Examination of naturally occurring polyacetylenes and α-terthienyl for their ability to induce cytogenetic damage[1]

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Summary. α-Terthienyl and 5 polyacetylenes were examined for chromosome damaging activity using Syrian hamster cells. None of these naturally occurring compounds induced sister chromatid exchanges and neither α-terthienyl nor phenylheptatriyne induced chromosome aberrations.

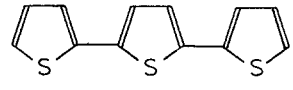
Many of the polyacetylenes and their thiophene derivatives, in the presence of longwave UV light (UV-A, 315-400 nm), have been shown recently to be toxic to a wide range of microorganisms and to human skin fibroblasts[2,3]. An example of the polyacetylenes is phenylheptatriyne (PHT) and for the thiophene derivatives, α-terthienyl (figure 1). These 2 compounds are also lethal to *Paramecium* and to *Drosophila* eggs under these conditions and nematocidal activity has been reported for α-terthienyl[4]. α-Terthienyl, but not the polyacetylenes, causes photodermatitis in human skin which is characterized by immediate severe erythema on exposure to sunlight and long lasting hyperpigmentation[6,7].

The striking photobiological activities of these compounds render them possible candidates for use as pesticides or topical antibiotics. Other naturally occurring photosensitizers, such as 8-methoxypsoralen (8-MOP), used currently in the treatment of psoriasis, may represent a hazard to health as they possess the ability to induce genetic damage and mutations[8]. There is some evidence that α-terthienyl, in the presence of UV-A light, damages DNA since it induces unscheduled DNA synthesis[9]. For these reasons, a careful examination of the genetic risk associated with natural polyacetylenes and thiophenes is desirable.

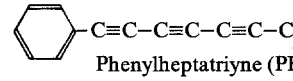
We have utilized chromosome aberration and sister chromatid exchange (SCE) formation in Syrian hamster cells (BHK-21) as indices of damage to the genome of mammalian cells. Chromosome aberrations are produced characteristically by agents which damage DNA and their quantitative analysis has been widely used for screening compounds suspected of possessing mutagenic and/or carcinogenic activity[10]. SCEs are reciprocal exchanges between replicating, or newly replicated sister chromatids. Like chromosome aberrations, they are produced in response to exposure to agents which damage DNA[11].

Materials and methods. The cytogenetic techniques used for analysing SCEs and chromosome aberrations have been described previously[12]. Briefly, BHK-21 cells (kindly supplied by Dr J.B. Hudson, Dept. of Microbiology, UBC) were cultured in Dulbecco's modified minimum essential medium (MEM, Gibco) supplemented with 15% fetal bovine serum (FBS, Microbiological Associates). Chemicals to be tested were dissolved in 95% ethanol and diluted in MEM to a final ethanol concentration of 1%. These solutions were applied to the cells which were incubated at 37 °C in the dark for 1 h and then irradiated for 30 min with 1.25 J/cm^2 of either UV-A or white light, depending upon the chemical being used. After the light treatment, cells were returned to MEM + 15% FBS. In the case of samples to be analyzed for SCEs, 5'-bromodeoxyuridine (BrdU, Sigma) was included in this medium and cells harvested after 26 h. Samples for chromosome aberrations were harvested after 9 h. After differential staining to produce harlequin-stained chromosomes[12], 25 metaphase cells were analyzed and this data used to compute the mean number of SCEs per cell. Fifty Giemsa-stained cells were examined in the determination of the percentage of cells containing one or more chromosome aberrations.

8-Methoxypsoralen (Sigma) was included with the compounds tested as representing a known mutagen, while methylene blue (BDH) and rose bengal (Eastman) were chosen to represent photosensitizing dyes[8]. The polyacetylenes used are naturally occurring. PHT was isolated from



α-Terthienyl

1 

Phenylheptatriyne (PHT)

2 $CH_3-CH=CH-(C\equiv C)_2-CH_2-CH=CH-(CH_2)_5-CH=CH_2$

3 $CH_3-(CH=CH)_2-C\equiv C-(CH=CH)_2-(CH_2)_4-CH_2OAc$

4 $CH_3-CH=CH-(C\equiv C)_2-(CH=CH)_2-CH=CH_2$

5 $CH_3-CH=CH-(C\equiv C)_3-CH=CH-CHOH-CH_2-OH$

Fig. 1. Structures of α-terthienyl and polyacetylenes.

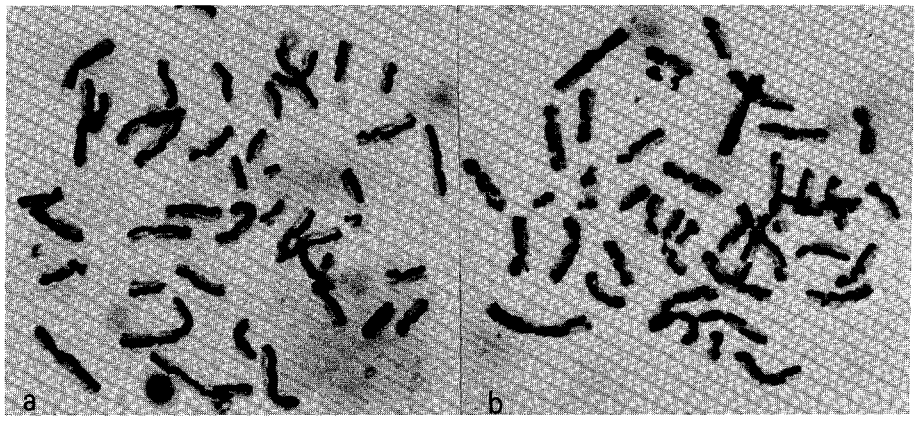


Fig. 2. Metaphase cells of Syrian hamster showing 'harlequin'-stained chromosomes. *a* Control cell with 10 SCEs. *b* Cell exposed to 8-methoxypsoralen and UV-A light within excess of 80 SCEs.

Effect of 8-MOP, α-terthienyl, polyacetylenes and photosensitive dyes on the SCE frequency of Syrian hamster cells

Chemical	Concentration (μg/l)	SCEs per metaphase*** No light	With light
8-MOP*	220	15.9 (1.9)	+
	66	16.5 (1.1)	106.4 (3.1)
	22	13.7 (1.6)	54.0 (1.6)
Control*	0	10.2 (1.0)	15.7 (1.4)
α-Terthienyl*	250	7.5 (0.4)	+ +
	25	8.2 (0.3)	+
	2.5	7.9 (0.4)	10.3 (1.3)
	0.25	8.5 (0.4)	10.4 (0.4)
	0.025	8.7 (0.4)	11.2 (0.4)
Control*	0	8.3 (0.6)	11.4 (0.5)
PHT*	5000	16.0 (3.1)	+ +
	1600	13.9 (1.2)	+
	500	12.7 (1.6)	20.6 (1.5)
	160	12.4 (0.8)	13.9 (1.2)
	50	14.1 (1.3)	16.6 (1.0)
	16	13.2 (1.3)	17.1 (2.2)
Control*	0	11.4 (1.0)	15.4 (1.1)
Compound 2*	1000	10.3 (0.6)	+
	300	11.8 (0.6)	13.7 (1.1)
	100	12.4 (1.0)	12.7 (0.5)
	30	10.4 (0.6)	17.3 (1.7)
	10	12.2 (1.2)	15.4 (1.0)
	3	11.2 (0.9)	15.1 (0.9)
Compound 3*	1000	+ +	+ +
	300	10.0 (0.7)	+ +
	100	10.8 (0.7)	+
	30	12.0 (1.0)	11.8 (0.8)
	10	10.3 (0.6)	13.3 (0.7)
	3	11.6 (0.8)	13.9 (0.7)
Compound 4*	1000	11.1 (0.4)	+ +
	300	12.2 (0.7)	+ +
	30	11.7 (0.6)	+ +
	3	9.7 (0.7)	13.0 (0.5)
	0.3	10.6 (0.6)	13.2 (1.0)
	0.03	10.2 (0.8)	11.8 (0.7)
Compound 5*	1000	+	+ +
	300	9.8 (0.6)	+ +
	100	9.6 (0.5)	+
	30	10.5 (0.5)	13.9 (0.7)
	3	9.8 (0.6)	14.3 (0.7)
	0.3	10.0 (1.0)	13.7 (1.1)
Control*	0	10.8 (0.6)	14.0 (0.7)
Methylene blue**	27,000	10.1 (0.6)	+ +
	8250	9.2 (0.5)	+
	2750	8.8 (0.4)	11.6 (0.7)
	825	9.1 (0.5)	9.0 (0.6)
	275	9.3 (0.5)	9.3 (0.4)
Rose bengal**	9740	9.7 (0.5)	+ +
	2920	9.1 (0.4)	+ +
	974	8.7 (0.6)	+
	292	9.3 (0.6)	9.2 (0.5)
	97	9.2 (0.5)	9.7 (0.4)
Control**	0	8.9 (0.4)	10.2 (0.5)

The data for 5 separate experiments are included along with the control values obtained in each. Toxic effects were quantitated on the basis of numbers of cells surviving to complete one or 2 divisions after chemical treatment: M1 and M2 mitotic cells respectively. No toxic effect = mitotic index of M2 cells greater than 2%; + = mitotic index of M2 cells = 0%, + + = mitotic index of M1 cells = 0%. * Irradiated with UV-A light: source = 2 Sylvania F15T8-BL fluorescent lamps; ** Irradiated with cool-white light: source = 2 Westinghouse F15T12/CW fluorescent lamps; *** SEM in parentheses.

Bidens pilosa L., compound 2 from *Chrysanthemum leucanthemum* L., compounds 3 and 4 from *Dahlia* spp. and compound 5 from *Centaurea scabiosa* L. Synthetic α-terthienyl was prepared by the method of Kooreman and Wynberg[13].

Results and discussion. Whereas the photoactive nature of the compounds tested was evident from the toxic effects observed, only 8-MOP was found to be capable of producing a marked increase in SCE frequency. The spontaneous SCE level of untreated BHK-21 cells ranged between 8.3 and 11.4 (table). The SCE frequency of cells irradiated with UV-A light varied between 11.4 and 15.4. Shortwave UV is known to induce SCEs[14] and this difference may reflect the emission of some UV of shorter wavelength from the light source used. 8-MOP, in the presence of UV-A light, produced approximately a 10-fold greater SCE frequency at a concentration of 66 μg/l than that of the non-irradiated cells. Although not inducing SCEs, synthetic α-terthienyl and compounds 3, 4 and 5 exhibited their toxicity over a concentration range of 25–100 μg/l compared with 220 μg/l of 8-MOP which was required to prevent mitotic activity (table). The appearance of harlequin-stained metaphases of 8-MOP treated and control cells are shown in figure 2.

Similar results were obtained from studies on chromosome aberrations. Neither α-terthienyl nor PHT induced an increase in the frequency of chromosome aberrations while low concentrations of 8-MOP were efficient in this respect. Exposure of cells to 220, 66 and 22 μg/l of 8-MOP, in the presence of UV-A, produced chromosome aberrations in 73, 56 and 21%, respectively, of metaphase cells analyzed. Neither α-terthienyl (over the concentration range 2.5–250 μg/l) nor PHT (over the concentration range 50–5000 μg/l) was effective in increasing the spontaneous frequency of chromosome aberrations.

Despite the data of others which indicate that α-terthienyl, in the presence of UV-A light, induces low levels of unscheduled DNA synthesis[9], our results suggest that chromosomal damage does not accompany the phototoxic effects of this compound, nor of the polyacetylenes. This is encouraging in view of the frequent exposure of gardeners and horticultural workers to plants which contain them. Furthermore, the prospect of including these biologically active compounds in the pharmacopoeia or applying them as antibiotic or pesticidal agents appears even more attractive.

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