

PHOTOTOXIC COUMARINS IN LIMES

H. N. NIGG*, H. E. NORDBY†, R. C. BEIER‡, A. DILLMAN§, C. MACIAS§ and R. C. HANSEN§

Citrus Research and Education Center, University of Florida, IFAS, 700 Experiment Station Road, Lake Alfred, FL 33850, USA, †Horticultural Research Laboratory, USDA, ARS, 2120 Camden Road, Orlando, FL 32803, ‡Food Animal Protection Research Laboratory, USDA, ARS, Route 5, Box 810, College Station, TX 77845, and §Department of Internal Medicine (Dermatology and Pediatrics), University of Arizona, College of Medicine, Tucson, AZ 85724, USA

(Accepted 22 December 1992)

Abstract—Coumarins in the rind and pulp of Persian and Key limes were quantified. In the rind of Persian limes, coumarin concentrations were in the order: limettin > bergapten > isopimpinellin > xanthotoxin > psoralen. In the rind of Key limes, psoralen and xanthotoxin were analytically absent; limettin was 10 times more concentrated than either bergapten or isopimpinellin, which were equal in concentration. Coumarin content in Persian lime pulp was in the order: isopimpinellin > limettin > bergapten > xanthotoxin > psoralen. For Key lime pulp, the concentrations of limettin, isopimpinellin and bergapten were equal; psoralen and xanthotoxin were not detected. Coumarins in lime pulp were 13 to 182 times less concentrated than those in the peel. Based on the amounts and types of coumarins, Persian limes appear to be potentially more phototoxic than Key limes. Although bergapten may be the main component of limes responsible for phytophotodermatitis, dermatological interaction assays with psoralen, bergapten, xanthotoxin and limettin should be conducted.

INTRODUCTION

Many species of citrus have caused dermatitis in humans (Mitchell and Rook, 1979; Pathak, 1974). Limes (*Citrus aurantifolia*) contain coumarins, furanocoumarins (psoralens) and pyranocoumarins (Gray and Waterman, 1978; McHale and Sheridan, 1989; Macheix *et al.*, 1990; Tatum and Berry, 1977). The photosensitizing psoralens have been reported to cause photodermatitis in humans (Berkley *et al.*, 1986; Pathak *et al.*, 1974; Schilcher, 1985; Seligman *et al.*, 1987; Song and Tapley, 1979; see Murray *et al.* (1982) for a review of the occurrence of these compounds in other plants).

The present study was prompted by experience with severe phytophotodermatitis in two young boys who made limeade in Tucson (AZ, USA) (A. Dillman, C. Macias and R. C. Hansen, University of Arizona, personal communication, 1992). Other cases of dermatitis and photodermatitis have been described in children handling limes (Gross *et al.*, 1987; Maryland Department of Health and Mental Hygiene, 1984). Sams (1941) reported 11 cases of dermatitis from Persian lime oil, of which three were documented cases of dermatitis caused by limeade preparation from Persian limes. Limes contain the photosensitizing compounds psoralen, bergapten and xanthotoxin (Fig. 1). Limes also contain isopimpinellin and limettin (Fig. 1) (Gray and Water-

man, 1978). Isopimpinellin is not a photosensitizer (Ashwood-Smith *et al.*, 1983; Hudson *et al.*, 1987). Limettin is 200 times less photoactive than bergapten on rabbit skin (Naganuma *et al.*, 1985) although it was not phototoxic at 1% on stripped human skin (Marzulli and Maibach, 1970). These coumarins have been quantified in various lime oils, but they have never been quantified in fresh rind and pulp from which limeade and other foods and drinks are prepared.

The purpose of the present study was to compare the levels of psoralen, bergapten, xanthotoxin, limettin and isopimpinellin in Arizona Persian limes, obtained from the same source as those affecting the patients, with the levels of these compounds in Florida Key limes.

MATERIALS AND METHODS

Chemicals. The standards used in this study were psoralen (7H-furo[3,2-g][1]benzopyran-7-one) (Sigma Chemical Co., St Louis, MO, USA), xanthotoxin (8-methoxypsoralen) (Biochemical Laboratories, Redondo Beach, CA, USA), bergapten (5-methoxypsoralen), limettin (5,7-dimethoxycoumarin) (Aldrich Chemical Co., Inc., Milwaukee, WI, USA) and isopimpinellin (5,8-methoxypsoralen) (isolated from *Ammi majus* as reported by Ivie, 1978). Standard purities of 98% and above were verified by HPLC and gas-liquid chromatography with flame-ionization detection (FID).

Sample preparations. In August, eight immature (mature in November; R. C. Hansen, University of

*To whom correspondence should be addressed.

Abbreviations: FID = flame-ionization detection; GLC = gas-liquid chromatography.

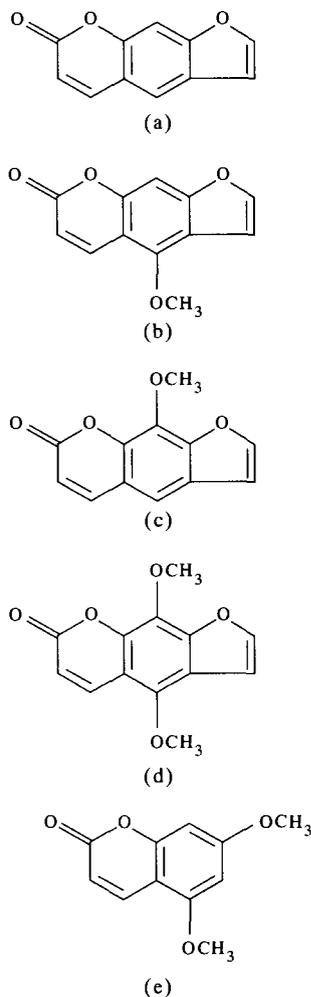


Fig. 1. Chemical structures of psoralen (a), bergapten (b), xanthotoxin (c), isopimpinellin (d) and limettin (e).

Arizona, personal communication, 1992) limes were picked (within 2 wk of the dermatitis incident) at random from the same tree in Tucson, where the two patients picked their limes. These were wrapped in aluminium foil, frozen and shipped express by air on dry ice to the University of Florida (Lake Alfred, FL, USA). On receipt, Persian limes were thawed, weighed and peeled. The peel and flesh were weighed. The individual peels were cut into small pieces and four 2-g subsamples were placed in 20-ml scintillation vials and stored at -17°C . The pulp was treated similarly except that the flesh was homogenized with a tissumizer (Tekmar, Cincinnati, OH, USA). Mature Key limes were obtained by H. E. Nordby in Key Largo (FL, USA) on 9 November, 1991. These were transported, held at 4°C for 4 days, and were processed as above.

Sample extraction. The method of Beier *et al.* (1983) was used. Briefly, 15 ml distilled water and 15 ml diethylether were added to either a peel or a pulp sample and homogenized for 1 min. The sample was centrifuged at 1000 *g* for 5 min and the ether

layer transferred to a 250-ml round-bottomed boiling flask. The pellet was homogenized twice with 10 ml diethylether and centrifuged as above. The combined ether extracts were evaporated to dryness at 40°C on a vacuum rotary evaporator. The residue was taken up in two 2-ml rinses of acetonitrile–water (60:40) and passed through a C_{18} Sep Pak column followed by 8 ml acetonitrile–water (60:40). The combined eluates, with the addition of 10 ml methanol, were evaporated to dryness at 40°C on a rotary evaporator. The residue was taken up in three 1-ml rinses of chloroform, and the combined rinses were passed through a Sep Pak silica gel followed by 8 ml ethyl acetate (7.5%) in chloroform. The combined eluents were dried on a nitrogen evaporator and then stored at -20°C until analysis.

Sample analysis. Analyses for quantification were performed on an Adsorbosphere reverse-phase phenyl column (150×4.6 mm, $5 \mu\text{m}$ particle size; Alltech Associates, Inc., Deerfield, IL, USA). The CN Radial Pak guard column was from Waters (Millipore, Bedford, MA, USA). Detection was at 254 nm with a Waters 490E multiwavelength detector. The flow rate was 1.0 ml water–acetonitrile (75:25)/min controlled with a Waters automated gradient controller and a Waters 6000 pump. A Waters 712 Wisp autoinjector was used to make 20- μl injections. Quantification was with a five-point standard curve constructed during and interspersed with the analytical runs, with the four standards injected as a combined mixture. For identity, three additional systems were used. A Tracor 540 gas–liquid chromatograph with a 30-m DB-1 megabore column ($1.5 \mu\text{m}$ film thickness; J & W Scientific, Folsom, CA, USA) was programmed from 50 to 270°C at $3^{\circ}/\text{min}$ with a 5-min hold at 270°C (the N_2 -carrier flow rate was 20 ml/min; the FID-detector and injection-port temperatures were 270°C and 210°C , respectively). A Perkin–Elmer 8320 capillary gas chromatograph equipped with a DB-5 column (J & W Scientific) was also used ($30 \text{ m} \times 0.25 \text{ mm}$, film thickness $0.25 \mu\text{m}$,⁶³ Ni electron-capture detector at 300°C , He-carrier rate at 1.0 ml/min, injector at 210°C and column temperature at 185°C). Finally, one rind extract was streaked on a silica gel G thin-layer chromatography plate (20×20 cm; Fisher Scientific, Pittsburgh, PA, USA) and chromatographed with 100% CHCl_3 . Coumarin zones were detected by UV visualization and were then removed and extracted with 2 ml methanol. These extracts were filtered through a $0.45\text{-}\mu\text{m}$ nylon syringe filter. Selected HPLC peaks, with the same retention times as known standards, and unknown peaks were also collected. Mass spectra of these samples and of coumarin standards were determined with a Hewlett–Packard 5971 gas–liquid chromatograph coupled with a mass spectroscope (Hewlett–Packard, Avondale, PA, USA) equipped with an HP-1 column ($12 \text{ m} \times 0.2 \text{ mm} \times 0.3 \mu\text{m}$). The oven was held at 160°C for 5 min following injection and then pro-

Table 1. Furanocoumarin contents in Persian and Key limes ($\mu\text{g/g}$ fresh weight)

Compound	Persian lime		Key lime	
	Rind	Pulp	Rind	Pulp
Psoralen	$3.9 \pm 3.4^*$	7 = ND, one fruit = 0.1	ND	ND
Xanthotoxin	5.9 ± 5.1	0.1 ± 0.1	ND	ND
Bergapten	128.7 ± 32.9	1.1 ± 0.9	$20.9 \pm 34.1^*$	0.4 ± 0.6
Isopimpinellin	53.7 ± 14.1	2.9 ± 2.5	22.0 ± 31.4	1.7 ± 2.0
Limettin	310.1 ± 136.3	1.7 ± 1.3	291.1 ± 85.4	2.8 ± 2.1

ND = not detected

*Values are means \pm SD of eight Persian limes and 13 Key limes.

grammed to 270°C (at $4^\circ\text{C}/\text{min}$); this temperature was held for 30 min. The transfer line and injection port were at 280°C . The mass analyser was at 70 eV, 180°C and 30 torr, with a He flow rate of 1.0 ml/min. The percentage of recovery of coumarins was assessed in two ways. Four rind subsamples from one lime were extracted as above with an additional fourth ether extraction. Each ether extract was processed and analysed separately. This was carried out to determine if all detectable coumarins had been extracted. After the fourth extraction, the remaining residue was fortified with 40 mg psoralen, 400 mg xanthotoxin, 800 mg bergapten and 400 mg isopimpinellin. These fortified residues were extracted with the regular method. Extracts were quantified by HPLC.

RESULTS

The retention times for psoralen, xanthotoxin, bergapten, limettin and isopimpinellin matched authentic standards on three GLC columns (HP1 = DB1, except for length) and one HPLC column. Except for psoralen, the quantified coumarin compounds were positively identified by GLC-mass spectrometry. Recoveries from the four fortified pre-extracted rind sub-samples were: psoralen $75.0 \pm 4.6\%$; xanthotoxin $81.5 \pm 1.5\%$; bergapten $73.2 \pm 2.0\%$ and isopimpinellin $86.5 \pm 2.5\%$. The overall efficiency of the first extraction of the four subsamples (where extracts were analysed separately) was very high and contained the following percentages of the total coumarin contents recovered (means \pm SD): psoralen $97.5 \pm 3.0\%$; xanthotoxin $97.9 \pm 0.6\%$; bergapten $98.4 \pm 0.5\%$ and isopimpinellin $98.4 \pm 0.4\%$. The fourth extraction was blank.

The average coefficient of variation for the four subsamples of each Persian lime sample was as follows: rind, 13.6% (psoralen), 8.7% (xanthotoxin), 6.5% (bergapten), 7.4% (isopimpinellin) and 7.5% (limettin); pulp, 6.7% (psoralen, one sample contained psoralen), 44.7% (xanthotoxin), 20.4% (bergapten), 12.5% (isopimpinellin) and 35.5% (limettin). In general, a smaller quantity resulted in greater variation in the subsamples. Overall, the methods described here, as adapted from Beier *et al.* (1983), were quite consistent with excellent recoveries of standards from fortified samples. Limettin was not used in recovery studies because it had not been

identified prior to HPLC analyses. Limettin quantity was estimated from xanthotoxin standard curves and by comparing limettin standard curves with those of xanthotoxin.

The average linear furanocoumarin contents of the eight Persian lime rind and pulp samples are reported in Table 1. In the rind, they were in the order: limettin > bergapten > isopimpinellin > xanthotoxin > psoralen. In the pulp, the order of furanocoumarin contents was: isopimpinellin > limettin > bergapten > xanthotoxin > psoralen.

Furanocoumarin content in the rind of Key limes was different from that of Persian limes. Psoralen and xanthotoxin were analytically absent from the rind of Key limes. Bergapten was variable, ranging from 0.37 to $99.96 \mu\text{g/g}$ fresh weight (average $20.9 \pm 34.1 \mu\text{g/g}$ fresh weight) (Table 1) compared with an average of $128.7 \pm 32.9 \mu\text{g/g}$ bergapten/g fresh weight (Table 1) in Persian lime rind. Furanocoumarin content of lime pulp ranged from not detectable to $2.9 \mu\text{g/g}$ fresh weight. On a percentage basis, isopimpinellin content was the highest. Isopimpinellin content in the pulp was 6% of what was observed in the rind of both Persian and Key limes.

DISCUSSION

Dermatitis caused by exposure to limes, lime oil and linear furanocoumarins has been reported (Gross *et al.*, 1987; Maryland Department of Health and Mental Hygiene, 1984; Roesyanto-Mahadi *et al.*, 1990; Sams, 1941; Schilcher, 1985). A variety of coumarins have been isolated from lime oil (Caldwell and Jones, 1945; Lawrence, 1982; McHale and Sheridan, 1989; Stanley and Jurd, 1971; Stanley and Vannier, 1967), but none was more phototoxic than psoralen, xanthotoxin and bergapten (Pathak *et al.*, 1960; Scott *et al.*, 1976). Relative to psoralen (100), xanthotoxin and bergapten had phototoxic activities of 37.5 and 27.5, respectively, on human skin, and all three were phototoxic on guinea pig skin (psoralen 100, xanthotoxin 71, bergapten 61) (Musajo and Rodighiero, 1962; Musajo *et al.*, 1974). The main compound in limes responsible for phytophotodermatitis appears to be bergapten, which had an average concentration of $1.1 \pm 0.9 \mu\text{g/g}$ fresh weight in the pulp and $128.7 \pm 32.9 \mu\text{g/g}$ fresh weight in the rind of Persian limes, and $20.9 \pm 34.1 \mu\text{g/g}$ fresh weight in Key lime rind. Psoralen was not detected in

seven of eight Persian lime pulp samples; this is reasonable since psoralen is presumed to be the biosynthetic precursor of the other linear furanocoumarins (Beier and Oertli, 1983; Brown *et al.*, 1970), and the level of xanthotoxin was only about one-seventh of that of bergapten in the pulp (Table 1).

The Persian lime is lemon-shaped, with a thicker peel and more oil in the cortex, and is larger than the Key lime. The latter is about the size of a hen's egg with a thick skin (Sams, 1941). The Persian lime is also referred to as Tahiti lime (Hodgson, 1967), whereas the Key lime is also called Mexican or West Indian lime (Hodgson, 1967). Our quantitative data may be the result of these morphological differences, or may be due to the maturity differences of the Persian and Key limes in this study (e.g. mature Key limes may contain less psoralen because it has been converted to other furanocoumarins in mature limes).

Bergapten, often found in fragrances, causes an adverse skin reaction at 0.001 to 0.002% (10–20 µg/g) (Marzulli and Maibach, 1970), and concentrations of linear furanocoumarins at 12.5 µg/g are known to cause contact dermatitis (Austad and Kavli, 1983). Concentrations as low as 8–10 µg total linear furanocoumarins/g in celery were responsible for phytophotodermatitis in grocery store workers (Beier, 1990). With squeezing, both the Key and Persian lime pulps, and presumably limeade, would contain about 3.0 µg furanocoumarins/g (psoralen + bergapten + xanthotoxin + limettin) as well as some fractions of rind limettin, bergapten, xanthotoxin and psoralen from broken oil glands. Some lime oils may contain 4640 µg limettin/g and 5080 µg isopimpinellin/g (Stanley and Vannier, 1967). Key lime oil from Dominica, Mexico, Peru and Haiti ranged from 1200 to 2400 µg bergapten/g and 3000 to 5700 µg isopimpinellin/g (McHale and Sheridan, 1989). Human exposure would depend on squeezing techniques, whether the limeade was stirred by hand, and personal hygiene.

Limettin was not phototoxic at a 1% concentration on the stripped skin of six human volunteers (Marzulli and Maibach, 1970). Limettin was not phototoxic to guinea pigs (Giles *et al.*, 1979; Naganuma *et al.*, 1985); however, it was phototoxic to rabbits (Naganuma *et al.*, 1985). Although there may be other possible but unstudied phototoxic compounds in limes (Towers, 1984), our data suggest that bergapten is most responsible for the development of lime dermatitis. However, humans may have differing sensitivities to these compounds, either individually or in combination. Because of the levels of limettin, psoralen and xanthotoxin in Persian lime rind, the reported phototoxic potential of limettin in rabbits and the similarity between limettin's chemical structure and that of psoralen, bergapten and xanthotoxin, dermatitis assays designed to evaluate the phototoxic interaction of psoralen, bergapten, xanthotoxin and limettin are warranted.

Acknowledgements—The authors thank S. Callahan and L. Mallory for their excellent technical assistance. Florida Agricultural Experiment Station Journal Series No. RO2593.

REFERENCES

- Ashwood-Smith M. J., Poulton G. A., Ceska O., Liu M. and Furniss E. (1983) An ultrasensitive bioassay for the detection of furocoumarins and other photosensitizing molecules. *Photochemistry and Photobiology* **38**, 113–118.
- Austad J. and Kavli G. (1983) Phototoxic dermatitis caused by celery infected by *Sclerotinia sclerotiorum*. *Contact Dermatitis* **9**, 448–451.
- Beier R. C. (1990) Natural pesticides and bioactive components in foods. *Reviews of Environmental Contamination and Toxicology* **113**, 47–137.
- Beier R. C., Ivie G. W., Oertli E. H. and Holt D. L. (1983) HPLC analysis of linear furocoumarins (psoralens) in healthy celery (*Apium graveolens*). *Food and Chemical Toxicology* **21**, 163–165.
- Beier R. C. and Oertli E. H. (1983) Psoralen and other linear furocoumarins as phytoalexins in celery. *Phytochemistry* **22**, 2595–2597.
- Berkley S. F., Hightower A. W., Beier R. C., Fleming D. W., Brokopp C. D., Ivie G. W. and Broome C. V. (1986) Dermatitis in grocery workers associated with high natural concentrations of furanocoumarins in celery. *Annals of Internal Medicine* **105**, 351–355.
- Brown S. A., El-Dakhkhny M. and Steck W. (1970) Biosynthesis of linear furanocoumarins. *Canadian Journal of Biochemistry* **48**, 863–871.
- Caldwell A. G. and Jones E. R. H. (1945) The constituents of expressed West Indian lime oil. *Journal of the Chemical Society*. pp. 540–543.
- Giles A., Tobin P. and Kornhauser A. (1979) 8-MOP and 5,7 dimethoxycoumarin phototoxicity, oral vs. topical. 7th Annual Meeting of the American Society of Photobiology Program. Abstract 146.
- Gray A. I. and Waterman P. G. (1978) Coumarins in the Rutaceae. *Phytochemistry* **17**, 845–864.
- Gross T. P., Ratner L., DeRodriguez O., Farrell K. P. and Israel E. (1987) An outbreak of phototoxic dermatitis due to limes. *American Journal of Epidemiology* **125**, 509–514.
- Hodgson R. W. (1967) Horticultural varieties of citrus. In *The Citrus Industry*. Edited by W. Reuther, H. J. Webber and L. D. Batchelor. Vol. I. Chapter 4. pp. 576–577. University of California, Div. Agr. Sci., Riverside, CA.
- Hudson J. B., Miki N. and Towers G. H. N. (1987) Isopimpinellin is not phototoxic to viruses and cells. *Planta Medica* **53**, 306–307.
- Ivie G. W. (1978) Linear furocoumarins (psoralens) from the seed of Texas *Ammi majus* L. (Bishop's Weed). *Journal of Agricultural and Food Chemistry* **26**, 1394–1403.
- Lawrence B. M. (1982) Progress in essential oils. Coumarins and psoralens in citrus oils. *Perfumer & Flavorist* **7**, 57–65.
- McHale D. and Sheridan J. B. (1989) The oxygen heterocyclic compounds of citrus peel oils. *Journal of Essential Oil Research* **1**, 139–149.
- Macheix J. J., Fleuriet A. and Billot J. (1990) *Fruit phenolics*. pp. 34–39. CRC Press, Boca Raton, FL.
- Maryland Department of Health and Mental Hygiene (1984) Outbreak of phototoxic dermatitis from limes. *Morbidity and Mortality Weekly Report* **34**, 462–464.
- Marzulli F. N. and Maibach H. I. (1970) Perfume phototoxicity. *Journal of the Society of Cosmetic Chemists* **21**, 695–715.
- Mitchell J. and Rook A. (1979) *Botanical Dermatology*. p. 608. Greengrass, Vancouver.
- Murray R. D. H., Mendez J. and Brown S. A. (1982) *The natural coumarins. Occurrence, Chemistry and Biochemistry*. pp. 471–594. John Wiley & Sons, New York.

- Musajo L. and Rodighiero G. (1962) The skin-photosensitizing furocoumarins. *Experientia* **18**, 153-161.
- Musajo L., Rodighiero G., Caporale G., Dall'Acqua F., Marciani S., Bordin F., Baocichetti F. and Bevilacqua R. (1974) Photoreactions between skin-photosensitizing furocoumarins and nucleic acids. In *Sunlight and Man, Normal and Abnormal Photobiologic Responses*. Edited by M. A. Pathak, L. C. Harber, M. Seiji and A. Kukita; T. B. Fitzpatrick, consulting editor. Chapter 23. pp. 369-387. University of Tokyo Press, Tokyo.
- Naganuma M., Hirose S., Nakayama Y., Nakajima K. and Someya T. (1985) A study of the phototoxicity of lemon oil. *Archives of Dermatological Research* **278**, 31-36.
- Pathak M. A. (1974) Phytophotodermatitis. In *Sunlight and Man, Normal and Abnormal Photobiologic Responses*. Edited by M. A. Pathak, L. C. Harber, M. Seiji and A. Kukita; T. B. Fitzpatrick, consulting editor. Chapter 32. pp. 495-513. University of Tokyo Press, Tokyo.
- Pathak M. A., Fellman J. H. and Kaufman K. D. (1960) The effect of structural alterations on the erythematous activity of furocoumarins: psoralens. *Journal of Investigative Dermatology* **35**, 165-183.
- Pathak M. A., Kramer D. M. and Fitzpatrick T. B. (1974) Photobiology and photochemistry of furocoumarins (psoralens). In *Sunlight and Man, Normal and Abnormal Photobiologic Responses*. Edited by M. A. Pathak, L. C. Harber, M. Seiji and A. Kukita; T. B. Fitzpatrick, consulting editor. Chapter 22. pp. 335-368. University of Tokyo Press, Tokyo.
- Roesyanto-Mahadi I. D., Geursen-Reitsma A. M., van Joost Th. and van den Akker Th. W. (1990) Sensitization to fragrance materials in Indonesian cosmetics. *Contact Dermatitis* **22**, 212-217.
- Sams W. M. (1941) Photodynamic action of lime oil (*Citrus aurantifolia*). *Archives of Dermatology and Syphilology* **44**, 571-587.
- Schilcher H. (1985) Effects and side-effects of essential oils. In *Essential Oils and Aromatic Plants*. Edited by A. Baerheim Svendsen and J. J. C. Scheffer. Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands.
- Scott B. R., Pathak M. A. and Mohn G. R. (1976) Molecular and genetic basis of furocoumarin reactions. *Mutation Research* **39**, 29-74.
- Seligman P. J., Mathias C. G. T., O'Malley M. A., Beier R. C., Fehrs L. J., Serrill W. S. and Halperin W. E. (1987) Phytophotodermatitis from celery among grocery store workers. *Archives of Dermatology* **123**, 1478-1482.
- Song P.-S. and Tapley K. J., Jr (1979) Photochemistry and photobiology of psoralens. *Photochemistry and Photobiology* **29**, 1177-1197.
- Stanley W. L. and Jurd L. (1971) Citrus coumarins. *Journal of Agricultural and Food Chemistry* **19**, 1106-1110.
- Stanley W. L. and Vannier S. H. (1967) Psoralens and substituted coumarins from expressed oil of lime. *Phytochemistry* **6**, 585-596.
- Tatum J. H. and Berry R. E. (1977) 6,7-Dimethoxycoumarin in the peels of *Citrus*. *Phytochemistry* **16**, 1091-1092.
- Towers G. H. N. (1984) Interactions of light with phytochemicals in some natural and novel systems. *Canadian Journal of Botany* **62**, 2900-2911.