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# Metabolism of 1,8-Cineole in Rat: Its Effects on Liver and Lung Microsomal Cytochrome P-450 Systems

K. Madhava Madyastha* and Anju Chadha

Bio-organic Section, Department of Organic Chemistry, Indian Institute of Science, Bangalore-560 012, India

The monoterpene cyclic ether, cineole (1,8-cineole, I) also known as eucalyptol, is a component of many essential oils and is widely distributed in nature. It is extensively used in pharmaceutical preparations for external application and also as a nasal spray. It was reported earlier that cineole when administered to sheep may be largely oxidized in the system (Scheline 1978). However the mode of metabolism of cineole is not known. Hence the present study was undertaken to investigate the metabolic fate of this ubiquitous terpenoid following its administration to rats by gastric intubation.

Reports on the effects of cineole have shown that administration of this cyclic ether to rats either subcutaneously or by aerosol route even at very low concentrations, induced the hepatic microsomal enzymes to a significant extent (Jori et al. 1969; Cinti et al. 1976). Although the aerosol route of administration results in high absorption of volatile compounds by mucous membranes of the respiratory tract, there has not been any attempt to determine their effect on lung microsomal enzymes. Hence it was of interest to compare the effects of cineole on both hepatic and lung microsomal enzymes when administered by the aerosol route.

## MATERIALS AND METHODS

NADPH, DTT, cytochrome c, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, methyl cellulose, cineole were purchased from Sigma Chemical Co., USA. Cineole was distilled under reduced pressure before use and it was 99% pure as judged by g.l.c. analyses (Madyastha and Renganathan 1983).

*To whom correspondence should be addressed.

UV and visible absorption spectra were recorded on Hitachi 557 double wavelength double beam spectrophotometer. The infra red spectra were recorded on a Perkin Elmer model 397 or 599 spectrophotometer. NMR spectra were recorded with a Varian T-60 spectrometer.

Male albino rats (IISc., strain) weighing 150-200 g were used in the present study. For in vivo metabolic studies, cineole was administered (800 mg/kg body wt.) once daily for 20 days by gastric intubation as a suspension in 1% methyl cellulose solution. Control rats were given only the vehicle (4 ml/kg). After dosing, control and experimental rats were housed separately in metabolism cages with free access to food and water. Urine was collected in bottles maintained at 0-4°C. Urine samples collected daily for 20 days following oral administration of cineole were adjusted to pH 3-4 and extracted with ether. The aqueous portion containing conjugated metabolites was then subjected to acid hydrolysis and extracted with ether (Chadha and Madyastha 1984). Both the ether extracts were separated into neutral and acidic fractions. The total acidic fraction was methylated using diazomethane (Chadha and Madyastha 1984).

Thin-layer chromatographic (TLC) analyses (silica gel G) of the metabolites were carried out using hexane-ethyl acetate (8:2, system I; 7.5:2.5, system II; 7:3, system III). Metabolites were visualized either by exposing the TLC plates to iodine or by spraying with 1% vanillin in 50% conc. $H_2SO_4$. Separation and purification of the metabolites were accomplished by using a silica gel column and hexane-ethyl acetate as the eluent.

The rats used for the inhalation experiments were housed in an air tight desiccator (15 l) with an inlet connected to a series of bottles as described earlier to purify the air and to pass the vapours of the terpenoid under study (Hashimoto et al. 1972). The rats were exposed to the terpenoid throughout with a break of one hour, every twelve hours, for cleaning operations. Animals used as controls were placed under identical conditions except for the absence of cineole. A flow rate of 1 l/min was maintained. The concentration of terpenoid in the air was found to be $\sim$ 10.0 µmoles/hr as judged by GLC analyses.

Lung and liver microsomes from experimental and control rats were prepared as described earlier (Chadha and Madyastha 1982). Protein concentrations were determined by the method of Lowry et al. (1951). The concentrations of cytochromes P-450 and $b_5$ in the liver microsomes were measured by the procedure of Omura and Sato (1964), whereas the modified method of Matsubara et al.

(1974) was used for their estimation in lung microsomes. The NAD(P)H-cytochrome c reductase activity was determined as reported earlier (Chadha and Madyastha 1982).

## RESULTS AND DISCUSSION

The acid methyl esters (250 mg) were subjected to TLC (System I) which showed the presence of one major ($R_F$ 0.18) and three minor compounds. The methyl esters were separated on a silica gel column (20 g) using a mixture of hexane-ethyl acetate as the eluent. The methyl ester ($R_F$ 0.18) was eluted with ethyl acetate hexane (1:9, v/v) in the pure form. This compound showed the following spectral characteristics: IR (liquid film) $\nu_{max}$ 3300 $cm^{-1}$ (tertiary hydroxyl) and 1740 $cm^{-1}$ (an ester group). NMR($CDCl_3$) $\delta$: 1.1(3H,s,C-7), 1.3(3H,s,C-9), 1.5-2.3(11H, broad multiplet, on $D_2O$ exchange accounted for 9H indicating the presence of 2 hydroxyl protons and ring protons which includes methylene and tertiary protons), 3.5(3H,s,carbomethoxy group). Based on the above spectral data, the compound was identified as methyl ester of 1,8-dihydroxy-10-carboxy p-methane (III). The minor acid methyl esters could not be identified due to paucity of material.

The TLC analysis (system I) of the neutral fraction (200 mg) showed the presence of one major ($R_F$ 0.41) and two minor metabolites. This fraction was subjected to column chromatography over silica gel (20 g) and the major metabolite was eluted with ethyl acetate-hexane (2:8, v/v). IR spectrum (liquid film) of this compound indicated the presence of a hydroxyl function (3420 $cm^{-1}$) and a gem-dimethyl group (1480, 1330 $cm^{-1}$. NMR($CDCl_3$) $\delta$: 1.07(3H,s), 1.165(3H,s) and 1.23(3H,s) for protons of three saturated methyl groups, 1.33-2.2(8H,m) for seven methylene protons and one hydroxyl proton, 3.6 (1H,m) for one hydroxyl methine proton. This metabolite was characterized as 2-hydroxy cineole (IV). The spectral data of this compound corresponded well with the values reported earlier for 2-hydroxy cineole (MacRae et al. 1979).

The neutral fraction (500 mg) obtained from hydrolysed urine on TLC analysis (system III) revealed the presence of two major ($R_F$ 0.4 and 0.37) and two minor metabolites. This fraction was subjected to column chromatography over silica gel (25 g) and the column was eluted in succession with 2, 4, 6, 8, 10 and 12% ethyl acetate in hexane. The fraction eluted with 10% ethyl acetate in hexane showed spectral characteristics (IR and NMR) identical to those of 2-hydroxy cineole (IV). The compound which eluted with 12% ethyl acetate in hexane showed IR absorptions largely similar to those of 2-hydroxy cineole (IV). However, this compound showed

Table 1 Effects of inhalation of 1,8-cineole on rat hepatic and pulmonary enzyme systems

| Days of treatment | Cyt. P450 (n mole/mg) | | Cyt. $b_5$ (n mole/mg) | | NADPH Cyt. c reductase (n mole/min/mg) | | NADH Cyt. c reductase (n mole/min/mg) | |
|---|---|---|---|---|---|---|---|---|
| | Liver | Lung | Liver | Lung | Liver | Lung | Liver | Lung |
| Control | 0.58 ± 0.04 | 0.036 ± 0.004 | 0.71 ± 0.11 | 0.052 ± 0.003 | 87.7 ± 5.1 | 26.9 ± 1.9 | 498 ± 32 | 205 ± 51 |
| 3 | 0.77 ± 0.06 | 0.038 ± 0.002 | 0.72 ± 0.13 | 0.049 ± 0.002 | 90.0 ± 12.6 | 27.8 ± 2.4 | 484 ± 25 | 198 ± 47 |
| 6 | 1.13 ± 0.09 | 0.038 ± 0.004 | 0.81 ± 0.14 | 0.051 ± 0.003 | 88.2 ± 12.9 | 26.3 ± 1.3 | 471 ± 37 | 214 ± 21 |
| 9 | 1.19 ± 0.08 | 0.040 ± 0.004 | 0.82 ± 0.15 | 0.048 ± 0.004 | 89.5 ± 14.5 | 28.8 ± 3.1 | 487 ± 23 | 197 ± 41 |

All values represent mean ± S.D., N=4 sets, each set with six rats. The values of the control rats used for three, six and nine days did not differ appreciably from one another and hence they were combined and the mean value ± S.D. is presented in the table.

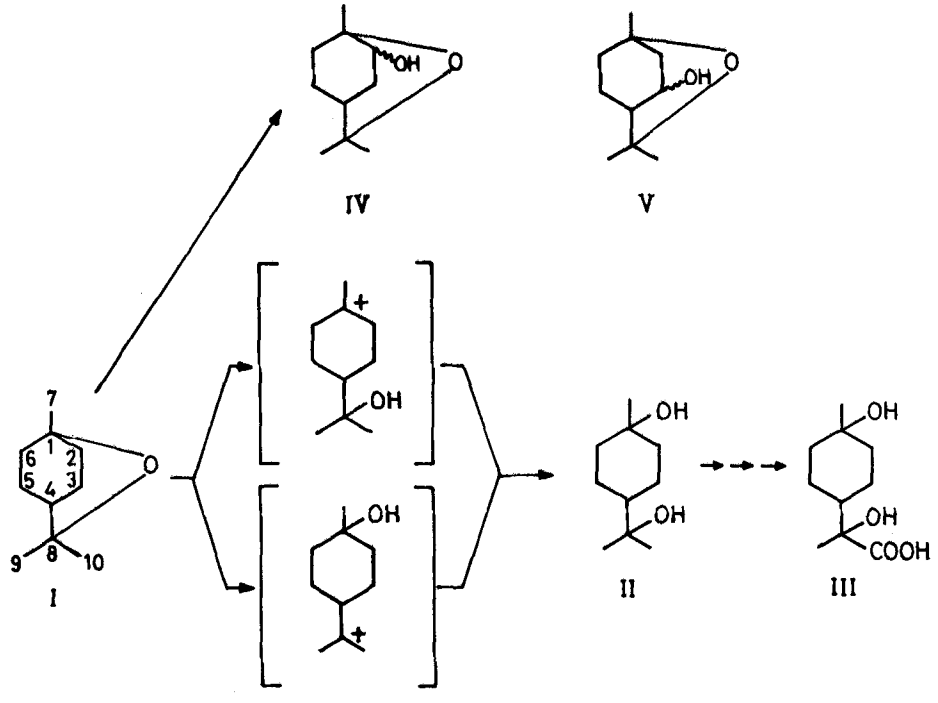


Fig. 1 Possible metabolic pathways of 1,8-Cineole in rats.

NMR signals different than that observed for 2-hydroxy cineole. The NMR spectrum ($CDCl_3$) δ: 1.07(3H,s), 1.2 (3H,s), 1.3(3H,s) - three different saturated methyl groups, 1.33-2.2(8H,m,seven methylene protons and one hydroxyl proton); 4.46(1H,m, one hydroxy methine proton). The spectral data is consistent with 3-hydroxy cineole (V) (Nishimura et al. 1982). The minor metabolites could not be identified.

Table 1 clearly indicates the effects of cineole on the pulmonary and hepatic microsomal cytochrome P-450 systems following its administration by the aerosol route. The hepatic cytochrome P-450 levels showed an increase of 32, 94 and 105% after 3, 6 and 9 days of pretreatment with this monoterpene cyclic ether. The cytochrome $b_5$ levels in the liver increased by 14% after six days of pretreatment and remained higher than the control values even at the end of nine days. In contrast to these observations, cineole did not elicit any significant effect on the lung microsomal enzymes (Table 1). The levels of NADPH and NADH cytochrome c reductases from both lung and liver microsomes seemed unaffected following the pretreatment with cineole by the aerosol route.

From the available evidences, it is rather difficult to predict the sequence of reactions taking place during the biotransformation of cineole. However, one can envisage the formation of 1,8-dihydroxy-10-carboxy-p-menthane (III) through the intermediacy of p-menthane-1,8-diol (II) and further metabolism is possibly initiated by the oxygenation of the C-10 methyl group resulting in the formation of p-menthane-1,8,10-triol which undergoes stepwise oxidation to the corresponding aldehyde and then to an acid (Fig. 1). The opening of the ether bridge in cineole could result in the formation of a p-menthanoid cation with a positive charge either at C-1 or C-8 which further gets readily neutralized by the attack of a hydroxide ion to yield p-menthane-1,8-diol (Fig. 1). The 2- and 3-hydroxy derivatives from cineole have been reported in bacterial (MacRae et al. 1979) and fungal systems (Nishimura et al. 1982) respectively. So it appears that the microbial systems are more specific while carrying out the hydroxylation of cineole unlike the mammalian system which hydroxylates at C-2 as well as C-3 position. Both these hydroxylated derivatives are excreted as conjugates.

Earlier workers (Jori et al. 1969) have reported the induction of hepatic microsomal enzymes after administration of cineole subcutaneously and by the aerosol route. But the quantitative change in the levels of hepatic cytochrome P-450 has not been determined. The

present studies have clearly demonstrated that administration of cineole by the aerosol route induces the liver microsomal cytochrome P-450 to a significant extent (Table 1). However, its levels in the lung microsomes do not show an apparent increase. The low response seen in the lung microsomes upon administration of cineole by aerosol route could be possibly due to the fact that the nasal mucosa absorbs cineole before it reaches the lung tissue and only a small amount of the terpenoids reaches the lungs which may not be enough to elicit a pronounced effect. The effects of cineole on the hepatic and lung microsomal enzymes seem to be very similar to those elicited by various acyclic monoterpene alcohols (Chadha and Madyastha 1984). However, cineole appears to be a better inducer of hepatic cytochrome P-450 than either geraniol or linalool (Chadha and Madyastha 1984).

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