UV-MEDIATED GENOTOXICITY OF FURANOQUINOLINE AND OF CERTAIN TRYPTOPHAN-DERIVED ALKALOIDS

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ABSTRACT.—Certain furanoquinolines of the Rutaceae and a number of the widely distributed β-carboline or harmane alkaloids are photosensitizers in near ultraviolet light (320-400 nm). These compounds have been shown to be phototoxic to yeasts and bacteria. In addition, two canthinones of the Rutaceae and the *N*-methylpyrolidine substituted harmane alkaloid, brevicolline (from *Carex*), display phototoxicity towards bacteria and fungi in near ultraviolet light. All of these alkaloids are phototoxic to Chinese hamster ovary cells, inhibiting mitosis and causing gross chromosomal changes. The target for their phototoxicity appears to be the cell nucleus.

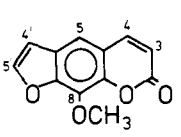
In our continuing survey of plants for natural photosensitizers that are of potential use in photochemotherapy, we have found that some alkaloids are phototoxic to bacteria and fungi. These alkaloids include certain furanoquinolines of the Rutaceae (1) and a number of simple β-carbolines or harmane alkaloids (2), which are very widely distributed, occurring in about 26 families of plants (3). β-Carbolines are also found in natural foods, charred meats (4), and animal cells and tissues (5). They have been detected in human and rat urine under conditions of alcohol loading (6).

Dictamnine, a furanoquinoline occurring in a number of Rutaceae, including *Skimmia japonica,* was selected by us for detailed study. It was shown to undergo monofunctional covalent binding to calf thymus DNA *in vitro*, to synthetic DNAs (7,8), and to fungal DNA *in vivo* (9). It seems clear that the target for the photodestructive effect of this alkaloid is the cell nucleus, as it is with the well-known photosensitizer, 8-methoxypsoralen (10). With the β-carbolines, however, less information is available; they display phototoxicity towards bacteria and fungi (2), and some have been shown to bind readily to native DNA and RNA (11,12). These results suggest that the β-carbolines also interact with the nucleus in the photosensitization process. None of the previous studies of the β-carbolines, however [except for that of McKenna and Towers (2)], has recognized that light modifies the chemical and, consequently, the biological activities of these alkaloids. We have now shown that the β-carbolines and certain other tryptophan-derived alkaloids, as well as furanoquinolines, inhibit mitosis and cause chromosomal damage to Chinese hamster ovary cells (CHO cells) in long-wave ultraviolet light (320-400 nm).

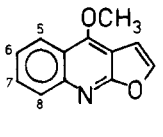
The additional tryptophan alkaloids are two canthinones of *Zanthoxylum* (Rutaceae) (13) and brevicolline, an *N*-methylpyrolidine substituted harmane alkaloid occurring in the sedge, *Carex brevicollis* (Cyperaceae) (14,15). Structures of these compounds are shown in figure 1. Canthinones also occur in the Amarantaceae (*Charpentiera*) (16) and the Simaroubaceae (*Picrasma*) (17), and may be quite widespread. Similar compounds have recently been identified in *Datura stramonium* (18).

RESULTS AND DISCUSSION

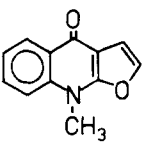
Table 1 shows the phototoxicity of 6-canthinone, 5-methoxy-6-canthinone and of brevicolline to species of bacteria and fungi; 8-methoxypsoralen was used as a reference photosensitizer. Like the β-carbolines and the furanoquinolines, these alkaloids are also photosensitizers, and this has to be taken into account in future experiments designed to test their biological activities. For example, Mitscher *et al.* (13) showed that the two canthinones, and particularly 6-canthinone, are quite effective antimicrobial agents when tested against a variety of bacteria, but the authors were not aware of the photo-



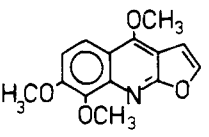
I. 8-Methoxypsoralen



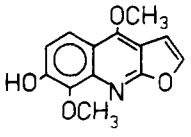
II. Dictamnine



III. Isodictamnine

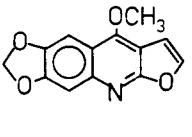


IV. Skimmianine

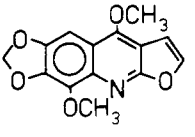


V. 7-Demethylskimmianine

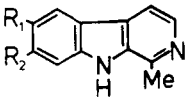
VI. Maculosidine



VII. Maculine

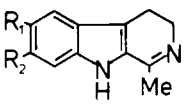


VIII. Flindersiamine



IX. R_1=H;R_2=H Harmane

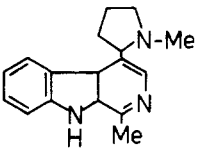
X. R_1=H;R_2=OMe Harmine



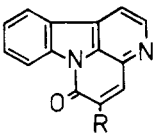
XI. R_1=H,R_2=OMe Harmaline

XII. R_1=H,R_2=OH Harmalol

XIII. R_1=OMe,R_2=H 6-methoxy-harmalan

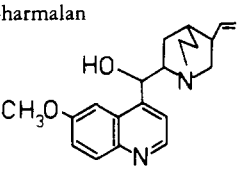


XIV. Brevicolline



XV. R=H 6-Canthinone

XVI. R=OCH 5-Methoxy-6-canthinone



XVII. Quinine

Photogenotoxic alkaloids

sensitizing ability of these compounds. Similarly, Wolters and Eilert found that at least 17 antimicrobial substances, including furanocoumarins and furanoquinolines, were produced in callus cultures of *Ruta graveolens* (19). The furanoquinolines, dictamnine, γ-fagarine, and skimmiane were found to be more active than the furanocoumarins. The activities of both classes of compounds are tremendously enhanced in light, however, and, had this been taken into account, their comparative analyses would undoubtedly have been different. Species of *Pseudomonas* are typically resistant to all the photosensitizers we have tested in previous studies (1,2,20), and again we find this to be the case with these newly discovered photosensitizers.

Table 2 shows the uv-mediated genotoxicity of all of these alkaloids to CHO cells. Their activities are considerably lower than those of 8-methoxypsoralen, which was included for comparative purposes. The harmane alkaloids were also tested with a 60-min irradiation treatment. The data (not shown) indicated that under these conditions, they were effective at approximately half the concentrations used in the experiments shown in table 2. The following furanoquinoline alkaloids were also found to cause either inhibition of mitosis or chromosomal aberrations after irradiation, but are not included in the table because the small amounts available precluded quantification: flindersiamine, maculine, maculosidine, isodictamnine, and 7-demethyl skimmianine. Atropine, strychnine, or vinblastine were also tested and, at the highest concentrations used, were found not to be genotoxic, with or without irradiation (data not shown). Quinine was phototoxic at 25 ppm and caused mitotic inhibition at 12.5 ppm on irradiation. Quinine is known to be a photosensitizer capable of generating singlet oxygen *in vitro* (21). Whether or not it is photogenotoxic cannot be determined from our experiments as no chromosomal damage was observed, merely mitotic inhibition. Arecoline, the

TABLE 1. Phototoxicity of alkaloids to microorganisms expressed as zone of inhibition of growth (in cm).

Dose	Microorganism	*Escherichia coli*		*Pseudomonas aeruginosa*		*Pseudomonas fluorescens*		*Bacillus subtilis*	
(μg/disc)	treatment compound	light[a]	dark[b]	light	dark	light	dark	light	dark
100	Atropine sulfate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
100	Strychnine sulfate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
100	Brevicolline	1.55	0.0	1.3	0.0	0.7	0.0	1.25	0.0
~10	6-Canthinone	0.7	0.0	0.0	0.0	0.0	0.0	0.86	1.6
~16.7	5 Methoxy-6-canthinone	0.7	0.0	0.0	0.0	0.0	0.0	0.8	0.8
10	8 MOP	1.4	0.0	0.0	0.0	0.0	0.0	1.4	0.0
		Streptococcus faecalis		*Staphylococcus aureus*		*Candida albicans*		*Saccharomyces cerevisiae*	
		light	dark	light	dark	light	dark	light	dark
100	Atropine sulfate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
100	Strychnine sulfate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
100	Brevicolline	1.4	0.0	1.55	0.0	0.95	0.0	0.7	0.0
~10	6-Canthinone	0.0	0.0	1.0	0.0	1.3	0.9	1.3	0.9
~16.7	5 Methoxy-6-canthinone	0.0	0.0	0.8	0.0	1.2	0.0	0.9	0.0
10	8 MOP	1.4	0.0	1.8	0.0	1.4	0.0	1.9	0.0

[a]Light=2 h of uv irradiation at 5 W/m^2.
[b]Dark control.

major alkaloid of betel-nut, causes chromosomal breakages in mouse bone-marrow cells, but light is apparently not a factor in the genotoxicity of this compound (22) nor of certain pyrrolizidine alkaloids, such as heliotrine or lasiocarpine, which induce chromosomal aberrations and mutations in V79 hamster cell cultures (23).

The fact that some of the β-carbolines are photogenotoxic suggests that, in all future work with these compounds and DNA, the influence of light will have to be considered. For example, Levitt *et al.* (24) showed that harmane and norharmane inhibited the mutagenicity of benzo(a)pyrene, whereas Nagao *et al.* (25) found that this mutagenicity was greatly enhanced by these compounds. Neither group mentions whether precautions were taken to exclude light in their experiments. The effects of light on chromosomal morphology shown in our experiments with β-carbolines are quite remarkable and have far-reaching implications, particularly when one considers that some of these compounds occur in animals (6), including man (26,27).

It should also be pointed out that some of these phototoxic alkaloids are potentially useful as probes of certain aspects of RNA and DNA structure, as has been demonstrated with furanocoumarins (28).

EXPERIMENTAL

MATERIALS.—β-Carbolines were purchased from Sigma Chemical Co., St. Louis, MO. 6-Canthinone and 5-methoxy-6-canthinone were gifts of Prof. Jack Beal, The Ohio State University. Brevicolline were kindly provided by Prof. Ed Leete, University of Minnesota, vinblastine by Prof. J. Kutney, University of British Columbia, and the furanoquinolines, except for dictamnine and skimmianine, by Prof. I. Spenser, McMaster University. Dictamnine was synthesized by Dr. Irma Panfil, and skimmianine was isolated from *Skimmia japonica* by Dr. B. Abeysekera of this laboratory.

Chinese hamster ovary cells were kindly provided by Prof. H. Stich, Cancer Research Centre, Vancouver.

The sources and strains of the microbial cultures have been described previously (29).

METHODS.—Phototoxicity tests on microorganisms were carried out as previously described (1,2). *Genotoxicity tests.* CHO cells were cultured in Eagle's minimum essential medium (MEM)

TABLE 2. Chromosomal aberrations in CHO cells treated with various photosensitizers, including 8-methoxypsoralen, in uv light or in dark.

Compound	Dose (ppm)	Light		Dark	
		No.	%	No.	%
Skimmianine	50.0	T	T	0	0
Skimmianine	25.0	1.6	68.3		
Skimmianine	12.5	1.3	61.9		
Skimmianine	6.2	1.3	59.9		
Skimmianine	3.1	0.8	45.0		
Skimmianine	0.8	0.2	10.9		
Skimmianine	0.4	0.1	3.0		
Skimmianine	0.2	0	0		
Dictamnine	5.0	MI	MI	0	0
Dictamnine	2.5	2.7	82.4		
Dictamnine	1.3	1.8	60.0		
Dictamnine	0.6	1.1	50.6		
Dictamnine	0.3	0	3.4		
Dictamnine	0.2	0	0		
8-Methoxypsoralen	0.3	T	T	0	0
8-Methoxypsoralen	0.2	MI	MI		
8-Methoxypsoralen	0.1	1.1	63.6		
8-Methoxypsoralen	0.04	0.9	38.8		
8-Methoxypsoralen	0.02	0.1	7.1		
Harmalol HCl	10.0	1.5	63.4	0	0
Harmalol HCl	5.0	0.7	43.2		
Harmalol HCl	2.5	0.3	22.1		
Harmalol HCl	1.25	0	0		
Harmol HCl	5.0	0.5	35.2	0	0
Harmol HCl	2.5	0.1	5.7		
Harmol HCl	1.25	0	0		
Harmaline	10.0	T	T	0	0
Harmaline	5.0	T	T		
Harmaline	2.5	T	T		
Harmaline	1.25	MI	MI		
Harmaline	0.62	MI	MI		
Harmaline	0.31	0.4	22.1		
Harmaline	0.15	0	2.3		
Harmine HCl hydrate	10.0	T	T	0	0
Harmine HCl hydrate	5.0	T	T		
Harmine HCl hydrate	2.5	T	T		
Harmine HCl hydrate	1.25	T	T		
Harmine HCl hydrate	0.62	0.4	21.7		
Harmine HCl hydrate	0.31	0.1	7.5		
Harmine HCl hydrate	0.15	0	2.4		
6-MeO-Harmalan	10.0	T	T	0	0
6-MeO-Harmalan	5.0	T	T		
6-MeO-Harmalan	2.5	T	T		
6-MeO-Harmalan	1.25	T	T		
6-MeO-Harmalan	0.62	T	T		
6-MeO-Harmalan	0.31	MI	MI		
6-MeO-Harmalan	0.15	1	45.2		
6-MeO-Harmalan	0.07	0	0		
6-MeO-Harman	10.0	T	T	0	0
6-MeO-Harman	5.0	T	T		
6-MeO-Harman	2.5	T	T		
6-MeO-Harman	1.25	MI	MI		
6-MeO-Harman	0.62	0.1	8.9		

TABLE 2. Continued.

Compound	Dose (ppm)	Light		Dark	
		No.	%	No.	%
6-MeO-Harman	0.31	0	0		
Harmane HCl	10.0	T	T	0	0
Harmane HCl	5.0	T	T		
Harmane HCl	2.5	T	T		
Harmane HCl	1.25	0.1	6.7		
Harmane HCl	0.62	0	0		
Norharmane	10.0	T	T	0	0
Norharmane	5.0	T	T		
Norharmane	2.5	T	T		
Norharmane	1.25	0.1	6.7		
Norharmane	0.62	0	0		
Brevicolline	12.5	T	T	0	0
Brevicolline	6.25	T	T		
Brevicolline	3.12	MI	MI		
Brevicolline	1.56	1.2	56.0		
Brevicolline	0.78	0.8	37.1		
Brevicolline	0.39	0.1	5.5		
Brevicolline	0.19	0	0		
6-Canthinone	125.0	T	T	0	0
6-Canthinone	62.5	T	T		
6-Canthinone	31.2	T	T		
6-Canthinone	15.6	T	T		
6-Canthinone	7.8	T	T		
6-Canthinone	3.9	MI	MI		
6-Canthinone	1.9	0.4	22.2		
5-MeO-6-Canthinone	83.5	T	T	0	0
5-MeO-6-Canthinone	41.7	T	T		
5-MeO-6-Canthinone	20.8	T	T		
5-MeO-6-Canthinone	10.4	T	T		
5-MeO-6-Canthinone	5.2	T	T		
5-MeO-6-Canthinone	2.6	MI	MI		
5-MeO-6-Canthinone	1.3	0	0.8		
Quinine	100.0	T	T	0	0
Quinine	50.0	T	T		
Quinine	25.0	T	T		
Quinine	12.5	T	T		
Quinine	6.3	MI	MI		

No. = Average number of exchanges and breaks per metaphase plate.
% = Percentage of metaphase plates with at least one chromosomal aberration.
MI = Mitotic inhibition (slide with less than 40 metaphases).
T = Toxic; no dividing cells.
Light treatment involved 60-min incubation in dark, followed by 30 min in light.
Dark treatment consisted of 90-min incubation in dark.

supplemented with 10% fetal calf serum (FCS) and streptomycin sulfate (29.6 μg/ml), penicillin G, N.F. sodium (125 μg/ml), kanamycin (100 μg/ml), fungizone (2.5 μg/ml), and 7.5% Na bicarbonate (1 mg/ml). Cells of stock cultures, grown in 240-ml plastic culture flasks (Falcon) at 37° in a CO_2 incubator at 100% humidity, were dispersed, centrifuged, and resuspended in fresh medium. For seeding, the suspension was diluted to an approximate density of 7×10^4 cells/ml. Of this suspension, 2 ml were seeded on a 22 mm^2 coverslip in a 35 × 10 mm Falcon plastic dish and kept in MEM with 10% FCS at 37° for 2 d to achieve a 60-80% confluency of cells. The compounds to be tested were dissolved in 95% ethanol or sterile water and diluted in MEM containing 2.5% FCS. The ethanol concentration in the first dilution did not exceed 1%. Subsequent twofold dilutions were made, and 1 ml of each was added to the Petri dishes after removing the tissue culture medium. Tests were carried out in duplicate, one series being irradiated

and the second being maintained in darkness. Irradiated cultures were first exposed to the chemicals for 1 h in the dark at 37°, after which they were irradiated for either 10, 30, or 60 min at ambient temperature at a distance of 20 cm from a bank of Sylvania black-light blue fluorescent lamps (F20T12-BLB) (maximum output at 350 nm). The light intensity was 5 W/m^2, as determined with a Model 65 YSI Kettering Radiometer. After irradiation test solutions were removed, the coverslips were washed twice with MEM, and fresh medium containing 10% FCS was added to the Petri dishes. Samples were incubated for 16 h. Four hours prior to harvesting, 0.2 ml of colchicine (0.01% in 2.5 MEM) was added. Cells were then treated with 1% Na citrate for 20 min, followed by fixation in Carnoy's solution for 20 min. Air-dried coverslips were stained with 2% acid orcein and mounted, and 100 metaphase plates were analyzed in each case for chromosome breakages and exhanges. (This technique was kindly taught to us by Dr. M.P. Rosin of Prof. Stich's laboratory.)

ACKNOWLEDGMENTS

We thank the Natural Sciences and Engineering Council of Canada for support of this research and also Prof. Stich and Dr. Rosin for help in the genotoxicity assays with CHO cells. We also thank those who kindly provided samples of alkaloids.

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Received 24 November 1982