Natural Toxicants in Human Foods: Psoralens in Raw and Cooked Parsnip Root
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fertility. Nonetheless, these hybrids have been successfully backcrossed to the parental *Nicotiana* species, particularly with NN + Su/Su plants as female parents. Only one clone of NN + Su/Su could be self-fertilized. Although seed viability was low, germinating seed has been recovered in all cases and has segregated for the Su leaf pigmentation character (Table 2). The data obtained thus far are consistent with segregation ratios expected in amphiploid interspecific hybrids of *Nicotiana* (*14*). The Su locus of *N. tabacum* is associated with the formation of single and double spots on light green Su/su *N. tabacum* plants (*15*). The appearance of double spots (dark green area adjacent to albino) on the light green surface of both NN + Su/Su and NSt + Su/Su plants implies genetic recombination between the wild species and the *N. tabacum* genome. Such recombination may facilitate introgression of traits such as disease resistance into cultivated tobacco. In addition, double spots have been observed on all light green plants of NN + Su/Su backcrossed to *N. nesophila* or to *N. tabacum*, but as in *N. tabacum*, have not been observed on dark green plants. Consequently, the constancy of genetic behavior of the Su locus and segregation of the Su gene in backcross and self-fertilized progeny of NN + Su/Su provide additional verification that the protoplast-derived light green plants are somatic hybrids.

No NR + Su/Su somatic hybrids have been recovered. This is consistent with the results of ovule culture techniques (*4*). The NR + Su/Su cell hybrids were observed to undergo mitosis, but no light green shoots were isolated. The NN + Su/Su and NSt + Su/Su somatic hybrids will be screened for resistance to other diseases. Production of somatic hybrids may complement other novel methods utilizing plant protoplasts to incorporate genetic variability into crop species (*16*).

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References and Notes

1. J. R. Stavely, in *Nicotiana: Procedures for Experimental Use*, R. D. Durbin, Ed. (U.S. Department of Agriculture, Washington, D.C., 1979), p. 87.
2. ———, G. W. Pittarelli, L. G. Burk, *J. Hered.* **64**, 265 (1973).
3. G. W. Pittarelli and J. R. Stavely, *ibid.* **66**, 311 (1978).
4. S. M. Reed and G. B. Collins, *ibid.* **69**, 76 (1966).
5. L. G. Burk and H. E. Heggestad, *Econ. Bot.* **20**, 76 (1966).
6. D. A. Evans, *Z. Pflanzenphysiol.* **95**, 459 (1979).
7. O. L. Gamborg, J. P. Shyluk, L. C. Fowke, L. R. Wetter, D. A. Evans, *ibid*, p. 255.
8. D. A. Evans, L. R. Wetter, O. L. Gamborg, *Physiol. Plant.* **48**, 255 (1980).
9. K. N. Kao and M. R. Michayluk, *Planta* **126**, 105 (1975).
10. H. H. Smith, K. N. Kao, N. C. Combatti, *J. Hered.* **67**, 123 (1976); P. Maliga, G. Lazar, F. Joo, A. H. Nagy, L. Menczel, *Molec. Gen. Genet.* **157**, 291 (1977).
11. L. O. Kunkel, *Phytopathology* **24**, 437 (1934).
12. F. O. Holmes, *ibid.* **28**, 553 (1938).
13. J. F. Chaplin, D. F. Matzinger, T. J. Mann, *Tob. Sci.* **10**, 81 (1966).
14. D. U. Gerstel, *Genetics* **45**, 1723 (1960).
15. D. A. Evans and E. F. Paddock, *Can. J. Genet. Cytol.* **18**, 57 (1976).
16. J. F. Shepard, D. Bidney, E. Shahin, *Science* **208**, 17 (1980).
17. We thank Joan Markiewicz for preparation of Fig. 1 and Drs. S. M. Reed and W. R. Sharp for helpful comments. Supported by USDA grant 7900065 and a SUNY UAC grant to D.A.E.

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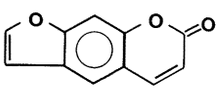
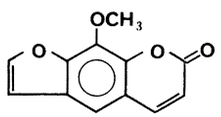
Natural Toxicants in Human Foods: Psoralens in Raw and Cooked Parsnip Root

Abstract. *Parsnip root contains three photoactive, mutagenic, and photocarcinogenic psoralens in a total concentration of about 40 parts per million. These chemicals are not destroyed by normal cooking procedures (boiling or microwave); thus humans are exposed to appreciable levels of psoralens through the consumption of parsnip and possibly other psoralen-containing foodstuffs. The toxicologic consequences to man of such exposure may be speculated on the basis of medicinal and laboratory studies, but epidemiologic data are not available.*

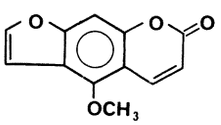
Man is exposed to thousands of natural and synthetic chemicals from a great number of environmental and nutritional sources. Many of these chemicals are necessary to sustain life itself, while others are harmless considering the levels encountered, their inherent toxicological properties, and the usually efficient array of detoxication and excretory mechanisms that act upon them in man. Still other chemicals represent significant toxicological hazards, and their interaction with man may result in clear and readily definable dangers such as the possibility of acute poisonings, effects on reproduction or other body functions, and genetic effects (mutations). The potential significance to man of naturally occurring toxicants in foods may be equal to or greater than that of man-made chemicals and must not be overlooked. We report here that roots of parsnip, *Pastinaca sativa*, a vegetable available in most supermarkets, contain appreciable levels of three phototoxic, mutagenic, and photocarcinogenic linear furocoumarins (psoralens) and that these chemicals are not destroyed by normal cooking procedures. Thus, humans are exposed to psoralens in the diet, with so far undefined toxicological consequences.



Psoralen



Xanthotoxin



Bergapten

Fig. 1. Linear furocoumarins (psoralens) from parsnip root.

California-grown parsnip roots were obtained from a local supermarket, they were washed thoroughly in tap water, and the ends and crowns were cut off. Each root was quartered lengthwise, then cut crosswise into approximately 1-cm pieces. Samples (100 g each) were cooked by boiling in water or by microwave radiation until tender (*1*). The cooked samples, as well as samples of uncooked parsnip root, were homogenized in water and extracted five times with ethyl acetate. Portions of the extracts equivalent to 5 g of parsnips were then analyzed by thin-layer chromatography (TLC) (*2*) for resolution of the psoralens present. Gel areas on the developed plates corresponding to authentic psoralen, xanthotoxin (8-methoxypsoralen), and bergapten (5-methoxypsoralen) (Fig. 1), as visualized under long-wavelength ultraviolet light, were eluted with diethyl ether and then subjected to gas-liquid chromatography (GLC) for quantitative measurements (*3*). The identities of the three psoralens

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Table 1. Concentrations of linear furocoumarins (psoralens) in parsnip and carrot root.

Sample	Concentrations (ppm ± standard deviation)		
	Psoralen	Xanthotoxin	Bergapten
	Parsnip root (whole)		
Raw	10.5 ± 0.5	26.1 ± 1.8	3.2 ± 0.3
Boiled	11.8 ± 1.8	28.8 ± 2.4	4.1 ± 0.4
Microwave	10.7 ± 0.6	27.9 ± 1.6	3.3 ± 0.5
	Carrot root (whole, raw)		
Texas	< 0.3	< 0.3	< 0.3
California	< 0.3	< 0.3	< 0.3

in the parsnip root extracts were confirmed by GLC–mass spectrometry (*4*).

Raw parsnip root contains appreciable levels of psoralen, xanthotoxin, and bergapten, with a cumulative concentration approaching 40 ppm (Table 1). Further, cooking parsnip root either by boiling or microwave radiation has no detectable effects on the concentration of these three psoralens (Table 1).

Because parsnip root might routinely be peeled or scrubbed before being eaten by humans, we considered the possibility that psoralens might be concentrated in the root epidermis. Analysis of parsnip root peelings (obtained with a common vegetable parer) showed that, in fact, levels of psoralens are substantially higher in the peeling than in the peeled root. However, such peelings constitute only a small fraction of the root, and peeling removed only about 30 percent of the amount of psoralens present. Thus, human exposure to these chemicals can be reduced somewhat, but not totally avoided by peeling the root before it is consumed as food.

Studies with carrot (*Daucus carota*), a vegetable related to parsnip, showed that raw whole carrot root apparently contains little if any psoralen, xanthotoxin, or bergapten. In the two samples analyzed, one California grown and the other Texas grown—both obtained from a local supermarket—concentrations of these psoralens were < 0.3 ppm, the sensitivity of the GLC method used (Table 1).

Psoralens occur in many plant species from several families (*5*, *6*), and they have been detected in different parts of some vegetables of the family Umbelliferae, including parsnip (*7*), celery (*8*), and parsley (*9*). There have been several documented cases of toxicological interactions with man attributed to psoralens in foods, but to our knowledge these episodes have been restricted to a form of photoinduced dermatitis among vegetable handlers and processors (*5*). Psoralens are potent photosensitizers and are highly mutagenic in the presence of activating long-wavelength ultraviolet light. They readily intercalate into DNA strands, where they form light-induced mono- or di-adducts with pyrimidine bases (*10*). Psoralens in the presence of light induce melanization in human skin, and they are used medicinally in the treatment of skin depigmentation (*10*). These chemicals are similarly effective in the treatment of psoriasis (*11*). Psoralens are photocarcinogenic in laboratory mammals (*12*), and there are strong indications of photocarcinogenicity associated with the medical use of psoralens in man (*13*). Psoralens at moderate levels are acutely toxic to mammals and certain other organisms even in the absence of light (*14*), and it is now known that psoralens are mutagenic in the dark (*15*). Recently, some investigators have concluded that psoralens present risks of sufficient magnitude to man such that medically unnecessary exposures should be avoided (*16*).

On the basis of the amounts of psoralens found in the parsnip samples studied here, it is apparent that consumption of moderate quantities of this vegetable by man can result in the intake of appreciable amounts of psoralens. Consumption of 0.1 kg of parsnip root could expose an individual to 4 to 5 mg of total psoralens, an amount that might be expected to cause some physiological effects under certain circumstances (*17*). In addition, some plants, including parsnip and celery, produce high concentrations of psoralens as phytoalexins in response to disease infection, and levels of psoralens in such cases can reach hundreds and perhaps thousands of parts per million (*8*, *18*). The effects of species variety or strain, stress agents, and other factors on the psoralen content of human food plants remain largely undefined; thus, we know very little at this time regarding the extent of human dietary exposure to these compounds.

Because of the known toxic, photoactive, mutagenic, and photocarcinogenic properties of psoralens, parsnip and other psoralen-containing food plants may present some toxicological risk to man. We believe, however, that it is not possible to accurately assess that risk, if it exists, on the basis of information available to date. Epidemiologic studies coupled with measurement of psoralens in consumed foodstuffs might provide such information.

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References and Notes

1. Samples (in 1000-ml glass beakers) were either boiled in 150 ml of water for about 10 minutes, or were cooked in a microwave oven without added water for 2.5 minutes.
2. Thin-layer chromatography was accomplished with silica gel chromatoplates (20 by 20 cm, 0.25-mm gel thickness, with fluorescent indicator, Brinkmann Silplate F-22) developed twice in chloroform. The R_F values were as follows: psoralen, 0.30; xanthotoxin, 0.19; bergapten, 0.26. A sample of psoralen was kindly supplied by M. A. Pathak, Harvard Medical School, Boston, Mass. Xanthotoxin was obtained commercially (Biochemical Laboratories, Redondo Beach, Calif.), and bergapten was that described in a previous study [G. Ivie, *J. Agric. Food Chem.* **26**, 1394 (1978)].
3. Gas-liquid chromatographic studies were done on a Tracor instrument with a flame ionization detector. The glass column (4 mm, inside diameter, by 1.68 m) was packed with 3 percent XE-60 on Gas Chrom Q and was heated isothermally at 165°C (psoralen) or 191°C (xanthotoxin and bergapten). Nitrogen carrier gas was approximately 100 ml/min. Retention times were as follows: psoralen, 4.9 minutes; xanthotoxin, 4.5 minutes; bergapten, 4.5 minutes. Although xanthotoxin and bergapten exhibited identical GLC retention times, this did not pose analytical problems because they were initially resolved on TLC.
4. Electron impact mass spectra were kindly recorded by J. Carter Cook, University of Illinois, Urbana.
5. M. Pathak, F. Daniels, Jr., T. Fitzpatrick, *J. Invest. Dermatol.* **39**, 225 (1962); G. Ivie, in *Effects of Poisonous Plants on Livestock*, R. Keeler, K. Van Kampen, L. James, Eds. (Academic Press, New York, 1978), p. 475.
6. R. Murray, *Fortschr. Chem. Org. Naturst.* **35**, 199 (1978).
7. I. Fahmy, *J. Pharm. Pharmacol.* **8**, 653 (1956); T. Sonie, *J. Am. Pharm. Assoc.* **45**, 426 (1956); W. Steck, *Can. J. Chem.* **47**, 3577 (1967).
8. L. Scheel, V. Perone, R. Larkin, R. Kupel, *Biochemistry* **2**, 1127 (1963).
9. L. Musajo, G. Caporale, G. Rodighiero, *Gazz. Chim. Ital.* **84**, 870 (1954); G. Rodighiero and G. Allegri, *Farmaco* **14**, 727 (1959).
10. B. Scott, M. Pathak, G. Mohn, *Mutat. Res.* **39**, 29 (1976).
11. E. Van Scott, *J. Am. Med. Assoc.* **235**, 197 (1976).
12. R. Hakim, A. Griffin, M. Knox, *Arch. Dermatol.* **82**, 522 (1960); P. Forbes, R. Davies, F. Urback, *Food Cosmet. Toxicol.* **14**, 303 (1976).
13. B. Reed, *Acta Derm. Venereol.* **56**, 315 (1976); R. Stern, L. Thibodeau, R. Kleinerman, J. Parrish, T. Fitzpatrick, *N. Engl. J. Med.* **300**, 809 (1979).
14. A. ElMofty, *J. Egypt. Med. Assoc.* **31**, 651 (1948); E. Spath and F. Kuffner, *Monatschrift* **69**, 75 (1936); M. Lauger, H. Martin, P. Muller, *Helv. Chim. Acta.* **27**, 71 (1944); T. Yajima, N. Kato, K. Munakata, *Agric. Biol. Chem.* **41**, 1263 (1977); M. Berenbaum, *Science* **201**, 532 (1978).
15. B. Bridges and R. Mottershead, *Mutat. Res.* **44**, 305 (1977); C. Clarke and M. Wade, *ibid.* **28**, 123 (1975).
16. M. Ashwood-Smith, G. Poulton, M. Barker, M. Mildenberger, *Nature (London)* **285**, 407 (1980).
17. The potent photosensitizer 4,5′,8-trimethylpsoralen is available by prescription in the United States as 5 mg tablets for use in long-wavelength ultraviolet light therapy of skin depigmentation (vitiligo).
18. C. Johnson and D. Brannon, *Phytochemistry* **12**, 2961 (1973).

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