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DICTAMNINE, AN ALKALOID WHICH CROSSLINKS DNA IN THE PRESENCE OF ULTRAVIOLET LIGHT

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SUMMARY

In the presence of ultraviolet light, the furoquinoline alkaloid, dictamnine, caused calf thymus DNA to become easily renaturable. The effect was less pronounced than for the furocoumarin, 8-methoxypsoralen. Ease of renaturation is evidence of the formation of interstrand crosslinks in DNA. The mechanism of crosslink formation by this alkaloid may be like that of 8-methoxypsoralen.

Recently we have found that the furoquinoline alkaloid, dictamnine, is phototoxic for microorganisms (1). This alkaloid which is found in plants of the family Rutaceae (2) has a structure similar to that of the linear furocoumarin, 8-methoxypsoralen (Fig. 1). Psoralens such as 8-methoxypsoralen are phototoxic, probably because of reaction with pyrimidine bases of DNA, which occurs in the presence of ultraviolet light. Two types of photoproducts are formed: monoadducts and biadducts. There is evidence that two monoadducts are produced; both the 3,4- and 4',5'- C=C bonds of psoralen can react with pyrimidines in DNA (3). Biadducts are created by reaction of a psoralen molecule with pyrimidine bases on either side of a DNA double helix. Biadducts thus damage DNA because they form interstrand crosslinks. (For reviews on psoralen action, see references (4) and (5).)

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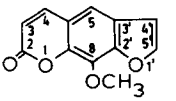
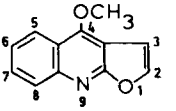


8-methoxypsoralen



Dictamnine

Fig. 1: Structures of 8-methoxypsoralen and dictamnine.

Since it seemed that dictamnine could react in a manner similar to that of 8-methoxypsoralen, we tested the alkaloid for its *in vitro* reaction with DNA. Our results show that dictamnine reacts with DNA to form interstrand crosslinks.

MATERIALS AND METHODS

Dictamnine was isolated in the laboratory of Dr. I.D. Spenser, Dept. of Chemistry, McMaster University (6). 8-Methoxypsoralen was obtained from Sigma Chemical Company, St. Louis, Mo. Each compound was dissolved in 95% ethanol. The concentration of dictamnine was determined spectrophotometrically. (The molar extinction coefficient of dictamnine at 308 nm is 7586 (7).) A solution of 8-methoxypsoralen was made by dissolving 20.7 mg in 95% ethanol to a volume of 100 ml.

Ten µg (or 120 µg) of each compound in ethanol was mixed with 2 ml of a solution of calf thymus DNA (Sigma, Type 1) in 0.02 M phosphate buffer. All phosphate buffers contained equimolar amounts of NaH_2PO_4 and Na_2HPO_4 (pH 6.4). The solution of DNA had an absorbance of 2.0 at 260 nm.

The solutions in plastic dishes (Falcon, 35 x 100 mm style) with lids, were incubated at 35° under a bank of four Sylvania (or Westinghouse) black light blue fluorescent bulbs (type F20T12-BLB) for 2½ hours. Dark control dishes were wrapped in aluminum foil. Solutions were shaken slowly during irradiation.

The method which we used to determine crosslinks in DNA is similar to that of Cole (8), who first showed that 8-methoxypsoralen reacts with DNA to form crosslinks. After irradiation, the solutions were transferred to test tubes, placed in boiling water for 10 min and then placed in an ice bath for 5 min. This procedure denatures native DNA, but allows renaturation of crosslinked DNA. Aliquots of the DNA solution were chromatographed on a column which contained 4 g of hydroxylapatite (Bio-Rad, DNA grade). DNA which contained crosslinks (and was renatured) and denatured DNA were separated by elution of the column with a linear gradient (200 ml) of 0.02 - 0.6 M phosphate buffer. The absorbance of the column eluate was monitored at 254 nm by the use of an ISCO monitor (model UA-5) and optical unit (type 6). Fractions of 70 drops (3.6 ml) were collected. Since the flow cell had a light path of 5 mm, absorbance measurements were doubled to obtain absorbance for an optical path of 1 cm.

RESULTS

DNA without crosslinks was totally denatured to single-stranded DNA by our boil-cool procedure, but DNA which contains crosslinks was renatured to form double-stranded DNA (8). Thus, any double-stranded DNA which reforms was evidence of crosslinks. Fig. 2 shows that dictamnine caused crosslink formation in calf thymus DNA. Double-stranded DNA which eluted between fractions 24 - 30 was formed in the presence of ultraviolet light and dictamnine or 8-methoxypsoralen (Fig. 2). However, in the absence of ultraviolet light, we observed only single-stranded DNA, which eluted between fractions 17 - 24. Fewer DNA molecules with interstrand crosslinks were formed by 120 µg of dictamnine than by 10 µg of 8-methoxypsoralen (Fig. 2). Only a very small proportion of the DNA was crosslinked by 10 µg of dictamnine. Ethanol alone, when added in the same amounts as for each previous experiment, did not photoreact with calf thymus DNA to produce crosslinks. Thus, dictamnine produced crosslinks, but not as effectively as 8-methoxypsoralen.

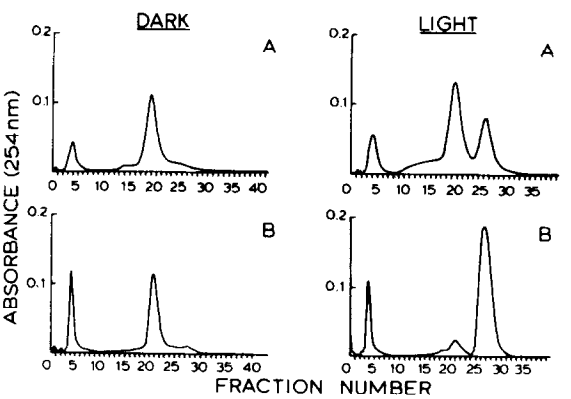


Fig. 2: Elution patterns of calf thymus DNA after hydroxylapatite chromatography. DNA was treated with dictamnine (120 µg, Fig. 2A) or 8-methoxypsoralen (10 µg, Fig. 2B) as described in Materials and Methods. DNA solutions (1.4 ml) were applied to hydroxylapatite columns. The absorbance scale is 0-0.2.

DISCUSSION

The 2,3- and 5,6- C=C bonds of dictamnine are comparable to the 3,4- and 4',5'- C=C bonds of 8-methoxypsoralen (Fig. 1). These double bonds of 8-methoxypsoralen have been shown to be responsible for its photoreaction with DNA (3). In crosslink formation, the 2,3- and 5,6- C=C bonds of dictamnine may also photoreact with pyrimidine rings in DNA. A preference of 8-methoxypsoralen for formation of a crosslink between thymine of one nucleotide pair and cytosine of the next pair (9) may also take place in the dictamnine photoreaction.

Dictamnine is phototoxic for Escherichia coli and Saccharomyces cerevisiae (1), which may be a result of the formation of DNA crosslinks in vivo. Dictamnine, through its action on DNA in the presence of ultraviolet light, may also contribute to the photodermatitis which is evoked by plants of the family Rutaceae (10).

A combination of 8-methoxypsoralen administration and ultraviolet light has been used for treatment of the skin disease, psoriasis. There is concern about 8-methoxypsoralen and ultraviolet light therapy since this treatment can cause skin tumors in mice (4), chromosome abnormalities in human lymphocytes (11) and mutations in animal cells (12), although psoralen-DNA crosslinks can be repaired in the skin of guinea pigs (13). It seems reasonable that before use of dictamnine in treatment of psoriasis, the toxicity and mutagenic potential of the compound should be compared with that of psoralens. Also, the extent and speed of repair of crosslinks which are induced by psoralens and dictamnine should be compared. It seems likely that if repair of dictamnine crosslinks is less than those of 8-methoxypsoralen, phototoxicity will be enhanced.

Indeed, 8-methoxypsoralen plus ultraviolet light is less phototoxic for the wild-type than for repair-deficient mutants of Saccharomyces (15). Comparisons between dictamnine and psoralen action will aid in the estimation of the potential hazard of administration of dictamnine.

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REFERENCES

1. Towers, G. H. N., Graham, E. A., Spenser, I. D. and Abramowski, Z. A., unpublished results.
2. Mester, I. (1973) Fitoterapia 44, 123-152.
3. Bordin, F., Musajo, L. and Bevilacqua, R. (1969) Z. Naturforsch. B, 24, 691-693.
4. Song, P. and Tapley, K. J. (1979) Photochem. Photobiol. 29, 1177-1197.
5. Scott, B. R., Pathak, M. A. and Mohn, G. R. (1976) Mutat. Res. 39, 29-74.
6. Monkovic, I., Spenser, I. D. and Plunkett, A. O. (1967) Can. J. Chem. 45, 1935-1948.
7. CRC Atlas of Spectral Data and Physical Constants for Organic Compounds, 2nd Edition (1975) (Grasselli, J. G. and Ritchley, W. M., eds.) Vol. 3, p. 190, CRC Press, Cleveland.
8. Cole, R. S. (1970) Biochim. Biophys. Acta 217, 30-39.
9. Dall'Acqua, F., Vedaldi, D., Bordin, F. and Rodighiero, G. (1979) J. Invest. Dermatol. 73, 191-197.
10. Mitchell, J. C. and Rook, A. (1979) Botanical Dermatology pp. 606-629. Lea & Febiger, Philadelphia.
11. Waksvik, H., Brogger, A. and Stene, J. (1977) Hum. Genet. 38, 195-207.
12. Burger, P. M. and Simons, J. W. I. M. (1979) Mutat. Res. 60, 381-389.
13. Chandra, P., Biswas, R. K., Dall'Acqua, F. Marciani, S., Baccichetti, F., Vedaldi, D. and Rodighiero, G. (1973) Biophysik 9, 113-119.
14. Carter, D. M., Pan, M., Gaynor, A., McGuire, J. S. and Sibrack, L. (1979) J. Invest. Dermatol. 73, 97-101.
15. Averbeck, D. and Moustacchi, E. (1975) Biochim. Biophys. Acta 395, 393-404.