Tetrahedron Letters No. 28, pp 2437 - 2440, 1977. Pergamon Press. Printed in Great Britain.

BIOTRANSFORMATION OF TERPENOIDS IN MAMMAL. I.
BIOTRANSFORMATION OF 3-CARENE AND RELATED COMPOUNDS IN RABBITS.

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(Received in Japan 7 May 1977; received in UK for publication 31 May 1977)

During the course of the investigation of the detoxication mechanism of terpenoids in mammals, we have found that some monoterpene hydrocarbons and a related compound to them have been stereoselectively transformed to hydroxylated products in rabbits. We report herein the biotransformation of 1-methylcyclohexene, (+)-, (-)- and (±)-2-pinenes, (-)-2(10)-pinene and (+)-3-carene.

Each compound was administered by a stomach tube as the suspension of Tween-80 (100 ml) to an unanesthetized male rabbit for four days after two days starvation. The urine was treated according to the method of LUU Bang et al.[1] The ether extract, after removal of acidic and phenolic fractions, gave a neutral fraction.

1-Methylcyclohexene (10 g), which has a common partial structure to those of some cyclic monoterpene hydrocarbons, was metabolized and gave two alcohols (973 mg). After fractionation with column chromatography on SiO_2, the main alcohol, 3-methyl-2-cyclohexen-1-ol[2] (6.8 %)[3] (1) was isolated. The absolute configuration of C-1 of 1 was deduced by the Horeau's method to be S.[4] In addition to 1, a small amount of 2-methyl-2-cyclohexen-1-ol[5] (2) was obtained as the metabolite.

2-Pinenes were administered and the main metabolite, verbenol[6] (3) was obtained as shown in Table 1. A trace of myrtenol[7] (4) was also identified in each case.

(-)-2(10)-Pinene ($[\alpha]_D$ -19.8°, 12 g) gave 753 mg of neutral metabolites. Column chromatography on SiO_2 of this part separated four alcohols, trans-pinocarveol[8] (5, 0.7 %), trans-10-pinanol[9] (6, 2.4 %), α-terpineol (7, $[\alpha]_D$ -51.0°, 0.3 %) and 1-p-menthen-7,8-diol (8, $[\alpha]_D$ -33.3°, 1.9 %).

(+)-3-Carene ($[\alpha]_D$ +20.7°, 10 g) gave 83 mg of neutral metabolites, which gave three alcohols on SiO_2 chromatography. The main alcohol was identified as m-mentha-4,6-dien-8-ol[10] (9, 11.2 %) by the spectra of MS, UV, NMR, NMDR and $Eu(FOD)_3$ experiment and by the formation

Table 1.

2-pinene $[\alpha]_D$,	administered weight	weight of neutral metabolite	verbenol $[\alpha]_D$,	yield*
-30.9°,	8 g	1.423 g	-31.2°,	17.6 %
+22.0°,[11]	10 g	0.318 g	± 0.0°,[11]	2.1 %
± 0.0°,	10 g	1.199 g	-11.3°,	8.1 %

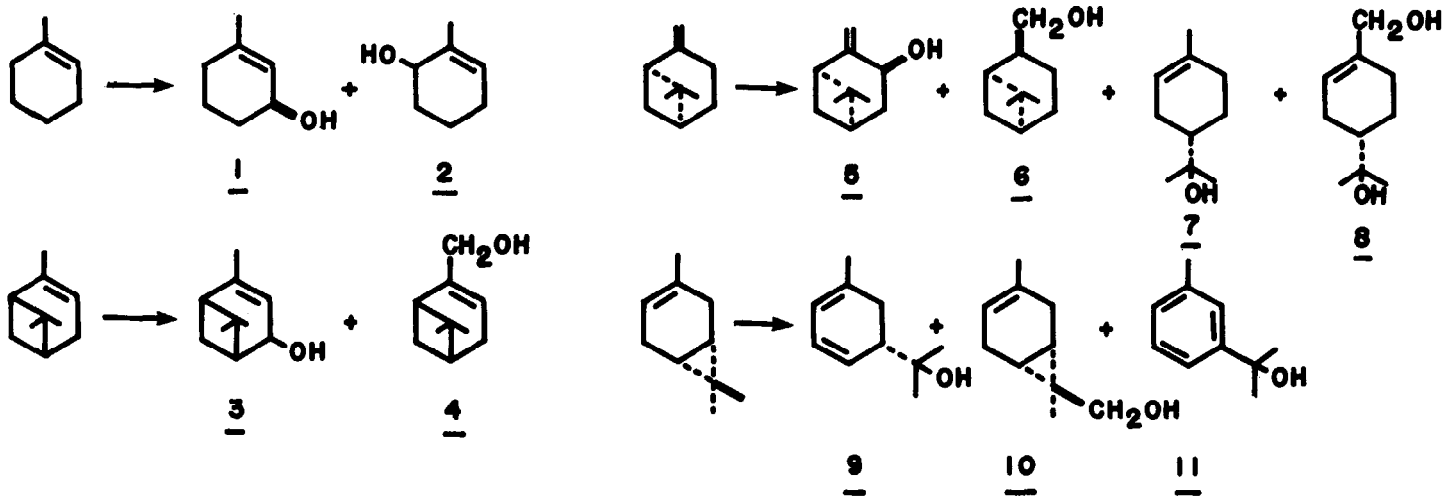
* Yield : verbenol / 2-pinene

of an adduct with maleic anhydride. This alcohol has not been found as a natural product. The minor alcohol was determined as the structure 10 (1.6 %) on the basis of IR, NMR and MS spectral data and also proved by the chemical correlation. The spectra of IR and NMR of 10 showing the absorption at 3350 cm^{-1} and a singlet of two protons at 3.33 ppm, respectively suggest this alcohol to be primary. This was further supported by the spectra of its acetate at 1720 cm^{-1} and a sharp singlet at 3.80 ppm. The NMR spectrum of 10 also posseses two methine protons (1.00 - 0.73 ppm) on the cyclopropane ring. The chemical shifts of C-8 and C-9 methyl groups of 3-carene are 0.80 and 1.07 ppm, respectively,[12] while that of one tertiary methyl group of 10 resonanced at 0.88 ppm, indicating the maintenance of C-8 methyl group and the location of hydroxyl group to be at C-9. Thus, the structure of this alcohol was established to be 3-caren-9-ol. 3-Caren-9-ol has neither been prepared by *in vitro* oxidation of 3-caren nor found in nature. The third alcohol was found in a trace amount and identified as *m*-cymen-8-ol[13] (11).

In the case of 1-methylcyclohexene and 2-pinenes, an endo-cyclic allylic oxidation occurred selectively rather than exo-cyclic one. The metabolism of 2-pinenes suggests that rather (-)-2-pinene is well transformed to (-)-verbenol than (+)-2-pinene. In 3-carene we could not detect the allylic oxidation products, 3-caren-2-ol or 3-caren-5-ol but it may be reasonable that the rearrangement of either of these alcohols gave *m*-mentha-4,6-dien-8-ol[14], which again might aromatize to *m*-cymen-8-ol. The formation of 3-caren-9-ol shows the first example of the stereoselective hydroxylation of the *gem*-dimethyl group on the cyclopropane ring. The similar biooxidation of *gem*-dimethyl group has been known in the metabolites of (+)-camphor[15] and retinoic acid[16] in mammals.

Recently, Renwick et al.[17] and Southwell[18] reported the metabolites of pinenes in a bark beetle and in koala, respectively. On the basis of the comparison of these metabolites with our results, it is considered that the detoxication processes of pinenes in insect and in each mammal are considerably different. Moreover it is interesting that 3-methyl-2-cyclohexen-1-ol and verbenol have been found as pheromones of bark beetles[2), 19] and we are currently investigating this point.

Ackowledgement : The authors wish to express their thanks to Dr. Yoshio Hirose, The Institute of Food Chemistry for the MS measurement and to Yasuhara Perfumary Co. Ltd. for the administered monoterpene hydrocarbons.



Alcohol 1 : MS M^+ 112($C_7H_{12}O$); NMR(90MHz) 5.47(HC-2), 4.12(HCOH), 2.36(OH), 1.68(H_3C-7); IR(neat) 3350-1160-1140-10750-1060-1030-990-855-810; $[\alpha]_D$ +1.64°; Acetate : MS M^+ 154($C_9H_{14}O_2$); NMR 5.37(HC-2), 5.17(HC-OAc), 2.00(OAc), 1.67(H_3C-7).

Alcohol 2 : NMR(90MHz) 5.56(HC-3), 3.96(HCOH), 1.70(H_3C-7).

Alcohol 3 : NMR 5.35(HC-3), 4.28(HCOH), 1.72(H_3C-10), 1.33 & 0.87(H_3C-8 & H_3C-9); Acetate ; NMR 5.35(HC-3), 2.03(OAc), 1.73 (H_3C-10), 1.34 & 0.93(H_3C-8 & H_3C-9).

Alcohol 5 : NMR 5.00 & 4.80(H_2C-10), 4.45(HC-3), 1.32 & 0.67(H_3C-8 & H_3C-9); $[\alpha]_D$ +28.2°.

Alcohol 6 : MS 139(M^+-15); NMR 3.50(H_2C-10), 1.26 & 0.95(H_3C-8 & H_3C-9); $[\alpha]_D$ -21.4°.

Alcohol 8 : 7-Acetate-8-ol : NMR 5.75(HC-2), 4.48(H_2C-7), 2.08(OAc), 1.20(H_3C-9 & H_3C-10).

Alcohol 9 : MS M^+ 152($C_{10}H_{16}O$); UV λ_{max}^{EtOH} 264nm(log ε 2.95); $[\alpha]_D$ -116.1°; NMR (90MHz) 5.84, 5.64 & 5.57(HC-4, HC-5 & HC-6),1.80 (H_3C-7), 1.20(H_3C-8 & H_3C-9); $Eu(FOD)_3$(52.0mmol, Δδ-ppm) HC-3(2.57), H_2C-2(1.59 & 1.35), H_3C-9 & H_3C-10(2.08, 2.01).

Alcohol 10 : NMR 5.27(HC-4), 3.33(H_2C-9), 2.17(OH), 1.62(H_3C-10), 0.88(H_3C-8); IR($CHCl_3$) 3350-1430-1370-1240-1020-820-780.
Acetate : MS M^+ 194($C_{12}H_{18}O_2$); NMR(90MHz) 5.22(HC-4), 3.80(H_2C-9), 2.04(OAc), 1.60(H_3C-10), 0.84(H_3C-8).

Alcohol 11 : NMR 7.40-6.35(aromatic 4H), 2.35(H_3C-7), 1.55(H_3C-8 & H_3C-9).

Refernces and Notes

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