or ascites plus normal mouse serum showed marked suppression (experiment 2, H and I).

In an attempt to characterize the immunosuppressive factor, we found that: (i) It was not virus (*7*), because ultracentrifugation at 100,000*g* for 50 minutes did not reduce its effect. (ii) It did not appear to be antigen or antibody (*8*), because there was no absolute antigenic specificity; for example, human breast carcinoma effusion had a similar effect and it did not precipitate with the γ-globulin fraction. Also, ascites fluid did not produce tumor immunity (data not shown). (iii) It was probably not α-globulin (*9*), since α-globulin levels were not elevated in the ascites, as compared to normal mouse serum. After precipitation with 50 percent ammonium sulfate, most of the reactivity was found in the second fraction (supernatant fraction) (Table 1, experiment 3). This reactive fraction was excluded from Sephadex G-50, and sodium dodecyl sulfate (SDS) gel electrophoresis showed three distinct protein bands: the major one (more than 90 percent) appeared to be albumin, and there were two weaker bands with molecular weights between 40,000 and 60,000. The immunosuppressive factor might be in one or both of these two weaker bands although at this stage of purification the protein would probably not be visible on the gel.

Previous studies of immunosuppressive factors were usually conducted with systems in vitro or did not deal directly with tumor immunity (*7–9*). Our studies indicate that a humoral factor with two distinct and possibly related biological activities can be demonstrated in tumor-bearing hosts, both man and mouse: tumor growth promotion and immunosuppression. Excessive production or inability to inhibit the production of these factors is correlated with progressive tumor growth in the tumor model studied here. On the other hand, the hosts that have escaped the immunosuppressive effect produce substances that neutralize the immunosuppressive factors and such counter factors may play an important role in the control of tumor growth.

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References and Notes

1. L. Thomas, in *Cellular and Humoral Aspects of Hypersensitive State*, H. S. Lawrence, Ed. (Holber, New York, 1959), p. 259.
2. J. P. Glynn, J. L. McCoy, A. Fefer, *Cancer Res.* **28**, 434 (1968).
3. The benign ascites fluid was collected from mice that did not develop the plasma cell tumors that usually result from pristane injection.
4. C. C. Ting, G. S. Bushar, D. Rodrigues, R. B. Herberman, *J. Immunol.* **115**, 1351 (1975); C. C. Ting, D. Rodrigues, G. S. Bushar, R. B. Herberman, *ibid.* **116**, 236 (1976); C. C. Ting, H. Kirchner, D. Rodrigues, J. Y. Park, R. B. Herberman, *ibid.*, p. 244.
5. C. C. Ting, *Cancer Res.* **36**, 3695 (1976).
6. ——— and G. D. Bonnard, *J. Immunol.* **116**, 1419 (1976).
7. R. G. Morteusen, W. S. Ceglowski, H. Friedman, *ibid.* **112**, 2077 (1974); G. D. Bonnard, E. K. Manders, D. A. Campbell, Jr., R. B. Herberman, M. J. Collins, Jr., *J. Exp. Med.* **143**, 187 (1976).
8. I. Hellström and K. E. Hellström, *Int. J. Cancer* **4**, 587 (1969); R. W. Baldwin, J. G. Bower, M. R. Price, *Br. J. Cancer* **28**, 16 (1973); B. Bonnavida, *J. Immunol.* **112**, 926 (1974); R. W. Baldwin, M. R. Price, R. A. Robins, *Nature (London)* **238**, 185 (1972).
9. S. R. Cooperband, A. M. Badger, R. C. Davis, K. Schmid, J. A. Mannick, *J. Immunol.* **109**, 154 (1972).
10. We thank R. Braylan for reviewing the histological specimens, E. Chu for supplying the pleural effusions, M. Potter and J. Pumphrey for supplying the benign ascites fluid, J. Dean of Bionetic Research Laboratories for preparing the assay with α-globulin, and L. Bruson for preparing the manuscript.

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Perilla Ketone: A Potent Lung Toxin from the Mint Plant, *Perilla frutescens* Britton

Abstract. *Perilla ketone, from the essential oil of* Perilla frutescens, *is a potent pulmonary edemagenic agent for laboratory animals and livestock. This finding would account for reported effects of the plant on grazing cattle. The use of perilla in oriental foods and medicinal preparations suggests possible hazards to human health as well.*

The discovery in our laboratory of potent lung-toxic 3-substituted furans in mold-damaged sweet potatoes (*Ipomoea batatas*) led to investigations of other naturally occurring compounds in this class. Of special interest were those produced in plants associated with foods of animals and man. The close chemical similarity of perilla ketone to the lung-toxic ipomeanols from sweet potatoes indicated that the former compound would likely exhibit similar toxic properties. We have found that both perilla ketone and crude extracts from the plant source *Perilla frutescens* show potent lung toxicity for experimental animals. This fact should serve to explain reported outbreaks of atypical pulmonary emphysema in cattle that grazed the plant and points to possible health hazards for humans using the plant or its essential oil in oriental food and medicinal preparations.

Perilla frutescens, known in the United States as "purple mint plant," "beefsteak plant," "perilla mint," and "perilla," is an import from Asia that was, apparently, a garden escapee and became widely distributed in midwestern, eastern, and southern United States (*1*). *Perilla frutescens* and related species are also found in certain European countries and have been used extensively in plant biochemistry and physiology studies in the United States (*2*), the Soviet Union (*3*), and elsewhere (*4*).

In Japan, species of *Perilla* and related plants grow wild, and several varieties are cultivated for various uses (*5*). The anthocyanins of highly tinted perilla leaves are extracted and used as coloring agents for green plums. Intact leaves may be used as condiments or flavoring agents in a variety of human foods and are often included in preparations of tempura deep-fried with batter. Perilla seeds are available as bird feed at pet shops in Japan and are used to flavor various foods, including pickled ginger. Volatile (essential) oil from the leaves and other parts of the plant (particularly *P. frutescens*, var. *crispa*, *forma viride*) is known in Japan as "Ao-shiso"; in 1958–59, approximately 5 to 7 metric tons of perilla oil were produced in Japan (*5*). "Ohara shiso" (variety *crispa* Decaisne) is reported to be the source of the Chinese drug called "soyo" that reportedly has several therapeutic applications in humans (*6*).

Perilla seed oil, which is commercially available, has been evaluated as a drying oil for paints and lacquers (*7*). Perilla seed oil cake, or meal, has been used both as a fertilizer and a high protein animal feed (*8*).

The volatile oils of different species and varieties of *Perilla* vary considerably as to main constituents. In some varieties of *P. frutescens*, 1-perillaldehyde is the principal component imparting a pungent aroma to the oil (*9*). The α-*syn*-oxime of 1-perillaldehyde (*10*), called perillartine, is a potent sweetening agent often added to Japanese tobacco. Other varieties may contain perilla ketone (**1**) (*11*), egomaketone (**2**) (*12*), and isoegomaketone (**3**, Fig. 1) (*13*) as predominant compounds in the plant essential oil. Chemical structures of these 3-substi-

tuted furans have been confirmed by synthesis (*14*). Their close chemical similarity to 4-ipomeanol (**4**, Fig. 1), a potent lung edemagenic agent for mold-damaged sweet potatoes (*15*), is quite evident. 2-Substituted furans of undetermined toxicity also have been noted in certain species of *Perilla* (*16*).

In the state of Oklahoma, *P. frutescens* has been implicated as a causative agent of a lethal respiratory disease syndrome (*17*) of cattle, a disease variously known as acute bovine pulmonary emphysema (ABPE), pulmonary adenomatosis, and atypical interstitial pneumonia. In Britain the term "fog fever" is commonly used to describe similar conditions in grazing animals (*18*). ABPE is apparently attributable to more than one causative agent since mold-damaged sweet potatoes (*Ipomoea batatas*) also are now well recognized as causing the disease (*15, 19*).

Several dry specimens of *P. frutescens* were collected for analysis in December 1976 in the vicinity of Nashville, Tennessee, along the banks of streams and in other low lying areas. The material consisted of leafless stems and infructescences with intact calyces, some of which contained the fruit nutlets. Ether extracts of the dried material contained perilla ketone in low concentration, as determined by gas-liquid chromatography, and the residues caused death of mice when administered by stomach tube or by intraperitoneal injection. The pulmonary disease found upon postmortem examination was essentially identical to that caused by 4-ipomeanol, consisting of extensive intra-alveolar edema and pleural effusion. Perilla ketone was not detected, however, in ether extracts of one sample of perilla seeds collected several months earlier in Oklahoma and of three taxonomically different samples commercially available in Japan. Japanese investigators, however, have reported finding compounds **1** and **3** in perilla seeds (*20*).

We have synthesized perilla ketone and determined the toxic reactions and values for the minimal lethal dose (LD_{50}) in both Notre Dame strain white mice and Swiss-Webster rats. The pulmonary response in mice was indistinguishable from that caused by 4-ipomeanol (*21*) and certain other 3-substituted furans. In addition to typical lung toxicity in rats, this species also showed evidence of peritoneal inflammation in response to intraperitoneal injection. The intraperitoneal LD_{50}'s in mice were approximately 6 and 2.5 mg/kg for males and females, respectively. The lethal oral doses in mice were

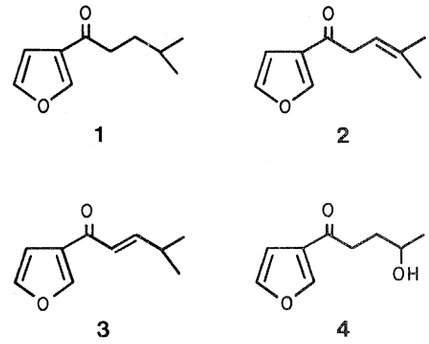

Fig. 1. Structures of the 3-substituted furans (**1, 2, 3**) from *Perilla frutescens* and *Ipomoea batatas* (**4**).

also proportionately low. The intraperitoneal lethal dose for the male rat was 10 mg/kg. The low values in mice indicate approximately a sixfold greater potency of perilla ketone compared with 4-ipomeanol by intraperitoneal injection. Although we have not yet studied egomaketone and isoegomaketone, it would not be surprising, on the basis of initial pulmonary toxicity studies of several closely related 3-substituted furans, if these constituents of perilla oil should also prove to be lung toxic.

Synthetic perilla ketone, diluted with dimethyl sulfoxide, was injected through the abdominal wall directly into the rumens of two Angus heifers weighing approximately 320 kg each. The first animal received approximately 3 mg/kg and the second 9 mg/kg; both doses were without observable adverse affects. However, in a subsequent experiment in which a 272-kg heifer was injected intravenously with 8 g of perilla ketone (approximately 30 mg/kg), the animal became ill within 10 hours and developed severe respiratory distress that led to death on day 3 after toxin injection. Postmortem examination revealed hepatization of the lungs and other characteristics of acute interstitial pneumonia with extensive pleural effusion.

Intravenous injection of 1 g of perilla ketone into a 56-kg male sheep (19 mg/kg) was followed by respiratory distress in the animal on day 3. By day 5, some improvement in the condition of the animal was noted, and it was killed for examination. The lungs, appearing to be the primary targets of the toxin, exhibited capillary congestion with intra-alveolar edema. The pulmonary studies with bovines, however, suggest they are probably less susceptible to the ill effects of perilla ketone than to 4-ipomeanol (*22*). The reverse situation obtains in laboratory rodents (*21*).

Our observations indicate that *P. frutescens* is potentially a major causative agent of ABPE for cattle in the United States and other countries where the plant grows wild. This toxicity can be attributed to perilla ketone, and in all probability the other 3-substituted furans contained in the essential oil of the plant will also be implicated. More important, use of this plant in human foods in Oriental countries should be questioned because of the obvious potential hazards to health.

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References and Notes

1. M. L. Fernald, in *Manual of Botany*, A. Gray, Ed. (American Book, New York, ed. 8, 1950), p. 1251.
2. T. L. Mounts and H. J. Dutton, *J. Am. Oil Chem. Soc.* **41**, 537 (1964).
3. S. V. Rushkovskii, *Biokhim. Fiziolog. Maslich. Rast.* **2**, 15 (1967).
4. T. Krzywacka and T. Rylska, *Acta Soc. Bot. Pol.* **29**, 11 (1960).
5. S. Arctander, *Perfume and Flavor Materials of Natural Origin* (Rutgers Univ. Press, Elizabeth, N.J., 1960), p. 520.
6. Y. Nagao, T. Komiya, S. Fujioka, T. Matsuoka, *J. Takeda Res. Lab.* **33**, 111 (1974) [as cited in *Chem. Abstr.* **81**, 117072p (1974)].
7. C. W. A. Mundy, *Oil Colour Trades J.* **91**, 917 (1937).
8. F. B. Morrison, *Feeds and Feeding* (Morrison, Ithaca, N.Y., ed. 22, 1956), p. 500.
9. E. Guenther, *The Essential Oils* (Van Nostrand, New York, 1949), vol. 3, p. 687.
10. E. M. Acton, H. Stone, M. A. Leaffer, S. M. Oliver, *Experientia* **26**, 473 (1970).
11. Y. Sebe, *J. Chem. Soc. Jpn.* **64**, 1130 (1943).
12. T. Ueda and Y. Fujita, *Chem. Ind. (London)* (1962), p. 1618.
13. H. Ito, *Yakugaku Zasshi* **84**, 1123 (1964).
14. J. E. Garst and B. J. Wilson, unpublished data.
15. B. J. Wilson, M. R. Boyd, T. M. Harris, D. T. C. Yang, *Nature (London)* **231**, 52 (1971).
16. Y. Fujita and T. Ueda, *Chem. Ind. (London)* (1960), p. 236.
17. D. T. Peterson, *Proceedings of the Symposium on Acute Bovine Pulmonary Emphysema (ABPE)* (University of Wyoming, Laramie, 1965), pp. R1–R13.
18. I. E. Selman, A. Wiseman, R. G. Breeze, H. M. Pirie, *Vet. Rec.* **99**, 181 (1976).
19. J. C. Peckham, F. E. Mitchell, O. H. Jones, Jr., B. Doupnik, *J. Am. Vet. Med. Assoc.* **160**, 169 (1972).
20. I. Hiroshi, *Yakugaku Zasshi* **84**, 1123 (1964).
21. M. R. Boyd, L. T. Burka, T. M. Harris, B. J. Wilson, *Biochim. Biophys. Acta* **337**, 184 (1974).
22. A. R. Doster, F. E. Mitchell, B. J. Wilson, unpublished data.
23. We thank Dr. D. Peterson for information on perilla poisoning of cattle and for a generous sample of perilla seeds. Appreciation is also expressed to Drs. Kazubo, Samejima and Junichiro Samejima for information on perilla varieties and for seed samples from Japan. Supported by a Center in Environmental Toxicology grant (ES 00267) and a research grant from the National Institute of Environmental Health Sciences (ES 00569-12), U.S. Public Health Service, to Vanderbilt University.

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