the rhesus monkey fetus during late gestation whereas they are not inducible by this glucocorticoid in fetal rat liver. Hepatic 7-ethoxyresorufin-*O*-deethylase activity is not inducible by dexamethasone in the fetal monkey even though this monooxygenase activity is present in adult monkey liver and is inducible by dexamethasone in postnatal rat liver.

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Induction of rat liver microsomal cytochrome P-450 by muscone (3-methylcyclopentadecanone)

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Muscone (3-methylcyclopentadecanone) is the ordorous principle of musk, a product usually made from the musk glands of male musk deer. Musk is a component in many perfumes and is considered to be a precious ingredient in traditional Chinese medicine. Although musk has been used for thousands of years for the treatment of various diseases and malfunctions, its biological activities have not been characterized. It has been reported that pretreatment of rats with musk or muscone increases the clearance of pentobarbital and shortens pentobarbital-induced sleeping time [1]. Such effects have also been observed with mice (X. Zhu, unpublished observations). It has been suggested that the effects are due to the induction of liver microsomal enzymes [1]. However, direct evidence for such induction was not available. In this communication, we report the induction of rat liver microsomal cytochrome P-450 by muscone.

Materials and methods

Chemicals. Isocitrate dehydrogenase, DL-isocitric acid, NADP, NADPH, and metyrapone were obtained from the Sigma Chemical Co. (St. Louis, MO). *p*-Nitroanisole was from the Eastman Organic Co. (Rochester, NY). *N*-Nitrosodimethylamine was purchased from the Aldrich Chemical Co. (Milwaukee, WI). Chemicals received as gifts were: chemically synthesized muscone from the Shangdong Pharmaceutical Co. (Jinan, Shandong, China), ethylmorphine-HCl from Merck & Co. (Rahway, NJ), and benzphetamine-HCl from the Upjohn Co. (Kalamazoo, MI).

Animals and microsomes. Male Sprague–Dawley rats (body weight 50–65 g) were obtained from Taconic Farms, Germantown, NY. They were fed a commercial laboratory chow (Ralston Purina Co., St. Louis, MO) and water *ad lib.* Muscone was dissolved in corn oil and administered by a single intraperitoneal injection 22–24 hr before the animals were killed. The animals in the control group received the vehicle only. In other induction studies, the rats received a daily intraperitoneal injection of phenobarbital (75 mg/kg in saline) or 3-methylcholanthrene (25 mg/kg in corn oil) for 3 days. Liver microsomes were prepared by differential centrifugation and washed once with a solution containing 154 mM KCl and 10 mM EDTA as described previously [2]. The microsomal samples were

stored frozen in small portions at −80° prior to use.

Enzyme assays and biochemical analyses. Protein and P-450 contents, NADPH-cytochrome *c* reductase activity, as well as demethylase activities with benzphetamine, ethylmorphine, *p*-nitroanisole, and nitrosodimethylamine were determined by previous methods [2, 3]. Aryl hydrocarbon hydroxylase was assayed by measuring the phenolic products of benzo[*a*]pyrene fluorometrically as described earlier [4]. Epoxide hydrolase activity was measured by the procedure of Dansette *et al.* [5] with 2 μM benzo[*a*]pyrene-4,5-oxide as the substrate. The rate of the reaction at 37° was followed by monitoring the fluorescence (excitation at 310 nm and emission at 385 nm) increase with a recording Perkin–Elmer spectrofluorometer. Glutathione *S*-transferase was determined according to Habig *et al.* [6]. The reaction mixture contained 1 mM glutathione, 1 mM 1-chloro-2,4-dinitrobenzene, liver 9000 *g* supernatant fraction (0.2 to 0.3 mg protein), and 0.1 M potassium phosphate, pH 6.5, in a total volume of 1 ml. The reaction rate at 25° was monitored by absorbance changes at 340 nm. All these assays were carried out in duplicate, and the difference between duplicate runs was <10%. Difference spectra were recorded with either an Aminco DW-2a or a Cary 17 spectrophotometer. Gel electrophoresis was performed with a discontinuous slab gel system similar to that of Laemmli, as described previously [3].

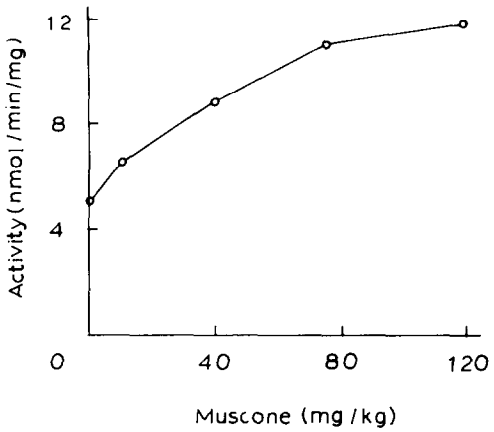

Fig. 1. Effect of different doses of muscone on the activity of liver microsomal benzphetamine demethylase. Muscone at doses indicated was given, i.p. 22 hr before sacrifice; each group consisted of four rats.

Results and discussion

Induction of P-450 and benzphetamine demethylase by muscone. Pretreatment of rats with muscone (75 mg/kg) for 1 day enhanced the microsomal benzphetamine demethylase activity 2.8-fold (Table 1). This was accompanied by a 54% increase in gross P-450 content and a slightly enhanced NADPH-cytochrome *c* reductase activity. The liver weight of the rat was not affected by the treatment but the microsomal protein yield from per gram liver was increased. Similar results were also observed in other experiments in our laboratories in both the United States and China.

The induction of microsomal benzphetamine demethylase by muscone was dose dependent. Significant induction was observed with 10 mg/kg, and it appeared to reach a plateau after 120 mg/kg (Fig. 1). The P-450 contents were also increased in a similar manner, showing a maximal 61% increase at 120 mg/kg. Slight induction in NADPH-cytochrome *c* reductase activity was also seen but a dose–response relationship was not established (data not shown). Significant growth alteration or signs of toxicity were not observed with the doses employed.

The induction was accompanied by the appearance or increase of a protein species with a molecular weight around 51,000. This is seen on electrophoresis gels in Fig. 2 by comparing Lanes C and D with Lanes A and B. The muscone-induced microsomal protein band pattern (e.g. Lanes C and D) was similar to that of phenobarbital-induced microsomes (Lane E) but dissimilar to that of 3-methylcholanthrene-induced microsomes (Lane F). These results are consistent with the idea that a P-450 species is induced by muscone. The intensity of this protein band appears to increase with the increasing dose of muscone (Lanes G to K), suggesting that the induction of this molecular species is dose dependent.

Spectral properties of the muscone-induced microsomes. The muscone-induced microsomes (in the presence of a reducing agent) showed a maximum at 450 nm in the carbon monoxide binding difference spectrum (data not shown). This property is similar to that of the phenobarbital-induced microsomes, but different from the 3-methylcholanthrene-induced microsomes which showed a maximum of 448 nm. To further characterize the muscone-induced P-450 species, the metyrapone-binding spectra of the microsomes were measured. Metyrapone has been reported to bind specifically to "phenobarbital-induced" P-450 isozymes showing a maximum at 446 nm in the binding spectrum [7, 8]. In our experiment, metyrapone binding peaks were observed with control, muscone-induced, and phenobarbital-induced microsomes, but the fraction of metyrapone-binding P-450 species differed markedly (data not shown). The control and phenobarbital-induced microsomes contained 12.4 and 52.0% of metyrapone-binding P-450 species, respectively, whereas the muscone-induced microsomes contained 57.7% of metyrapone-binding P-450 isozymes. With purified enzymes, Ryan *et al.* [9] reported that metyrapone bound to P-450b and P-450e, both phenobarbital-induced forms, but not to other isozymes examined. The presently observed result on metyrapone binding further delineates the similarity between the muscone- and phenobarbital-induced microsomes.

Table 1. Effects of muscone pretreatment on microsomal monooxygenase enzymes

Treatment	Microsomal protein (mg/g liver)	P-450 (nmoles/mg)	NADPH-cytochrome *c* reductase (units/mg)	Benzphetamine demethylase (nmoles/min/mg)
Control	12.93, 13.07	0.79, 0.81	216, 228	3.16, 3.36
Muscone	15.33, 16.33	1.19, 1.27	234, 254	8.79, 9.43

Rats were treated with muscone 75 mg/kg (one i.p. injection) 22 hr before being killed. Livers from two rats were pooled to make one microsomal sample. The values from these two samples are reported. One unit of NADPH-cytochrome c reductase activity corresponds to the reduction of 1 nmole cytochrome *c* per min at ambient temperature.

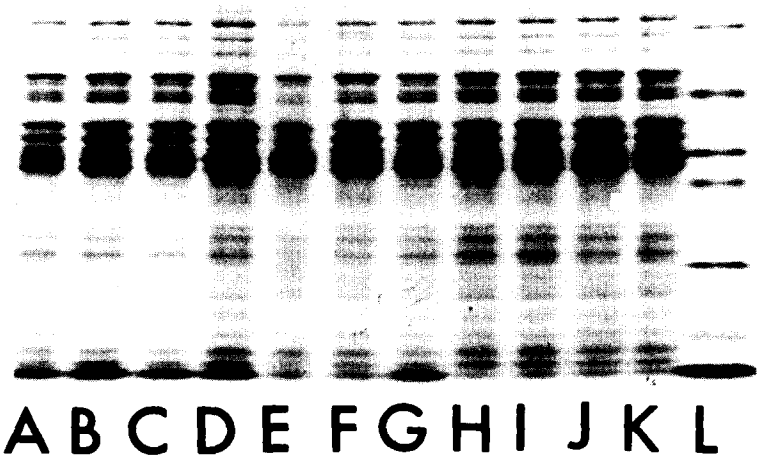

Fig. 2. Gel electrophoresis of microsomal proteins. The microsomal samples in different lanes are: control (Lanes A and B), muscone (75 mg/kg)-induced (C and D), phenobarbital-induced (E), and 3-methylcholanthrene-induced (F). Lanes G to K contain microsomes from rats treated with muscone at doses of 0, 10, 40, 75 and 120 mg/kg, respectively, corresponding to the results in Fig. 1. Lane L contains protein molecular weight standards: from bottom to top, α-lactalbumin (14,400 daltons at the buffer front), trypsin inhibitor (20,100), carbonic anhydrase (30,000), ovalbumin (43,000), P-450 (52,000), albumin (67,000) and phosphorylase b (94,000).

Effects of muscone pretreatment on other enzyme activities. To further examine the alteration of the microsomal monooxygenase system by muscone, the metabolism of other substrates was investigated (Table 2). Pretreatment with muscone increased the NADPH-dependent ethylmorphine and *p*-nitroanisole demethylase activities 1.7- and 1.6-fold respectively. Activities of aryl hydrocarbon hydroxylase and nitrosodimethylamine demethylase were also measured to assess the effects of muscone on the metabolism of carcinogens (Table 2). Aryl hydrocarbon hydroxylase was increased (by 31%) but nitrosodimethylamine demethylase was decreased (by 20%). Significant alterations in microsomal epoxide hydrolase and cytosolic glutathione *S*-transferase activities were not observed.

Binding to P-450 and inhibition of benzphetamine demethylase activity by muscone. When muscone was added to microsomes, a "type I" binding spectrum [10] with a peak of 391 nm and a trough at 422 nm was seen (data not

Table 2. Effect of muscone pretreatment on drug metabolism enzymes

Treatment	Ethylmorphine demethylase	*p*-Nitroanisole demethylase	Aryl hydrocarbon hydroxylase	Nitrosodimethylamine demethylase	Epoxide hydrolase	Glutathione *S*-transferase
Control	5.24	1.58	0.40	2.94	4.24	346
Muscone	9.09	2.59	0.53	2.34	3.09	372

Rats were treated with muscone (75 mg/kg, i.p.) 22 hr before being killed. Enzyme activities are expressed as nmoles/min/mg protein, and each value is the mean of two experiments. The variations between the two experiments were <10%. For assaying the demethylase activities, the incubation mixture contained 0.6 to 0.8 mg protein in 0.5 ml of incubation mixture and 5 mM ethylmorphine, 1 mM *p*-nitroanisole, or 4 mM nitrosodimethylamine. Conditions for other enzyme assays are described in Materials and Methods.

shown). A similar "type I" binding spectrum can also be produced by the addition of muscone to purified P-450 prepared from phenobarbital-treated rats as described previously [11]. The results suggested that muscone is similar to many "type I" substrates of the monooxygenase system in binding to P-450 and causing a low- to high-spin transition of the heme group [11, 12]. The metabolism of muscone by microsomes was not measured. When added to the incubation mixture, muscone was found to be an inhibitor of benzphetamine demethylase and a 50% inhibition was observed with about 7 mM muscone (Fig. 3).

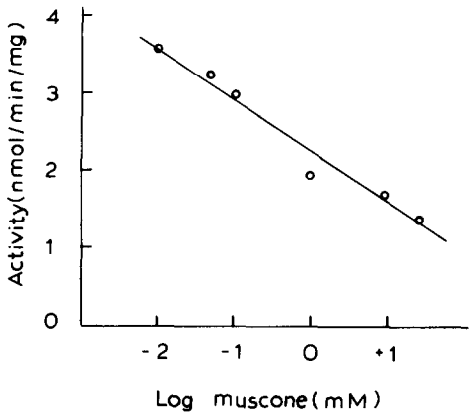

Fig. 3. Inhibition of benzphetamine demethylase activity by muscone *in vitro*. The assay mixture contained liver microsomes (0.6 mg protein) from control rats and 1 mM benzphetamine. Muscone at the concentrations indicated was added in 10 μl of acetone in a 0.5-ml incubation mixture.

Many environmental chemicals are known to affect the microsomal monooxygenase system [13, 14]. Depending upon the chemicals used, different forms of P-450 isozymes can be induced; these isozymes are different in their primary structure, catalytic activity, and other properties [14]. The present report demonstrates that muscone is also an inducer of this system. It appears that muscone induces a P-450 species that is similar to the phenobarbital-inducible P-450 isozymes. This tentative conclusion is supported by studies on substrate specificity, carbon monoxide and metyrapone binding spectra, and molecular weight. Phenobarbital is known to induce more than one form of P-450 in rats [9, 14]. It is not known whether this is also the case with muscone. The mechanism of P-450 induction by muscone (as well as its relationship to phenobarbital induction) remains to be investigated. Concerning other microsomal enzymes, muscone does not induce epoxide hydrolase, a property differing from that of phenobarbital [15]. When added to the incubation mixture, muscone inhibits microsomal benzphetamine demethylase activity and presumably also other monooxygenase activities. However, because of the rather high concentration needed for this incubation, muscone is not considered to be a potent inhibitor of the microsomal monooxygenase system.

In comparison to the amount of muscone required for the induction, the quantities of human exposure due to perfume appear to be low. Muscone has been used recently in the treatment of coronary disease in China at doses of 3–5 mg per administration (to adults of body weight from 45 to 75 kg; the result of the clinical trial has not been published). If we assume that humans and rats have the same response to muscone, a single dose of this compound is also too low to have any significant effect on P-450 isozymes. There is no published report on the doses of muscone commonly used in traditional Chinese medicine or on whether its use would affect the microsomal enzymes in humans. The present report suggests the possibility that, when used at high doses, muscone may induce certain P-450 isozymes which may, in turn, alter the metabolism of drugs and endogenous substrates.

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