diacetate—3-Carene-9,10-diol (77.6 mg) was acetylated with acetic anhydride (0.5 ml) and pyridine (1 ml) to give a diacetate (42.9 mg), $[\alpha]_D$ +8.4° (c, 4.0); mass spectrum: *m/z* (%) 252 (M^+, $C_{14}H_{20}O_4$, 1), 192 (3), 132 (78), 117 (base), 91 (53), and 43 (52); IR: ν 1740, 1370, 1240, and 1035 cm^{-1}; ^{1}H-NMR: δ 5.67 (m, 1H), 4.47 (s, 2H, 10-CH_2), 3.85 (s, 2H, 9-CH_2), 2.08 (s, 6H, two CO_2CH_3), 0.87 (s, 3H, 8-CH_3), and 1.00–0.87 (2H, cyclopropane).

3-Carene-10-carboxylic Acid (XXI) as Methyl Ester—A minor acidic metabolite was isolated from the near fraction of XIX*a*, and it was identified as methyl chaminate (XXI*a*) from the spectral data, $[\alpha]_D$ +3.88° (c, 2.57); UV: $\lambda_{max}^{ethanol}$ 220 (log ϵ 3.6) nm; mass spectrum: *m/z* (%) 180 (M^+, $C_{11}H_{16}O_2$, 37), 137 (78), 121 (53), and 105 (base); IR: ν 1710, 1650, 1250, and 1070 cm^{-1}; ^{1}H-NMR (90 MHz): δ 6.90 (m, 1H, 4-CH), 3.73 (s, 3H, 10-CO_2CH_3), 2.80–1.8 (4H), 1.8–1.5 (2H, cyclopropane), 1.06 (s, 3H, 9-CH_3), and 0.73 (s, 3H, 8-CH_3) (31, 32).

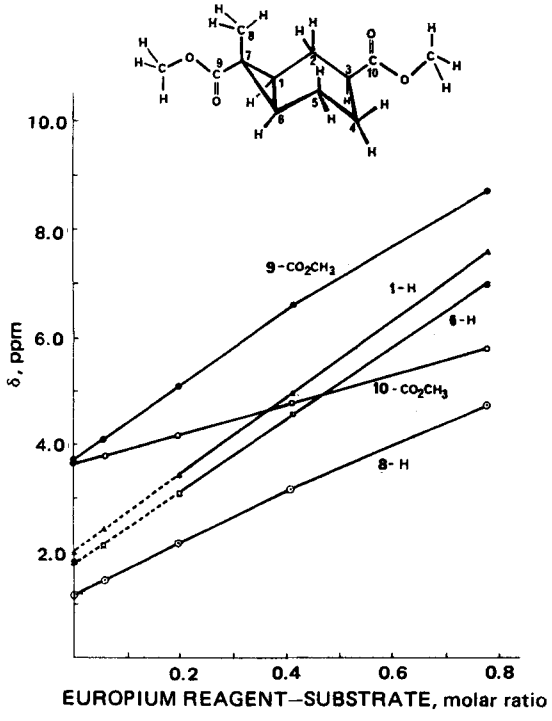


Figure 4—*Lanthanide-induced shifts with europium reagent in the ^{1}H-NMR spectrum of dimethyl 3-carane-9,10-dicarboxylate (XXVIIIa).*

3-Caren-10-ol—Methyl chaminate (XXI*a*) (45.0 mg) was reduced with aluminum lithium hydride (15.3 mg) at 0° to give an allylic alcohol (39.5 mg); ^{1}H-NMR: δ 5.50 (bs, 1H), 3.93 (bs, 2H, CH_2OH), 1.00 (s, 3H, 9-CH_3), and 0.73 (s, 3H, 8-CH_3).

3-Caren-10-ol-9-carboxylic Acid (XX) as Methyl Ester—In addition to the three acidic metabolites, one acidic compound was estimated by GLC–mass spectrometric analysis. This compound has the molecular ion at *m/z* 196, meaning $C_{11}H_{16}O_3$. From this molecular formula and the base peak of *m/z* 112, two structures can be proposed as metabolites, methyl 3-caren-10-ol-9-carboxylate and methyl 3-caren-9-ol-10-carboxylate. The mass spectrum of the compound did not show the same cracking pattern, even partially, as XVIII and XXI*a*. Thus, methyl 3-caren-10-ol-9-carboxylate (XX*a*) seems to be reasonable; mass spectrum: *m/z* (%) 196 (M^+, $C_{11}H_{16}O_3$, 2), 181 (4), 167 (13), 165 (8), 153 (6), 137 (12), 136 (13), 135 (17), 121 (20), 119 (19), 112 (base), 107 (33), 95 (20), 93 (27), 91 (24), and 79 (18).

Metabolites of (−)-*cis*-Carane—The neutral metabolites of (−)-*cis*-carane (XXVII) were acetylated, and one compound was isolated as the acetate; its structure remains to be established. One compound that resisted acetylation was isolated by TLC and identified as 1,4,4-trimethylcycloheptan-1-ol (XXX) from the spectral data (33).

1,4,4-Trimethylcycloheptan-1-ol (XXX)—$[\alpha]_D$ −14.4° (c, 2.2); mass spectrum: *m/z* (%) 156 (M^+, $C_{10}H_{20}O$, 1), 141 (41), 123 (39), and 71 (base); IR (liquid film): ν 3375, 2960, 2860, 1470, 1375, 1365, 1110, 930, and 865 cm^{-1}; ^{1}H-NMR: δ 1.20 (s, 3H) and 0.87 (s, 6H). The ^{13}C-NMR data support the structure (XXX) (Table IV).

Carane-9,10-dicarboxylic Acid (XXVIII) as Dimethyl Ester—Acidic metabolites of carane in acetone were refluxed with dimethyl sulfate in the presence of sodium bicarbonate, and the neutral products were separated into pure compounds by preparative GLC. Compound XXVIII showed the parent peak at *m/z* 226 of $C_{12}H_{18}O_4$ in mass spectrum, and other spectral data (1740 cm^{-1}; 3.60 and 3.63 ppm) showed the loss of the secondary methyl group of carane and the appearance of two carbomethoxyl groups. The positions and the orientations of the metabolized methyl groups were determined by the lanthanide-induced shift experiment (Fig. 4). According to the lanthanide increase, two carbomethoxyl groups showed two completely different slopes, indicating the stereochemically separated position of these two groups. One group was tertiary, and the other was secondary. One carbomethoxyl group showed the same sharp slant as the two cyclopropane protons, and one *tert*-methyl group of the three-membered ring slanted more sharply. Hence, dimethyl 3-carane-9,10-dicarboxylate (XXVIII*a*) was established.

Moreover, the hydrogenation of dimethyl 3-carene-9,10-dicarboxylate (XXII*a*) (75.2 mg) in the presence of palladium-on-charcoal at room temperature for 1 day gave only one product, whose spectrum agreed with that of the diester from carane. This finding means that hydrogen attacks the unsaturated diester from the less-hindered lower side and, thus, the 10-CO_2CH_3 group orients toward the equatorial axis (Fig. 4); XXVIII*a*: $[\alpha]_D$ −17.3° (c, 1.5); mass spectrum: *m/z* (%) 226 (M^+, $C_{12}H_{18}O_4$, 6), 194 (87), 167 (10), 166 (39), 151 (12), 135 (85), 134 (50), and 107 (base); IR (liquid film): ν 1740, 1438, 1310, 1265, 1195, 1170, 1135, 1110, 1010, and 770 cm^{-1}; ^{1}H-NMR: δ 3.63 (s, 3H), 3.60 (s, 3H), and 1.19 (s, 3H).

Metabolites of Myrcene—Three neutral and two esterified acidic metabolites of myrcene (XXXI) were isolated chromatographically.

Myrcene-3(10)-glycol (XXXII) (40.7%)—The main neutral metabolite showed the molecular ion at *m/z* 170 of $C_{10}H_{18}O_2$ in the mass spectrum. This alcohol (3380 cm^{-1}) was converted into the monoacetate (1735 cm^{-1}) with a hydroxyl group remaining (3655 and 3500 cm^{-1}). Consequently, it was shown that the major metabolite of myrcene has

two hydroxyl groups. Furthermore, this diol was changed to a conjugated ketone (UV: $\lambda_{max}^{ethanol}$ 215 nm) with activated manganese dioxide in acetone. The parent peak of the ketone appeared at *m/z* 138 of $C_9H_{14}O$, indicating the loss of one carbon atom. The ^{1}H-NMR spectral data of this ketone showed *ABC*- or *AMX*-type olefinic three protons with the signals of an isopropenyl group. These facts ruled out the presence of methyl ketone in this conjugated ketone. Thus, the positions of the two hydroxyl groups were determined to be C-3 and C-10.

The ^{13}C-NMR spectrum (Table IV) of XXXII, which corresponds to that of linalool (34), further supports the structure of XXXII. The diol also was converted into its acetonide to confirm the 1,2-glycol. Compound XXXII showed $[\alpha]_D$ ±0° (*c*, 2.9); mass spectrum: *m/z* (%) 170 (M^+, $C_{10}H_{18}O_2$, trace), 152 (4), 139 (24), 121 (47), and 69 (base); IR: ν 3380, 3090, 1450, 1380, 1260, 1200, 1195, 1150, 1140, 1000, 930, 842, and 838 cm^{-1}; ^{1}H-NMR (90 MHz): δ 5.82 (q, 1H, J_{XA} = 17.5 Hz, J_{XM} = 10.0 Hz), 5.35 (q, 1H, J_{AX} = 17.5 Hz, J_{AM} = 2.0 Hz), 5.20 (q, 1H, J_{MX} = 10.0 Hz, J_{MA} = 2.0 Hz), 5.10 (bt, 1H, J = 6.0 and 1.8 Hz), 3.47 (s, 2H, CH_2O, $w_{1/2}$ = 5 Hz), 2.55 (s, 2H, two OH), 1.67 (s, 3H, $w_{1/2}$ = 3.5 Hz, 9-CH_3), and 1.59 (s, 3H, 8-CH_3); ^{1}H-NMR (dimethyl sulfoxide-d_6): δ 5.87 (1H, J_{XA} = 17.0 Hz, J_{XB} = 10.0 Hz), 5.17 (1H, J_{AX} = 17.0 Hz, J_{AB} = 3.0 Hz), 4.97 (1H, J_{BX} = 10.0 Hz, J_{BA} = 3.0 Hz), 4.37 (bt, 1H, J = 6.0 Hz, CH_2OH), 4.17 (bs, 1H, *tert*-OH), 3.23 (bs, 2H, $w_{1/2}$ = 4.2 Hz, CH_2O), 1.63 (s, 3H), and 1.55 (s, 3H). By the addition of deuterium oxide to this solution, the signals at 4.37 and 4.17 ppm were lost, and the peak at 3.23 became sharp ($w_{1/2}$ = 2 Hz).

Myrcene-3(10)-glycol-10-acetate—Mass spectrum: *m/z* (%) 194 (M^+ − H_2O, trace), 152 (1), 139 (19), 134 (56), 119 (78), 87 (10), 83 (22), 69 (base), 55 (49), 43 (77), and 41 (22); IR: ν 3655, 3500, 3075, 2950, 2910, 2845, 1735, 1640, 1450, 1410, 1380, 1260, 1240, 1145, 1100, 1040, 993, 913, and 850 cm^{-1}; ^{1}H-NMR: δ 6.00–4.93 (4H), 4.05 (q, 2H, J = 15.0 and 11.0 Hz, CH_2OCOCH_3), 2.10 (s, 3H), 1.72 (s, 3H, 9-CH_3), and 1.63 (s, 3H, 8-CH_3).

10-Normyrcen-3-one—The glycol (XXXII) (105.1 mg) was dissolved in petroleum ether (5 ml) containing activated manganese dioxide (0.5 g) and stirred for 3 days at room temperature. The products were purified by column chromatography to give a ketone (16.9 mg); mass spectrum: *m/z* (%) 138 (M^+, $C_9H_{14}O$, 7), 123 (25), 95 (53), 55 (base), and 41 (46); IR: ν 2960, 2920, 2850, 1700 (weak), 1680, 1620, 1422, 1380, 1360, 1182, 1098, 990, 965, and 818 cm^{-1}; ^{1}H-NMR: δ 6.36–6.22 (2H, *AMX*), 5.77 (q, 1H, J = 8.0 and 4.0 Hz, AM*X*), 5.10 (bt, 1H, J = 7.0 Hz, 6-CH), 2.93–1.93 (4H), 1.67 (s, 3H, 9-CH_3), and 1.60 (s, 3H, 8-CH_3).

Acetonide of XXXII—The glycol (XXXII) (38.3 mg) was dissolved in anhydrous acetone (5 ml) containing anhydrous cupric sulfate (28.2 mg) and stirred for 21 hr at room temperature. After filtration of the reaction mixture, acetone was evaporated under reduced pressure to give 15.9 mg of an acetonide; mass spectrum: *m/z* (%) 195 (M^+ − 15, $C_{12}H_{19}O_2$, 3) and 69 (base); ^{1}H-NMR: δ 6.10–4.93 (4H), 2.62 (2H, CH_2O), 1.67 (s, 3H), 1.60 (s, 3H), and 1.27 [ss, 6H, $C(CH_3)_2$]; IR: ν 1360–1380 (very strong) cm^{-1}.

Myrcene-1,2-glycol (XXXIV) (20.8%)—The mass spectrum of the second neutral metabolite, $C_{10}H_{18}O_2$ (M^+, 170), showed the presence of a dimethyl allyl group [1.73, 1.63 (s, each 3H), and 5.13 (m, 1H); *m/z* 69 (base)] and an *exo*-cyclic methylene group [4.97 and 5.13 (s, each 1H) ppm]. The IR spectrum revealed hydroxyl absorption at 3575 and 3450 cm^{-1}. This alcohol was converted into its diacetate (1735 cm^{-1}), whose ^{1}H-NMR showed two sets of route protons of primary and secondary acetoxyl groups (4.14, 2H, and 5.38, 1H). The presence of the two hydroxyl groups of XXXIV was determined further by ^{1}H-NMR in dimethyl sulfoxide-d_6, namely, the signals of 4.63 (d) and 4.38 (t), assignable to CH(*OH*) and CH_2OH, respectively, which disappeared upon the addition of deuterium oxide. Thus, it was confirmed that the vinyl group of myrcene was metabolized to a glycol, and the formation of 1,2-glycol acetonide strongly supports this determination.

The biotransformed XXXIV showed the same ^{1}H-NMR spectrum as that of the synthetic one (35); $[\alpha]_D$ ±0° (*c*, 2.5); mass spectrum: *m/z* (%) 170 (M^+, $C_{10}H_{18}O_2$, 1), 152 (2), 121 (8), 119 (11), 109 (21), 101 (17), 95 (13), 85 (12), 81 (12), 79 (11), 70 (12), and 69 (base); IR: ν 3575, 3400, 1010, 910, and 840 cm^{-1}; ^{1}H-NMR: δ 5.13 and 4.97 (s, each 1H, =CH_2), 5.13 (m, 1H, C=CH), 4.17 [m, 1H, C*H*(OH)], 3.63 (m, 2H, CH_2OH), 1.70 (s, 3H, 9-CH_3), and 1.63 (s, 3H, 8-CH_3); ^{1}H-NMR (dimethyl sulfoxide-d_6): δ 4.63 [d, 1H, J = 5 Hz, CH(*OH*)] and 4.38 (t, 1H, J = 6 Hz, CH_2OH), which were replaceable with deuterium oxide.

Diacetate of XXXIV—The 1,2-glycol (19.3 mg) was acetylated in the usual manner to give the diacetate (13.2 mg), $[\alpha]_D$ −0.08° (*c*, 2.5); mass spectrum: *m/z* (%) 194 (M^+ − 60, $C_{12}H_{18}O_2$, 4), 151 (10), 134 (86), and 119 (base); IR: ν 3020, 1735, 1650, 980, 950, and 915 cm^{-1}; ^{1}H-NMR (90 MHz): δ 5.38 [q, 1H, J = 7.5 and 4.0 Hz, =C–C*H*($OCOCH_3$)], 5.09 and 4.97 (s, each 1H, =CH_2), 5.20–4.90 (bs, 1H, =CH), 4.17 and 4.14 (t, each 1H, –CH_2OCOCH_3), 2.08 (s, 3H), 2.13 (s, 3H), 1.68 (s, 3H), and 1.61 (s, 3H).

Acetonide of XXXIV—The 1,2-glycol (34.9 mg) was stirred with anhydrous cupric sulfate (0.2 g) in acetone for 16 hr at room temperature. After filtration and evaporation, an acetonide (10.3 mg) was obtained; mass spectrum: *m/z* (%) 194 (M^+ − H_2O, trace), 152 (2), 139 (19), 134 (56), 119 (78), 69 (base), and 43 (77).

Uroterpenol (XXXVI) as Acetate (11.8%)—From the acetates of myrcene neutral metabolites, uroterpenol monoacetate was isolated in a minor yield by preparative TLC. Its mass spectrum agreed with that of an authentic sample (36). The IR (3425 and 1720 cm^{-1}) and ^{1}H-NMR [5.38 (1H), 4.05 (2H of CH_2OH), and 1.67 (allylic methyl)] data support this structure; mass spectrum: *m/z* (%) 194 (M^+, $C_{12}H_{20}O_3$, 8), 139 (32), 134 (base), 119 (35), 105 (17), 95 (37), and 43 (84); IR (liquid film): ν 3425, 1720, 1430, 1370, 1240, and 1040 cm^{-1}; ^{1}H-NMR: δ 5.38 (bs, 1H), 4.05 (s, 2H), 2.12 (s, 3H), 1.67 (s, 3H), and 1.15 (s, 3H).

2-Hydroxymyrcene-1-carboxylic Acid (XXXV) as Methyl Ester—An acidic metabolite was isolated as the methyl ester, and the mass spectrum showed the molecular ion at *m/z* 198 of $C_{11}H_{18}O_3$. Its IR spectra showed the presence of a hydroxyl group at 3450 cm^{-1} and −CH(*OH*) at 3.06 ppm (d, J = 6 Hz), and the isopropenyl and 3(10)-endmethylene groups remained unchanged. The hydroxyl proton signal at 60 MHz disappeared in the case of 90 MHz, and the −C*H*(OH) signal became broad. Thus, the structure of this hydroxyl ester was determined to be methyl 2-hydroxymyrcene-1-carboxylate (XXXV*a*), $[\alpha]_D$ ±0° (*c*, 1.1); mass spectrum: *m/z* (%) 198 (M^+, $C_{11}H_{18}O_3$, 2), 180 (2), 173 (3), 138 (13), and 69 (base); IR (liquid film): ν 3450, 1730, 1370, 1260, 1210, 1080, 1050, 982, and 900 cm^{-1}; ^{1}H-NMR: δ 5.18 and 5.04 (s, each 1H, =CH_2), 5.20–5.00 (bs, 1H, C=CH), 4.60 [d, 1H, J = 6 Hz, C*H*(OH)], 3.06 [d, 1H, J = 6 Hz, CH(*OH*)], 3.82 (s, 3H), 2.15 (2H), 2.11 (2H), 1.70 (s, 3H, 9-CH_3), and 1.63 (s, 3H, 8-CH_3).

3-Hydroxymyrcene-10-carboxylic Acid (XXXIII) as Methyl Ester—The alternative hydroxyacid, 3-hydroxymyrcene-10-carboxylic acid, was estimated tentatively from its GLC–mass spectrometric spectrum as a methyl ester; mass spectrum: *m/z* (%) 198 (M^+, $C_{11}H_{18}O_3$, 2), 180 (18), 165 (22), 139 (26), 121 (55), 116 (92), 93 (22), 83 (39), 69 (base), 55 (74), and 41 (50).

Metabolites of *p*-Cymene—In *p*-cymene (XXXVII), the neutral metabolites were isolated by preparative TLC; acidic metabolites were identified by the GLC–mass spectrometric spectra.

p-*Cymen-9-ol (XXXVIII) (50%)*—Mass spectrum: *m/z* (%) 150 (M^+, $C_{10}H_{14}O$, 18), 120 (12), 119 (base), 117 (12), 91 (25), 77 (8), and 65 (6); IR (liquid film): ν 3370, 1520, 1040, 1020, and 819 cm^{-1}; ^{1}H-NMR: δ 7.15 (s, 4H), 3.66 (d, 2H, J = 7 Hz), 2.83 [m, 1H, C*H*(CH_3)$_2$], 2.35 (s, 3H), and 1.27 (d, 3H, J = 7 Hz). These data were coincident with those of *p*-cymen-9-ol (XL) in the literature (37).

α-p-Tolylpropionic Acid (XXXIX) as Methyl Ester—The spectral data agreed with those of methyl α-*p*-tolyl propionate (37); mass spectrum: *m/z* (%) 178 (M^+, $C_{11}H_{14}O_2$, 42), 120 (74), and 91 (base); IR (liquid film): ν 1735, 1518, 1280, 820, and 715 cm^{-1}; ^{1}H-NMR: δ 7.15 (s, 4H), 3.65 (s, 3H), 3.44 (q, 1H, J = 6 Hz, 8-CH), 2.33 (s, 3H), and 1.48 (d, 3H, J = 6 Hz).

p-*Cymen-8-ol (XXVI) (28%)*—Mass spectrum: *m/z* (%) 150 (M^+, $C_{10}H_{14}O$, 9), 135 (base), 132 (19), 117 (17), 115 (10), 105 (4), 91 (25), 77 (6), 65 (10), and 43 (base); IR (liquid film): ν 3400, 1518, 1024, 968, 868, 820, and 725 cm^{-1}; ^{1}H-NMR: δ 7.34 (q, 2H, J = 8 Hz), 7.21 (d, 2H, J = 8 Hz), 2.38 (s, 3H), and 1.58 (s, 6H). All of these spectra agreed with those of *p*-cymen-8-ol (XXVI) (19, 23).

Another four acidic metabolites were estimated tentatively on the basis of mass spectra. These compounds were not isolated because of the small amounts.

α-Tolyl-α-hydroxylpropionic Acid (XL) as Methyl Ester—Mass spectrum: *m/z* (%) 176 (M^+ − H_2O, base), 164 (16), 163 (10), 149 (9), 145 (26), 131 (5), 92 (6), and 91 (10).

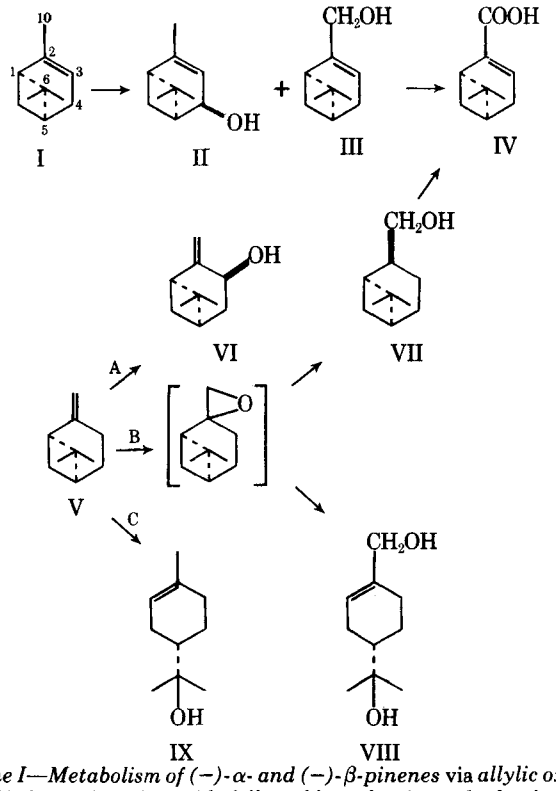
α-Tolylacrylic Acid (XLI) as Methyl Ester—Mass spectrum: *m/z* (%) 176 (M^+, $C_{11}H_{12}O_2$, 55), 146 (12), 145 (base), 117 (12), 116 (8), 115 (27), and 91 (13).

p-*Isopropylbenzoic Acid (XLII) as Methyl Ester*—Mass spectrum: *m/z* (%) 178 (M^+, $C_{11}H_{14}O_2$, 38), 163 (base), 119 (65), and 91 (29).

p-*1-Hydroxyisopropylbenzoic Acid (XLIII) as Methyl Ester*—Mass spectrum: *m/z* (%) 176 (M^+ − H_2O, base), 162 (32), 145 (51), and 91 (10).

DISCUSSION

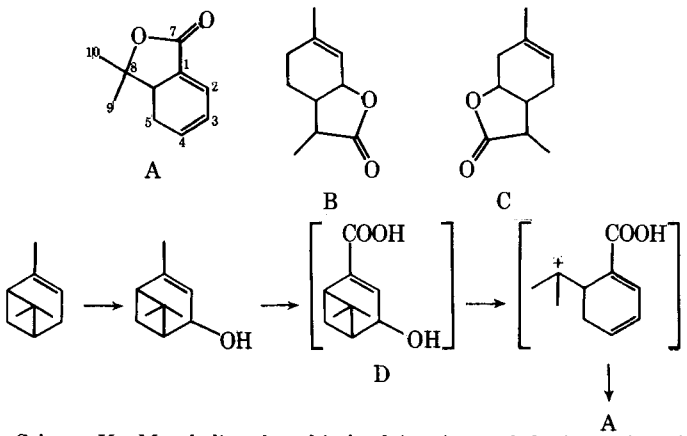
Scheme I shows the metabolic routes of α- and β-pinenes. The main



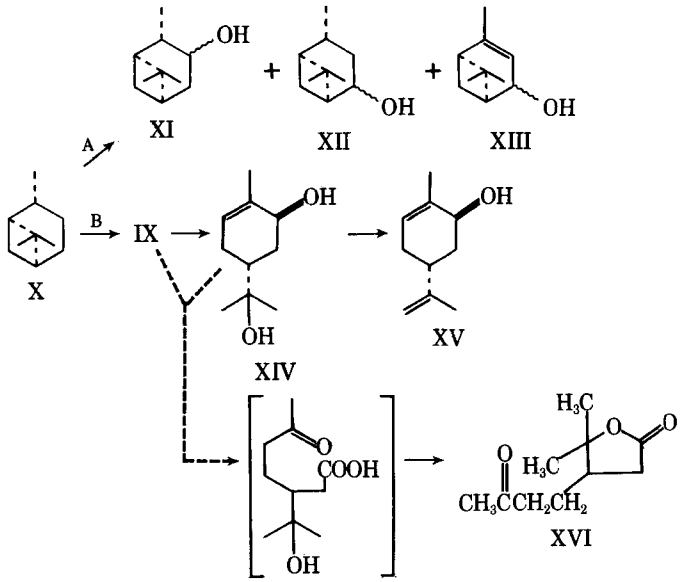
Scheme I—Metabolism of (−)-α- and (−)-β-pinenes via *allylic oxidation (A), formation of epoxide followed by reduction or hydration (B), and ring cleavage of the four-membered ring (C).*

urinary metabolites of α-pinene in rabbits were the verbenols. The purities and optical rotations of the verbenols are shown in Table II. In the case of (−)-α-pinene (I), the purity of verbenol by GLC of neutral metabolites was 99%. On the other hand, the purities of verbenols from (+)- and (±)-α-pinenes were 67 and 68%, respectively. This finding means that the biotransformation of (−)-α-pinene in rabbits is remarkably efficient in the preparation of (−)-*trans*-verbenol (II). Another method of the chemical preparation of (−)-*trans*-verbenol was reported by Mori (38). In addition, the relation of the signs and values of the optical rotations between the administered α-pinenes and the obtained verbenols suggests the stereoselective hydroxylation of α-pinene. As minor metabolites of α-pinene, two allylic products, myrtenol (III) and myrtenic acid (IV), were obtained.

In (−)-β-pinene (V), among three metabolic routes (A, B, and C in Scheme I), Route B is thought to be the main route judging from the yields of 10-pinanol (VII) and 1-*p*-menthene-7,8-diol (VIII) (39 and 30%, respectively). This route includes epoxide formation of the end-methylene group. Such epoxide formation also was found in the metabolism of camphene in rabbits (13), and it was confirmed by the administration of camphene epoxide to obtain camphanol (39). Myrtenic acid might be



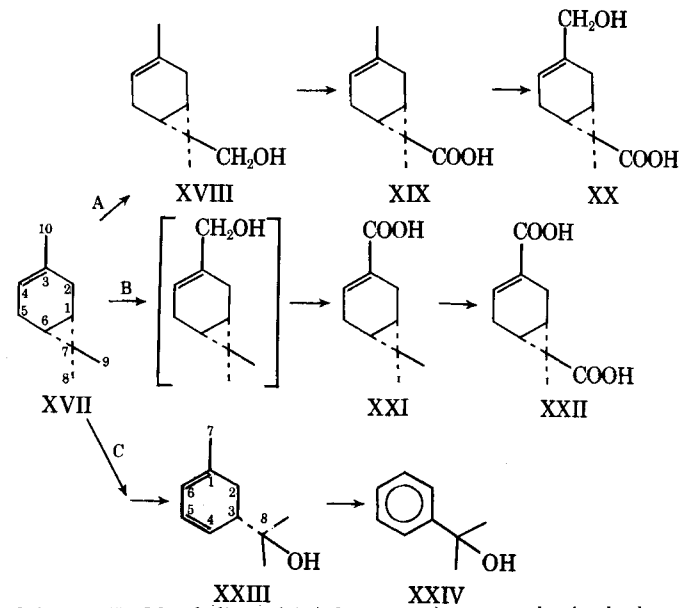
Scheme II—Metabolites found in koala's urine and the formation of Lactone A.



Scheme III—Metabolism of (−)-cis-pinane via *simple hydroxylation at secondary carbon atoms (A) and ring cleavage of the four-membered ring and hydration (B).*

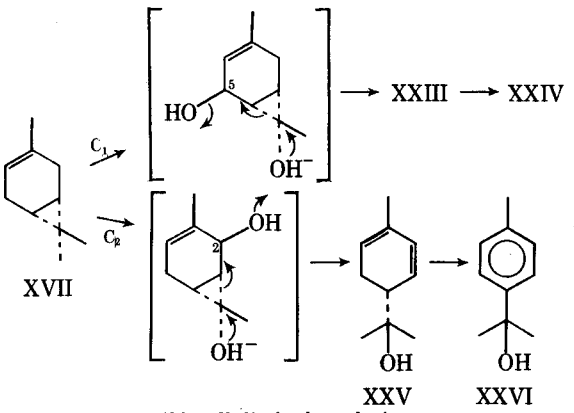
derived through this route from β-pinene. The additional routes, A and C, are estimated from the corresponding metabolites. On the other hand, the koala fed a diet of *Eucalyptus punctata* containing α- and β-pinenes metabolized the lactones A, B, and C (Scheme II) (9), and later it was pointed out that A is yielded through 4-hydroxymyrtenic acid (D, Scheme II) (40). The presence of this acid (D) was suggested as a metabolite of α-pinene in rabbits[10]. In the present study, however, it was not detected even in GLC–mass spectrometry, so it is concluded that it is a species-specific metabolite of α-pinene in mammals.

In pinane (Scheme III), Route B was the main metabolic route [α-terpineol (IX) (43%)]. In the first step, it can be estimated that eradication of a hydride ion occurred. The three compounds of Route A [3-pinanol (XI), 4-pinanol (XII), and verbenol (XIII)] were clearly estimated as metabolites on the basis of the isolated oxidation products (3-pinanone, 4-pinanone, and verbenone, respectively) of the neutral portion. A lactonic compound (XVI) might be yielded as an artifact from α-terpineol



Scheme IV—Metabolism of (+)-3-carene via *stereoselective hydroxylation at* gem*-dimethyl followed by carboxylation (A), allylic oxidation at an allylic methyl group (B), and allylic hydroxylation at a secondary carbon atom (C).*

[10] R. Mechoulam, Hebrew University of Jerusalem, Jerusalem, Israel, personal communication.



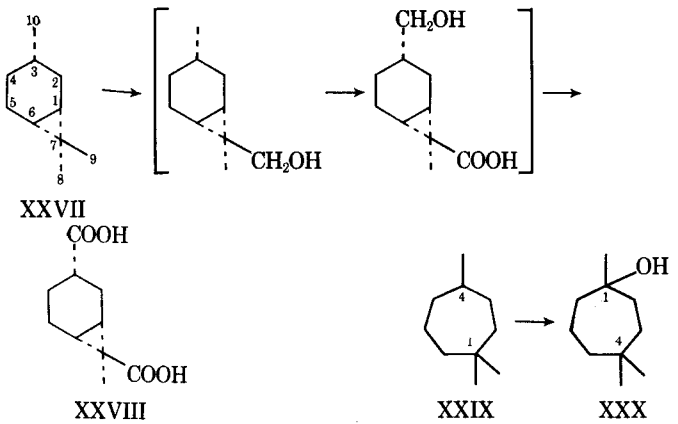
Scheme V—Two possible allylic hydroxylation routes at secondary carbon atoms (C-2 and C-5) of 3-carene.

(IX) or sobrerol (XIV) by Jones oxidation of a neutral portion of the metabolites.

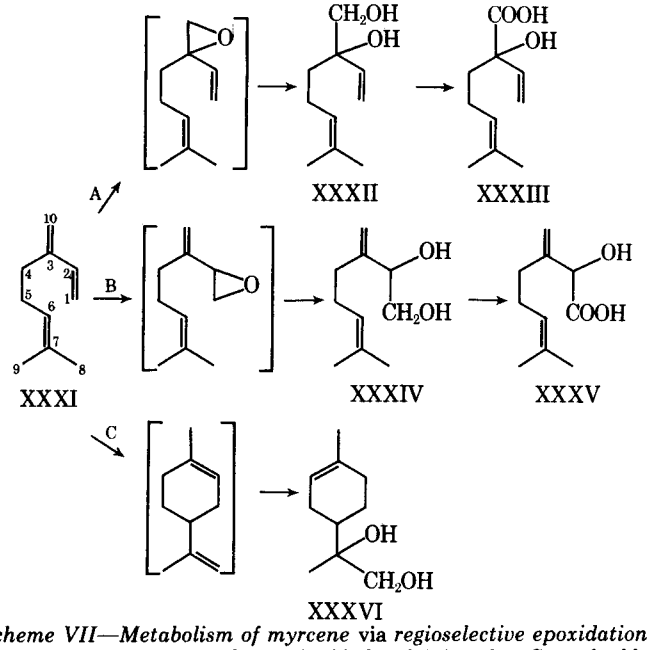
As shown in Scheme IV, 3-carene (XVII) was stereoselectively hydroxylated and carboxylated. In this case, *m*-mentha-4,6-dien-8-ol (XXIV) was a main urinary neutral metabolite (71.8%) along with its aromatized *m*-cymen-8-ol (XXIV) (<1%). The formation of 3-caren-9-ol (XVIII) in Route A in Scheme IV is explained as allylic hydroxylation due to the cyclopropane ring. Four compounds were isolated as acidic metabolites: 3-carene-9-carboxylic acid (XIX), 3-carene-9,10-dicarboxylic acid (XXII), chamic acid (XXI), and 3-caren-10-ol-9-carboxylic acid (XX). Metabolites XVIII, XIX, XX, and XXII in Scheme IV have not been reported yet in literature.

Routes A and B show the stereoselective hydroxylation and carboxylation of the *gem*-dimethyl group. Such reactions have not been found in the chemical oxidation of 3-carene, indicating the enzymatic specificity in the metabolism. In Route C, two metabolic pathways (C_1 and C_2) can be proposed as the *endo*-cyclic allylic hydroxylation (Scheme V). It is reported that 1,3-rearrangement of the hydroxyl group in 3-caren-5-ol to *m*-mentha-4,6-dien-8-ol (XXIII) occurs easily due to the cyclopropane ring (41). Thus, Route C_1 in Scheme V includes 3-caren-5-ol as an intermediate. In the same manner, Route C_2 [formation of 3-caren-2-ol, followed by the rearrangement to a dienol (XXV) and the formation of an aromatic alcohol (XXVI)] may be postulated, but neither XXV or XXVI was found in this experiment. Consequently, Route C_2 is ruled out. Therefore, the position of C-5 in 3-carene is thought to be more easily hydroxylated than C-2 by enzymatic systems in the liver of a rabbit. The same example can be seen in the metabolism of 1-methylcyclohexene in rabbits (12).

In carane, the C-9 and C-10 methyl groups were metabolized by enzymes (Scheme VI). The carboxylation of the *gem*-dimethyl group is said to be stereoselective and is one kind of allylic oxidation due to the cyclopropane ring; the secondary C-10 methyl group of carane was metabolized as in the case of carboxylation of the secondary methyl of patchoulol (6). Therefore, oxidation of the C-10 methyl group of carene does not always seem to be affected by the double bond. This is the first case in which carane-9,10-dicarboxylic acid has been reported. As a



*Scheme VI—Metabolism of (−)-*cis*-carane via stereoselective gem-dimethyl and secondary methyl hydroxylation and carboxylation in 3-carane.*

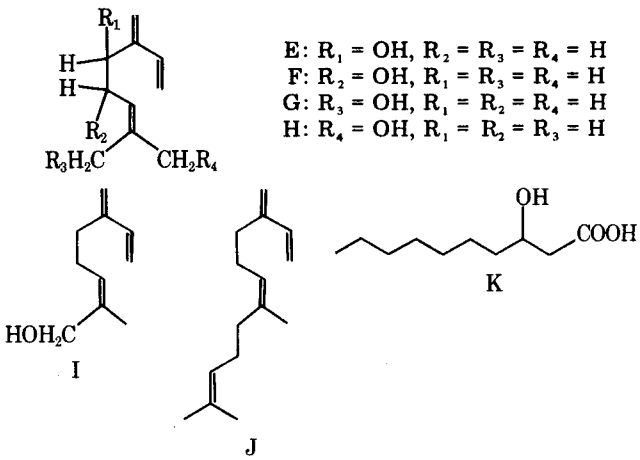


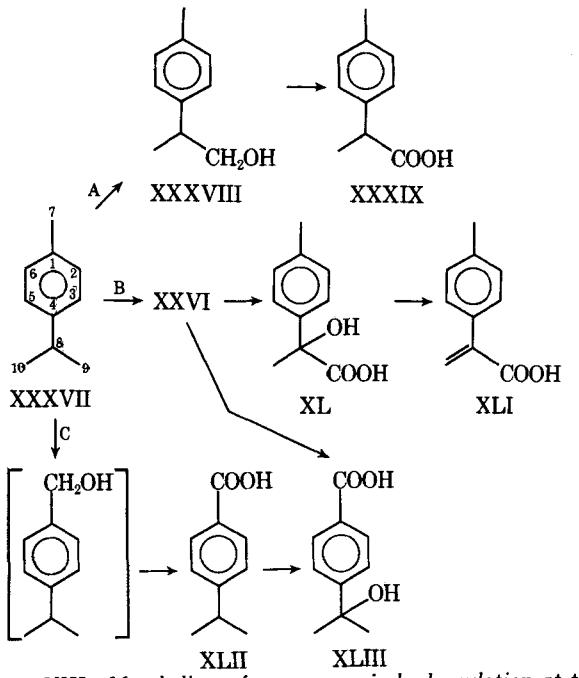
Scheme VII—Metabolism of myrcene via regioselective epoxidation followed by hydration at C-3,10 double bond (A) and at C-1,2 double bond (B).

neutral metabolite of carane, 1,4,4-trimethylcycloheptan-1-ol (XXX) was identified by spectral data. This alcohol can be explained as a metabolite from 1,1,4-trimethylcycloheptane (XXIX), which was reported as a main reduction product of 3-carene in some conditions (42) and whose minor presence in the administered carane was confirmed by comparison of GLC–mass spectrometry of authentic samples. The formation of this tertiary alcohol was reported in methylcyclohexane metabolism (43, 44).

Scheme VII shows the metabolism of myrcene (XXXI). Among these metabolites, two compounds had not been reported previously [myrcene-3(10)-glycol (XXXII) and 2-hydroxymyrcene-1-carboxylic acid (XXXV)], and myrcene-1,2-glycol (XXXIV) was newly obtained as a natural product. The formation of two glycols was due to the hydration of the corresponding epoxides formed as intermediates. The yield of 3(10)-glycol was higher than 1,2-glycol, indicating the facile enzymatic attack to the fixed 3(10)-double bond. The optical rotation of these glycols each showed a value of zero, meaning that there is an equal chance of attack by enzymes to the double bond from both sides. The formation of uroterpenol (XXXVI) may proceed through limonene, which was clearly derived from myrcene in the acidic conditions of rabbit stomachs.

At first, four allylic alcohols (E, F, G, and H) were expected as the neutral metabolites of myrcene. However, none of these compounds was obtained. The fact that the allylic positions (C-4, C-5, C-8, and C-9) of myrcene were not hydroxylated shows the regioselective hydroxylation by enzymes in rabbits. This metabolism of myrcene offers one type of biotransformation of β-farnesene (J), a linear sesquiterpene hydrocarbon having the same partial structure as myrcene.





Scheme VIII—Metabolism of p-*cymene* via *hydroxylation at the ω position (A), at the ω-1 position (B), and at the benzyl position (C).*

Biotransformation of aromatic hydrocarbons has been studied extensively in relation to carcinogenic activity. However, the metabolism of *p*-cymene (XXXVII), which is often found in essential oils, remains to be established in mammals. As shown in Scheme VIII, seven metabolites were identified or estimated. The intraperitoneal administration of *p*-cymene gave the same metabolites as did oral administration, suggesting that all terpenoids used were biotransformed not microbiologically but through liver enzymatic systems. Cumene was metabolized in rabbits and gave 2-phenyl-2-propanol (40%), 2-phenyl-1-propanol (25%), and 2-phenylpropionic acid (25%) (45). In addition, isopropyl diphenyl was metabolized in many mammals (dog, monkey, rat, and human), and species-different metabolic routes were proposed previously (46). Those studies and the present results establish the metabolism of aromatic hydrocarbons having an aliphatic group as follows:

1. Aromatic hydrogen atoms are not replaced by hydroxyl groups.
2. In the metabolism of methyl and isopropyl groups on aromatic hydrocarbons, the isopropyl group is more easily hydroxylated or carboxylated (Routes A and B) than the benzyl methyl group (Route C), judging from their yields.

On the other hand, in the microbial transformation of *p*-cymene, the carboxylation of the benzyl methyl group and the hydroxylation of aromatic hydrogens were reported (37). Thus, the biotransformation of *p*-cymene in rabbits and microorganisms are considerably different. The metabolism of ethyl benzene was reported and a similar pathway was proposed (47).

From the point of biological activity, mammals (rabbits) and insects (bark beetles) show an interesting relationship in the metabolism of monoterpenoids. Namely, verbenol and pinocarveol also were reported as oxidation products of α- and β-pinenes, respectively, in a bark beetle, *Dendroctonus frontalis* (48). Moreover, (−)-*cis*- and (+)-*trans*-verbenols have pheromonal activity in *Ips paraconfusus* and in *Dendroctonus brevicomis*, respectively (49). In addition, myrcene was metabolized to ipsdienol (Structure F) and ipsenol (Structure I), both known as pheromones that are widely used in Scandinavia (50), in *Ips* spp. (51, 52). *p*-Cymen-8-ol (XXVI) also was found from the frass of a longhorn beetle, *Hylotrupes bajalus* (53). The high yield (50% of the dose) of *p*-cymen-8-ol following intraperitoneal administration offers a new preparation method for this pheromone.

2-Hydroxymyrcene-1-carboxylic acid (XXXV) has a structure similar to myrmicacin (Structure K) (54), so it is expected to have some biological activity.

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Quantitative TLC Determination of Epimeric Ratios of 16-Methyl 17-Ketone Oxidation Products of Dexamethasone and Related Drugs

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Abstract □ A TLC system sensitive enough to detect, identify, and allow quantitation of the 16-methyl α- and β-epimers of 9-fluoro-11β-hydroxy-16-methylandrosta-1,4-diene-3,17-dione is described. These epimeric 17-ketones may be present as impurities in dexamethasone, betamethasone, or related drugs. A spectrodensitometer with a TLC scanning attachment was used, and results from densitometry compared favorably with quantitation by high-performance liquid chromatography, as was described recently. TLC was convenient in the rapid examination of drug samples for the detection and identification of epimeric 17-ketones and for the determination of the α to β ratio of such epimers. Various applied photographic techniques for documenting TLC data are described.

Keyphrases □ TLC—detection and identification of 16α- and 16β-methyl epimers of 9-fluoro-11β-hydroxy-16-methylandrosta-1,4-diene-3,17-dione, impurities in dexamethasone, betamethasone, and related drugs □ Dexamethasone impurities—16α- and 16β-methyl 17-ketone oxidation products, identification and quantitative detection by TLC □ Epimers—16α- and 16β-methyl 17-ketone oxidation products, possible impurities in dexamethasone, betamethasone, and related drugs, TLC detection and identification of α- and β-epimers

The TLC or partition chromatography of C_{18} steroid estrogens (1–3), C_{19} androstanes (4–6) such as androgenic hormones, C_{21} pregnanes (7–10) including cortisone, and the related oxygenated derivatives of these families is documented. The C_{22} steroids such as dexamethasone and betamethasone and their C_{20} oxidation products have not received such detailed study.

Interest has developed in qualitative and quantitative studies of these epimeric C_{20} oxidation products by high-performance liquid chromatography (HPLC) (11) and TLC (12) because of their presence as impurities in dexamethasone (11–13) and dexamethasone sodium phosphate (I) drug samples. Inherently, the C_{22} steroids and their 17-oxo derivatives possess a structural feature not exhibited by the C_{19} and C_{21} steroids and their oxygenated derivatives, namely, an optical center resulting from the additional methyl group at the C-16 position. These epimeric optical isomers were subjected to HPLC and TLC in this study.

BACKGROUND

The 17-oxo derivative, *i.e.*, 9-fluoro-11β-hydroxy-16-methylandrosta-1,4-diene-3,17-dione (epimers IIα and IIβ), recently was reported to be present (12) at ~2% as an impurity in drug products from five of six dexamethasone manufacturers for the Canadian market. In addition, a massive contamination (50%) by the α- and β-epimers of this ketone (IIα and IIβ) was reported (11) in a commercial sample of dexamethasone sodium phosphate (I) solution for injection. In that study (11), it was found by HPLC that this 17-keto oxidation product was actually an epimeric mixture of the 16α- and 16β-methyl compounds and that the α to β ratio could be determined approximately. Identification of the 16-methyl 17-ketone epimers (IIα and IIβ) by TLC systems similar in nature to the reversed-phase HPLC system was attempted, but the TLC systems failed to separate the α- and β-epimers; dexamethasone acetate, a related compound, consistently exhibited the same R_f value as the epimers (11).

Another report (12) indicated that separation between the 16α- and 16β-methyl epimers (IIα and IIβ) can be effected by TLC (R_f 0.60 and 0.59, respectively). The epimeric corticosteroids, dexamethasone and betamethasone, which differ in configuration at the C-16 optical center, were separated by paper chromatographic liquid partition (14). General techniques for photographic documentation of data collected on TLC plates were reported (15).

A method to determine the α to β epimeric ratio of the impurity is important because the components of the impurity are diastereoisomeric with different physiological properties and, consequently, different effects on the patient. An overview of possible physiological effects of such contamination, including references, was given previously (11).

The aim of the present work was to develop a TLC technique that allows identification of epimeric 16-methyl 17-ketones (IIα and IIβ) in samples of drugs related to dexamethasone. Furthermore, the method must determine the α to β ratio of the impurity. In the first part of the

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