Phytochemistry, Vol. 20, No. 5, pp. 1001–1004, 1981.
Printed in Great Britain.

0031-9422/81/051001-04 $02.00/0

ULTRA-VIOLET MEDIATED CYTOTOXIC ACTIVITY OF β-CARBOLINE ALKALOIDS

DENNIS J. MCKENNA and G. H. N. TOWERS

Department of Botany, University of British Columbia, Vancouver, B.C., Canada

(*Received* 30 *June* 1980)

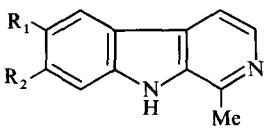
Key Word Index—β-Carbolines; alkaloids; phototoxicity.

Abstract—Six β-carboline alkaloids were screened for phototoxicity using yeast and bacterial bioassay systems. Five of the compounds tested showed various degrees of activity. The remaining compound, 6-methoxy-tetrahydroharman, was devoid of activity.

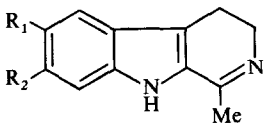
INTRODUCTION

The β-carboline alkaloids (Fig. 1) are distributed among a diversity of plant families and genera [1,2]. Harmine, harmaline, and tetrahydroharman have been implicated as active constituents of the hallucinogenic beverage *ayahuasca* [3,4] used by numerous aboriginal tribes in the Amazon Basin. Harmine, harmaline and related compounds are also known to be potent reversible inhibitors of monoamine oxidase [5] and this property may be partly responsible for some of their CNS effects. Certain β-carboline alkaloids have also been reported [6] to bind readily to native DNA, but not to denatured DNA, while binding to RNA was about 40% of that observed for native DNA. At least one β-carboline alkaloid, 6-methoxy-tetrahydroharman (**6**) has been found as a naturally occurring pineal metabolite [7].

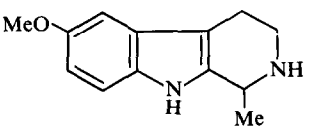
This report presents the preliminary results of our investigations into the phototoxicity of some β-carboline alkaloids, using bacterial and yeast bioassay systems. Cells, organisms and biologically important molecules can be damaged or destroyed when incubated in the light in the presence of photosensitizing compounds such as acridine orange, riboflavin, phenylthiazines and furocoumarins such as 8-methoxy-psoralen [8]. The overall structural resemblance of β-carbolines to certain tricyclic photosensitizers such as the psoralens or acridines provided the initial rationale for the present investigation into their phototoxic properties.



1 R_1 = H, R_2 = H, harman
2 R_1 = H, R_2 = OMe, harmine



3 R_1 = H, R_2 = OMe, harmaline
4 R_1 = H, R_2 = OH, harmalol
5 R_1 = OMe, R_2 = H, 6-methoxy-harmalan



6 6-methoxy-tetrahydroharman

Fig. 1. Structure of β-carboline alkaloids.

RESULTS AND DISCUSSION

Initial bioassays were carried out on *Saccharomyces cerevisiae* and *Escherichia coli*, using six β-carboline alkaloids purchased from commercial sources. 8-Methoxy-psoralen (8-MP), which gives a positive phototoxic reaction with most organisms tested, served as a control. The organisms were spread on agar in petri dishes and exposed to the compounds by placing filter paper discs dosed with appropriate aliquots on to the plates. Cultures were first incubated briefly in the dark to allow diffusion of the compounds into the medium, then exposed to UV and re-incubated for 12–14 hr (see Experimental). A clear halo of inhibited growth around the paper disc was scored as a positive reaction. Controls were treated identically but were maintained in the dark throughout. Doses of 10 μg/disc were used for the initial trials. At this level, *S. cerevisiae* gave a uniformly negative response to all β-carbolines tested, but a positive response to the control, 8-MP; with *E. coli*, however, positive phototoxic responses were observed for four of the β-carbolines, viz. harman (**1**), harmine (**2**), harmaline (**3**) and 6-methoxy-harmalan (**5**), as well as for the control. Harmalol (**4**), which differs from harmaline by having a hydroxyl substituted on the aromatic ring in place of a methoxy, was inactive at the 10 μg level, as was 6-methoxy-tetrahydroharman (**6**), which differs from the corresponding harmalan (**5**) in having a fully saturated piperidine ring.

Table 1. Phototoxicity of β-carboline alkaloids in *E. coli*: effect of varying doses

Dose:	10 μg				25 μg				50 μg			
Replicates:*	UV-1	UV-2	D-1	D-2	UV-1	UV-2	D-1	D-2	UV-1	UV-2	D-1	D-2
Compound:												
Harmine	−	+	−	−	++	+	−	−	++	++	−	−
Harmaline	+	+	−	−	++	++	−	+	++	++	−	−
Harmalol	−	−	−	−	+	+	−	−	++	++	−	−
Harman	−	−	−	−	−	−	+	++	++	++	++	−
6-Methoxy-harmalan	+	+	−	−	++	++	−	+	++	++	+	+
6-Methoxy-tetra-hydroharman	−	−	−	−	−	−	−	−	−	−	−	−
8-Methoxy-psoralen (control)	++	+	−	−	++	++	−	−	++	++	−	−

*UV-1, UV-2 denote UV-exposed replicates; D-1, D-2 denote dark control replicates; + = positive reaction, zone of inhibition < 10 mm; ++ = positive reaction, zone of inhibition > 10 mm dia.; − = no reaction.

In the next series of trials, the same six compounds were tested on *E. coli* at three different dose levels, viz. 10, 25, and 50 μg per disc (Table 1). Phototoxic activity was again observed for harmaline and 6-methoxy-harmalan at all dose levels. Harmine (**2**) exhibited a positive response for one 10 μg replicate, and a negative response for the other; positive responses were observed for this compound in both replicates at the higher doses however. A positive phototoxic reaction was observed for harman in only one 50 μg replicate; harman (**1**) also inhibited growth to some degree in the two 25 μg dark controls, and in one 50 μg dark control, and thus appears to have 'antibiotic' as well as slight phototoxic activity. 6-Methoxy-harmalan was phototoxic at all three dose levels and also possessed some antibiotic activity at the higher dose levels; its saturated isomer, 6-methoxy-harman, was completely inactive at all dose levels. Harmalol again showed no activity at the 10 μg level but showed positive responses at both higher levels. These data suggest that this harmaline analogue may be potentially as phototoxic as harmaline but the presence of the polar hydroxyl group in harmalol may interfere with diffusion across the cell membrane to the site of activation. Higher concentrations of harmalol may enable enough of the compound to cross the membrane to exert an effect at the active site.

The next set of experiments tested saturating doses (50 μg/disc) of the six β-carboline alkaloids on a selection of Gram-positive and Gram-negative bacteria, and on two yeasts (Table 2). Again differential responses were observed. Harmaline and 6-methoxy-harmalan again proved to be the most photoactive of the compounds tested, giving positive responses in all organisms tested except *Pseudomonas fluorescens*. This organism was also negative to 8-methoxy-psoralen, the control compound, and could well have specialized membranes, proteins, or other components which afford it protection from most photoactive compounds. 6-Methoxy-tetrahydroharman was again completely inactive for all organisms tested. Harmine, harman, and harmalol all gave variable activity, and in general were active only in the most photosensitive organisms (those sensitive to the greatest numbers of compounds).

Thus, differences in phototoxicity were found to be related to structure, dosage, and to the type of organism tested. The information related to different doses (Table 1) and the type of organism tested (Table 2) suggest that one of the primary determinants of photoactivity in these compounds is related to membrane permeability. A secondary determinant is related to the degree of unsaturation of the piperidine ring. 6-Methoxy-harmalan, (**5**) was consistently the most photoactive of any compounds tested, while 6-methoxy-tetrahydroharman (**6**) which lacks a 1–2 double bond, was completely inactive. The presence of a 3–4 double bond reduces activity (cf. harmine and harman with harmaline and harmalol). These observations are particularly interesting since 6-methoxy-harmalan and 6-methoxy-tetrahydroharman may occur in a precursor–product relationship as natural hormonal components of the mammalian pineal gland, an organ known to be sensitive to light [16–18]. The photoactivity of β-carbolines of this type suggest that they may function as pineal photoreceptors. Further studies of the phototoxic properties of these β-carbolines are being undertaken in our laboratory.

EXPERIMENTAL

Yeasts were cultured on Sabaroud's medium, bacteria were cultured on Difco nutrient agar. Plates were spread according to the method of ref. [19]. Test and control compounds were absorbed on to sterile filter paper discs (7 mm dia.) in 10 μg aliquots using an Eppendorf pipette. Standard solns of ref. compounds (in MeOH) of the appropriate concn (1.0, 2.5 or 5.0 mg/ml) were used. Following evapn of the solvent, the discs were applied to the agar plates using sterile technique. Two experimental replicate plates and two control plates were used for each treatment. 8-MP was used as a control on all plates. Control plates were wrapped in foil to prevent light exposure, and otherwise treated identically to experimental replicates. Cultures were incubated for 30 min at 30° prior to UV exposure to allow diffusion of the compounds into the surrounding medium, then irradiated with UV (300–400 nm, Sylvania F20T12BLB) at 35° for 1 hr. Placement of the lamp was *ca* 12 cm from the agar surface. Following exposure, cultures were incubated at 35° for 12–14 hr, then scored for phototoxic response. A clear zone of inhibition surrounding the disc was interpreted as a positive reaction (+); zones with dia. greater than 10 mm were scored as ++.

Table 2. Phototoxicity of β-carboline alkaloids: inhibition of bacterial and yeast growth in UV light

Compound (50 μg/disc):	Harmine		Harmaline		Harmalol		Harman		6-Methoxy-harmalan		6-Methoxy-th-harman†		8-Methoxy-psoralen	
Treatment*:	UV	Dark	UV	Dark	UV	Dark	UV	Dark	UV	Dark	UV	Dark	UV	Dark
Gram + bacteria														
Bacillus subtilis	+	−	+ +	−	−	−	−	−	+	−	−	−	+ +	−
Streptococcus faecalis	−	−	+ +	−	−	−	−	−	+ +	−	−	−	+ +	−
Staphylococcus albus	+ +	−	+ +	−	+ +	−	+	−	+ +	−	−	−	+ +	−
Gram − bacteria														
Pseudomonas fluorescens	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Proteus mirabilis	+ +	−	+ +	−	+ +	−	+	−	+ +	−	−	−	+ +	−
Yeasts														
Candida albicans	−	−	±	−	−	−	−	−	+	−	−	−	+ +	−
Saccharomyces cerevisiae	±	−	+ +	−	+	−	+	−	+ +	−	−	−	+ +	−

*Two replicates/treatment for each compound and organism tested. + = positive reaction, zone of inhibition 10 mm or less; + + = positive reaction, zone of inhibition > 10 mm; − = no reaction.

†6-Methoxy-tetrahydroharman.

Acknowledgements—The authors are grateful to K. Downum for helpful suggestions and instructions in the phototoxicity bioassay procedure. Thanks also to L. Johns for providing the figure, and Z. Abramowski for technical assistance. We thank the National Science and Engineering Council of Canada for financial support of this work.

REFERENCES

1. Manske, R. H. F. (1965) in *The Alkaloids, Chemistry and Physiology*, Vol. 8, Chap. 3. Academic Press, New York.
2. Raffauf, R. A. (1970) *A Handbook of Alkaloids and Alkaloid-Containing Plants*. Wiley-Interscience, New York.
3. Fuentes, J. A. and Longo, V. G. (1971) *Neuropharmacology* **10**, 15.
4. Rivier, L. and Lindgren, J. E. (1972) *Econ. Botany* **26**, 101.
5. Deulofeu, V. (1967) in *Ethnopharmacologic Search for Psychoactive Drugs* (Efron, D. H., Holmstedt, B. and Kline, N. S., eds.) U.S. Public Health Serv. Publication No. 1645.
6. Pinkley, H. V. (1969) *Lloydia* **32**, 305.
7. Schultes, R. E. (1972) *Harv. Bot. Mus. Leafl.* **23**, 137.
8. Naranjo, C. (1967) in *Ethnopharmacologic Search for Psychoactive Drugs* (Efron, D. H., Holmstedt, B. and Kline, N. S., eds.) p. 385. U.S. Public Health Serv. Publication No. 1645.
9. Pennes, H. H. and Hoch, P. H. (1957) *Am. J. Psychiat.* **113**, 887.
10. Undenfriend, S., Witkop, B., Redfield, B. G. and Weisbock, H. (1958) *Biochem. Pharmacol.* **1**, 160.
11. Der Marderosian, A. H., Pinkley, H. V. and Dobbins, M. F. (1968) *Am. J. Pharmac.* **140**, 137.
12. Smythies, J. R. and Antun, F. (1969) *Nature (London)* **223**, 1061.
13. Farrel, G. and McIsaac, W. M. (1961) *Arch. Biochem. Biophys.* **94**, 543.
14. McIsaac, W. M. (1961) *Biochim. Biophys. Acta* **52**, 607.
15. Spikes, J. D. (1977) in *The Science of Photobiology* (Smith, K. C., ed) Chap. 4. Plenum, New York.
16. Moore, P. Y., Heller, A., Wurtman, R. J. and Axelrod, J. (1967) *Science* **155**, 220.
17. Snyder, S. H., Axelrod, J., Fischer, J. E. and Wurtman, R. J. (1964) *Nature (London)* **203**, 98.
18. Axelrod, J., Wurtman, R. J. and Snyder, S. H. (1965) *J. Biol. Chem.* **240**, 949.
19. Daniels, F. (1965) *J. Invest. Dermatol.* **44**, 259.