ON THE INTERACTION BETWEEN JATROPHONE AND DNA

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1. Introduction

Jatrophone (fig.1) is a macrocyclic diterpene first isolated [1] from an alcoholic extract of *Jatropha gossypiifolia* L. (Euphorbiaceae) which shows a strong inhibitory activity in vitro against cells obtained from human carcinoma of the nasopharynx (KB) and in vivo against animal tumor systems, such as sarcoma 180, Lewis lung carcinoma, P-388 lymphocytic leukemia and the Walker 256 intramuscular carcinosarcoma. In fact, extracts of *Jatropha gossypiifolia* and related species have been employed in the treatment of tumors [2]. The stereochemistry and structure of Jatrophone was elucidated by chemical and X-ray crystallographic studies [3] and the strategy for total synthesis reported [4]. α-Methylene γ-lactone function is essential to the tumor inhibitor activity [5,6]; nucleophilic addition of thiol groups on proteins to the α,β-unsaturated lactone may be involved as in the case of RNA polymerase with loss of enzymatic activity [7].

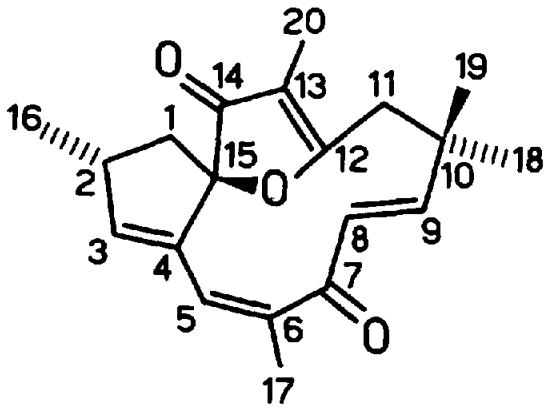

Fig.1. Molecule of Jatrophone.

Jatrophone is insoluble in water so that its pharmacological application is almost limited. To promote its solubility in water and to gain insight on the possible binding with DNA we attempted to bind it to two DNA species, from *Escherichia coli* and *Micrococcus lysodeikticus*.

This paper deals with the results of spectroscopic studies concerning the Jatrophone–DNA systems.

2. Materials and methods

Jatrophone, isolated from extracts in hexane of *Jatropha* species, was purified on silica gel column (70–270 mesh; Macherey Nagel, Düren) with chloroform–hexane (70/30, v/v) as eluant; the solid residue obtained by evaporation of the chloroform–hexane extract, repeatedly crystallized from hexane, yield colourless needles, m.p. 152.5–154°C; $[\alpha]_{589}^{25°}$ = 352.1 and $[\alpha]_{579}^{25°}$ = 373.8 (0.2352% in 95% ethanol, Uvasol grade, Merck); ϵ_M = 10 960 l . mol^{-1} . cm^{-1} at 282 nm and ϵ_M = 13 300 l . mol^{-1} . cm^{-1} at 220 nm (7.52×10^{-4} M in 95% ethanol, Uvasol grade, Merck). The absorption and circular dichroism (CD) spectra in Tris–HCl 0.01 M–ethanol (90/10, v/v, pH 7.30) solution are shown in fig.2.

X-Ray photographs, recorded on a single crystal with Cu Kα radiation, gave unit cell parameters in good agreement with those reported and the same space group [3]. Mass spectra agreed with the presence of the Jatrophone molecule. The purity of the Jatrophone crystals was checked by HPLC, obtaining a very sharp single peak. The concentration of Jatro-

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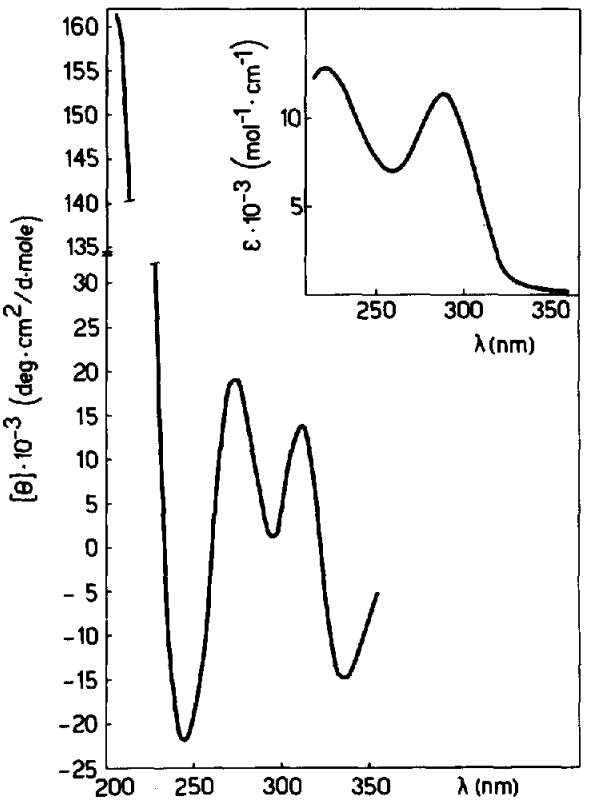

Fig.2. CD spectrum of Jatrophone 5.3×10^{-5} M, in Tris–HCl 0.01 M–ethanol (90/10, v/v) solution (pH 7.30). The absorption spectrum of Jatrophone at the same condition, is shown upper right.

phone in the Jatrophone–DNA solutions was determined on the basis of the molar ellipticity values of Jatrophone at 335,337.5 and 340 nm in Tris–HCl 0.01 M–ethanol solution ($[\Theta]_{335} = -15\,125$; $[\Theta]_{337.5} = -15\,130$; $[\Theta]_{340} = -14\,152$ deg . cm^2 . $dmol^{-1}$). These molar ellipticity values are not concentration-dependent and unaffected by the DNA binding within the concentration range studied here. Notice, however, that in the Jatrophone–DNA solutions the probable absence of ethanol may slightly influence the molar ellipticity.

Escherichia coli (type VIII) and *Micrococcus lysodeikticus* (type XI) DNA were products of Sigma (St Louis MO). Stock solutions of DNA were freshly prepared in 0.01 M Tris–HCl (pH 7.30), exhaustively dialysed against the same buffer and used immediately or, stored frozen at –20°C. The following extinction coefficients with respect to nucleotide were used for concentration determination: *Escherichia coli* DNA, 6500 l . $mol.^{-1}$. cm^{-1} at 258 nm [8] *Micrococcus lysodeikticus* DNA, 6300 l . $mol.^{-1}$. cm^{-1} at 260 nm [9]. Doubly glass-distilled water was used throughout.

The binding of Jatrophone to DNA was accomplished as follows: A 7×10^{-4}–7×10^{-3} M solution of Jatrophone in ethanol (Uvasol, Merck) was added portion-wise (10–20 μl) to a cooled (2–4°C) DNA aqueous solution ($3–4 \times 10^{-4}$ M). The ethanol contained in the solution was always in an amount of 7% v/v. The solution was kept for 100 h with shaking at 2–5°C using a Griffin flask shaker. Lyophilization of the solution and resolubilization in Tris–HCl 0.001 M (pH 7.30) with subsequent, after 24 h, centrifugation at 10 000 rev./min at 5°C, allowed to obtain solution without ethanol.

Attempts to bind even a moderate amount of Jatrophone to DNA by means of the solvent-partition method [10], using ethyl acetate–hexane mixtures, were unsuccessful.

Absorption measurements were carried out with Beckman DK-2A and Cary 219 spectrophotometers. Circular dichroism (CD) spectra were recorded on a Cary 61 spectropolarimeter; the slit width was programmed to ensure wavelength accuracy of better than 0.5 nm. The calculated curves both for the absorption and CD spectra were computed by assuming the contribution of Jatrophone and DNA simply additive. Optical rotations were obtained using a Perkin-Elmer 141 M spectropolarimeter. X-Ray measurements were performed by Weissenberg and precession cameras using a 1001 Iso-Debyeflex generator of the Rich Seifert Co.

3. Results and discussion

Aqueous solutions with different Jatrophone/nucleotide ratios were studied by absorption and CD spectra. The samples were prepared by using the same concentration of the two DNA species and adding the same amount of Jatrophone. Thus, nearly equal Jatrophone/nucleotide ratios were obtained for both DNAs at variance with the trend observed for the tingenone–DNA systems [11]. The range of the ratios examined is included within ~0.02 and ~0.66. As an example, two spectra, representative of the Jatrophone–DNA behaviour at low and high ratios, are reported for each DNA. Fig.3 shows the observed and calculated absorption spectra of samples containing a Jatrophone molar concentration of 1.8×10^{-5},

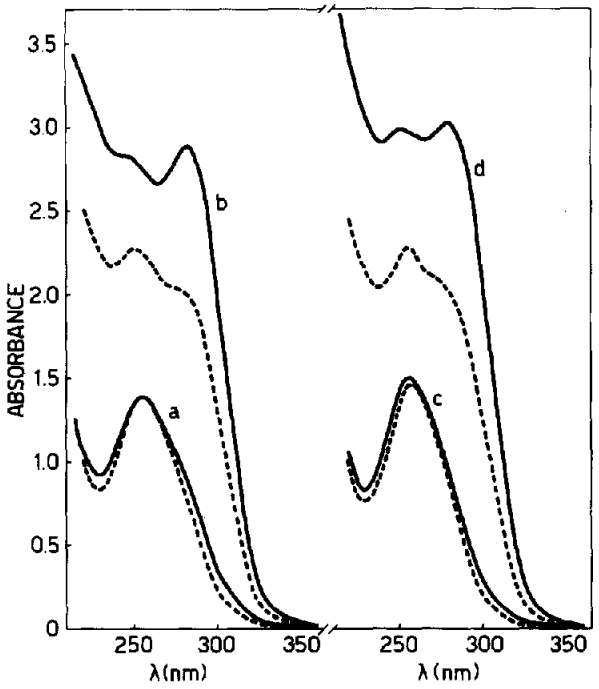

Fig.3. Absorption spectra of Jatrophone with: (a,b) *Micrococcus lysodeikticus* DNA; (c,d) *Escherichia coli* DNA. The Jatrophone/nucleotide ratios are: (a) 0.09; (b) 0.66; (c) 0.08; (d) 0.63; (——) observed spectra; (– – –) calculated spectra.

1.38×10^{-4}, 1.6×10^{-5} and 1.3×10^{-4} for curves a–d, respectively. Curves b and d indicate that a remarkable hyperchromic effect occurs when a great amount of Jatrophone interacts with DNA. From other spectra, recorded for Jatrophone/nucleotide intermediate ratios (not shown), it is clear that the hyperchromicity increases with the Jatrophone/nucleotide ratio. This trend is evidence of the binding of Jatrophone to DNA, which improves its stability raising the melting temperatue (fig.4) where the hyperchromicity $(A_T - A_{T_0}/A_{T_0}) \times 100$ *vs* the temperature is reported. A_T and A_{T_0} are the absorptions at 260 nm of a solution at a given temperature and at a reference temperature (27°C), respectively. Unfortunately, since Jatrophone is insoluble in water–Tris–HCl 0.01 M its contribution to the total absorbance could not be subtracted. However, the A_{260} of Jatrophone in ethanol–Tris–HCl 0.01 M (pH 7.32)(10–90, v/v) does not vary within 27–78°C, so that its constant contribution can be neglected. The melting temperature, calculated as the temperature corresponding to half of the maximum value of the hyperchromicity, increases with the Jatrophone/nucleotide ratio from 61.6–64.6°C for the *Escherichia coli* DNA and from 70.9–74.6°C for the *Micrococcus lysodeikticus* DNA. A comparison with the behaviour of tingenone [11] shows that a much smaller amount of this compound causes a higher increase of the melting temperature. This may be interpreted in terms of a weaker binding of Jatrophone to DNA with respect to that of tingenone. Thus it seems plausible to suppose that Jatrophone interacts with DNA on a limited surface, so that the DNA may be surrounded by pendant molecules.

Another evidence of the Jatrophone–DNA bind-

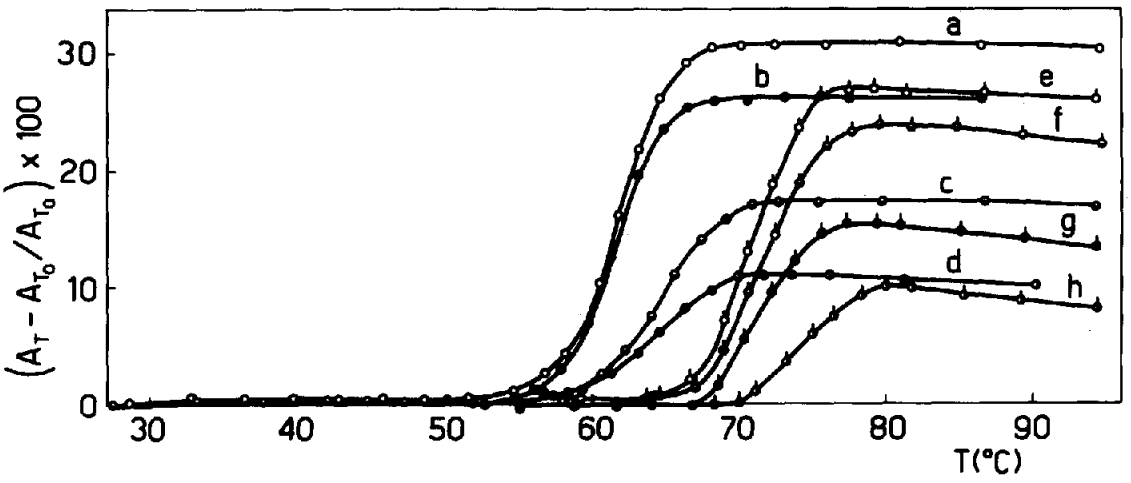

Fig.4. Thermal profiles of Jatrophone with *Escherichia coli* (a–d) and *Micrococcus lysodeikticus* (e–h) DNA aqueous solutions 0.01 M Tris–HCl (pH 7.32). The DNA molar concentration is 2.07×10^{-4} and the Jatrophone/nucleotide ratios are 0,0.08,0.30,0.63,0, 0.09,0.42 and 0.66 for samples a–h, respectively.

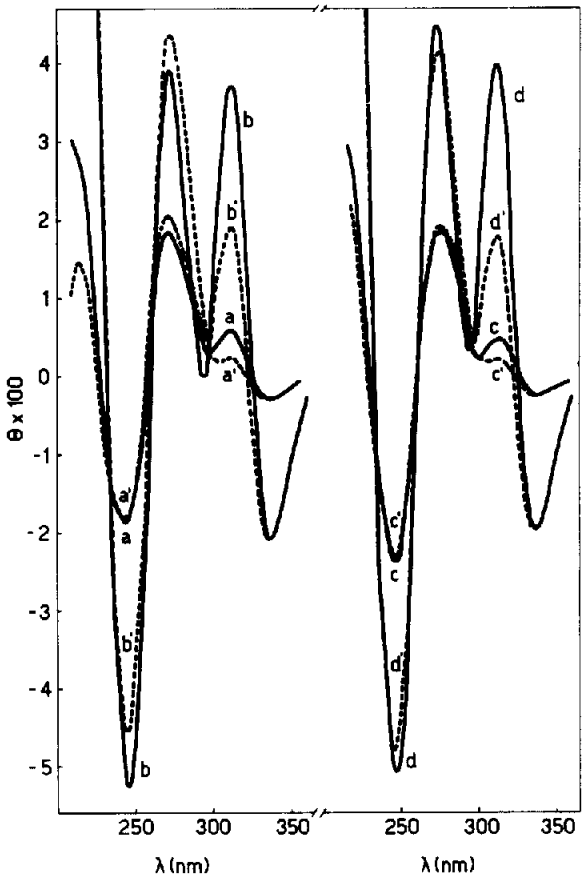

Fig.5. Observed (——) and calculated (– – –) CD spectra of Jatrophone with *Micrococcus lysodeikticus* DNA (a,a',b,b') and *Escherichia coli* DNA (c,c',d,d') aqueous solutions 0.01 M Tris–HCl (pH 7.32). The DNA molar concentration is 2.07×10^{-4} and the Jatrophone/nucleotide ratios are as in fig.3.

ing is shown in fig.5, where the CD band at ~312 nm can be taken as representative for Jatrophone, since DNA does not contribute at this wavelength. Hyperellipticity is observed for all the samples and the molar ellipticity of Jatrophone–DNA greatly differs from that of Jatrophone ($[\Theta]_{312}$ = 13 917 deg . cm^2 . dmol^{-1}, fig.1). This assertion can be verified by inspection of fig.6, showing the trend of the molar ellipticity of Jatrophone–*Micrococcus lysodeikticus* DNA for some Jatrophone concentration. Increasing the Jatrophone content the [Θ] value tends to decrease for the samples a–d and, subsequently, rises for the sample e. This behaviour may be ascribed to different types of interaction involving the Jatrophone molecules. Two explanations seem to be more plausible:

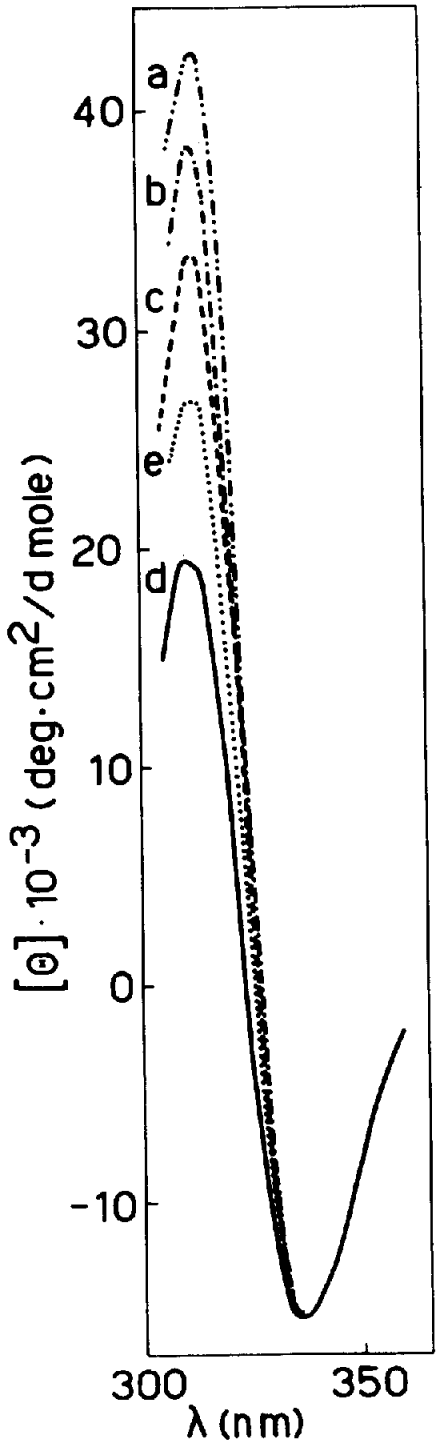

Fig.6. CD spectra of Jatrophone–*Micrococcus lysodeikticus* DNA aqueous solutions 0.01 M Tris–HCl (pH 7.32). The DNA molar concentration is 2.07×10^{-4} and those of Jatrophone are: (a) 5.5×10^{-6}; (b) 7.5×10^{-6}; (c) 1.8×10^{-5}; (d) 8.7×10^{-5}; (e) 1.4×10^{-4}.

1. The formation of 2 types of binding of Jatrophone to DNA (for example, two hydrogen bonds between the DNA phosphate groups and the C_7 and C_{14} keto groups of Jatrophone).
2. One type of binding between Jatrophone and DNA and Jatrophone–Jatrophone interactions when these molecules begin to crowd together on the DNA outer surface.

These results may be important to account for the biological activity of Jatrophone besides the mechanism of the nucleophilic addition of the sulfhydryl groups [5,7].

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